GermanStemCellNetwork

2nd International Annual Conference

of the German Stem Cell Network (GSCN)
November 3-5, 2014

German Cancer Research Center (DKFZ)
Heidelberg

Program & Abstracts

www.gscn.org

Federal Ministry of Education and Research

VDI Technologiezentrum

dkfz. GERMAN CANCER RESEARCH CENTER IN THE HELMHOLTZ ASSOCIATION

50 Years – Research for A Life Without Cancer

GermanStemCellNetwork

2nd International Annual Conference German Stem Cell Network (GSCN)

German Cancer Research Center (DKFZ) Heidelberg November 3 – 5, 2014

Organization:
German Stem Cell Network (GSCN)
c/o Max Delbrück Center (MDC)
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13125 Berlin

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web: www.gscn.org

Dear Stem Cell Colleagues,

It is a great pleasure to welcome you to the **2nd International Annual Conference of the German Stem Cell Network (GSCN)** at the German Cancer Research Center (DKFZ) in Heidelberg.

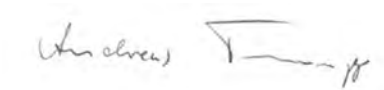
The most important development during 2014 has undoubtedly been the continued commitment of the Bundesministerium für Bildung und Forschung (BMBF) to support the GSCN for three more years. This was possible due to the overwhelming German-wide support from all of our members and the success of the 1st Annual GSCN Conference in Berlin last autumn. Today, we are very excited to start the second GSCN conference with a packed program and even more cutting-edge research as well as a variety of workshops.

The 2nd International Annual Conference of the GSCN provides an opportunity for scientists, clinicians, educators and industry professionals to share new data, learn from peers, and discover global advances within the stem cell field. Topics cover the latest findings in stem cell biology, from genetic and epigenetic mechanisms of reprogramming and maintenance of pluripotency, to the molecular basis of self-renewal and differentiation. The conference will foster the interaction between researchers working on different types of stem cells, including embryonic stem cells, stem cells during development and in adult organs as well as cancer stem cells. Moreover, the application of stem cells in regenerative therapies, disease modeling and drug development will represent the translational and applied angle of the field. Finally, sessions on strategic issues relating to stem cell research such as career development, public outreach, funding policies and clinical trials using stem cells will complete the program.

With many speakers selected from the best abstracts, the conference is a platform for the entire scientific community providing unique opportunities especially for young talented scientists. The program is flanked by a few selected Keynote lectures given by international leaders in their field. The GSCN conference will be directly followed by the Joint Satellite Symposium on Neural Stem Cells which lasts until November 6th (free of charge for GSCN Conference participants).

We are looking forward to three busy days packed with input, discussion, new insights and new contacts, the refreshing of old connections and a constantly growing fascination for our research with the most exciting cells we know of: stem cells. I am delighted to see all of you in my hometown of Heidelberg and wish you a stimulating and productive meeting.

Best wishes,



Andreas Trumpp (GSCN Acting President)

For the program committee

Daniel Besser (Berlin) • Thomas Braun (Bad Nauheim) • Oliver Brüstle (Bonn) • Micha Drukker (Munich) • Ulrich Martin (Hannover) • Ana Martin-Villalba (Heidelberg) • Andreas Trumpp (Heidelberg)

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Conference information

Venue:
DKFZ Communication Center, Heidelberg
Im Neuenheimer Feld 280
69120 Heidelberg

Date:
Monday, November 3 to Wednesday, November 5, 2014

Registration

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

The registration fee includes attendance at all scientific sessions, November 3 – 5, conference documents, name badge, final program and abstract book, conference bag, Networking Evening, coffee breaks, lunch, dinner and free internet access.

Posters:
Posters will be displayed during the meeting in 2 sessions in the exhibition area.
The size of a single poster should not exceed 1m x 1,20m (width / height).
You will find the number of your poster in this abstract volume. According to this number, you may mount your poster in the exhibition area.

Posters scheduled for **Poster session I** (Poster numbers P001 – P097) can be viewed on Monday, November 3, 2014. The posters in this session should be set-up on Monday, 12:00 h – 16:00 h and removed latest on Tuesday 13:00 h.

Posters scheduled for **Poster session II** (Poster numbers P098 – P187) can be viewed on Tuesday, November 4, 2014. The posters in this session should be set-up on Tuesday, 13:00 h – 17:00 h and removed latest on Wednesday 14:00 h.

Internet:
Internet access via Wireless LAN is free of charge in the Communications Center.
Username: g-guest46; Password: mosamoli

GSCN navigator:
Install the official app for the German Stem Cell Network (GSCN) and event guide for the GSCN Conference 2014 on your smartphone:

- Overview of the program and portfolio
- Create your own program for the event (MyPlan): bookmark the sessions to attend
- Networking tool: find other participants and schedule meetings with them
- Use map to navigate to the venue

Information on the workshops at the GSCN Conference 2014

Banking of human pluripotent stem cells – workshop
ZMBH, Tuesday, November 4th; 14:30 – 15:30 h
(Chair: Andreas Kurtz / Joana Namorado)

Human embryonic stem cells and induced human pluripotent stem cells (PSC) have opened a wide range of possibilities in therapeutical settings and for disease modeling and drug development. Large scale banking of these cells has become a major trend worldwide. Several PSC banking projects in Europe are funded by the European Commission and pharmaceutical industry for preclinical drug assessment, mode-of-action studies and therapeutic use. Global registration of pluripotent stem cells will be instrumental for the efficient future use of these resources. This workshop will show the current efforts on derivation, banking and registration of human PSCs.

Clinical relevance of animal models in stem cell research and regenerative medicine
Lecture Hall, Wednesday, November 5th; 11:00 – 12:30 h
(Chair: Georg Duda / Frank Emmrich)
(Workshop of the 'Regenerative Medicine Initiative Germany (RMIG)')

Suitable animal models are of central importance to the development of effective therapies, especially in the field of stem cell research and regenerative medicine. In basic research, lower vertebrates and small rodents under standard 'Safety Pathogen Free' housing conditions are the preferred model organisms, while large animal models as well as small animal models represent an important step in the preclinical evaluation of cell-based therapies. The institutes in the RMIG are working to improve the quality and predictability of pre-clinical animal models for stem cell and regenerative therapies. Based on the expertise in the institutes, a panel aims to enter into a critical discussion on the clinical relevance of animal model systems.

Big pharma and the stem cell field – matching expectations?
K 1+2, Wednesday, November 5th; 11:00 – 12:30 h
(Organizers: Ira Herrmann / Oliver Brüstle)
(Workshop of the Stem Cell Network North Rhine Westphalia)

Disease modeling and drug development are considered major deliverables of translational stem cell research. However the expectations of the pharmaceutical industry from this rapidly evolving area have yet to be defined. This workshop features experts from drug discovery and compound screening programs of large pharmaceutical companies. It's objective is to provide the stem cell community with a real world view on this vibrant topic and enable future interactions in this scientifically and economically fulminating field.

Panel:
Oliver Brüstle; *LIFE & BRAIN GmbH, Bonn*
Ralf Heilker; *Boehringer Ingelheim Pharma GmbH & Co. KG, Div. Research Germany*
Jörg Hüser; *Bayer Pharma AG, Lead Discovery Wuppertal*
Bert Klebl; *Lead Discovery Center GmbH, Dortmund*
Moderation: *Ira Herrmann; Stem Cell Network NRW, Düsseldorf*

GSCN Travel Awards

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

- Cantas Alev (RIKEN Center for Developmental Biology, Kobe, Japan)
- Janine Müller (University of Bielefeld)
- Susanne Rahmig (Technical University Dresden)
- Yuval Rinkevich (Stanford University, California, United States)

GSCN
GermanStemCellNetwork

Poster awards

The **poster awards** are supported by **Peprotech GmbH**.

There will be two poster awards for each poster session:

Poster session I: Monday, November 3rd, 2014, 16:15 – 18:15 h

- Pluripotency and embryonic stem cells (P001 – P017)
- Programing and Reprograming (P018 – P038)
- Stem cells in development (P039 – P061)
- Somatic stem cells (P062 – P072)
- Hematopoietic stem cells (P073 – P097)

PEPROTECH
Manufacturer of Quality Cytokine Products

Even numbers: will be presented from 16:15 – 17:15 h
Uneven numbers: will be presented from 17:15 – 18:15 h

Poster session II: Tuesday, November 4th, 2014, 17:15 – 19:15 h

- P098 – P129: Stem cells in regenerative therapies
- P130 – P161: Stem cells in disease modeling and drug development
- P162 – P178: Stem cells in disease: Cancer stem cells
- P179 – P187: Computational stem cell biology

Even numbers: please present your poster from 17:15 – 18:15 h
Uneven numbers: please present your poster from 18:15 – 19:15 h

Explaining science and motivating people – outreach activities

Presentation of outreach materials and formats by the working group 'Public engagement and outreach activities' (*Tobias Cantz / Ira Herrmann*)

Tuesday, November 4th, 13:15 – 14:15,
room K 1+2 *and*

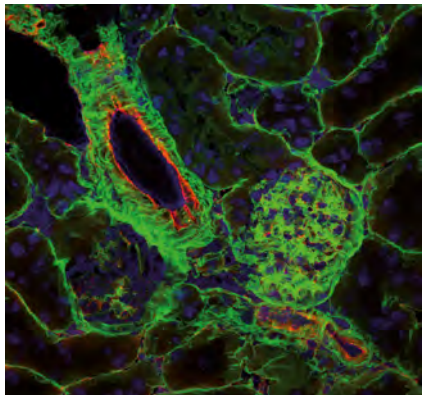
Wednesday, November 5th, 13:45 – 14:45,
room K1+2

Stop by and get to know different tools, activities, materials and games to communicate stem cell research to different audiences.
The next 'open science day' or 'night of sciences' in your institute is just around the corner!

Public outreach event

Stammzellen als Chance – Realität und Perspektiven

Podiumsdiskussion zur aktuellen Forschung in Labor und Klinik



Mittwoch, 5. November, 18:30 – 20 Uhr,
Hörsaal DKFZ

Krebs- und neurodegenerative Erkrankungen wie Alzheimer und Parkinson nehmen weltweit zu. Die Stammzellforschung weckt große Hoffnungen bei Patienten: Doch wo steht die Stammzellforschung? Bei der Therapie von Leukämien gehört die Stammzelltransplantation zum erfolgreichen Behandlungsrepertoire. Für andere Erkrankungen dienen Stammzellen als Modell in der Forschung oder werden gar als bedeutend für den Krankheitsverlauf, z.B. bei bestimmten Krebsarten, gehandelt.

Drei Pioniere der Stammzellforschung stellen Zusammenhänge dar und diskutieren ihre aktuellen Projekte mit dem Publikum:

- Prof. Dr. Magdalena Götz, München
- Prof. Dr. Anthony Ho, Heidelberg
- Prof. Dr. Andreas Trumpp, Heidelberg

Moderation: Dr. Stefanie Seltmann, DKFZ

In collaboration with:

SCHERING STIFTUNG

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Photos: © Schlosshotel Molkenkur GmbH

Social Event

The **Networking Evening** with dinner buffet and DJ is open for all participants and will take place on Tuesday, November 4th, 20:00 h in the restaurant **Molkenkur**.

Address:
Schlosshotel Molkenkur
Klingenteichstr. 31
69117 Heidelberg

Supported by:

CellGenix

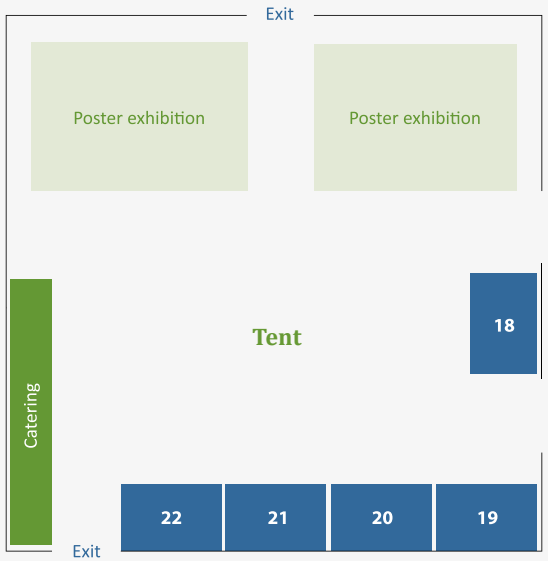
Free shuttle buses to network evening. Buses will leave at 19:30 h from the Communication Center.

Floor plan

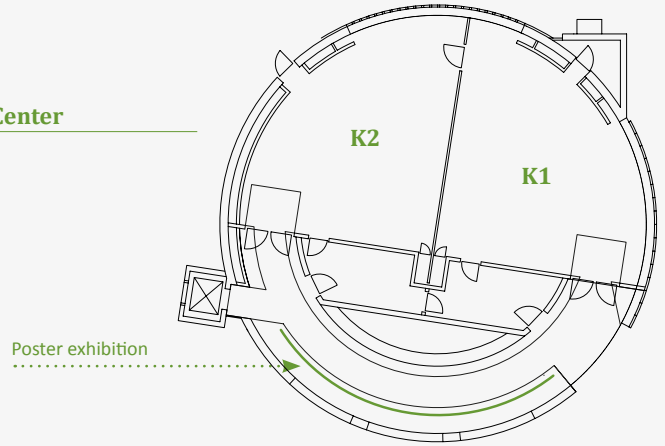
Legend

Company	booth	Company	booth
AMS Biotechnology (Europe) Ltd	6	Lonza Cologne GmbH	16
BD Biosciences	5	Miltenyi Biotec GmbH	22
Bio Froxx GmbH	7	Nanostring Technologies Germany GmbH	2
Biomol GmbH	4	PELOBIOTECH GmbH	11
CellGenix GmbH	12	PeproTech GmbH	13
Cenibra GmbH	18	PromoCell GmbH	21
Diagenode s.a. BELGIUM EUROPE	19	STEMCELL Technologies SARL	3
Eppendorf AG	15	Takara Bio Europe, SAS	20
ESSEN BioScience Ltd	8	Thermo Scientific	10
Fluidigm Europe B.V.	14	VWR International GmbH	17
Labotect GmbH	1		
Life Technologies GmbH	9		

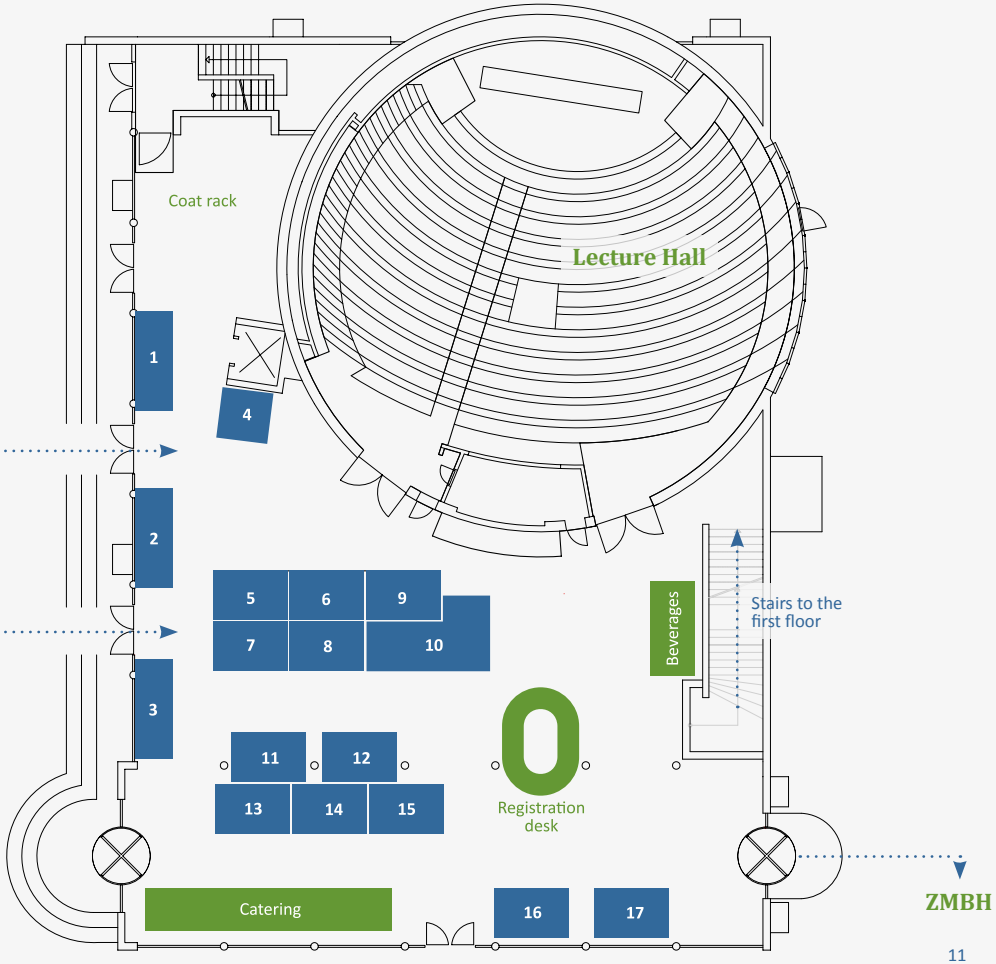
Tent



First floor – Communication Center



Ground floor – Communication Center



Program overview

MONDAY, November 3rd, 2014

	Lecture hall	K1	K2	ZMBH
11:00 – 12:00	Registration			
12:00 – 12:30	Opening			
12:30 – 13:15	Keynote lecture I Eduard Batlle			
13:15 – 14:00	Keynote lecture II Philippe Menasché			
14:00 – 14:30	Coffee break / industry exhibition			
14:30 – 16:00	Concurrent scientific working group session I			
	Stem cells in regenerative therapies I	Stem cells in disease: cancer stem cells	Computatational stem cell biology	Somatic stem cells
16:00 – 16:15	Coffee break / industry exhibition			
16:15 – 18:15	Poster session I	P001 – P017: Pluripotency and embryonic stem cells P018 – P038: Programing and reprograming P039 – P061: Stem cells in development P062 – P072: Somatic stem cells P073 – P097: Hematopoietic stem cells		
18:15 – 19:15	Dinner			
19:15 – 20:45	GSCN General Membership meeting			
20:45 – 21:30	Informal GSCN Get-together			

TUESDAY, November 4th, 2014

	Lecture hall	K1+2	ZMBH
09:00 – 10:30	Concurrent scientific working group session II		
	Stem cells in disease modeling and drug development	Pluripotency and embryonic stem cells	Stem cells in development
10:30 – 11:15	Coffee break / industry exhibition		
11:15 – 12:50	Industry session: “Technologies from GSCN industry partners”		
	PeproTech Eppendorf CellGenix	Life Technologies apceth STEMCELL Technologies	Nanostring Fluidigm Lonza
12:50 – 14:30	Lunch buffet / industry exhibition / poster viewing		
13:15 – 14:15		Outreach materials for stem cell research	
14:30 – 15:30	Concurrent strategic working group session A		Workshop
	Carreer Development	Clinical trials and regulatory affairs I	Large scale banking of human pluripotent stem cells
15:30 - 15:45	Short break		
15:45 – 16:45	Concurrent strategic working group session B		
	Stem cell technologies	Clinical trials and regulatory affairs II	Funding opportunities for young and advanced scientists
16:45 – 17:15	Coffee break / industry exhibition		

16:45 – 17:45	Meet-the-expert table: Genetic engeneering (DKFZ Mensa)	
17:15 – 19:15	Poster session II	P098 – P129: Stem cells in regenerative therapies P130 – P161: Stem cells in disease modeling and drug dev. P162 – P178: Stem cells in disease: Cancer stem cells P179 – P187: Computational stem cell biology
19:15 – 20:00	Free bus shuttle to networking evening	
20:00 – 01:00	Networking Evening with dinner buffet at Schlosshotel “Molkenkur” (Klingenteichstr. 31, 69117 Heidelberg)	

WEDNESDAY, November 5th, 2014

	Lecture hall	K1+2	DKFZ SR 4th floor
09:00 – 10:30	Concurrent scientific working group session III		
	Hematopoietic stem cells	Programming and reprogramming	Stem cells in regenerative therapies II (Meet at the registration desk)
10:30 – 11:00	Coffee break / Industry exhibition		
			ZMBH
11:00 – 12:30	Concurrent workshops		
	Clinical relevance of animal models in stem cell research and regenerative medicine	Big pharma and the stem cell field – matching expectations?	Proceedings of the GSZ
12:30 – 12:45	Short break		
12:45 – 13:30	Keynote lecture III Shahragim Tajbakhsh		
13:30 – 15:00	Lunch buffet / industry exhibition		
13:45 – 14:45	Meet-the-expert table: Laser printing & Reprogramming technologies (DKFZ Mensa)		
13:45 – 14:45		Outreach materials for stem cell research	
15:00 – 15:15	Poster award ceremony		
15:15 – 15:30	Outlook and closing remarks		
15:30 – 16:15	Keynote lecture IV Jürgen Knoblich		
16:15 – 17:00	Keynote lecture V Amelia Eisch		
17:00 – 17:45	Keynote lecture VI Sebastian Jessberger		
17:45	End of GSCN conference		
17:45 – 18:30	Coffee break GSCN satellite event		
18:30 – 20:00	GSCN public outreach event: Stammzellen als Chance – Realität und Perspektiven		
20:00 – 21:00	Reception public outreach event		

LEGEND

- General meeting
- Opening / Closing
- Workshops
- Keynote lectures
- Concurrent scientific sessions
- Concurrent strategic sessions
- Public event
- Networking evening
- Outreach information
- Meet-the-expert table
- Poster Session
- Industry session

Program

MONDAY, November 3rd

11:00 – 12:00 Registration and light lunch

Opening

Lecture hall:

12:00 – 12:05 Welcome to the German Cancer Research Center (DKFZ):
Otmar Wiestler (Chairman and Scientific Director, DKFZ)

12:05 – 12:15 *Andreas Trumpp* (Acting President, GSCN)

12:15 – 12:30 Opening remarks
Bärbel Brumme-Bothe (Department Head "Life Sciences", BMBF)

12:30 – 13:15 **Keynote lecture I**
K1 - Exploring the hierarchical organization of colorectal cancer
Eduard Batlle; Institute for Research in Biomedicine Barcelona, Spain
(Chair: *Andreas Trumpp*)
supported by Eppendorf AG

13:15 – 14:00 **Keynote lecture II**
K2 - Myocardial regeneration by ESC-derived cardiac progenitors
Philippe Menasché; Hospital European George Pompidou, Paris, France
(Chair: *Ulrich Martin*)

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

Concurrent scientific working group session I

Lecture hall: Stem cells in regenerative therapies I (chair: *Ulrich Martin / Hans-Dieter Volk*)

14:30 – 14:50 Stem cells in regenerative therapies – Introduction
Ulrich Martin; Rebirth / Hannover Medical School

14:50 – 15:05 T01 - Intranasal delivery of migratory neural stem/progenitor cells: A non-invasive passage to therapeutically target intracerebral brain tumors
Nils Ole Schmidt; University Medical Center, Hamburg-Eppendorf

15:05 – 15:20 T02 - MicroRNA-181a promotes human neuronal differentiation by down-regulating genes involved in neural stem cell maintenance
Laura Stappert; Institute of Reconstructive Neurobiology, Bonn

15:20 – 15:35 T03 - Pulmonary transplantation of multipotent- or pluripotent-stem cell derived macrophages as a novel treatment option for pulmonary alveolar proteinosis
Nico Lachmann; Hannover Medical School

15:35 – 15:50 T04 - Human iPSC-derived MSC-like progenitor cells: functional differences from primary MSCs affecting their use in cartilage regenerative medicine
Solvig Diederichs; University Hospital Heidelberg

15:50 – 16:00 Group discussion

Room K 1: Stem cells in disease: cancer stem cells (chair: *Thomas Brabletz / Andreas Trumpp*)

14:30 – 14:50 Concepts of cancer stem cells – Introduction
Andreas Trumpp; German Cancer Research Center, Heidelberg

14:50 – 15:05 T05 - Shp2 Signaling is essential to the suppression of senescence in mammary gland cancer stem cells in mice
Linxiang Lan; Max Delbrück Center, Berlin

15:05 – 15:20 T06 - Humanization of mouse models for human breast cancer using mesenchymal stem cells
Maxine Silvestrov; Experimental Pharmacology & Oncology (EPO) GmbH, Berlin

15:20 – 15:35 T07 - Targeting the kinome to induce differentiation in human breast cancer stem cells
Jochen Maurer; University Medical Center Freiburg

15:35 – 15:50 T08 - CD151+ cells drive tumor-initiation, hierarchical growth and proliferation-associated signaling in patient-derived models of serous ovarian cancer
Franziska Zickgraf; German Cancer Research Center, Heidelberg

15:50 – 16:00 Group discussion

Room K 2: Computational stem cell biology (chair: *Georg Fuellen / Ingo Roeder*)

14:30 – 14:45 Computational stem cell biology – Overview and introduction
Ingo Roeder; Dresden University of Technology

14:45 – 14:55 T09 - What makes stem cells move? Studying stem cell migration in vitro (back-to-back presentation with T10)
Michael Ansorge; Leipzig University

14:55 – 15:05 T10 - What makes stem cells move? Studying stem cell migration in silico (back-to-back presentation with T09)
Axel Krinner; Dresden University of Technology

15:05 – 15:20 T11 - Characterizing transcriptional cell-to-cell heterogeneities during stem cell differentiation
Fabian Theis; Helmholtz Center Munich

15:20 – 15:35 T12 - A network-based strategy to direct cell fate determination
Antonio del Sol; Luxembourg Center for Systems Biomedicine

15:35 – 15:50 T13 - Computational identification of pluripotency networks
Maryam Nazarieh; Saarland University, Saarbrücken

15:50 – 16:00 Group discussion

ZMBH (ground floor): **Somatic stem cells** (chair: *Thomas Braun / Ana Martin-Villalba*)

14:30 – 14:50 Studies on somatic stem cells – an introduction
Thomas Braun; Max Planck Inst. f. Heart and Lung Res., Bad Nauheim

14:50 – 15:05 T14 - The RNA helicase DDX6 regulates cell-fate specification in neural stem cells via miRNAs
Sarah Nicklas; Luxembourg Center for Systems Biomedicine

15:05 – 15:20 T15 - Angiogenesis controls neural stem cell expansion by regulating tissue oxygenation and HIF signaling
Christian Lange; VIB KU Leuven Campus, Belgium

15:20 – 15:35 T16 - Regulation of asymmetric/symmetric stem cell division in human epidermis
Katharina Nöske; German Cancer Research Center, Heidelberg

15:35 – 15:50 T17 - Clonal heterogeneity within the exocrine pancreas
Damian Wollny; German Cancer Research Center, Heidelberg

15:50 – 16:00 Group discussion

16:00 – 16:15 Coffee break / industry exhibition

16:15 – 18:15 Poster session I: P001 – P097 supported by Fluidigm Europe B.V.
(posters will be displayed in the tent)
P001 – P017: Pluripotency and embryonic stem cells
P018 – P038: Programing and reprograming
P039 – P061: Stem cells in development
P062 – P072: Somatic stem cells
(posters will be displayed in front of room K 1+2)
P073 – P097: Hematopoietic stem cells

Even numbers: please present your poster from 16:15 – 17:15
Uneven numbers: 17:15 – 18:15

18:15 – 19:15 Dinner

Lecture hall:

19:15 – 20:45 GSCN General Membership Meeting
20:45 – 21:30 Get-together

TUESDAY, November 4th

Concurrent scientific working group session II

Lecture hall: Stem cells in disease modeling and drug development
(chair: *Oliver Brüstle / Karl-Ludwig Laugwitz*) supported by Lonza Cologne GmbH

09:00 – 09:20 The use of stem cells in disease modeling and drug development – Overview
Oliver Brüstle; Institute of Reconstructive Neurobiology, Bonn

09:20 – 09:35 T18 - Probing mitochondrial DNA disorders using iPSC-derived neural progenitors
Alessandro Prigione; Max Delbrück Center, Berlin

09:35 – 09:50 T19 - Modeling Dravet syndrome in iPS cell-derived neurons
Matthias Hebisch; Institute of Reconstructive Neurobiology, Bonn

09:50 – 10:05 T20 - The functionality of stem cell derived neurons: How to define it?
Sebastian Illes; Inst. of Molecular Reg. Medicine, Salzburg, Austria

10:05 – 10:20 T21 - Modeling and pharmacological rescue of ion channel diseases enabled by improved cardiac induction of human pluripotent stem cells in 2D and 3D formats
Boris Greber; Max Plank Institute for Molecular Biomedicine, Münster

10:20 – 10:30 Group discussion

Room K 1+2: Pluripotency and embryonic stem cells (chair: *James Adjaye / Mathias Treier*)

09:00 – 09:20 Pluripotency and embryonic stem cells: Introduction and overview
James Adjaye; University Hospital of Düsseldorf

09:20 – 09:35 T22 - Primate-specific endogenous retrovirus driven transcription defines naïve-like stem cells
Jichang Wang; Max Delbrück Center, Berlin

09:35 – 09:50 T23 - A novel lncRNA-protein interaction characterizes mouse embryonic stem cell fate
Debojyoti Chakraborty; Dresden University of Technology

09:50 – 10:05 T24 - NANOG and CDX2 pattern distinct subtypes of human mesoderm during exit from pluripotency
Sasha Mendjan; Cambridge Stem Cell Institute, United Kingdom

10:05 – 10:20 T25 - Insights from the streak: identification of novel genes associated with germ-layer formation and mesendoderm differentiation
Cantas Alev; RIKEN Center for Developmental Biology, Kobe, Japan

10:20 – 10:30 Group discussion

ZMBH (ground floor): **Stem cells in development** (chair: *Jan Lohmann / Francesca Spagnoli*)

09:00 – 09:15 Stem cells in development – Overview
Jan Lohmann; Centre for Organismal Studies Heidelberg

09:15 – 09:30 T26 - Tet-dependent processing of 5-methylcytosine protects DNA methylation canyons against hypermethylation
Achim Breiling; German Cancer Research Center, Heidelberg

09:30 – 09:45 T27 - PIEZO2 is required for mechanotransduction in human ES-cell-derived touch receptors
Katrin Schrenk-Siemens; University of Heidelberg

09:45 – 10:00 T28 - Transcriptional and epigenetic dynamics underlying cell lineage commitment
Filippo M. Cernilogar; Ludwig Maximilian University, Munich

10:00 – 10:15 T29 - The histone 3 lysine 4 methyltransferase Mll2 primes the Nxt2 promoter for neural differentiation
Katrin Neumann; Dresden University of Technology

10:15 – 10:30 T30 - Signals from the injury niche increase fate potential of adult neural stem cells
Enric Llorens Bobadilla; German Cancer Research Center, Heidelberg

10:30 – 11:15 Coffee break / industry exhibition

Industry session: "Technologies from GSCN industry partners"

Lecture hall: Main supporters

11:15 – 11:20 Introduction (*Andreas Trumpp*)

11:20 – 11:50 C01 - High throughput screening using iPSC-based models
Jared Sterneckert; CRT Dresden, representing PeproTech GmbH

11:50 – 12:20 C02 - Controlling expansion and cardiomyogenic differentiation of human pluripotent stem cells in scalable suspension culture
Robert Zweigerdt; MH Hannover, representing Eppendorf AG

12:20 – 12:50 C03 - Raw materials in the manufacture of advanced therapies medicinal products: Quality attributes and quality assurance
Bernd Leistler; CellGenix GmbH

Room K 1+2: Supporters

11:15 – 11:20 Introduction (*Michael Cross*)

11:20 – 11:50 C04 – Efficient neural and cardiac differentiation systems for iPSC
Mohan C. Vemuri; Life Technologies GmbH

11:50 – 12:20 C05 - Cell-based gene delivery leverages conventional immunotherapy for cancer; Treatment of advanced gastrointestinal cancer in a phase I/II trial with modified autologous MSC_apceth_101
Volker Scherhammer; apceth GmbH & Co. KG

12:20 – 12:50 C06 - Scalable enzyme-free protocols for the isolation and maintenance of human induced pluripotent stem cells without mechanical colony scraping
Alexandra Blak; STEMCELL Technologies SARL

ZMBH (ground floor): **Supporters**

11:15 – 11:20 Introduction (*Torsten Tonn*)

11:20 – 11:50 C07 - The future is counting – Digital analysis of RNA, DNA and protein biomarkers
Maik Pruess; NanoString Technologies Germany GmbH

11:50 – 12:20 C08 – Primate iPS cells as tools for evolutionary analyses
Wolfgang Enard; Ludwig Maximillians University, Munich; representing Fluidigm Europe B.V.

12:20 – 12:50 C09 – Building bridges from research to therapy: A roadmap for the successful generation of clinical-grade iPSCs
Thomas Fellner; Lonza Cologne GmbH

12:50 – 14:30 Lunch buffet / industry exhibition / poster viewing
supported by STEMCELL Technologies SARL

13:15 – 14:15 **Room K 1+2:** Explaining science and motivating people – Outreach materials for stem cell research (*Tobias Cantz / Ira Herrmann*)

Concurrent strategic working group session A

Lecture hall: Career development (chairs: *Hartmut Geiger / Insa Schröder*)

14:30 – 14:45 Careers in industry, *Andreas Bosio;* Miltenyi GmbH, Berg. Gladbach

14:45 – 15:00 Careers in science administration, *Hella Lichtenberg;* DLR Bonn

15:00 – 15:15 Careers in academia, *Hartmut Geiger / Insa Schröder*

15:15 – 15:30 Panel discussion

Room K 1+2: Clinical trials and regulatory affairs I
(chair: *Andreas Kurtz / Torsten Tonn / Hans-Dieter Volk*) supported by apceth GmbH & Co. KG

14:30 – 14:50 Development of iPS cell-based cardiac therapy
Ulrich Martin, MH Hannover

14:50 – 15:10 Regulatory challenges of stem cell-based medicinal products
Egbert Flory; Paul Ehrlich Institute, Langen

15:10 – 15:30 Group discussion

ZMBH (ground floor): **Large scale banking of human pluripotent stem cells – workshop**
(chair: *Andreas Kurtz / Joana Namorado*)

14:30 – 14:45 Biomedical applications of iPS cell technology
Angel Raya; CMRB Barcelona

14:45 – 15:00 The need for large-scale iPSC collections - a pharma perspective
Alex Gutteridge; Pfizer

15:00 – 15:15 Delivery of human pluripotent stem cells for therapy: Phase IV of the UK Stem Cell Bank Project
Lyn Healy; UK Stem Cell Bank, South Mimms, U.K.

15:15 – 15:30 The relaunch of the human pluripotent stem cell registry (hESCreg)
Andreas Kurtz; BCRT Berlin

15:30 – 15:45 Short break

Concurrent strategic working group session B

Lecture hall: Stem cell technologies (chair: *Andreas Bosio / Frank Emmrich*)

15:45 – 15:50 Introduction: *Andreas Bosio,* Miltenyi GmbH, Berg. Gladbach

15:50 – 16:05 Methods of genetic engineering
Claudio Mussolino; University of Freiburg

16:05 – 16:20 Reprogramming technologies
Micha Drukker; Helmholtz Center Munich

16:20 – 16:35 Methods of laser cell printing
Boris Chichkov; Laser Center Hannover (LZH)

16:35 – 16:45 Group discussion

Room K 1+2: Clinical trials and regulatory affairs II
(chair: *Andreas Kurtz / Torsten Tonn / Hans-Dieter Volk*)

15:45 – 16:05 Placenta expanded mesenchymal (PLX) cells– preclinical data for preparing clinical trials
Lena Pinzur; PluriStem Therapeutics Inc., Haifa, Israel

16:05 – 16:25 PLX cells – from preclinical to clinical studies – what did we learn so far from biomarker studies?
Hans-Dieter Volk; Berlin-Brandenburg Center for Reg. Therapies (BCRT)

16:25 – 16:45 Panel discussions

ZMBH (ground floor): **Funding opportunities for young and advanced scientists**
(chair: *Hartmut Geiger / Albrecht Müller*)

15:45 – 16:30 Funding opportunities of the DFG for stem cell scientists at various career stages
Eckard Picht; German Research Foundation (DFG), Bonn

16:30 – 16:45 Group discussion

16:45 – 17:15 Coffee break / industry exhibition

16:45 – 17:45 **DKFZ Mensa:** Meet-the-expert table: *Stem cell technologies – genetic engineering (Claudio Mussolino) limited to 10 registered participants*

17:15 – 19:15 **Poster session II: P098 – P187**
(posters will be displayed in the tent)
P098 – P129: Stem cells in regenerative therapies
P130 – P161: Stem cells in disease modeling and drug development
(posters will be displayed in front of room K 1+2)
P162 – P178: Stem cells in disease: Cancer stem cells
P179 – P187: Computational stem cell biology

Even numbers: please present your poster from 17:15 – 18:15
Uneven numbers: 18:15 – 19:15

19:15 – 20:00 Free bus shuttle to Networking Evening
(busses will depart from the DKFZ main entrance)

20:00 – 01:00 Networking Evening with dinner buffet at Schlosshotel "Molkenkur"
Klingenteichstr. 31, 69117 Heidelberg (www.molkenkur.de)
(Opening: *Oliver Brüstle*) (included in the registration fee)

supported by CellGenix GmbH

WEDNESDAY, November 5th

Concurrent scientific working group session III

Lecture hall: Hematopoietic stem cells (chair: *Anthony Ho / Claudia Waskow*)

09:00 – 09:20 Introduction "Hematopoietic stem cells"
Claudia Waskow; Technical University Dresden

09:20 – 09:35 T31 - Stroma-derived osteopontin regulates hematopoietic stem and progenitor cells function upon aging
Novella Guidi; University of Ulm

09:35 – 09:50 T32 - Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylome analysis
Daniel Klimmeck; German Cancer Research Center, Heidelberg

09:50 – 10:05 T33 - Kit deficiency regulates stable human hematopoietic stem cell engraftment in mice
Susann Rahmig; Technical University Dresden

10:05 – 10:20 T34 - STAT5-regulated miRNA193b controls hematopoietic stem and progenitor cell expansion by fine tuning cytokine signaling
Nadine Hätscher; University Hospital Frankfurt

10:20 – 10:30 Group discussion

Room K 1+2: Programming and reprogramming (chair: *Micha Drukker / Frank Edenhofer*)

09:00 – 09:15 Introduction to re-programming
Frank Edenhofer; University of Würzburg

09:15 – 09:30 T35 - Selective gene delivery into human iPS cells based on CD30-targeted lentiviral vectors
Thorsten Friedel; Paul Ehrlich Institute, Langen

09:30 – 09:45 T36 - Generation of iPS cells from young vs. aged cell sources – influence of donor age on reprogramming efficiency and genetic stability of iPS clones
Katarzyna Osetek; LEBAO / Hannover Medical School

09:45 – 10:00 T37 - Mesenchymal stem cells derived from iPS cells from aged individuals acquire fetal characteristics
Lucas Spitzhorn; University Hospital of Düsseldorf

10:00 – 10:15 T38 - TRIM32, a dual player regulating the entry and exit from pluripotency
Lamia'a Bahnassawy; University of Luxembourg

10:15 – 10:30 T39 - Robust generation of cardiomyocytes from human iPS cells requires precise modulation of Bmp and Wnt signaling
Asifiqbal Kadari; Julius Maximilians University, Würzburg

DKFZ SR 4th floor: Stem cells in regenerative therapies II (chair: *Michael Cross / Frank Emmrich*)
H2.04.073

The meeting point for this session is at the registration desk!

09:00 – 09:15 Introduction to session II stem cells in regenerative therapies
Michael Cross; University of Leipzig

09:15 – 09:30 T40 - Efficient generation of functionally defined iPSC-derived human neural stem/progenitor cells and oligodendrocytes
Giacomo Frati; San Raffaele Scientific Institute, Milan, Italy

09:30 – 09:45 T41 - Aging affects the mesenchymal stem cell derived oligodendrogenic / remyelination activities: implication for a potential therapy of multiple sclerosis
Francisco J. Rivera; Paracelsus Medical University, Salzburg, Austria

09:45 – 10:00 T42 - The european project REBORNE: Multipotent mesenchymal stromal cells and biomaterial for bone healing
Alexander Erle; Ulm University Hospital

10:00 – 10:15 T43 - Identification, isolation and targeted inhibition of a fibroblast lineage responsible for scarring and cancer stroma
Yuval Rinkevich; Stanford Institute for Stem Cell Biology and Regenerative Medicine, U.S.A.

10:15 – 10:30 T44 - Proofing the (Un)Proven: Proven or approved stem cell therapies – that is the question
Bianca Büchner; Center for Ethics and Law in the Life Sciences (CELLS), Hannover

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

Concurrent workshops

Lecture hall: Clinical relevance of animal models in stem cell research and regenerative medicine (chair: *Georg Duda / Frank Emmrich*)
(Workshop of the 'Regenerative Medicine Initiative Germany (RMIG)')

11:00 – 11:20 Introduction: Animal models in research for regenerative medicine
Axel Haverich; Rebirth / Hannover Medical School

11:20 – 11:30 Cell transplantation into pre-clinical models of retinal degeneration
Marius Ader; Center for Regenerative Therapies Dresden

11:30 – 11:40 Medical devices and the application of stem cells
Konrad Kohler; zrm, Tübingen University Hospital

11:40 – 11:50 Regenerative medicine for an aging society: How relevant are our current animal models?
Georg Duda; BCRT Berlin

11:50 – 12:00 Heterogeneity, age, comorbidities and the translational failure – can we do better in projecting from preclinical stem cell studies to clinical trials?
Johannes Boltze; TRM Leipzig

12:00 – 12:10 Hurdles for clinical translation of regenerative stem cell therapy – lessons learned from the cardiac Phase III PERFECT multicenter trial
Gustav Steinhoff; RTC Rostock

12:10 – 12:30 Panel discussion

Room K 1+2: Big pharma and the stem cell field – matching expectations?
(organizer: *Oliver Brüstle / Ira Herrmann*)
(Workshop of the Stem Cell Network North Rhine Westphalia)

Panel:
Oliver Brüstle; LIFE & BRAIN GmbH, Bonn
Ralf Heilker; Boehringer Ingelheim Pharma GmbH & Co. KG, Div. Research Germany
Jörg Hüser; Bayer Pharma AG, Lead Discovery Wuppertal
Bert Klebl; Lead Discovery Center GmbH, Dortmund
Moderation: *Ira Herrmann;* Stem Cell Network NRW, Düsseldorf

ZMBH (ground floor): **Proceedings of the German Society for Stem Cell Research (GSZ e.V.)**
(chair: *Jürgen Hescheler*)

11:00 – 11:10 Introduction

11:10 – 11:30 Sinus nodal cells from pluripotent stem cells
Robert David; Reference and Translation Center for Cardiac Stem Cell Therapy (RTC), Rostock

11:30 – 11:50 Chondrogenic lineage determination during differentiation of mouse embryonic stem cells
Jürgen Rohwedel; University of Lübeck

11:50 – 12:10 Use of transcriptomics and epigenetic approaches in human ESC-based developmental neuro toxicology (DNT) testing
Tanja Waldmann; University of Konstanz

12:10 – 12:30 Long term culture of iPSC-derived cardiomyocytes on mechanically-defined substrates
Carlos Heras; University of Cologne

12:30 – 12:45 Short break

Lecture hall:

12:45 – 13:30 **Keynote lecture III**
K3 - Molecular and cellular regulation of muscle stem cells during development and regeneration
Shahragim Tajbakhsh; Institute Pasteur, Paris, France
(Chair: *Oliver Brüstle*)

13:30 – 15:00 Lunch buffet / industry exhibition
supported by Nanostring Technologies Germany GmbH

13:45 – 14:45 **Room K 1+2:** Explaining science and motivating people – Outreach materials for stem cell research (*Tobias Cantz / Ira Herrmann*)

13:45 – 14:45 **DKFZ Mensa:** Meet-the-expert table: *Stem cell technologies – laser printing* (*Boris Chichkov*) and – *reprogramming technologies (Micha Drukker) limited to 10 registered participants each*

Closing ceremony

Lecture hall:

15:00 – 15:15 Poster award ceremonies and announcement of industry quiz winners (*Daniel Besser; GSCN*)
supported by PeproTech GmbH

15:15 – 15:30 Outlook and closing remarks
Incoming president: *Thomas Braun*

15:30 – 16:15 **Keynote lecture IV – Joined session with the 6th BMBF symposium – Adult neural stem cells** (Chair: *Magdalena Götz / Ana Martin-Villalba*)
K4 - Modeling human brain development and disease in 3D culture
Jürgen Knoblich; Institute of Molecular Biotechnology (IMBA)
Vienna, Austria
supported by Life Technologies GmbH

16:15 – 17:00 K5 - Adult hippocampal neurogenesis: What are new neurons good for? And what is good for them?
Amelia Eisch; UT Southwestern, Dallas, U.S.A.

17:00 – 17:45 K6 - A mechanism for the segregation of age in mammalian neural stem cells
Sebastian Jessberger; University of Zurich, Switzerland

End of GSCN conference

Announcements:

18:30 – 20:00 Public outreach event of the GSCN in collaboration with the Schering Stiftung and the DKFZ (in German)
Stammzellen als Chance – Realität und Perspektiven
Podiumsdiskussion zur aktuellen Forschung in Labor und Klinik

Panel: *Magdalena Götz* (LMU München), *Anthony Ho* (Uni. Heidelberg), *Andreas Trumpp* (DKFZ)
Moderation: *Stefanie Seltmann* (DKFZ)

20:00 – 21:00 Reception public outreach event

and

November 6th, 2014 – GSCN Satellite Event: 6th BMBF Symposium – Adult neural stem cells
The program of the satellite event is available at the reception desk.

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Stem Cell Network North Rhine Westphalia

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Speaker abstracts

2nd Annual Conference
German Stem Cell Network
November 3 – 5, 2014

Keynote lectures: K1 – K6

Abstract No. K1

Exploring the hierarchical organization of colorectal cancer

Eduard Batlle[1]

[1]ICREA & Oncology Program. Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain

The inner layer of the intestinal tube, the intestinal epithelium, is in a constant process of renewal. Hundreds of millions of terminally differentiated intestinal cells are replaced by new cells every day during the life of an adult organism. This tremendous regenerative power is ultimately sustained by a small population of intestinal stem cells (ISCs). We have recently discovered that most human colorectal cancers (CRCs) are constituted by cell populations with phenotypes similar to either ISCs or intestinal differentiated cells organized into well-defined compartments. ISC-like cells purified from primary human CRC samples generate tumors in immunodeficient mice with high efficiency and display both self-renewal and differentiation capacity whereas differentiated CRC cells are not capable of propagating the disease. We propose that the acquisition of an ISC-like gene program is a central process in the development of metastatic and recurrent CRC. Our observations also imply the existence of a tumor cell hierarchy that imposes distinct phenotypes and behaviors on CRC cells. Here, I will discuss our latest research regarding the mechanisms that control the renewal and differentiation of CRC stem cells. We have recently performed an shRNA screen for genes controlling the stem cell phenotype in CRC. This strategy has led us to identify a transcriptional circuit dedicated to suppress the expansion of tumor stem cells at the onset of CRC.

Abstract No. K2

Myocardial regeneration by ESC-derived cardiac progenitors

Philippe Menasché[1]

[1]Hôpital Européen Georges Pompidou, Paris, France

The rationale for the use of embryonic stem cells (ESC) in patients with heart failure stems from the assumption that regeneration of scarred myocardium likely requires the supply of cells endowed with a true cardiomyogenic differentiation potential. This approach is made possible by the pluripotentiality of ESC which allows to drive their fate in vitro towards a cardiac lineage. Consequently, our approach has been to generate SSEA-1-positive cardiac progenitors which, experimentally, have proven their ability to differentiate into cardiomyocytes in a clinically relevant scenario of allogeneic transplantation in nonhuman primates. Asides from ethical issues, the clinical translation of this ESC-based program has entailed a stepwise approach including the following steps: (1) the expansion of a clone of pluripotent hESC to generate cell banks under Good Manufacturing Practice conditions (GMP); (2) a growth factor-induced cardiac specification; (3) the purification of committed cells by immunomagnetic sorting to yield a SSEA-1-positive cell population strongly expressing the early cardiac transcription factor Isl-1; (4) the incorporation of these cells into a fibrin scaffold intended to be epicardially delivered onto the infarcted area and the assessment of the functional benefits of this cell construct in a rat model of chronic myocardial infarction subjected to an extended follow-up; (5) a safety assessment focused on (i) the loss of teratoma-forming cells by in vitro (transcriptomics) and in vivo (cell injections in immunodeficient mice) measurements, (ii) the absence of cytogenetic abnormalities and (iii) the absence of microbiological contamination; and (6) the characterization of clinically relevant release criteria of the final cell product. Put together, these data have led to an approval for a first-in-man clinical trial of transplantation of these SSEA-1+ progenitors in patients with a severely impaired post-infarction cardiac function and otherwise candidates for a coronary artery bypass operation.

Abstract No. K3

Molecular and cellular regulation of muscle stem cells during development and regeneration

Shahragim Tajbakhsh[1]

[1]Institut Pasteur, Stem Cells & Development, CNRS URA 2578, 25 rue du Dr. Roux, Paris, 75015, France, shahragim.tajbakhsh@pasteur.fr

The regulatory cell state of muscle stem cells is determined by multiple parameters during prenatal and post-natal growth. We have proposed that distinct gene regulatory networks give rise to skeletal muscle stem cells in different anatomical locations and we have used genetically modified mice to determine how stem and progenitor cells contribute to myogenesis. In the adult, we have observed a that the muscle stem cell pool is heterogeneous during quiescence and proliferation. A major regulator of cellular quiescence in this tissue is Notch signaling, which is lost rapidly following cell cycle reentry, then restored during self-renewal. We and others have reported that Notch signaling could mediate the stability of the niche by regulating extracellular matrix molecules. During muscle regeneration, muscle stem cells adopt distinct modes of cell division. To examine their properties, single cells were investigated in a context where extrinsic cues were modulated in single cells using micropattern technology. The distinct regulatory cell states of muscle stem cells will be discussed in the context on their functional capacity for muscle regeneration.

Abstract No. K4

Modeling human brain development and disease in 3D culture

Jürgen A. Knoblich[1]

[1] IMBA - Institute of Molecular Biotechnology of the Austrian Academy of Science
Vienna 1030, Austria

The human brain is highly unique in size and complexity. While many of its characteristics have been successfully studied in model organisms, recent experiments have emphasized unique features that can not easily be modeled in animals. We have therefore developed a 3D organoid culture system derived from human pluripotent stem cells that recapitulates many aspects of human brain development. These cerebral organoids are capable of generating several brain regions including a well-organized cerebral cortex. Furthermore, human cerebral organoids display stem cell properties and progenitor zone organization that show characteristics specific to humans. Finally, we use both RNAi and patient specific iPS cells to model microcephaly, a human neurodevelopmental disorder that has been difficult to recapitulate in mice. This approach reveals premature neuronal differentiation with loss of the microcephaly protein CDK5RAP2, a defect that could explain the disease phenotype. Our data demonstrate an *in vitro* approach that recapitulates development of even this most complex organ, which can be used to gain insights into disease mechanisms.

Abstract No. K5

Adult hippocampal neurogenesis: What are new neurons good for? And what is good for them?

Amelia J. Eisch, Ph.D. [1]

[1]Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX, USA

About 50 years ago, it was discovered that new neurons are made throughout life in the mammalian hippocampus. Since then new neurons have been implicated in a variety of hippocampal functions, including memory and mood control. I will highlight recent and unpublished research from my laboratory suggesting adult neurogenesis is important for our understanding, treatment, and perhaps prevention of psychiatric disorders, like depression and addiction. I will also discuss the emergence of new molecular, cellular, and network level approaches to manipulate neurogenesis and hippocampal function in laboratory animals, and comment on the future of such approaches for translational application, particularly outside the scope of early life and into maturity. Finally, in emphasizing recent work from our laboratory and other on the heterogeneity of the hippocampal neural stem cell population *in vivo*, this presentation will provide a current view not only of “what new neurons are good for” and “what is good for them”, but also how neural stem cell manipulations influence neurogenesis and hippocampal function.

Abstract No. K6

A mechanism for the segregation of age in mammalian neural stem cells

Sebastian Jessberger[1]

[1] *Laboratory of Neural Plasticity, Brain Research Institute, University of Zurich, 8093 Zurich, Switzerland*

Neural stem cells (NSCs) generate new neurons in distinct regions of the mammalian brain throughout life. This process, called adult neurogenesis, is critically involved in certain forms of learning and memory. In addition, failing or altered neurogenesis has been associated with a number of neuro-psychiatric diseases such as major depression and epilepsy. However, the cellular mechanisms underlying life-long self-renewal of NSCs remain unknown. Here we show that NSCs in vitro and within the developing forebrain generate a diffusion barrier during cell division allowing for the retention of certain aging/senescence factors within the stem cell. Thus, the data presented here describe a novel mechanism how age is asymmetrically distributed in the context of somatic stem cell division that may allow for the selective extension of stem cell-based plasticity of the adult mammalian brain.

Selected presentations

2nd Annual Conference
German Stem Cell Network
November 3 – 5, 2014

Selected presentations: T01 – T44

(the PXXX number indicates an accompanying poster presentation)

T01 Intranasal delivery of migratory neural stem/progenitor cells: A non-invasive passage to therapeutically target intracerebral brain tumors
Nils Ole Schmidt

T02 MicroRNA-181a promotes human neuronal differentiation by down-regulating genes involved in neural stem cell maintenance
Laura Stappert

T03 Pulmonary transplantation of multipotent- or pluripotent-stem cell derived macrophages as a novel treatment option for Pulmonary Alveolar Proteinosis
Nico Lachmann

T04 Human iPSC-derived MSC-like progenitor cells: functional differences from primary MSCs affecting their use in cartilage regenerative medicine
Solvig Diederichs

T05/P168 Shp2 signaling is essential to the suppression of senescence in mammary gland cancer stem cells in mice
Linxiang Lan

T06 Humansation of mouse models for human breast cancer using mesenchymal stem cells
Maxine Silvestrov

T07 Targeting the kinome to induce differentiation in human breast cancer stem cells
Jochen Maurer

T08/P178 CD151+ cells drive tumor-initiation, hierarchical growth and proliferation-associated signaling in patient-derived models of serous ovarian cancer
Franziska M. Zickgraf

T09 What makes stem cells move? Studying stem cell migration in vitro
Michael Ansorge

T10 What makes stem cells move? Studying stem cell migration in silico
Axel Krinner

T11 Characterizing transcriptional cell-to-cell heterogeneities during stem cell differentiation
Fabian Theis

T12 A network-based strategy to direct cell fate determination
Antonio del Sol

T13/P186 Computational identification of pluripotency networks
Maryam Nazarieh

T14/P066 The RNA helicase DDX6 regulates cell-fate specification in neural stem cells via miRNAs
Sarah Nicklas

T15/P049 Angiogenesis controls neural stem cell expansion by regulating tissue oxygenation and HIF signaling
Christian Lange

T16/P067 Regulation of asymmetric/symmetric stem cell division in human epidermis
Katharina Nöske

T17 Clonal heterogeneity within the exocrine pancreas
Damian Wollny

T18 Probing mitochondrial DNA disorders using iPSC-derived neural progenitors
Alessandro Prigione

T19 Modeling Dravet syndrome in iPS cell-derived neurons
Matthias Hebisch

T20 The functionality of stem cell derived neurons. How to define it?
S*ebastian Illes*

T21/P038 Modeling and pharmacological rescue of ion channel diseases enabled by improved cardiac induction of human pluripotent stem cells in 2D and 3D formats
Boris Greber

T22 Primate-specific endogenous retrovirus driven transcription defines naïve-like stem cells
Jichang Wang

T23/P007 A novel lncRNA-protein interaction characterises mouse embryonic stem cell fate
Debojyoti Chakraborty

T24 NANOG and CDX2 pattern distinct subtypes of human mesoderm during exit from pluripotency
Sasha Mendjan

T25 Insights from the streak: identification of novel genes associated with germ-layer formation and mesendoderm differentiation
Cantas Alev

T26/P039 Tet-dependent processing of 5-methylcytosine protects DNA methylation canyons against hypermethylation
Achim Breiling

T27 PIEZO2 is required for mechanotransduction in human ES-cell-derived touch receptors
Katrin Schrenk-Siemens

T28 Transcriptional and epigenetic dynamics underlying cell lineage commitment
Filippo M. Cernilogar

T29 The histone 3 lysine 4 methyltransferase Mll2 primes the Nxt2 promoter for neural differentiation
Katrin Neumann

T30 Signals from the injury niche increase fate potential of adult neural stem cells
Enric Llorens-Bobadilla

T31 Stroma-derived osteopontin regulates hematopoietic stem and progenitor cells function upon aging
Novella Guidi

T32 Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylome analysis
Daniel Klimmeck

T33 Kit deficiency regulates stable human hematopoietic stem cell engraftment in mice
Susann Rahmig

T34 STAT5-regulated miRNA193b controls hematopoietic stem and progenitor cell expansion by fine tuning cytokine signaling
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T35 Selective gene delivery into human iPS cells based on CD30-targeted lentiviral vectors
Thorsten Friedel

T36 Generation of iPS cells from young vs. aged cell sources – influence of donor age on reprogramming efficiency and genetic stability of iPS clones
Katarzyna Osetek

T37/P033 Mesenchymal stem cells derived from iPS cells from aged individuals acquire fetal characteristics
Lucas Spitzhorn

T38 TRIM32, a dual player regulating the entry and exit from pluripotency
Lamia'a Bahnassawy

T39 Robust generation of cardiomyocytes from human iPS cells requires precise modulation of Bmp and Wnt signaling
Asifiqbal Kadari

T40 Efficient generation of functionally defined iPSC-derived human neural stem/progenitor cells and oligodendocytes
Giacomo Frati

T41 Aging affects the mesenchymal stem cell derived oligodendrogenic / remyelination activities: Implication for a potential therapy of multiple sclerosis
Francisco J. Rivera

T42 The European Project REBORNE: Multipotent mesenchymal stromal cells and biomaterial for bone healing
Alexander Erle

T43 Identification, isolation and targeted inhibition of a fibroblast lineage responsible for scarring and cancer stroma
Yuval Rinkevich

T44 Proofing the (un)proven: Proven or approved stem cell therapies – that is the question
Bianca Buechner

Abstract No. T01

Intranasal delivery of migratory neural stem/progenitor cells: A non-invasive passage to therapeutically target intracerebral brain tumors

Nils Ole Schmidt [1,], Matthias Reitz [1,*], Lasse Dührsen [1], Marvin Henze [1], Jan Sedlacik [1], Seung U. Kim [2], Kristoffer Riecken [1], Boris Fehse [1], and Manfred Westphal [1]*

[1]University Medical Center Hamburg-Eppendorf
[2]University of British Columbia Hospital, Vancouver, Canada
*Presenting author

Optimized cell delivery to the CNS remains a challenge since the direct intracerebral injection is an invasive method with low transplantation efficiency. We investigated the feasibility of intranasal administration of neural stem/progenitor cells (NSPC) as an alternative, non-invasive and direct passage for the delivery of stem cells to target malignant glioma. Tumor targeting and migratory pathways of murine and human NSPC were investigated by intravital 7T magnetic resonance imaging, luciferase imaging and in histological time course analyses in the intracerebral U87, NCE-G55T2 and syngenic Gl261 glioblastoma mouse models. Intranasally administered NSPC displayed a rapid, targeted tumor tropism with significant numbers of NSPC accumulating specifically at the intracerebral glioma site within six hours after intranasal delivery. Histological time series analysis revealed NSPC to migrate within the first 24 hours mainly via olfactory pathways but also by systemic distribution via the microvasculature of the nasal mucosa. A gradient of CXCL12 expression was observed in the migratory pathways, implicating a CXCL12/CXCR4-dependent mechanism of NSPC migration. Therapeutic efficency was assessed by using genetically modified NSPC to stably express a novel, codon-optimized HSV-thymidine kinase (A168H) mutant (TK.007). Repeated intranasal application of neural stem cell-mediated enzym (TK.007)/prodrug (GCV) therapy inhibits intracerebral glioma growth up to 95% with significantly improved survival times. Our findings establish that the directional distribution of cells accumulating intra- and peritumorally makes the intranasal delivery of NSPC a promising non-invasive and convenient alternative delivery method during the disease process, which can be exploited for the treatment of invasive malignant glioma with the possibility of multiple dosing regimens.

Abstract No. T02

MicroRNA-181a promotes human neuronal differentiation by down-regulating genes involved in neural stem cell maintenance

Laura Stappert [1,], Beate Roese-Koerner [1], Monika Veltel [1], Lodovica Borghese [1], and Oliver Brüstle [1]*

[1]Institute of Reconstructive Neurobiology
*Presenting author

Recent evidence indicates that microRNAs (miRNAs), which are post-transcriptional gene expression regulators, can be placed in midst of the regulatory mechanisms underlying neural stem cell maintenance, differentiation and neuronal subtype specification. However, given the large number of miRNAs expressed in neural stem cells, our understanding of miRNA-based regulation is still in its infancy. Here, we focused on miR-181a, a ubiquitous regulator of development highly expressed in the neural lineage. Using pluripotent stem (hPS) cell-derived long-term self-renewing neuroepithelial-like stem cells (lt-NES cells) we set out to investigate the impact of miR-181a on neuronal differentiation. Based on gain- and loss-of-function analyses we found that miR-181a, similar to miR-124, is required for shifting lt-NES cells from a self-renewing phenotype towards neuronal differentiation. Furthermore, overexpression of miR-181a in differentiating lt-NES cells induced a bias towards TH-positive dopaminergic-like neurons. On a mechanistic level, miR-181a might contribute to neuronal differentiation by targeting neural stem cell-associated genes such as NOTCH2, LIN28/B and Musashi2, the expression of which was reduced upon miR-181a overexpression. MicroRNA-181a-induced down-regulation of LIN28A/B also resulted in a shift of the LIN28-let-7 feedback loop towards higher expression of let-7, which itself is a well-known promoter of neuronal differentiation. Taken together, this study uncovers a mechanistic role of miR-181a in regulating the expression of genes implicated in neural stem cell maintenance and promoting neuronal differentiation. These findings might prove useful to refine the propagation and directed differentiation of human neural stem cells into medically relevant neuronal subtypes.

Stappert L, Borghese L*#, Roese-Koerner B, Weinhold S, Koch P, Terstegge S, Uhrberg M, Wernet P, Brüstle O#. MicroRNA-based promotion of human neuronal differentiation and subtype specification. PLoS One 8(3):e59011(2013)*

Roese-Koerner B, Stappert L*, Koch P, Brüstle O#, Borghese L#. Pluripotent stem cell-derived somatic stem cells as tool to study the role of microRNAs in early human neural development. Curr Mol Med 13(5):707-722 (2013)*

Abstract No. T03

Pulmonary transplantation of multipotent- or pluripotent-stem cell derived macrophages as a novel treatment option for Pulmonary Alveolar Proteinosis

Nico Lachmann [1,], Christine Happle [2], Mania Ackermann [1], Jelena Skuljec [2], Doreen Lüttge [1], Theresa Buchegger [1], Christian Hennig [2], Thomas Rodt [3], Jens Bankstahl [4], Gesine Hansen [2], and Thomas Moritz [1]*

[1]Hannover Medical School, Institut of Experimental Hematology
[2]Hannover Medical School, Dep. of Pediatric Pneumology, Allergology and Neonatology
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Hereditary pulmonary alveolar proteinosis (herPAP) constitutes a rare lung disease caused by mutations in the granulocyte/macrophage-colony-stimulating-factor (GM-CSF) receptor genes (CSF2RA or CSF2RB). Pathological hallmarks are disturbed differentiation of alveolar macrophage (AM), massive alveolar proteinosis, and life-threatening respiratory insufficiency. So far, treatment is symptomatic only, including repetitive whole lung lavage in general anaesthesia. We here introduce pulmonary cell transplantation (PCT) of stem cell derived macrophage-progenitors (MPs) as a novel, cause directed, and long-lasting therapy for herPAP.

In a Csf2rb-/- mouse-model, PCT yielded selective pulmonary engraftment of donor cells detected by flow- and chipcytometry. Profound reduction of alveolar-protein levels and significant improvement of clinical parameters such as lung function parameter and lung densities on computer tomography (CT) scans were observed for more than nine months. Subsequent in situ analysis revealed in vivo differentiation towards an AM typical phenotype, characterized by flow-cytometry, poor antigen presentation, high phagocytic activity. Moreover, in a humanized herPAP mouse model, PCT of human CD34+ -derived MPs profoundly improved symptoms, led to long-term human cell engraftment, reduced alveolar-fluid proteins by 50-70%, and significantly improved herPAP related signs on CT scans for at least six months.

As induced pluripotent stem cells (iPSC) appear as an innovative and readily available autologous cell source, we have generated hematopoietic cells from healthy human iPSCs. Hematopoietic differentiation of iPSCs yielded macrophages of typical morphology and phenotype (CD14+, CD11b+, CD45+) displaying GM-CSF dependent functions such as CD11b activation, GM-CSF uptake, and downstream signalling via STAT5. Transplantation of those cells let to exclusive engraftment in lungs of humanized herPAP mice for up to one month.

Thus, we here describe a novel, cause directed treatment approach to herPAP based on PCT of multi- or pluripotent-stem cell derived MPs, which may serve as a proof-of-principle to extend current HSC-based therapy concepts to strategies targeting mature, long-lived cells.

Abstract No. T04

Human iPSC-derived MSC-like progenitor cells: functional differences from primary MSCs affecting their use in cartilage regenerative medicine

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Human induced pluripotent stem cells (hiPSCs) are highly attractive for cartilage regenerative medicine, because they can be derived autologously in potentially unlimited amounts and comprise the inherent capacity to give rise to stable hyaline cartilage. On their way from the pluripotent state to a differentiated chondrocyte, iPSCs pass through a meta-stable mesenchymal stage that potentially resembles mesenchymal stromal cells (MSCs). This is most intriguing, since the derivation of MSC-like progenitors from pluripotent stem cells would combine the advantage of unlimited proliferative capacity of iPSCs with the well-known properties of MSCs. However, characterization of such MSC-like cells is currently inadequate, especially with regard to the question of whether these cells are equivalent or identical to MSCs. To address this question, we generated iMPCs (iPS-derived MSC-like progenitor cells) using 4 different strategies and compared these cells with primary bone marrow-derived hMSCs. All protocols yielded iMPCs with typical MSC/fibroblastic morphology, exhibiting the MSC-typical surface marker profile and expressing a general mesenchymal transcript profile. Moreover, iMPCs were capable to differentiate in vitro along the osteogenic, chondrogenic, and adipogenic lineages. Yet, iMPCs were not identical with BMSCs and displayed specific deviations on gene expression level. Importantly, they were less responsive to traditional MSC differentiation protocols, indicating that they require adapted stimuli for in vitro differentiation. Interestingly, during hiPSC differentiation into iMPCs, intermediate cell types showed a phase-dependent response towards chondrogenic stimuli implying that temporal regulation of differentiation events might be the key strategy for efficient in vitro induction of chondrogenesis in hiPSCs.

Diederichs S, Tuan RS (2014) Functional comparison of human-induced pluripotent stem cell-derived mesenchymal cells and bone marrow-derived mesenchymal stromal cells from the same donor. Stem Cells Dev 23:1594-1610

Abstract No. T05/P168

Shp2 signaling is essential to the suppression of senescence in mammary gland cancer stem cells in mice

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In this study, we have used techniques from cell biology, biochemistry, and genetics to investigate the role of the tyrosine phosphatase Shp2 in MMTV-PyMT mouse mammary gland tumors. Genetic ablation or pharmacological inhibition of Shp2 induces senescence in cancer stem cells (CSCs), as determined by the activation of senescence-associated β-gal (SA-β-gal), p53, and histone 3 trimethylated lysine 9 (H3K9me3). Senescence induction leads to the inhibition of CSC self-renewal of and the blockage of tumor formation. A signaling cascade was identified that acts downstream of Shp2 to counter senescence in CSCs: Src, Focal adhesion kinase and Map kinase inhibit senescence by activating the expression of Sphase kinase-associated protein 2 (Skp2), Aurora kinase A (Aurka), and the Notch ligand Delta-like 1 (Dll1), which block cyclin-dependent kinase inhibitor 1B (p27) and p53. Remarkably, the expression of Shp2 and of selected target genes highly predicts human breast cancer outcomes. We conclude that therapies which rely on senescence induction in CSCs by inhibiting Shp2 or controlling target gene products may be useful in blocking breast cancer.

Aceto N, et al. (2012) Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells via activation of key transcription factors and a positive feedback signaling loop. Nat Med 18: 529-537.

Grossmann KS, Rosario M, Birchmeier C, Birchmeier W (2010) The tyrosine phosphatase Shp2 in development and cancer. Adv Cancer Res 106: 53-89.

Abstract No. T06

Humansation of mouse models for human breast cancer using mesenchymal stem cells

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Mesenchymal stem cells (MSCs) migrate to breast carcinomas, where they involve in building up the tumor-associated stroma. In recent years, the importance of tumor-associated stroma for cancer initiation, development, local invasion and metastases has become in the focus of research. We studied the influence of MSCs on the in vitro and in vivo growth, engraftment and vascularisation of breast cancer cells. The breast cancer cell line MDA-MB231 was cultured directly with human adipose tissue derived MSCs in vitro and changes in morphology were monitored. Subsequently, alterations of protein and gene of MSC, angiogenesis, proliferation and stromal markers were investigated by flow cytometry and real-time RT-PCR. Estrogen receptor-negative MDA-MB231 or estrogen receptor-positive MCF7 breast cancer cells were injected together with MSCs subcutaneously and orthotopically into immunodeficient mice. The development and growth of tumors and metastasis was monitored. Tumors were surgically removed and changes in protein and gene expression of angiogenesis, proliferation and stromal markers were determined by flow cytometry and RT-PCR. We observed that in vitro human MDA-MB231 mammary ductal adenocarcinoma cells interact with human adipose tissue derived MSCs trough paracrine signaling whereas MSC lose their stem cell character and differentiate. RNA expression of CD31 and a-smooth-muscle-actin indicates the formation of human endothelial cells and cancer-associated fibroblasts. In vivo, tumor growth and cell dissociation (metastasis) of breast cancer cells is moderately stimulated through the addition of MSCs. During in vivo co-culture, MSCs lose their stem cell character and differentiate. Summarizing, our results indicate that human MSCs interact with breast carcinoma epithelia cells. They migrate from a tumor associatedstroma supporting proliferation, migration and neoangiogenesis.

Abstract No. T07

Targeting the kinome to induce differentiation in human breast cancer stem cells

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Conventional chemotherapy attacks the bulk of the tumor indiscriminately with the aim to kill tumor cells. The notion that some of these cells are cancer stem cells, which are quiescent, more resistant, able to self-renew and give rise to differentiated progeny, challenges classical treatments. Differentiation therapy on cancer stem cells was first mentioned in the 1980s. The idea to push a cell stuck in a mutagen induced proliferative state towards its "natural" differentiation is very attractive. It would make a killing-agent obsolete since the differentiated cell would remain in a stable, non-proliferative state. Unable to sustain tumor growth the cancer would deteriorate over time. We hypothesized that we could target a signaling pathway to terminally differentiate breast cancer stem cells, to acquire either a luminal or myoepithelial fate. Differentiated cells would be limited in their self-renewal capacity and would not be able to form tumors in vivo. To test this hypothesis, we treated an established triple negative breast cancer cell line (MDA-MB-468)(5) with a lentiviral shRNA library to knockdown individual kinases, using well established Keratin immunohistochemistry to identify differentiated cells. We identified over 30 kinases that can influence cancer stem cell fate. Here we present a novel role for ALPK1 and ERN1 both working through the same pathway to elicit a differentiation response in primary breast cancer stem cells upon inactivation. ERN1 and ALKP1 knockdown cells form fewer tumors than control cells. The tumors that do form grow slower and show a more epithelial differentiated pattern. This finding could set the stage for new cancer treatments by targeting cancer stem cells for differentiation.

Abstract No. T08/P178

CD151+ cells drive tumor-initiation, hierarchical growth and proliferation-associated signaling in patient-derived models of serous ovarian cancer

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Cancer stem cells (CSC) have been reported in several cancer entities. However, for serous ovarian cancer (SOC), there is limited evidence for CSC populations and the molecular mechanisms regulating tumor-initiation and stemness are poorly understood. Ovarian Cancer is the leading cause of gynecological cancer-related deaths. Currently, the 5-year survival rate of advanced-stage ovarian cancer is only 27 % (Siegel et al. 2014), emphasizing the need for improved personalized treatment and therapies targeted at CSC. We established primary SOC xenografts and derived serum-free culture models from these to identify novel CSC markers. In a large-scale antibody screen, we identified the cell surface protein CD151, a member of the tetraspanin family as a putative marker candidate. We show that CD151+, but not CD151- SOC cells have tumor-propagating potential in a mouse xenograft assay. Transcriptional profiling and Western blot analysis suggested upregulation of pathways connected to proliferation, drug resistance and stemness specifically in CD151+ cells. Knockdown of CD151 not only resulted in the downregulation of these signaling pathways, but also completely abrogated the tumor-propagating potential of CD151+ SOC cells. Taken together, our results uncover CD151 as an integrator of signaling pathways required for tumor-initiating activity of human serous ovarian cancer functionally contributing to the cellular heterogeneity observed in this tumor entity.

Siegel, R., et al. (2014). "Cancer statistics, 2014." CA: a cancer journal for clinicians 64(1): 9-29.

Abstract No. T09 (back-to-back presentation with T10)

What makes stem cells move? Studying stem cell migration in vitro

Michael Ansorge [1,*], *Weijia Wang* [2], *Axel Krinner* [3], *Peter Zandstra* [2], *Ingo Roeder* [3], and *Tilo Pompe* [1]

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It is commonly agreed that maintenance of adult stem cells depends on complex environmental settings - their 'stem cell niche'. Numerous studies have sought to unveil its components for the hematopoietic system in the bone marrow. Most of them have focused on single components, while less work is found on the dynamics of hematopoietic stem cells (HSC) in and between niche environments.

We consider migration a key mechanism for stem cell regulation as it means to actively choose a particular environment. Thus, we studied the effects of a set of cytokines and growth factors on migration and proliferation of HSC in vitro within a bio-inspired environment. Human cord blood-derived long-term repopulating HSC were harvested seeded onto fibronectin-coated silicone micro-wells and tracked for at least 24h. A ubiquitous cytokine background in the medium - SCF alone vs. combination with TPO and Flt3l - was used based on recent HSC expansion studies. After an initial tracking period in just medium, mobilization agents such as G-CSF, GM-CSF, angiopoietin, UM729 or osteopontin were added to study their effect on migration and proliferation on top of the given cytokine background and tracking was continued.

Under most conditions the effect of the mobilization agent was smaller than the effect of the cytokines in the medium. Cell migration was accelerated in parallel to proliferation for all conditions, but more profound with G-CSF and GM-CSF. This correlation of motility and proliferative activity was not found for individual cells.

Our experiments contribute to dissecting key elements of HSC regulation and represent one of the building blocks to further study the HSC system: 1. in vitro investigations in our controllable biomimetic environments, 2. in vivo studies to reveal HSC dynamics in the real stem cell niche and 3. in silico modeling for hypothesis generation and guidance of experiments.

Abstract No. T10 (back-to-back presentation with T09)

What makes stem cells move? Studying stem cell migration in silico

Axel Krinner [1,*], *Michael Ansorge* [2], *Weijia Wang* [3], *Narges Rashidi* [4], *Cristina Lo Celso* [4], *Peter Zandstra* [3], *Tilo Pompe* [2], and *Ingo Roeder* [1]

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Tracking of individual cells in vitro and in vivo generates phenomenological data on migration and proliferation characteristics. To overcome this level we used an individual cell-based model that represents cells as moving, interacting and proliferating/dying agents. It is built modular in the sense that models for migration and internal state can be coupled in different combinations for direct comparison. The hidden internal state determines both, the cell's motility and proliferative activity. We compared different models for cell migration with the data generated in the migration assay presented in the in vitro counterpart of this contribution. In this assay the effects of different cytokines and mobilization ligands on HSC migration and proliferation/death were quantified. By fitting the model to this data, parameter sets were adjusted to reproduce the results for various medium/mobilization ligand combinations. The model suggests the increase in proliferation and motility to be linked to a continuous adaptation to the culture conditions. This adaptation process is a noise-driven and cell-individual process. Additionally the model is applied to in vitro and in vivo data of murine HSC. We tracked mHSC in the same biomimetic environments without adding cytokines/mobilization ligands. These tracks were compared with tracks of labeled mHSC that were transplanted into lethally irradiated mice and imaged in the calvarium. Fitting the model to both data sets suggests a niche environment induced reduction of persistence time in vivo and also provides two possible mechanisms for cell niche interaction. The individual cell-based model thus reproduces cell motility and proliferation for the studied cytokines/media and provides a model formulation for the correlation of both processes. Furthermore, it is well suited for bridging the gap between in vitro and in vivo data.

Abstract No. T11

Characterizing transcriptional cell-to-cell heterogeneities during stem cell differentiation

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Cell-to-cell variations in gene expression underlie many biological processes. Currently more and more experimental tools are becoming available in order to observe these variations, and to draw conclusions on underlying processes. However, given these experimental advances, we are now facing a series on computational questions dealing with these data, since classical analysis tools are often tailored to population averages. Here I will discuss the computational analysis of single-cell qPCR and RNAseq expressions using nonlinear dimension reduction applied to stem cell differentiation. The analysis is based on a recently proposed framework based on Gaussian process latent variable models, which we extend to guarantee that small distances are preserved for cells from the same developmental stage [Buettner & Theis, Bioinformatics 2012; Buettner et al, Bioinformatics 2014]. Based on our recent characterization of transcriptional networks during blood stem cell differentiation [Moignard et al, Nat Cell Biol 2013], we show how to identify subpopulations using mixture models, extended in order to allow for censoring inherent to the qPCR measurements. We then are able to recapitulate the differentiation hierarchy by quantifying cell-to-cell distances in the transcript space using diffusion map embedding. In summary, we show that single-cell expression allows for a robust and far more detailed description of the often heterogeneous differentiation decisions than using population averages.

Abstract No. T12

A network-based strategy to direct cell fate determination

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A bistable toggle switch constitutes a molecular mechanism that has been shown to determine cellular commitment and provides stability to transcriptional programs of binary cell fate choices. Furthermore, it has been proposed that the balanced expression pattern of both transcription factors generates a metastable state corresponding to the stem/progenitor cell, whereas the two steady states corresponding to the two daughter cells are manifested when one of the transcription factors dominates the other. More recently, it has been proposed a model for explaining mesendodermal and ectodermal specification of embryonic stem cells (1). According to this model balanced expression of OCT4 and SOX2, which mutually activate each other, maintains the pluripotent state. Here we propose this concept as a general mechanism for stem/progenitor cell differentiation, which could involve more complex circuit architectures. We present a computational methodology to identify candidate genes, whose perturbations determine cell fate during cellular differentiation. This methodology, which relies on cell-type specific gene regulatory network inference and analysis (2), requires as input only transcriptome data of progenitor and daughter cells. Furthermore, we propose that positive feedback loops (PFLs) (a bistable toggle switch is a special case of PFLs) that contain these pairs of lineage specifiers are responsible for determining cell fates and maintaining the stem/progenitor state. Our predictions included experimentally validated pairs in ESCs, hematopoietic stem cells, and mesenchymal stem cells. In addition, we predicted candidate pairs of opposing lineage specifiers in neural stem cells, whose overexpression by using lentiviral transfection led to the expected cell lineage determination. To our knowledge, this is the first computational method that systematically predicts lineage specifiers of cellular differentiation without prior knowledge of potential candidate genes and involved pathways. Given the increasing interest of cell fate determination in regenerative medicine, our method can assist researchers in the filed in designing experimental strategies.

Abstract No. T13/P186

Computational identification of pluripotency networks

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Stem cells are distinguished from other cells based on their ability to differentiate into different cell types. Experimental studies have shown that the transcription factors which maintain the pluripotency network share many target genes [1]. Computational identification of a set of transcription factors which govern the full pluripotency network need to take into account the typical topology of gene regulatory networks (GRNs). The existence of several genes with high out-degrees suggests that this small set of core genes may have a major role in regulating maintenance of pluripotency or onset of differentiation. Thus we suggest to consider the task of identifying a set of transcription factors as an analogue of an optimization problem, namely that of constructing a Minimum Connected Dominating Set (MCDS) [2]. We present a novel approach to identify master regulatory genes responsible for cell cycle or cell fate transition. We applied our method to established GRNs of E.coli and S.cerevisiae and compared the results with a MCDS solution for the yeast cell cycle genes that was predicted from gene expression data of yeast genes during a time series of cell cycle intermediates. Our knowledge about the pluripotency network in embryonic stem cell is presently quite limited. Here we used pluripotency network for mouse published by Som et al. which consists of 574 molecular interactions until June 2010, involving 274 mouse genes/proteins. We applied the approximation algorithm for determining the MCDS on this data. The obtained MCDS consists of 42 genes, where the 7 genes Pou5f1, Nanog, Sox2, Nr5a2, Stat3, Tcf3, Klf4 are the top genes with highest connectivity.

Kim, J., Chu, J., Shen, X., et al. 2008. An extended transcriptional network for pluripotency of embryonic stem cells. Cell. 132, 1049-1061.

Nazarieh, M., Helms, V. 2014. Identificational of Master Regulatory Genes as a Minimum Connected Dominating Set. submitted.

Abstract No. T14/P066

The RNA helicase DDX6 regulates cell-fate specification in neural stem cells via miRNAs

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Stem cells have the ability to either self-renew, thereby maintaining their stem cell status, or to undergo differentiation. The balance between stem cell maintenance and differentiation is tightly regulated by a complex interplay of various signalling pathways, cell fate determinants and non-coding RNAs, including microRNAs. One important cell fate determinant during embryonic and adult neurogenesis is the TRIM-NHL family member TRIM32. In neural stem cells, TRIM32 inhibits proliferation and induces neuronal differentiation by two mechanisms. On the one hand, TRIM32 ubiquitinates the transcription factor c-Myc, thereby targeting it for proteasomal degradation and inducing cell-cycle exit. On the other hand, TRIM32 associates with the RNA-induced silencing complex and increases the activity of certain miRNAs such as Let-7a. However, the exact mechanism of miRNA regulation by TRIM32 during neuronal differentiation remains elusive. Here, we used a mass spectrometry approach to identify novel protein-protein interaction networks centred on TRIM32 during neuronal differentiation. We found that TRIM32 associates with proteins involved in neurogenesis and RNA-related processes, such as the RNA helicase DDX6, which has been implicated in microRNA regulation. We demonstrate, that DDX6 colocalises with TRIM32 in neural stem cells and neurons and that it increases the activity of Let-7a. Furthermore, we provide evidence that DDX6 is necessary and sufficient for neuronal differentiation and that it functions in cooperation with TRIM32.

Abstract No. T15/P049

Angiogenesis controls neural stem cell expansion by regulating tissue oxygenation and HIF signaling

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The neural stem cell niche controls the expansion and differentiation of neural stem cells to safeguard brain formation during development and brain homeostasis in adulthood. Blood vessels are part of the neurogenic niche in the developing and adult brain, but their functional significance for the regulation of neural stem cell differentiation and the mechanisms involved, remain poorly understood. We show that blood vessel formation in the developing cortex quenches hypoxia-inducible factor (HIF)-dependent gene transcription and coincides with induction of neural stem cell differentiation in time and space. Moreover, specific inhibition of normal brain angiogenesis maintains HIF activity and increases neural stem cell expansion at the expense of differentiation. Conversely, HIF is essential for proper cortex formation because genetic knockout of HIF1a increases neural stem cell differentiation during cortical development. Our findings establish a novel oxygen-dependent mechanism how blood vessels regulate neural stem cell differentiation during development and show for the first time that a lowered oxygen level triggers neural stem cell expansion in vivo.

Abstract No. T16/P067

Regulation of asymmetric/symmetric stem cell division in human epidermis

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Human epidermis is maintained by balancing stem cell (SC) maintenance/proliferation with subsequent differentiation. It was long suggested that the fate of epidermal SCs is decided by asymmetric cell division, with one daughter remaining as a SC and the other becoming a transit amplifying cell which proliferates and finally differentiates. This SC hierarchy is, however, questioned lately. Clayton et al. proposed in 2007 that all basal cells have progenitor cell function and expand by symmetric or asymmetric division. Accordingly, only basal cells have proliferative potential. At present, it remains elusive which hypothesis is correct and how these different division types are regulated. To monitor the mitotic behaviour of human epidermal SCs in an in vivo-like situation we use a long-term organotypic culture model. This allows for SCs to be followed for several weeks (label-chase approach). We could show that the slowly cycling putative SCs establish as label-retaining cells in the basal layer of the regenerative epidermis and apply this model to investigate their mode of division with markers specific for asymmetric division. First, and divergent from the present dogma, our data suggest that mitosis may not be restricted to the basal layer but occurs also in suprabasal layers. It now needs to be addressed how suprabasal proliferation is regulated. Second, we found different division angles in basal and in suprabasal mitoses, with most cells dividing in horizontal or oblique orientation. This is in contrast to different studies stating that mitosis occurs either mostly in horizontal or, depending on the tissue, in vertical direction. Third, we could show for the first time an asymmetric distribution of the cell fate determinant numb in mitotic human keratinocytes. A CRISP/R-Cas9 knockdown of NUMB is underway to explore the effect of NUMB deletion on human epidermal development and regeneration in the 3D organotypic culture model.

Abstract No. T17

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 heterogeneity of the acinar cell compartment. This notion led to the hypothesis that there might be previously neglected hierarchy within the acinar cell compartment since stem-/progenitor cells were proposed to be the cell of origin for many tumors. Here we use multicolor lineage tracing to study the clonal contribution of cells within the acinar cell population. We find substantial differences in the proliferation dynamics of single clones among acinar cells. As a complementary in vitro approach to assess clonal heterogeneity, we examine the organoid-forming capacity of these cells. In this assay, we identified a unique subpopulation of acinar cells with the ability to give rise to organoids. Thus, although the acinar population, similarly to the beta cells among the endocrine cells, is often considered as a homogeneous population we find clonally heterogeneous contribution to growth and maintenance of pancreas homeostasis.

Abstract No. T18

Probing mitochondrial DNA disorders using iPSC-derived neural progenitors

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The advance in treating mitochondrial DNA (mtDNA) disorders is hampered by the lack of viable cellular and animal models. Induced pluripotent stem cells (iPSCs) may bring unprecedented opportunities through the generation of cells harboring the same nuclear and mitochondrial genome of the patients. Given the detrimental effect of mtDNA mutations on neuronal functionality, the employment of neural committed cells would be particularly relevant. Here, we explore the possibility to employ iPSC-derived neural progenitor cells (NPCs) as a novel model system for studying mtDNA disorders. NPCs were efficiently generated from human iPSCs and hESCs using a direct small molecule based approach. Global transcriptomics was used to confirm the neural identity of our NPCs with respect to published NPCs and map their developmental stage on brain atlas repositories. Electron microscope studies, cell proliferation assay, gene expression analysis, quantitative calcium imaging, and bioenergetic profiling demonstrated that NPCs exhibit mitochondrial maturation and a metabolic shift towards oxidative metabolism. Deep sequencing of mtDNA established that the parental mtDNA profile was maintained during reprogramming (both using retroviruses and episomal plasmids) and upon neural induction. We then generated patient-derived NPCs carrying a mtDNA mutation within the MT-ATP6 gene. Importantly, mutation-associated neural phenotypes could be identified, including mitochondrial hyperpolarization, plasma membrane depolarization, and elevated free-radical production. Based on these detected dysfunctions, NPC-based high content screenings (HCS) strategies are currently being developed to discover compounds capable of reverting the disease phenotypes. Yeast high-throughput studies have been used in the past to suggest potential new treatments against MT-ATP6 mutations. Our proposed system would have the critical advantage of exhibiting the correct genetic and metabolic features, potentially enabling the identification of more effective therapies. Patient-derived NPCs might therefore represent a novel model system allowing the personalized discovery of innovative treatment strategies for debilitating mitochondrial DNA encephalopathies.

Abstract No. T19

Modeling Dravet syndrome in iPS cell-derived neurons

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Dravet syndrome (DS) is a debilitating, congenital form of childhood epilepsy. Most patients carry mutations in the SCN1A gene leading to insufficient functional Nav1.1 protein, a pore-forming α-subunit of voltage-gated sodium channels. Coinciding with Nav1.1 expression in developing GABAergic interneurons, myoclonic seizures develop within the first 3-18 months of life. To model DS in vitro, fibroblasts from patients harboring monoallelic loss-of-function mutations of SCN1A were reprogrammed to induced pluripotent stem cells and differentiated into long-term self-renewing neuroepithelial stem (lt-NES) cells. Upon growth-factor withdrawal, lt-NES cells predominantly differentiate into GABAergic neurons with anterior hindbrain identity (Koch et al., 2009). DS-specific neurons differentiated for 6 weeks exhibit voltage-dependent inward and outward currents, action potential generation and spontaneous synaptic activity. RT-qPCR and Western blot analyses of patient-specific neurons revealed a 50% reduction in wild-type SCN1A expression. Recent work suggests that the intracellular domain of an accessory channel subunit (β2) regulates Nav1.1. We set out to explore whether ectopic β2-ICD expression can be used to elevate Nav1.1 levels in human DS neurons. Thus, a DS lt-NES cell line was transduced with a lentiviral doxycycline-inducible construct coding for β2-ICD fused to GFP via a 2A peptide and sorted for GFP+ cells to near purity. Autocatalytic construct cleavage was confirmed by Western blot analysis, and nuclear import of transgenic β2-ICD could be verified by 3D microscopy. The β2-ICD transgenic cell line maintained typical neural stem cell markers and could differentiate into GABAergic neurons. After transgene induction a 4-8-fold increase in Nav1.1 protein levels was detected in 6-week-old neuronal cultures by Western blot analysis. In addition, biotinylation studies showed a quantitative increase of Nav1.1 α-subunit levels on the cell surface. These results indicate a regulatory activity of β2-ICD on sodium channel homeostasis in authentic human neurons, which might be exploited to counteract Nav1.1 deficiency in DS-specific neurons.

Koch P., Opitz T., Steinbeck J. A., Ladewig J. and Brüstle O. (2009). A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. PNAS 106:3225-30

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Abstract No. T20

The functionality of stem cell derived neurons: How to define it?

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The aim of cell-replacement therapies for the diseased CNS is to restore functions. Here, pluripotent stem cell (PSC)-derived neuronal progenies have been and still are one of the main cell candidates considered for such therapies. Brain function is based on the ability of neurons to self-organise into functional interconnected neuronal assemblies, which generate concerted activities. Thus, stem cell derived neurons have to fulfill several criteria on the single cell as well as on the network level. Since decades, genetic, immunocytochemistry as well as patch-clamp approaches are used to characterize the functionality of PSC-derived neurons at the single cell level. In the recent years, the stem cell field move forward to assess the functionality of PSC-derived neurons on the systemic level. For this issue, multi-site electrophysiological recordings and calcium imaging were applied on PSC-derived neuronal cultures to address the question if also in vitro generated neurons self-organize into functional neuronal networks. The presentation will provide an overview on the current approaches to define the functionality of PCS-derived neuronal assemblies. Furthermore novel insights about the origin of autonomous activities in PSC-derived neuronal networks will be presented and their possible implications for cell-replacement therapies will be discussed.

Abstract No. T21/P038

Modeling and pharmacological rescue of ion channel diseases enabled by improved cardiac induction of human pluripotent stem cells in 2D and 3D formats

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Directed cardiac differentiation of human pluripotent stem cells (hPSCs) enables disease modeling, investigation of human cardiogenesis, as well as large-scale production of cardiomyocytes for translational purposes. Multiple cardiomyocyte differentiation protocols have been developed to individually address specific requirements of these diverse applications. However, there is no universal high-efficiency procedure for generating cardiomyocytes both in 2D and 3D culture formats, and undefined or complex media additives compromise functional analysis or cost-efficient upscaling. Using systematic combinatorial optimization, we have developed a novel cardiac induction procedure. This implied differentiation in simple serum and serum albumin-free basal media, mediated by a minimal set of signaling pathway manipulations at moderate factor concentrations. The method was applicable both to 2D and 3D culture formats as well as to multiple hPSC lines. Global time-course gene expression analyses over extended time periods and in comparison with human heart tissue was used to monitor culture-induced maturation of the resulting cardiomyocytes (CMs). This suggested that hPSC-CMs obtained with our procedure reach a rather stable transcriptomic state after approximately four weeks of culture, which correlated well with functional maturation assays. These findings were applied to modeling genetic ion channel diseases using patient-specific hPSCs. We show that in vitro maturation of hPSC-CMs is a pre-requisite for faithful disease modeling and that this system is a powerful means for identifying and validating putative drugs to correct disease phentoypes.

Abstract No. T22

Primate-specific endogenous retrovirus driven transcription defines naïve-like stem cells

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Naïve embryonic stem cells (ESCs) hold great promise for research and therapeutics as they have broad and robust developmental potential. While such cells are readily derived from mouse blastocysts, to date it has been impossible to easily isolate human equivalents, although human naïve-like cells have been artificially generated (rather than extracted) by coercion of human primed ES cells by modifying culture conditions or through transgenic modification. Here we show that a sub-population within cultures of human ESCs (hESCs) and induced pluripotent stem cells (hiPSCs) manifest key properties of naïve state cells. These naïve-like cells can be genetically tagged, and are associated with elevated transcription of HERVH, a primate-specific endogenous retrovirus (ERV). HERVH elements provide functional binding sites for a combination of naïve pluripotency transcription factors, including LBP9, recently recognized as relevant to naivety in mice. LBP9/HERVH drives hESC-specific alternative and chimeric transcripts, including pluripotency modulating long non-coding RNAs (lncRNAs). Disruption of LBP9, HERVH and HERVH-derived transcripts compromises self-renewal. These observations define HERVH expression as a hallmark of naïve hESCs, and establish novel primate-specific transcriptional circuitry, regulating pluripotency.

Abstract No. T23/P007

A novel lncRNA-protein interaction characterises mouse embryonic stem cell fate

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ES cells are of particular interest to researchers owing to their properties of replicating indefinitely in culture and giving rise to germ layers that eventually constitute an organism. These unique abilities make them perfect models to study essential cellular developmental processes and also contribute to the understanding of the molecular pathways that ultimately lead to diseases like cancer. The maintenance of stem cell characteristics (also known as pluripotency), like other processes, is orchestrated by a host of different factors in which lncRNAs are slowly emerging as important players. Although there are thousands of lncRNAs identified, only a few have been implicated in pluripotency. Using endoribonuclease-prepared small interfering RNAs (esiRNAs), we used a loss of function approach to discover lncRNAs involved in pluripotency. By combining RNAi and localization, we report the discovery of a novel lncRNA Panct1 which through interaction with other factors takes part in the ES cell pluripotency programme. In the process of characterization of Panct1, we have also identified and partially characterized a potential DNA binding protein called CXORF23 which act in cohort with the lncRNA. Together, they appear to have a cell cycle specific interaction profile and implicate several genes in the OCT4 pathway. These discoveries hint towards the presence of more such lncRNA protein interactions and further widen our understanding of stem cell biology.

Abstract No. T24

NANOG and CDX2 pattern distinct subtypes of human mesoderm during exit from pluripotency

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SUMMARY

Mesoderm is induced at the primitive streak (PS) and patterns subsequently into mesodermal subtypes and organ precursors. It is unclear whether mesoderm induction generates a multipotent PS progenitor or several distinct ones with restricted subtype potentials. We induced mesoderm in human pluripotent stem cells with ACTIVIN and BMP or with GSK3-beta inhibition. Both approaches induced BRACHYURY+ mesoderm of distinct PS-like identities, which had differing patterning potential. ACTIVIN and BMP-induced mesoderm patterned into cardiac but not somitic subtypes. Conversely, PS precursors induced by GSK3-beta inhibition did not generate lateral plate and cardiac mesoderm and favored instead somitic differentiation. The mechanism of these cell fate decisions involved mutual repression of NANOG and CDX2. Although NANOG was required for cardiac specification but blocked somitic subtypes, CDX2 was required for somitic mesoderm but blocked cardiac differentiation. In sum, rather than forming a common PS progenitor, separate induction mechanisms distinguish human mesoderm subtypes.

Mendjan, S., Mascetti, V.L., Ortmann, D., Ortiz, M., Karjosukarso, D.W., Ng, Y., Moreau, T., and Pedersen, R.A. (2014). NANOG and CDX2 Pattern Distinct Subtypes of Human Mesoderm during Exit from Pluripotency. Cell Stem Cell.

Pedersen, R.A., Mascetti, V., and Mendjan, S. (2012). Synthetic organs for regenerative medicine. Cell Stem Cell 10, 646–647.

Abstract No. T25

Insights from the streak: identification of novel genes associated with germ-layer formation and mesendoderm differentiation

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The primitive streak of amniotes represents the anatomical correlate of gastrulation, the process during which the three principal germ layers, endoderm, mesoderm and ectoderm emerge from the pluripotent epiblast. Using the chick model and Affymetrix Genome Array based transcriptome analysis we characterized the spatiotemporally dynamic gene expression profiles of the anterior-posterior and medial-lateral extension of the primitive streak as well as its neighboring epiblast and underlying hypoblast. In addition to unraveling an intricate gene expression network present along the axis of the primitive streak, likely involved in and essential for the dorso-ventral patterning of mesoderm, we could also identify multiple novel markers of early mesendoderm differentiation including neuropilin 1 (NRP1) and leukemia inhibitory factor receptor alpha (LIFR) expressed selectively in the hypoblast and early endoderm. Utilizing human ES and iPS cells we could further show that NRP1 and LIFR are also robust markers of in vitro endoderm differentiation of human pluripotent stem cells and can be used for the enrichment of endodermal progenitors, which could be further differentiated to airway epithelial cells and utilized to ameliorate an in vivo murine lung injury model. Using our large scale embryonic transcriptome data sets, we were furthermore able to identify novel and so far uncharacterized pan-mesodermal markers which are specifically expressed in the primitive streak in vivo and tightly regulated during differentiation of murine and human pluripotent stem cells in vitro.

Alev C, Wu Y, Kasukawa T, Jakt LM, Ueda HR, Sheng G. Transcriptomic landscape of the primitive streak. Development. 2010 Sep 1;137(17):2863-74.

McIntyre BA, Alev C, Mechael R, Salci KR, Lee JB, Fiebig-Comyn A, Guezguez B, Wu Y, Sheng G, Bhatia M. Expansive generation of functional airway epithelium from human embryonic stem cells. Stem Cells Transl Med. 2014 Jan;3(1):7-17.

Abstract No. T26/P039

Tet-dependent processing of 5-methylcytosine protects DNA methylation canyons against hypermethylation

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DNA methylation plays an important role during lineage specification in the developing mammalian embryo that is accompanied by extensive reprogramming of the epigenome, resulting in the repression of stemness specific factors and the transcriptional maintenance of activated lineage-specific genes. As the presence of 5-methylcytosine (5mC) at or near promoter regions is mostly incompatible with transcription, methylated cytosines have to be removed or converted to enable active transcription. The Ten eleven translocation (Tet) family of enzymes converts 5mC to 5-hydroxymethylcytosine (5hmC) which promotes passive DNA demethylation and functions as intermediate in the process of active DNA demethylation.

In order to clarify the physiological roles of the three Tet proteins we have generated Tet1 and Tet2 double-knockout (DKO) mice and Tet1/2/3 triple-knockout (TKO) embryonic stem cells (ESCs) and examined their developmental potential. Combined deficiency of all three Tets depleted 5hmC completely and impaired differentiation. DKO ESCs were also depleted of 5hmC, but retain pluripotency. A fraction of double mutant embryos exhibited various developmental defects and perinatal lethality. Global gene expression and methylome analyses of TKO embryoid bodies showed in particular promoter hypermethylation and correlated deregulation of genes implicated in embryonic development and differentiation. These findings suggest a requirement for Tet- and 5hmC-mediated DNA demethylation for the proper regulation of gene expression during ESC differentiation and development. Using whole-genome bisulfite and transcriptome sequencing of wildtype and DKO mouse embryonic fibroblasts we found further severe epigenetic programming defects in DKO cells, with a key feature being the hypermethylation of DNA methylation canyons harbouring promoter regions of developmental genes. Our results suggest an important regulatory role of Tet-dependent DNA demethylation in the maintenance of DNA methylation canyons to prevent invasive DNA methylation into promoter regions of developmental genes.

Abstract No. T27

PIEZO2 is required for mechanotransduction in human ES-cell-derived touch receptors

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Mechanotransduction, the conversion of mechanical force into electrochemical signals, is the basis for several sensory phenomena such as hearing, balance and touch sensation. Touch receptors are highly specialized sensory neurons that innervate our skin and endow us with the capacity to discriminate shape and fine texture of objects. *In vivo*, touch receptors, also referred to as low-threshold mechanoreceptors (LTMRs), are intermingled with sensory neurons finely tuned to detect a variety of other (non-mechanical) thermal and chemical stimuli. The heterogeneity of sensory ganglia and very low abundance of LTMRs have hampered their isolation and characterization. Moreover, human LTMRs are not accessible at all for functional examination. Thus, the molecules that allow touch receptors to rapidly transduce small mechanical stimuli have remained elusive. Two molecules that recently have been implicated in sensing mechanical force in different cellular contexts are the large transmembrane proteins Piezo1 and 2. Here we show that the mechanosensitivity of human ES-cell-derived touch receptors depends on PIEZO2. In order to examine human touch receptor function, we recapitulated sensory neuron development *in vitro* and established a multi-step differentiation protocol to generate LTMRs via the intermediate production of hES-cell-derived neural crest cells, the sensory neuron progenitors. The generated neurons express a highly distinct set of LTMR specific genes and convert mechanical stimuli into electrical signals, their most salient characteristic *in vivo*. Strikingly, mechanosensitivity is lost following CRISPR/Cas9-mediated *PIEZO2* gene deletion.
This is the first time, to our knowledge, that highly specialized, fully functional human touch receptors have been generated *in vitro*. This approach may help to elucidate developmental programs underlying sensory neuron diversity and may also facilitate mechanistic analysis of different sensory subtypes.

Abstract No. T28

Transcriptional and epigenetic dynamics underlying cell lineage commitment

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Mouse Embryonic stem (ES) cells are pluripotent and have the potential to generate all cell lineages of an embryo. Under normal physiological conditions most cell types are unable to de-differentiate and to give rise to other cell lineages. However by forced expression of pluripotency-associated transcription factors, it is possible to reprogram a terminally differentiated cell to an ES-like state. Importantly the reprogrammed cells will have an epigenetic landscape very similar to the one of blastocyst-derived ES cells meaning that cell reprogramming has to be accompanied by a global re-setting of the epigenome. This suggests that the cell identity is the result of the crosstalk between specific transcription factors and the epigenetic landscape. How this interplay between transcription factors and epigenetic machineries is regulated in the context of differentiation is still largely unclear. We addressed this question by studying the transition from ES cells to definitive endoderm cells. We investigated the genome-wide transcriptional and epigenetic dynamics in FACS-sorted cells corresponding to defined differentiation states. Our results show that the switch in signaling from Lif/Bmp4 to Wnt/Activin leads to up-regulation of lineage-specific transcription factors. Master transcription factors, such as Foxa2, activate a new subset of enhancers leading to activation of the endoderm network. In this context Foxa2 acts as a pioneering factor that leads to increased DNA hydroxymethylation on its binding sites. However, additional transcription factors that bind in the vicinity are necessary to convert Foxa2 binding sites into functional enhancers. Thus our data support a model by which differential gene expression programs are driven by combinatorial binding of transcription factors on lineage-specific enhancers.

Abstract No. T29

The histone 3 lysine 4 methyltransferase Mll2 primes the Nxt2 promoter for neural differentiation

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Methylation of lysine residues in histone tails is an intensively studied epigenetic signal that regulates transcription throughout development. Methylation of histone 3 lysine 4 (H3K4) is usually associated with promoters of actively transcribed genes while H3K27 or H3K9 methylation marks silenced genes. Yeasts possess only one H3K4 methyltransferase, Set1. In contrast, there are six enzymes capable of catalyzing this modification in mammals implying a certain specialization or division of labor. We studied the functions of the mouse H3K4 methyltransferase paralogs Mixed Lineage Leukemia 1 (Mll1) and Mll2 during neural differentiation.

Mll2 is required for differentiation of embryonic stem (ES) cells to neural stem (NS) cells while Mll1 is not. However, Mll2 activity was only required briefly when ES cells started to differentiate. Mll1 could replace Mll2 from the primed epiblast stem (EpiS) cell state on. We identified Nuclear transport factor 2-like export factor 2 (Nxt2) as essential Mll2 target gene. Mll2 H3K4 trimethylated the Nxt2 promoter in ES cells in order to allow for transcriptional upregulation during subsequent neural differentiation. Additionally, Mll2 prevented apoptosis of differentiating cells by regulating B cell leukemia/lymphoma 2 (Bcl2) levels. Overexpression of Nxt2 together with Bcl2 partially rescued the neural differentiation defect of Mll2 knockout ES cells. Importantly, once established in NS cells, the expression of Nxt2 became independent of promoter H3K4 trimethylation. Thus, Mll2 and its target gene Nxt2 represent an example for H3K4 methylation functioning as priming mechanism rather than for fine-tuning or maintenance of transcription levels.

Abstract No. T30

Signals from the injury niche increase fate potential of adult neural stem cells

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Adult neural stem cells (NSCs) in the subventricular zone (SVZ), unlike multipotent embryonic progenitors, only give rise to restricted neuronal subtypes, namely inhibitory interneurons in the olfactory bulb. However, after brain injury this fate restriction can be partially overcome. The factors of the 'injury niche' that positively influence neurogenesis and that may be responsible for fate reprogramming remain largely unknown. Here we use global forebrain ischemia as a model to study progenitor-driven regeneration in the adult mouse brain. We show that NSCs contribute astrocytes, oligodendrocytes and neurons after ischemic injury. Notably, some NSCs are re-specified to produce striatal medium spiny neurons only after injury. Genome-wide transcriptional profiling of NSCs and their surrounding niche revealed that innate immunity pathways operate in the early stages of the regenerative response. We further studied how the DNA methylome integrates injury signals to mediate cell plasticity by whole genome bisulfite sequencing. We find that injury induces selective demethylation at subtype-specific neuronal genes in NSCs, suggesting epigenetic priming for future differentiation. Altogether, we show that cues from the injury niche promote a permissive transcriptional state that endows adult NSCs with increased fate plasticity.

Abstract No. T31

Stroma-derived osteopontin regulates hematopoietic stem and progenitor cells function upon aging

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Hematopoietic stem cells (HSCs) are located within bone marrow (BM) in a specific microenvironment referred to as the stem cell niche. HSCs as well as more differentiated hematopoietic cells interact in the BM with non-hematopoietic stroma cells (CD45- cells). This interaction is critical for hematopoiesis, regulating cell proliferation, self-renewal, differentiation and location. HSC aging was thought to be a primarily stem cell intrinsic driven mechanisms. Only more recently also aging mechanisms extrinsic to HSCs are being discovered and appreciated. We thus hypothesized that HSC niches age, and that these aging-associated changes impair HSCs function. Heterochronic transplantation of young or old BM cells into young or old recipients revealed that an aged environment supports aging-associated lineage skewing of both young and aged HSCs (myeloid over lymphoid), a decrease in the overall level of engraftment and an increase in the frequency of long term HSCs (LT-HSCs). In the same way, a young environment is responsible for an attenuation of a large number of phenotypes associated with aged HSCs. These data imply that HSC aging is, besides intrinsic effects, also determined by aging of the niche. Osteopontin (OPN) is a secreted glycoprotein expressed by osteoblasts located close to the endosteum. OPN was decreased in aged stroma, both in terms of expression and secretion. We then tested whether the decrease of OPN in stroma upon aging might be causatively linked to aging of HSCs. Transplantation of either young or aged BM cells into young OPN KO recipients revealed that similarly to results obtained when transplanted into aged recipients, young HSCs transplanted into OPN KO stroma resulted in a significant decrease in stem cell engraftment and an increase of the frequency of LT-HSCs and CMPs implying a causal role for reduced OPN in aged stroma for regulating aging-associated phenotypes of HSCs.

Abstract No. T32

Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylome analysis

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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. Despite intense research over the last decades the molecular basis of essential HSC features such as self-renewal and quiescence remains poorly understood. To determine the molecular programs employed by HSCs and MPPs, we performed an extensive global analysis combining quantitative proteome, transcriptome (RNA-seq) and DNA-methylome analyses on five FACS-sorted HSC and MPP populations - HSC (Linneg Sca-1+ cKit+ ,LSK, CD34- Flt3- CD150+ CD48-), MPP1 (LSK CD34+ Flt3- CD150+ CD48-), MPP2 (LSK CD34+ Flt3- CD150+ CD48+), MPP3 (LSK CD34+ Flt3- CD150- CD48+) and MPP4 (LSK CD34+ Flt3+ CD150+ CD48+) - as previously described in our laboratory (Wilson et al., Cell, 2008). Integration of Proteomics and RNA-seq analyses identified more than 6,000 proteins and 27,000 genes and revealed striking consistency between RNA and protein levels, arguing for limited but potentially important post-transcriptional regulation at the transition from HSCs to MPPs. Further, the presented first proteome analysis of refined HSC-MPPs revealed potential novel HSC markers. HSCs are defined by stage-specific expression clusters including Wnt and Lin28-Hmga signaling, the imprinted-gene-network, Hox genes, retinoic acid metabolism. This study provides a comprehensive genome-wide resource for functional exploration of the molecular, cellular and epigenetic processes operational at the pinnacle of the hematopoietic hierarchy.

Wilson et al., (2008). Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135, 1118-1129.

Abstract No. T33

Kit deficiency regulates stable human hematopoietic stem cell engraftment in mice

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Humanized mice are a promising tool for the study of human hematopoietic stem cell (HSC) function and immunobiology. But despite the number of available mouse models stable engraftment of substantial numbers of human HSCs and the continuous generation of myeloid cell types from those HSCs remain difficult to achieve. To improve stable stem cell engraftment which we considered a prerequisite of continuous myeloid cell generation, we generated three novel recipient mouse strains, BALB/c Rag2-/- Il2rg-/- KitWv/Wv (BRgWv), NOD/SCID Il2rg-/- (NSG) KitWv/Wv (NSGWv) and NSG KitW41/W41 (NSGW41) that all carry defective Kit receptors rendering endogenous mouse HSCs functionally impaired. We found that introduction of a loss-of-function Kit receptor into mice provides human Kit-proficient HSCs an advantage over endogenous murine cells. All three mouse strains support high and stable engraftment of human HSCs in the long-term without the need for previous irradiation. We detect considerable numbers of human HSCs and observe high reconstitution of the recipient mice with cells of the lymphoid, myeloid and erythroid lineages. Particularly in NSGW41 mice, we observe improved reconstitution of human myeloid cell types possibly because the Kit receptor is important for progenitor cell expansion. Robust numbers of myeloid cells are found in the bone marrow and spleen of transplanted mice. We conclude that Kit-signaling regulates HSC engraftment across the human-mouse species barrier and that Kit deficient mice bear broad potential for the study of human HSC function including self-renewal and differentiation but also mechanisms of innate immunity.

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. T34

STAT5-regulated miRNA193b controls hematopoietic stem and progenitor cell expansion by fine tuning cytokine signaling

Nadine Haetscher [1,*], *Yonatan Feuermann* [1], *Susanne Wingert* [1], *Maike Rehage* [1], *Christian Weiser* [2], *Frederic Thalheimer* [1], *Hanibal Bohnenberger* [3], *Timm Schroeder* [4], *Hubert Serve* [1], *Thomas Oellerich* [1], *Lothar Hennighausen* [5], and *Michael A. Rieger* [1]

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Hematopoietic stem cells (HSCs) regenerate the blood system life-long. Their number must be strictly controlled to avoid either exhaustion or excessive expansion leading to leukemia. Not only proteins but also regulatory RNAs are necessary to maintain HSCs. Hematopoiesis requires the right composition of microRNAs (miRNA), small, non-coding RNAs with the ability to simultaneously target multiple mRNAs for the orchestration of complex gene expression networks.

We identified miR193b to be induced in HSCs by the self-renewal promoting cytokine thrombopoietin via STAT5 signaling. To understand the physiological function of miR193b in HSCs, we generated a miR193b knock-out mouse. These mice showed a selective expansion of functional HSCs over time that were fully capable of multilineage blood reconstitution. MiR193b-deficient HSCs were less quiescent than wildtype HSCs in homeostasis, and were therefore less resistant to repeated hematopoietic stress. Further, they showed a higher expansion in vitro, not by changing the cell cycle or death rate, but by slowing differentiation. Conversely, ectopic miR193b expression restricted HSC expansion and abolished their repopulation potential. Videomicroscopy-based tracking of HSCs and their progeny revealed an increased cell death and slowed cell cycle upon miR193b expression. MiR193b-deficient HSCs and progenitor cells (HSPCs) showed a higher basal level of cytokine receptor signaling, and alterations in the signaling kinetics upon stimulation. Differential RNA-sequencing of HPSCs with and without miR193b confirmed the regulation of signaling-related gene clusters and revealed potential mRNA targets. We found the receptor tyrosine kinase ckit to be directly targeted by miR193b, suggesting that miR193b modulates signaling in HSPCs by regulating cytokine receptor expression.

Here we show a regulatory circuit that prevents excessive HSC self-renewal by upregulation of miR193b upon self-renewal promoting thrombopoietin–Mpl-STAT5 signaling, that in turn restricts cytokine signaling by targeting cytokine receptor expression. Thereby, we revealed the essential function of miR193b as a modulator of HSC self-renewal.

Abstract No. T35

Selective gene delivery into human iPS cells based on CD30-targeted lentiviral vectors

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Pluripotent stem cells (ESC, iPS) hold great promise for regenerative medicine. Human induced pluripotent stem cells (iPSC) can be generated by reprogramming differentiated somatic cells by delivering a defined set of transcription factors. The densely packed iPSC colonies can be identified by their typical morphology and cell surface markers such as SSEA-4 and CD30 (Abujarour et al., 2013). Reprogrammed cells are extremely heterogeneous in their dedifferentiation state and tend to spontaneously differentiate. Tools to genetically modify selectively the pluripotent cells are therefore needed for therapeutic as well as basic research. We have recently developed a novel technology for targeting lentiviral vectors (LVs) to cell surface receptors of choice (Anliker et al., 2010). Here we applied an engineered CD30-targeted vector, HRS3opt2-LV, for gene delivery into human iPSCs (hiPSCs). Efficiency, functionality and specificity were proven by assessing HRS3opt2-LV on CD30-positive and -negative cell lines. HRS3opt2-LV mediated stable gene transfer on CD30-positive cells only. When added to hiPS cell cultures, exclusively cells being positive for the pluripotency marker Oct-4 were transduced. At a dose of MOI=0.3 up to 20% of the SSEA-positive cells were genetically modified with genes encoding GFP and puromycin-resistance. Cultivation and differentiation of these cells proved that transduction by HRSopt2-LV neither impacted their growth behavior nor their pluripotency. When added to fibroblast cultures during the reprogramming process, all cells transduced by HRS3opt2-LV became NANOG positive. Our data show that CD30 is an early marker for cells that become fully reprogrammed iPSCs. HRS3opt2-LV may thus serve as a novel tool for the genetic manipulation of established hiPS cell lines as well as 'iPS cells-to-be' already during reprogramming.

Abstract No. T36

Generation of iPS cells from young vs. aged cell sources – influence of donor age on reprogramming efficiency and genetic stability of iPS clones

Katarzyna Osetek [1,*], *Alexandra Haase* [1], *Gudrun Göhring* [2], *Doris Steinemann* [2], *Philippe Chouvarine* [3], *Lutz Wiehlmann* [3], *Claudia Pommerenke* [4], *Gabriela Salinas-Riester* [4], *Cagatay Günes* [5], *Karl Lenhard Rudolph* [5], *Axel Schambach* [6], and *Ulrich Martin* [1]

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Many reports suggest that aging is associated with reduced cell proliferation as well as accumulation of genomic and mitochondrial mutations (1,2). This may lead to decreased quality of iPS cell based clinical products. Therefore, the aim of our study was to investigate to what extent the age of somatic cell donors influences the generation of iPS cells and their genetic integrity. Endothelial cells (ECs) and fibroblasts were isolated from neonates (cord blood, umbilical vein, foreskin) and adults (peripheral blood, saphenous vein, lung, skin). Population doublings were measured in early passage ECs and microarray analysis of primary cells was performed. Cells were reprogrammed with lentiviral vectors expressing Thomson or Yamanaka factors. Generated iPS clones were characterized for pluripotency marker expression and in vitro differentiation potential. Primary cells and iPS clones were karyotyped and subjected to aCGH analysis. Exom sequencing was performed on iPS clones, deep sequencing of PCR products is currently applied to clarify the origin of detected SNPs. Telomere length analyses are ongoing. ECs could be isolated from adult peripheral blood only from a small fraction of samples. Significantly higher proliferation and reprogramming rates with Thomson and Yamanaka constructs were observed in cells derived from neonatal sources. In contrast, it was difficult or impossible to generate iPS cells from adult sources with Thomson factors. Gene Set Enrichment Analysis revealed the enrichment of gene sets related to cell cycle, genome repair and telomere maintenance in primary cells that showed high reprogramming rates. Generated iPS clones reactivated endogenous pluripotency markers and were differentiated into derivatives of ecto-, meso- and endoderm. Our data demonstrate that somatic cells derived from neonatal sources with higher proliferation rates show higher reprogramming rates. Further data on the genetic integrity of iPSCs derived from juvenile compared to adult cells will be provided at the meeting.

1. Schneider EL and Mitsui Y (1976) The relationship between in vitro cellular aging and in vivo human age. Proc Natl Acad Sci; 73:3584–3588

2. Busuttil RA et al. (2007) Intra-organ variation in age-related mutation accumulation in the mouse. PLoS One; 2(9):e876.

Abstract No. T37/P033

Mesenchymal stem cells derived from iPS cells from aged individuals acquire fetal characteristics

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The *in vitro* expansion and application potential of human bone marrow derived mesenchymal stem cells (hBM-MSCs) derived from aged donors are limited by their short life span in culture and restricted differentiation potential. Recent studies have reported the derivation of hMSCs (iMSCs) from induced pluripotent stem cells (iPSCs) as a possible solution. However, little is known whether the age-associated phenotype is reverted into a younger state when iPSCs derived from hBM-MSCs of aged donors are re-differentiated into iMSCs. To obtain new insights into the potential roles of age in deriving iMSCs from iPSCs we induced pluripotency in hBM-MSCs from fetal femur (55 days post conception) and aged donors (60-70 years) and subsequently differentiated them into iMSCs. Higher levels of ROS, phosphorylated γH2AX and slower proliferation rates were detected in hBM-MSCs from aged individuals. Normal Karyotypes were detected in BM-MSCs of both age groups. Fetal hBM-MSCs could be reprogrammed more efficiently and faster compared to hBM-MSCs from aged donors using either retroviral or episomalbased reprogramming. hBM-MSCs and their corresponding iMSCs both fetal and aged expressed a typical MSC surface marker pattern and multipotency. iMSCs from aged parental hBM-MSCs acquired morphologies, senescent phenotypes and transcriptomes similar to that of fetal hBM-MSCs and iMSCs. Additionally, hBM-MSCs and their corresponding (iMSCs) both fetal and aged shared similar (GROα, IL-8, Serpin E1) secretome profiles. In summary we have demonstrated that (a) the efficiency of inducing pluripotency in hBM-MSCs is dependent on donor age. (b) The transcriptomes of iPS cells derived from both fetal and aged BM-MSCs are more similar to that of hESCs than the parental cells. (c) iMSCs irrespective of donor age re-acquire features typical of BM-MSCs. In conclusion, derivation of iMSCs by-passes the shortfalls associated with the expansion of native MSCs and these cells have tremendous potential in regenerative medicine.

Abstract No. T38

TRIM32, a dual player regulating the entry and exit from pluripotency

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Induced pluripotent stem cells (iPSCs) have revolutionized the world of regenerative medicine. Despite that fact, the exact molecular mechanisms underlying the generation and the differentiation of iPSCs remain elusive. Here, we investigated the role of the cell fate determinant TRIM32 in modulating these cell fate transitions. TRIM32 has been shown to be essential for the induction of neuronal differentiation in neural stem cells. It can achieve this by poly-ubiquitinating cMyc, targeting it for degradation and thereby resulting in inhibition of cell proliferation and induction of differentiation. In addition, TRIM32 has the potential of indirectly regulating other stemness factors -such as Oct4 and Sox2- through miRNA mediated processes. To elucidate the role of TRIM32 in regulating somatic cell reprogramming we analysed the speed and efficiency of iPSCs generation of TRIM32-knock-out mouse embryonic fibroblasts (MEFs) in comparison to their wild-type counterparts. TRIM32 knock-out MEFs produced a higher number of iPSC colonies denoting the role of TRIM32 in inhibiting this cellular transition. Further characterization of the generated iPSCs showed that TRIM32 is dispensable for pluripotency maintenance but essential for the proper differentiation of these cells. Accordingly, TRIM32 knock-out iPSCs show perturbed differentiation kinetics. Global gene expression analysis revealed that during differentiation, an Oct4 centred network in wild-type cells is replaced by an E2F1 centred network in the TRIM32 deficient cells. Through a series of biochemical assays we could show that this is the consequence of the ability of TRIM32 to directly regulate the degradation of Oct4 through its ubiquitin ligase activity. In summary, the here presented data reveal that TRIM32 directly regulates at least two of the four Yamanaka Factors (cMyc and Oct4), indicating that TRIM32 is an important regulator of cell fate transitions.

Abstract No. T39

Robust generation of cardiomyocytes from human iPS cells requires precise modulation of Bmp and Wnt signaling

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Cardiovascular disorders remain major causes of mortality in the world. A main shortcoming of innovative therapeutic concepts represents the lack of autologous cell sources and robust functional integration into the host tissue and the vascularization of the implant. Here we developed a novel protocol of robust cardiac differentiation of human iPS cells by systematically modulating BMP and Wnt signaling. We show efficient derivation of beating cardiomyocytes from multiple iPS lines. In particular we demonstrate cardiomyocyte differentiation within 15 days with an efficiency of up to 90 % as judged by flow cytometry staining against cardiac troponin T. hiPS-derived cardiomyocytes (iPS-CM) derived were functionally validated by alpha-actinin staining, transmission electron microscopy as well as electrophysiological analysis. Finally we explored the possibility to derive iPS-CM-based cardiac 3D tissues by seeding a single cell suspension of iPS-CM cells onto decellularized small intestinal submucosa scaffolds patches. 2 days after seeding we observed recovery of spontaneous beating in various regions on the patches. 3 days later the whole patch exhibited synchronous beating. Our data demonstrates robust and efficient cardiac differentiation of human iPS cells and highlights the possibility to establish a 3D myocardial tissue patch that can potentially serve as vital implant or in vitro test system for various applications. Exposure to defined biochemical and/or biophysical signals will allow to modulate the survival and maturation of functional cardiac tissue. We expect our 3D cardiac model to provide a robust basis for cardiotoxicity studies, disease modeling as well as cardiac cell replacement therapies.

Abstract No. T40

Efficient generation of functionally defined iPSC-derived human neural stem/progenitor cells and oligodendocytes

Giacomo Frati [1,*], *Vasco Meneghini* [1], *Silvia De Cicco* [1], *Chiara Cavazzin* [1], *Marco Luciani* [1], *Marianna Paulis* [2], *Angelo Lombardo* [1], *Francesca Sanvito* [1], *Anna Villa* [2], *Luigi Naldini* [1], and *Angela Gritti* [1]

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CNS-directed cell therapy strategies hold great promises for the treatment of demyelinating diseases. Pre-clinical studies have identified oligodendroglial progenitors (OPCs) and neural stem/progenitor cells (NSCs) as potential therapeutic cells. However, technical, ethical and safety issues posed by human OPCs and fetal-derived allogeneic hNSCs (hfNSCs) have hampered clinical development. Induced pluripotent stem cells (iPSCs) might overcome these issues, allowing the generation of autologous hNSCs and/or OPCs for translational purposes once their safety is verified. We have recently optimized a protocol to differentiate hiPSCs (generated from reprogramming of normal donor fibroblasts) into self-renewing, long-term proliferating NSCs (hiNSCs) that are phenotypically and functionally similar to hfNSCs. Our preliminary data suggest stable integration, little proliferation and multilineage differentiation of hiNSCs upon intracerebral transplantation in immunodeficient mice, contrary to their parental iPSC lines, which give rise to extensive teratomas. Also, we have established robust protocols to obtain hiNSC-derived OPCs (hiOPCS; OLIG2, NG2, A2B5) and mature oligodendrocytes (APC, GalCer, CNPase). At 35 days in vitro the oligodendroglial population represents up to 30-40% of total cells in culture, while neurons and astrocytes represent lower than 20% and lower than 4%, respectively. Importantly, no co-expression of neuronal or glial antigens and OPC markers was observed, suggesting robust lineage specification. We are currently performing FACS-based purification of hiOPCs for molecular characterization and transplantation experiments in murine models of demyelination. These studies will help defining whether hiNSCs and hiOPCs may represent safe, renewable, and effective transplantable cell sources, this being a prerequisite for potential therapeutic use.

Abstract No. T41

Aging affects the mesenchymal stem cell derived oligodendrogenic / remyelination activities: Implication for a potential therapy of multiple sclerosis

Francisco J. Rivera [1,*], *Chao Zhao* [2], *Ginez A. González* [2], *Anna O´ Sullivan* [3], *Roman Wodnar* [3], *Martina Feichtner* [3], *Eleni Oberbauer* [3], *Simona Lange* [3], *Alerie Guzman de la Fuente* [2], *Oihana Errea* [2], *Gabriele Brachtl* [4], *Richard Greil* [4], *Eva Rohde* [4], *Sebastien Couillard-Despres* [3], *Robin J.M. Franklin* [2], and *Ludwig Aigner* [3]

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Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) involving oligodendrocyte and myelin loss leading to severe neurological deficits. Mesenchymal stem cells (MSCs) display immunomodulatory and neuroprotective activities and promote oligodendrocyte differentiation of CNS progenitors and are thus interesting candidates for autologous cell therapy in MS. MS frequently has an onset in young adults, and thereafter progresses throughout adult life. It is therefore important to know whether MSCs retain their pro-oligodendrogenic activity throughout life, especially given the age-related decline in CNS remyelination efficiency. To address this question we isolated neural stem / progenitor cells (NSPCs) and MSCs from young (2 months old) and old (17-20 months old) rats and expanded them in vitro. NSPCs were exposed to MSCs-derived conditioned medium (MSC-CM) and oligodendrogenesis was evaluated by immunofluorescence and luciferase assay. We observed that soluble factors derived from aged MSCs display a reduced oligodendrogenic activity on NSPCs. Also, NSPCs obtained from aged donors present a lower oligodendrogenic respond to soluble factors derived from MSCs. Thus, aging decreases both; the MSCs derived oligodendrogenic activity and the oligodendrogenic potential of NSPCs. As oligodendroglial progenitor cells (OPCs) represent the main cellular source for myelin repair, we exposed also OPCs to young and old MSC-CM. In consistence with the previous findings, we found that aged MSCs have a reduced pro-oligodendrogenic effect on OPCs. Finally, only young MSC-CM enhanced endogenous remyelination in lysolecithin-induced demyelinated cerebellar slices, while soluble factors derived from old MSCs failed to induce such as increase. In summary, aging reduces MSC-derived oligodendrogenic activity and abolishes their remyelination potential. Thus, in autologous transplantation setting, MSCs are likely to gradually loose their pro-regenerative properties throughout disease duration and, therefore, autologous MSC-therapies should be done as early as possible, or non-autologous grafting of young MSCs, should be considered for MS treatment.

Abstract No. T42

The European project REBORNE: Multipotent mesenchymal stromal cells and biomaterial for bone healing

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The European project REBORNE targets healing of long non-union bone fractures by application of human bone marrow-derived Multipotent Mesenchymal Stromal Cells (BMMSC) and MBCP+ granules as biomaterial. 23 institutions, among them 12 clinical centers, in 8 European countries are involved. We have established and published one-step as well as two-step large-scale protocols for GMP-compliant ex vivo expansion of BMMSC, using pooled human platelet lysate as cell culture growth supplement. Our Advanced Therapy Medicinal Product has been characterized extensively by flow cytometry, cytokine/chemokine production, and differentiation capacity. Its potency for bone healing has been shown in animal models, including mouse, rabbit, sheep, and pig. 4 clinical studies are ongoing. To date 25 patients have been treated. Our contribution will cover main characteristics of REBORNE: large-scale clinical-grade MSC production, differentiation capacity, safety, animal models, and preliminary results of the clinical trials. This project is funded by the 7th Framework Programme of the European Commission.

Abstract No. T43

Identification, isolation and targeted inhibition of a fibroblast lineage responsible for scarring and cancer stroma

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Abstract: Dermal fibroblasts represent a heterogeneous population of cells with diverse features that remain largely undefined due to a lack of functional subclasses. Here we reveal the presence of multiple lineages of dermal fibroblasts within the dorsal back. Genetic lineage tracing and transplantation assays demonstrates that the bulk of connective tissue deposition during embryonic development, cutaneous wound healing, radiation fibrosis, and cancer stroma formation is carried out by a single, somitic-derived fibroblast lineage. Reciprocal transplantation of distinct fibroblast lineages between the dorsal back and oral cavity induced ectopic dermal architectures that mimic their place–of-origin. These studies demonstrate that intra and inter-site diversity of dermal architectures are set embryonically and maintained postnatally by distinct lineages of fibroblasts. Lineage-specific cell ablation using transgenic-mediated expression of the simian diphtheria toxin receptor in conjunction with localized administration of diphtheria toxin led to diminished connective tissue deposition in wounds and significantly reduced melanoma growth in the dorsal skin of mice. Using flow cytometry and in silico approaches, we identify CD26/DPP4 as a surface marker that allows for the isolation of this fibrogenic, scar-forming lineage. Small molecule-based inhibition of CD26/DPP4 enzymatic activity during wound healing results in diminished cutaneous scarring. The identification and prospective isolation of these lineages holds promise for translational medicine aimed at in vivo modulation of their fibrogenic behavior.

Rinkevich et al., 2012 (Nature Cell Biology)

Rinkevich et al., 2014 (Cell Reports)

Abstract No. T44

Proofing the (un)proven: Proven or approved stem cell therapies – that is the question

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In 2013, the EU Regulation No. 1394/2007 governing advanced therapies such as gene therapy, somatic cell therapy, and tissue engineering got challenged by judicial and executive decisions in Italy, which allowed the use of unproven stem cell treatments to seriously diseased children known as the “Stamina Case” or Durisotto v. Italy. In Durisotto v. Italy the European Court of Human Rights was asked to decide whether or not the fundamental human rights to life and health and the respect for private and family life is violated when access to the unproven stem cell therapy “Stamina” was denied. Solid clinical and methodological data, as one of the requirements for the possibility of compassionate use, were not available. Thus, the court stated that the effectiveness of the method is not scientifically proven and processes are pending. The court ruled that denying access to an unproven therapy is not discriminating the patient, even though other courts might have granted access. However, the court took the fact that the method is not scientifically proven for granted. What it means, to be “scientifically proven”, remains unclear. Thus, it is deemed to be necessary to examine and clarify the meaning of having an unproven stem cell therapy vs. an approved therapeutic by regulators. In order to do so, it has to be clarified why stem cell therapies are or meant to be treated differently than chemical drugs or other biologics. We examined and challenged the concept of “(un)proven stem cell therapies” vs. “approved” stem cell therapies in the European legal and ethical context to foster sound science and to ensure the quality of clinical data gathered in future clinical investigations in light of international harmonization approaches.

Company presentations

2nd Annual Conference
German Stem Cell Network
November 3 – 5, 2014

Company presentations: C1 – C9

PEPROTECH
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Abstract No. C1

High throughput screening using iPSC-based models

Jan Bruder [1,*], *Peter Reinhardt* [2], *Lydia Wagner* [2], *Hans R. Schöler* [1], and *Jared Sterneckert* [2]

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Cell-based models that accurately predict drug efficacy in vivo are urgently needed – in particular for neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS). Induced pluripotent stem cells (iPSCs) are ideally suited to generate these models because they are derived from patients with observable phenotypes and known genotypes. A major challenge in generating these models is to develop efficient protocols for directing the differentiation or self-renewal of specific cell types. In cooperation with Peprotech, we provide an example of using high-throughput screening (HTS) to identify combinations of recombinant proteins to control stem cell behavior. In addition, we recently developed an iPSC-based model of ALS. Using HTS, we identified compounds targeting a novel kinase that protected motor neurons from degeneration.

Abstract No. C2

Controlling expansion and cardiomyogenic differentiation of human pluripotent stem cells in scalable suspension culture

Robert Zweigerdt [1,*], *Henning Kempf* [1], *Ruth Olmer* [1], *Christina Kropp* [1], *Michael Rückert* [1], *Monica Jara-Avaca* [1], *Diana Robles-Diaz* [1], *Annika Franke* [1], *David Elliott* [2], *Daniel Wojciechowski* [1], *Martin Fischer* [1], *Angelica Roa Lara* [1], *George Kansah* [1], *Ina Gruh* [1], *Axel Haverich* [1], and *Ulrich Martin* [1]

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To harness the potential of hPSCs, abundant provision of their differentiated progenies is required. Here, hPSC expansion as matrix-independent aggregates in suspension culture (3D) was combined with cardiomyogenic differentiation using chemical Wnt-Pathway modulators. A multi-well screening approach was scaled-up to stirred tank bioreactors applying controlled feeding strategies (Batch and Cyclic Perfusion) followed by pre-optimized differentiation.

We found that the size of aggregates is not the prevailing factor regarding divergent differentiation outcomes, but physical and physiological culture parameters that shape aggregate development in the expansion phase. Global profiling revealed culture-dependent expression of BMP agonists/antagonists, suggesting their decisive role on cell fate determination. Furthermore, metallothionein was discovered as a potentially stress-related marker in hPSC culture.

Optimized conditions in 100 ml bioreactors enabled the production of 40 million cardiomyocytes (>80% purity), the majority of which displayed ventricular-like action potentials and were directly applicable for bioartificial cardiac tissue formation, a potential strategy for heart repair, drug discovery and safety pharmacology.

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

Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in vitro. Kensah G, Roa Lara A, Dahlmann J, Zweigerdt R, Schwanke K, et al. Eur Heart J. 2013 Apr;34(15):1134-46.

Abstract No. C3

Raw materials in the manufacture of advanced therapies medicinal products: Quality attributes and quality assurance

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Cytokines, growth factors and media are commonly used in the processing of cells for therapeutic applications. The quality of these materials (also called ancillary materials, AM) is crucial for the quality, safety and efficacy of the finished therapeutic product.

In recent years regulatory agencies recognized an increasing need for guidance for raw materials used for the production of ATMPs and started developing guidelines that outline general risk-mitigation strategies and qualification programs which can be used to select appropriate reagents (like USP General Chapter <1043> “ancillary materials for cell, gene, and tissue-engineered products”) or which outline specific quality attributes for cytokines and growth factors (like USP Chapter <92> “growth factors and cytokines used in cell therapy manufacturing”).

Despite arising guidance in this area, manufacturers of AM have to identify critical quality requirements to meet increasing quality and safety concerns. The origin, composition, manufacturing process, QC methods and release specifications of AM have to be defined carefully in close cooperation with regulatory agencies and ATMP manufacturers. The use of GMP grade and animal-derived component-free (ADCF) ancillary materials will significantly reduce qualification and validation efforts of cell therapy manufacturers and help to ensure consistency, safety and purity of the final cell therapy products

www.cellgenix.com

Abstract No. C4

Efficient neural and cardiac differentiation systems for iPSC

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The promise of human pluripotent stem cells will be realized only when these cells are successfully coaxed into different cell types found in the human body, through the process of directed differentiation. This is critical to getting the desired cell types and numbers needed for drug screening, translational cell therapy and regenerative medicine applications. Most of the existing methods of differentiation are suboptimal, involving laborious mechanical and manual steps leading to issues of reproducibility and reduced efficiency in downstream processing of functionally mature lineages. The complex developmental process of differentiation and the challenges associated need to be efficiently deciphered in order to successfully direct the hPSC differentiation to target cell types.

In this presentation, challenges associated with hPSC differentiation to neural and cardiac lineages and how these can be solved with tools and cGMP cell culture media systems for efficient and scalable differentiation of these two critical cell lineages is presented.

Abstract No. C5

Cell-based gene delivery leverages conventional immunotherapy for cancer; Treatment of advanced gastrointestinal cancer in a phase I/II trial with modified autologous MSC_apceth_101

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This is the first clinical trial with MSC_apceth_101, a suspension of autologous bone-marrow derived mesenchymal stromal/stem cells (MSC) which are expanded and modified in vitro by introducing a gene for the expression of HSV-Thymidine Kinase (HSV-TK). MSC_apceth_101 accumulate in tumor tissue after intravenous administration. Subsequently the prodrug Ganciclovir (GCV) is given and is converted by HSV-TK into the active cytotoxic compound GCV-triphosphate, a process which is restricted to the tumor tissue due to the preferential migration of MSC to injured tissue. The bone marrow aspiration is performed at least 10 weeks before the intended administration of the IMP under a separate approved protocol.
This study consists of a phase I and a phase II.
In phase I (= run-in phase) the safety of MSC_apceth_101 is being investigated in 6 patients mainly with liver metastases due to colorectal carcinoma (“advanced patients” or patient group 1). 3 out of these 6 patients will receive dose level 1 (1.5 x106 cells/kg in total [range: 1 - 2x106 cells/kg]) followed by 3 patients given dose level 2 (3x106 cells/kg [range: 2 - 4x106 cells/kg]). It is the aim of phase I to define a dose level on the basis of safety data to be used in phase II.
In phase II, 16 patients with advanced gastrointestinal adenocarcinoma will be enrolled. In addition to patient group 1 as defined in phase I, patients will be recruited who are characterized by their need for preoperative therapy with respect to the primary tumor and for the reduction of tumor size prior to tumor surgery (= patient group 2). 8-10 additional patients will be recruited in group 1 and 6-8 patients in group 2 during phase 2. In total 22 patients will be recruited in both phases.

Abstract No. C6

Scalable enzyme-free protocols for the isolation and maintenance of human induced pluripotent stem cells without mechanical colony scraping

Alexandra Blak [1,*] , *Erik B. Hadley* [1], *Jessica Norberg* [1], *Allen C. Eaves* [1,2], *Terry E. Thomas* [1], and *Sharon A. Louis* [1]

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Defined maintenance culture media such as mTeSR™1 and TeSR™-E8™ efficiently promote the expansion of human pluripotent stem cells (hPSC) in the absence of feeder cells. However, a key limitation has been the need to mechanically scrape the entire culture surface to generate cell aggregates for passaging. We developed an enzyme-free passaging reagent, ReLeSR™, for detachment of hPSC aggregates without selection of differentiated cells, scraping, or complicated manipulation to obtain the desired aggregate size. ReLeSR™ can be used for hPSC cultures maintained in culture flasks and other larger closed vessels where the use of a cell scraper is not possible, thus facilitating culture scale-up and automation. ReLeSR™ was tested over 10 passages with multiple hPSC lines. Briefly, the culture medium was aspirated, and ReLeSR™ was added to each well and immediately removed. After 6-8 minutes of incubation, fresh medium was added, and the plate was placed on a vortex mixer for 2-3 minutes to dislodge the cell aggregates prior to re-plating. The performance of the cells treated with ReLeSR™ was evaluated by cell morphology, expression of hPSC-associated markers, cell expansion (number of aggregates harvested at passage end divided by number of aggregates seeded) and differentiation ability. Cells passaged using ReLeSR™ displayed the expected colony morphology, and >90% of cells maintained expression of undifferentiated cell markers TRA-1-60 and OCT-3/4. Cell expansion over ten passages was very high when using ReLeSR™: expansion per passage was 52-fold for H1/mTeSR™1, 86-fold for H1/TeSR™-E8™ and 48-fold for WLS-4D1/TeSR™-E8™. The resulting cells were found to be capable of differentiating to cell types representing all three germ layers. In conclusion, ReLeSR™ is a convenient passaging reagent for hPSCs and that can be used in protocols for maintaining high quality cultures in mTeSR™1 or TeSR™-E8™ without the need for scraping, thus facilitating scale-up and automation.

Abstract No. C7

The future is counting – digital analysis of RNA, DNA and protein biomarkers

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*Presenting author

The nCounter Analysis System utilizes a digital color-coded barcode technology that is based on direct multiplexed measurement of biomarkers. For gene expression it offers high levels of precision and sensitivity. The technology uses molecular "barcodes" and single molecule imaging to detect and count hundreds of unique transcripts in a single reaction.
Each color-coded barcode is attached to a single target-specific probe corresponding to a biomarker of interest. Mixed together with controls, they form a multiplexed CodeSet.

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

With protocols starting from 100 ng or less of total RNA, raw cell or blood lysate, and Formalin-Fixed Paraffin-Embedded (FFPE) extracts, the nCounter Gene Expression CodeSets offer the ultimate in sample input flexibility, all with excellent performance.
The introduction of the nCounter® Single Cell Gene Expression Assay greatly expands the types of studies that can be analyzed on the nCounter Analysis System, allowing researchers to address biological questions that have previously been impossible to answer. Entire biological pathways and custom gene signatures can now be studied for single cells without the necessity to match the gene number to the format of a microfluidic PCR consumable.

Fluidigm

Abstract No. C8

Primate iPS cells as tools for evolutionary analyses

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representing Fluidigm Europe B.V.

Comparisons across species allow inferences about conserved and species-specific traits. This is important to understand evolution, but it is also of practical relevance for purposes such as identifying functional regions in a genome or protein sequence. Extending this approach from the sequence level to the level of molecular phenotypes to achieve such goals as identifying species-specific and conserved regulatory networks has the potential to yield major benefits, and comparisons to primates are especially relevant because of the possible implications for our own species. Induced pluripotent stem cells (iPSCs) allow experimental access to cell types and differentiation stages that are difficult or impossible to investigate in humans and primates. Hence, iPSCs from primates should allow us to compare inaccessible cellular differentiation programs in humans and their closest relatives. I will report on recent progress and insights gained from evolutionary comparisons using primate iPSCs and point out how single-cell transcriptome sequencing will be crucial for meaningful comparisons across differentiating iPSCs.

An introduction to the Fluidigm technology, titled “Exploring the dynamics of Biology One Cell at a Time“, will be delivered by Mark Lynch, Single-Cell Biology Specialist Europe, at the beginning of this presentation.

Lonza

Abstract No. C9

Building bridges from research to therapy: A roadmap for the successful generation of clinicalpgrade iPSCs

Thomas Fellner [1*]

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In 2007, Dr. Shinya Yamanaka became the first to successfully convert adult human cells to induced pluripotent stem cells (iPSCs). These cells have similar characteristics to embryonic stem cells (ESCs) including the potential to become any cell type in the body. Therefore, it is thought that human iPSCs (hiPSCs) can be utilized as the starting material for the manufacture of cell therapies to treat a multitude of diseases. While human ESCs are limited to allogeneic therapies, hiPSCs can be used for the development of both allogeneic and autologous therapies; the latter having the advantage of using a patient’s own cells for the generation of hiPSCs. However, before iPSC-based therapies can become technically and economically viable, several hurdles need to be overcome. From a clinical manufacturing perspective, the challenges are quite different depending on the type of therapy (allogeneic vs. autologous), phase of development (clinical phase I, phase II, phase III and commercial phase), and the clinical indication. Our presentation will expand on these challenges and address the critical steps necessary to enable the use of hiPSC-derived cells in a clinical setting. These fundamental steps include: (1) Establishing a tissue sourcing protocol that includes informed consent and donor eligibility. (2) Developing a robust process that is compliant with current Good Manufacturing Practices (cGMP) regulations for the generation and maintenance of clinical-grade iPSCs. (3) Defining a strategy for characterization and release testing of hiPSC Master Cell Banks (MCBs). In addition we will provide a status update on the clinical manufacturing of iPSCs we are working on as part of a contract Lonza has in place with the National Institutes of Health Center for Regenerative Medicine (USA).

Poster presentations

2nd Annual Conference
German Stem Cell Network
November 3 – 5, 2014

Poster session I: P001 – P097

(posters will be displayed in the tent)
P001 – P017: Pluripotency and embryonic stem cells
P018 – P038: Programing and reprograming
P039 – P061: Stem cells in development
P062 – P072: Somatic stem cells
(posters will be displayed in front of room K 1+2)
P073 – P097: Hematopoietic stem cells

Poster session II: P098 – P187

(posters will be displayed in the tent)
P098 – P129: Stem cells in regenerative therapies
P130 – P161: Stem cells in disease modeling and drug development
(posters will be displayed in front of room K 1+2)
P162 – P178: Stem cells in disease: Cancer stem cells
P179 – P187: Computational stem cell biology

Poster session I: P001 – P097

P001 – P017: Pluripotency and embryonic stem cells

(posters will be displayed in the tent)

P001 Induction and maintenance of naïve and primed human pluripotent stem cells
Raed Abu Dawud

P002 Large-scale hematopoietic differentiation of human iPSC provides granulocytes or macrophages for cell replacement- and genetic-therapies
Mania Ackermann

P003 Investigation of calcium signals in human pluripotent stem cells and in their derivates
Ágota Apáti

P004 Role of Wnt secretion in mouse embryonic stem cells maintenance and teratoma growth
Iris Augustin

P005 Identification and pluripotency of mouse spermatogonial stem cells
Hossein Azizi

P006 Role of Pcgf6 in the maintenance of pluripotency and improvement of pluripotent reprogramming efficiency
Matthias Becker

T23/P007 A novel lncRNA-protein interaction characterises mouse embryonic stem cell fate
Debojyoti Chakraborty

P008 Epigenetic Biomarker for Pluripotency Based on Three CpG Sites
Roman Goetzke

P009 Image-based quantification and mathematical modeling of spatial heterogeneity in ESC colonies
Maria Herberg

P010 Improved Cyropreservation and Recovery Solutions for Pluripotent Stem Cells & Difficult-to-Preserve Primary Cells
Katja Hufschmid

P011 Inhibition of H3K27-specific demethylase activity during murine ES cell differentiation induces DNA damage response
Sascha Huppertz

P012 Impact of feeding strategies on scalable expansion of human pluripotent stem cells in stirred tank bioreactors
Christina Kropp

P013 A versatile and robust xeno- and serum-free cultivation system for human pluripotent stem cells
Annett Kurtz

P014 Utility of hPSC Scorecard™ Assay in assessment of functional pluripotency of cells across the iPSC workflow
Roland Leathers

P015 A New Method to pick targeted ES Cell Clones more efficiently
*Ellen N*a

P016 Loss of Myc activity induces cellular dormancy in embryonic stem cells mimicking the status of diapause embryos
Roberta Scognamiglio

P017 Karyotypic abnormalities in human iPSCs: a result of LINE1 retrotransposition?
Anett Witthuhn

Abstract No. P001

Induction and maintenance of naïve and primed human pluripotent stem cells

Raed Abu Dawud [1,*], *Michael Thelen* [1], *Laura Schneider* [1], *Petra Reinke* [1], and *Andreas Kurtz* [1]

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*Presenting author

Human pluripotent stem cells are invaluable for regenerative therapies. In order to exploit their regenerative potential, it is not only mandatory to understand and establish differentiation mechanisms. It is of enormous importance to understand and control the undifferentiated state as well. Pluripotency exists at least in two states, the naïve ground state and the primed state, which is developmentally more advanced. This clear distinction is well established in murine system and is currently being established in human pluripotent cells. We set out to shed light on human pluripotency, in particular the human naïve state, by investigating the impact of different levels on primed human pluripotent stem cells and their induction into naïve pluripotent cells. We manipulated the cells on the levels of metabolome and epigenome, i.e. to amplify glycolysis and render the cells euchromatic rather than heterochromatic, respectively. In addition we have used a novel cocktail consisting of growth factors and inhibitors to alter signaling transduction. Our results strongly indicate that both states, the naïve and the primed state, benefit from adjustments made, that affect the metabolome, epigenome and signaling resulting in improved morphology, cologenicity, proliferation and the expression of key pluripotency markers indicative of both pluripotency states, naïve and primed.

Abstract No. P002

Large-scale hematopoietic differentiation of human iPSC provides granulocytes or macrophages for cell replacement- and genetic-therapies

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

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Hematopoietic differentiation of pluripotent stem cells (PSC) holds great promise for the development of novel cell replacement and gene-therapy strategies. In the past, interest has been directed primarily at reconstituting stem cells, a cell type as of yet problematic to generate. Recently, however, also long-lived mature myeloid cells have been described and transplantation of those may open new therapeutic scenarios. To prove this concept, we subjected hiPSC to an embryoid body (EB)-based myeloid differentiation protocol employing IL-3 in combination with M-CSF or G-SCF to generate of a so-called “myeloid cell forming complexes (MCFC)”. This MCFC produced >95% pure monocyte/macrophages (iPSC-MΦ) and/or granulocytes (iPSC-gra) from day 14 onwards over a period of 3-5 months at a quantity of 0.4-2.0 million cells/week per 3.5 cm well. Of note, generation of myeloid cells was driven by a MCFC-resident CD34+ population of progenitor/stem cells.Detailed characterization of mature myeloid cells demonstrated a typical monocyte/macrophage-morphology of iPSC-MΦ by cytospins and expression of CD45, CD11b, CD14, CD163, and CD68. In addition, iPSC-MΦ had the ability to phagocytose latex-coated beads similar to peripheral blood (PB) macrophages polarized to M2 and secreted MCP1, IL6, IL8, and IL10 upon LPS stimulation. iPSC-gra showed typical surface expression of CD45, CD11b, CD16, CD15, CD66b and a differential count containing pro-myelocyte (3%), myelocyte (5%), meta-myelocyte (30%), bands (22%), eosinophils (2%), basophils (1%), and segmented-neutrophils (37%). Moreover, iPSC-gra were able to migrate towards an IL8 or fMLP gradient, form neutrophil extracellular traps, and up-regulate NADPH activity and ROS production upon PMA stimulation to a similar degree as PB granulocytes. In summary, we provide a novel hematopoietic differentiation protocol for iPSCs recapitulating key events of physiologic hematopoiesis and allowing for the prolonged large-scale production of myeloid lineage cells. Thus, this protocol appears particularly suited for cell-replacement strategies or to study hematopoietic development.

Abstract No. P003

Investigation of calcium signals in human pluripotent stem cells and in their derivates

Ágota Apáti [1,], Katalin Pászty [2], Adrien Péntek [1], Tamás I. Orbán [1], Zsuzsa Erdei [1], Kornélia Szebényi [1], Ágnes Enyedi [2]*, and *Balázs Sarkadi [1]*

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Calcium plays major role in the life of the cells and thus measuring the changes in intracellular calcium concentration is important for studying signal transduction pathways and a wide variety of cellular responses. Human embryonic stem cells (hESCs), based on their normal genetic background and unlimited proliferation capacity, provide new possibilities to explore the development and differentiation of various cell types of the human body. Still, the calcium signaling events have been only rarely studied in pluripotent stem cells and in their differentiated progenies. In our recent work we demonstrated that the use of the fluorescent calcium indicator dye (Fluo-4), or a genetically engineered calcium indicator protein (G-CaMP2), and confocal microscopy allow sensitive and reliable measurements of calcium modulation in hESCs and hESC-derived cardiac, neural and mesenchymal tissues. We stably expressed G-CaMP2 in hESCs by using a transposon-based gene delivery system, and tested the effects of various ligands in undifferentiated hESCs and their differentiated offsprings. The calcium signals induced by various compounds were comparable to those in Fluo-4 loaded cells. Characteristics of both the spontaneous and ligand-induced Ca2+-signals, as well as their pharmacological modification could be successfully examined in these model cells by fluorescence imaging.

This work was supported by the Hungarian Scientific Research Fund [grant number NK83533]; Hungarian Brain Research Program [KTIA_13_NAP-A-I/6] and by the National Development Agency [grant numbers KTIA_AIK_12-1-2012-0025, KMR_12-1-2012-0112].

A. Apati et al. Characterization of calcium signals in human embryonic stem cells and in their differentiated offspring by a stably integrated calcium indicator protein. Cell Signal 25 (2013) 752-9.

A. Apati, K. Paszty, Z. Erdei, K. Szebenyi, L. Homolya, and B. Sarkadi, Calcium signaling in pluripotent stem cells. Mol Cell Endocrinol 353 (2012) 57-67.A.

Abstract No. P004

Role of Wnt secretion in mouse embryonic stem cells maintenance and teratoma growth

Iris Augustin [1,], Diah Dewi [1]*, and *Michael Boutros [1]*

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*Presenting author

The Wnt signaling pathway plays an important role in the specification and maintenance of precursor cell and stem cell lineages in various tissues. Evi/Wls is an essential component of the Wnt secretion machinery and is involved in the secretion of all Wnt proteins. Consequently, the genetic modulation of Evi globally affects Wnt signaling providing a unique experimental tool to modulate Wnt signaling through regulation of Wnt ligand secretion. Transgenic mouse embryonic stem cells (ESC) either deficient for Evi (Evi-LOF) or overexpressing Evi (Evi-GOF) were investigated with respect to their in vitro and in vivo stemness characteristics. Evi-LOF ESCs revealed reduced self-renewal, whereas Evi-GOF ESCs had increased self-renewal capabilities following LIF-withdrawal, supporting the concept that Wnt secretion maintains ESCs in their undifferentiated state. Likewise, lineage differentiation was promoted to cardiomyocyte differentiation in Evi-GOF ESCs whereas multiple germ layer formation was impaired in Evi-LOF ESCs. Upon transplantation in mice, ESCs differentiated in an uncoordinated manner, forming teratomas that gave rise to multiple tumorous embryonic tissues. Evi-LOF teratomas showed impaired tumor growth with hemorrhagic tissue. Interestingly, expression profiling of Evi-GOF teratomas revealed a strong alteration in the expression of genes involved in immune response modulation. Corresponding, T cell infiltration was reduced in Evi-GOF tumors suggesting an altered immune cell recruitment and function. Taken together, our study identified Evi-mediated Wnt secretion as a tumor promoting factor that modulates the tumor microenvironment.

Abstract No. P005

Identification and pluripotency of mouse spermatogonial stem cells

Hossein Azizi [1,*], *Sabine Conrad* [2], *Ursula Hinz* [1], *Behrouz Asgari* [3], *Daniel Nanus* [1], *Heike Peterziel* [4], *Akbar Hajizadeh Moghaddam* [5], *Hossein Baharvand* [3], and *Thomas Skutella* [1]

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*Presenting author

Spermatogonial stem cells (SSCs) are able to differentiate to sperm cells in order to transfer genetic information to the next generation. In the seminiferous tubules of the testis SSCs, sertoli cells and differentiating cells during spermatogonesis are present. In our studies we successfully established two types of SSCs with morphology-based selection (type I and type II) from testicular culture both from neonate and old Oct4-GFP transgenic mice. We have been successful to expand type I SSCs on mouse embryonic feeder (MEF) and type II SSCs on both SNL and primary testicular stroma cells (TSCs) feeder for long term culture. Immunocytochemistry, Flowcytometry and Fluidigm real time RT-PCR results showed that type I SSCs clearly express germ cells markers while type II SSCs partially expressed the typical germ cell profile of SSCs. Electron microscopic analysis revealed that type I SSCs have similar or homogenous morphology comparable with undifferentiated SSCs that are localized on the basement membrane of the seminiferous tubules (high nucleus/cytoplasm ratio) while type II SSCs have a different morphology (small nucleus/cytoplasm ratio). After transplantation of type II SSCs in busulphan treated NOD SCID mice we observed localization of GFP labeled cells in the basal compartment of the seminiferous tubule and observed GFP labeled sperms in the epididymis. The most obvious molecular differences between type I and type II SSCs were reprogramming to mouse embryonic stem cells-like cells, which occurred only during a special time window after initiation of type I SSCs culture from neonate and adult promoter-reporter Oct4-GFP transgenic mouse. These different types of SSCs could provide an ideal cell system for studying both germ and pluripotency profiles and provide a new strategy for isolation of SSCs from neonate and old mice.

Abstract No. P006

Role of Pcgf6 in the maintenance of pluripotency and improvement of pluripotent reprogramming efficiency

Matthias Becker [1,*], *Daniela Zdzieblo* [1], *Xiaoli Li* [1], *Qiong Lin* [2], *Damir Illich* [3], *Martin Zenke* [2], and *Albrecht Müller* [1]

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Polycomb group (PcG) proteins comprise evolutionary conserved factors with essential functions for embryonic development and adult stem cells. PcG proteins constitute two main multiprotein polycomb repressive complexes (PRC1 and PRC2) that operate in a hierarchical manner to silence gene transcription. Functionally distinct PRC1 complexes are defined by Polycomb group RING finger protein (Pcgf) paralogs. So far, six Pcgf paralogs (Pcgf1-6) have been identified as defining components of different PCR1 type complexes. Paralog-specific functions are not well understood. Here we show that Pcgf6 is the only Pcgf paralog with high expression in undifferentiated ESCs. Upon differentiation Pcgf6 expression declines. Following Pcgf6 kockdown (KD) in ESCs the expression of pluripotency genes decreased, while mesodermal- and spermatogenesis-specific genes were de-repressed. Concomitantly with the elevated expression of mesodermal lineage markers, Pcgf6 KD ESCs showed increased hemangioblastic and hematopoietic activities upon differentiation suggesting a function of Pcgf6 in repressing mesodermal-specific lineage genes. Consistant with a role in pluripotency, Pcgf6 replaced Sox2 in the generation of germline-competent iPS cells. Further, Pcgf6 KD in MEFs reduced the formation of ESC-like colonies in OSKM-driven reprogramming. Together, these analyses indicate that Pcgf6 is non-redundantly involved in maintaining the pluripotent nature of ESCs and it functions in iPS reprogramming.

Please find abstract P007 under ‘Selected presentations’ T23/P007

Abstract No. P008

Epigenetic Biomarker for Pluripotency Based on Three CpG Sites

Roman Goetzke [1,], Michael Lenz [2], Arne Schenk [3], Claudia Schubert [4], Jürgen Veeck [4], Hatim Hemeda [1], Steffen Koschmieder [4], Martin Zenke [4], Andreas Schuppert [2],* and *Wolfgang Wagner [1]*

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Several methods are used for quality control of induced pluripotent stem cells (iPSCs), e.g. analysis of iPSC-colony morphology, surface marker expression, analysis of individual genes or gene expression profiles, multilineage differentiation potential in vitro, or teratoma assays. There is a clear trade-off between cost and labor intensive methods on the one hand and reliability on the other hand. Pluripotency is also reflected by a characteristic epigenetic makeup in DNA methylation (DNAm) profiles (Shao K et al., 2013). Here, we describe a surrogate assay for pluripotency which is based on the DNAm level at only three specific CpG sites. Two of these CpG sites were selected based on their discriminatory power in 258 DNAm profiles (63 pluripotent, 195 non-pluripotent; 450k Illumina BeadChips, www.ncbi.nlm.nih.gov/geo/). They become either methylated or demethylated in iPSCs and their combination is referred to as “Epi-Pluri score”. In addition, a third CpG associated with the pluripotency-associated gene POU5F1 (OCT4) was considered. This epigenetic signature was validated on independent DNAm datasets (264 pluripotent and 1951 non-pluripotent samples; 27k Illumina BeadChips) with 99.9% specificity and 98.9% sensitivity. Notably, the method could also discriminate partially or improperly reprogrammed cells. Subsequently, we established pyrosequencing assays to specifically analyze DNAm at these CpGs. The results allowed reliable classification of 12 pluripotent cell lines and 30 non-pluripotent cell lines. DNAm changes at these three CpGs were subsequently analyzed in the course of differentiation of iPSCs towards mesenchymal stromal cells (Frobel J et al., 2014) demonstrating that particularly the CpG site in POU5F1 demarcates early differentiation events. There was a moderate association between DNAm changes and differential gene expression of the corresponding genes. Taken together, the DNAm level of three specific CpG sites provides a simple and robust biomarker for analysis of pluripotency with high sensitivity and specificity.

Shao K, et al. (2013). Induced Pluripotent Mesenchymal Stromal Cell Clones Retain Donor-Derived Differences in DNA Methylation Profiles. Molecular Therapy 21, 240-250.

Frobel J, et al., (2014). Epigenetic Rejuvenation of Mesenchymal Stromal Cells Derived from Induced Pluripotent Stem Cells. Stem Cell Reports, accepted for publication.

Abstract No. P009

Image-based quantification and mathematical modeling of spatial heterogeneity in ESC colonies

Maria Herberg [1,], Thomas Zerjatke [1], Walter de Back [2], Ingmar Glauche [1],* and *Ingo Roeder [1]*

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Pluripotent embryonic stem cells (ESCs) have the potential to differentiate into cells of all three germ layers. This unique property has been extensively studied on the intracellular, transcriptional level. However, cultured ESCs form clusters of distinct size and shape, and establish spatial structures that are essential for the maintenance of pluripotency. Even though it is recognized that the cells’ arrangement and local interactions play a role in fate decision processes, the relations between transcriptional and spatial patterns have not yet been studied.

We present a systems biology approach which combines quantitative live-cell image analysis and mathematical modeling of ESC growth. In particular, we develop measures on the morphology and on the spatial clustering of ESCs with different expression levels and apply them to images of both in vitro and in silico cultures. Using the same quantitative measures, we are able to compare model scenarios with different assumptions on cell-cell adhesion and intercellular feedback mechanisms directly with experimental data.

Applying our methodology to microscopy images of cultured pluripotent ESCs, we demonstrate that the emerging colonies are highly variable regarding both morphological and spatial fluorescence patterns. Moreover, we can show that clusters of ESCs with a high self-renewing capacity are preferentially located in the interior of a colony structure. Currently we are extending our approach to study the dynamics of pattern formation during ESC differentiation. The integrated approach combining image analysis with mathematical modeling allows us to infer cellular and intercellular mechanisms behind the emergence of observed patterns that cannot be derived from images directly.

Abstract No. P010

Improved Cyropreservation and Recovery Solutions for Pluripotent Stem Cells & Difficult-to-Preserve Primary Cells

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Pluripotent stem cells 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 for cryopreservation of early passage cells that minimize loss of viability, maximize post-thaw recovery, and minimize unwanted differentiation are essential components to PSC, as well as primary cell, workflows. While many cryopreservation reagents afford high viability immediately post-thaw, significant apoptosis and necrosis is often observed following the first 24 hours post-thaw, decreasing the effective viability, reducing cell numbers and adding additional stress and selective pressure to cultures. This extends the time post-thaw cells must be cultured prior to use in downstream experiments. Using a series of Design of Experiments and mathematical modeling methods, we describe the development of a xeno-free cryomedium for use in cryopreservation of PSCs and ESCs, and a chemically defined post-thaw recovery supplement for use in recovery of PSCs, ESCs, as well as difficult-to-preserve primary cells. When used together, we show this system provides >80% direct post-thaw viability of PSCs with >70% cell survival following 24 hours post-plating. As a result of increased post-thaw survival rate, cells recover faster and are ready to passage sooner than with current solutions, while maintaining pluripotency and normal karyotype over 10 passages. Additionally, the post-thaw recovery supplement was tested in combination with other cryopreservation reagents which lead to markedly improved 24 hour post-thaw viability of difficult-to-preserve primary cells, including primary cortical neurons and human corneal epithelial cells.

Abstract No. P011

Inhibition of H3K27-specific demethylase activity during murine ES cell differentiation induces DNA damage response

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Pluripotent embryonic stem (ES) cells are characterized by their capacity to self-renew indefinitely while maintaining their potential to differentiate into all cell types of an adult organism. Both the undifferentiated and differentiated states are characterized by specific gene expression programs which are determined at the chromatin level. The repressive H3K27me3 chromatin mark is stringently regulated in undifferentiated and differentiating ES cells. In this study, by employing a small molecule inhibitor (GSK-J4) and by targeted gene knockdown/knockout we analyzed the function of the H3K27me2,3-specific demethylases KDM6A and KDM6B in undifferentiated and differentiating ES cells. Surprisingly, we observed that inhibition of the H3K27 demethylase activity induced DNA damage, activation of the DNA damage response (DDR) and cell death in differentiating but not in undifferentiated ES cells. Lack of H3K27me3 attenuated the GSK-J4-induced DDR in differentiating Eed KO ES cells suggesting a critical role for H3K27me3 in DDR. Collectively our findings indicate that during ES cell differentiation KDM6A and KDM6B apart from regulating gene expression patterns have additional functions in preventing DNA damage.

Abstract No. P012

Impact of feeding strategies on scalable expansion of human pluripotent stem cells in stirred tank bioreactors

Christina Kropp [1,*], *Ruth Olmer* [1], *Diana Robles-Diaz* [1], *Ulrich Martin* [1], and *Robert Zweigerdt* [1]

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*Presenting author

The application of human pluripotent stem cells (hPSCs) and their derivates in regenerative medicine and drug discovery will require the constant supply of high cell numbers generated by robust and well defined processes. This cannot be achieved by conventional adherent 2D culture systems. Previous studies revealed the expansion of 3D aggregates in suspension as the most promising alternative. Stirred tank bioreactors are well monitored and controlled systems to optimize and up-scale suspension cultures and are widely used in pharmaceutical biotechnology for the production of recombinant proteins with mammalian cell lines. Recently, the expansion of single cell inoculated hPSCs as cell-only-aggregates in suspension (Zweigerdt et al. 2011), as well as the transfer of this technology into stirred tank bioreactors was shown (Olmer et al. 2012). Thereby an operation mode referred to as “batch-feeding”, where the culture medium is manually replaced daily, was applied. Process analysis, however, showed that these culture conditions resulted in linear growth kinetics rather than exponential cell growth. Perfusion, an alternative feeding strategy, is characterized by continuous exchange of depleted medium by fresh medium using a cell retention device to maintain cells inside the bioreactor. Using perfusion feeding allows better automation and consequently improved control of the culture environment including parameters such as pO2, pH, and the concentration of nutrients. Furthermore, the autocrine medium conditioning in the perfusion mode might support process efficiency and cell yield. Here we present that perfusion feeding results in more homogenous process characteristics and a more exponential growth pattern yielding ~3 x 10^8 cells in 100 mL culture at the process endpoint representing a ~46% improvement compared to batch-feeding. Notably, cells generated by both process strategies retained expression of pluripotency-associated markers as revealed by flow cytometry and global gene expression analysis. A detailed, multi parametric comparison of both feeding strategies will be shown.

Zweigerdt R, Olmer R, Singh H, Haverich A, Martin U. Scalable expansion of human pluripotent stem cells in suspension culture. Nat Protoc 2011;6:689-700.

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.

Abstract No. P013

A versatile and robust xeno- and serum-free cultivation system for human pluripotent stem cells

Annett Kurtz[1,*], *Andrea Bretz* [1], *Judith Finkbeiner* [1], *Frank Jüngerkes* [1], *Christiane Oleszynski* [1], *Jana Fassunke* [2], *Anne Schultheis* [2], *Reinhard Büttner* [2], *Laure Chatrousse* [3], *Christina Kropp* [4], *Ruth Olmer* [4], *Robert Zweigerdt* [4], *Thomas D. Rockel* [1], *Andreas Bosio* [1], *Mathilde Girard* [3], and *Sebastian Knöbel* [1]

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Pluripotent stem cells (PSC) have traditionally been cultivated on mouse embryonic feeder (MEF) cells which contribute to maintenance of pluripotency and deposit extracellular matrix components conferring cell attachment. Though constituting a relatively robust cultivation environment when monitored rigorously, mEF based systems are prone to lot-to-lot variances. Furthermore, the xenogeneic nature of mEF cells and commonly used media components is not compliant with current efforts to establish clinically compatible protocols for maintenance and differentiation of PSC. Different compositions have been devised in order to maintain pluripotency in feeder-independent conditions. However, most media require extensive adaption periods when cells are transferred from feeder-dependent to feeder-free culture conditions. We have optimized a xeno- and serum free media formulation that a) allows rapid adaption to feeder-free conditions, i.e. culture on Matrigel or Vitronectin, b) enables robust and efficient expansion of bona fide PSC as single cells as well as cell-clusters for more than 10 passages while maintaining pluripotency as evidenced by marker expression, in vitro differentiation potential, teratoma formation and karyotyping, c) allows rapid culture initiation after cryopreservation and genetic manipulation, and e) supports episomal reprogramming of human fibroblasts. Furthermore, preliminary observations suggest that the medium supports formation of aggregates from single cell inoculated hPSCs and their expansion in suspension culture. The formulation will allow a rapid translation into a clinical grade medium designed following the recommendations of USP (1043) on ancillary materials and will be suitable for clinical grade expansion of PSC.

Abstract No. P014

Utility of hPSC Scorecard™ Assay in assessment of functional pluripotency of cells across the iPSC workflow

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Improvements in induced pluripotent stem cell (iPSC) reprogramming technologies have led to the generation of patient-derived stem cells from various sources and conditions, creating valuable tools in drug discovery and future cell therapies. The steep challenge of characterizing these resulting iPSCs is minimally addressed by current methods that rely on a combination of in vitro and in vivo cellular methods. Molecular analysis methods offer an appealing solution for rapid, quantitative, and comprehensive characterization.
We had earlier reported the development of a hPSC ScorecardTM Panel comprising a 94-gene TaqMan® panel. The accompanying cloud-based analysis software computes the signature for self-renewal and lineage markers for test samples and compares the data against a pluripotent reference standard to generate scores. Over two hundred samples were analyzed using the ScorecardTM to determine pluripotency along several stages of the iPSC workflow. Established clones were subjected to spontaneous embryoid bodies to assess for trilineage differentiation potential. Established clones were further used for directed differentiation into specific lineages and the optimal combination of cytokines and length of differentiation was determined using ScorecardTM. To further simplify the process and minimize sample size, methods were developed for direct use of cell lysate in the assay without compromising the quality of the results.
These results collectively demonstrate the simplicity, ease and consistency of this method to predict functionality, thus offering a much needed uniform standardization and qualification of pluripotent cell lines.

Abstract No. P015

A New Method to pick targeted ES Cell Clones more efficiently

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One way to generate a KO-mouse line is the usage of genetically modified ES cells. In brief: the ES cells are electroporated with a DNA construct and grown under selective conditions to enrich the clones with the desired genetic modification. The surviving clones are then separated manually to genotype them. The individual clones have to be scraped from the plate (picked) in PBS and the cells afterwards separated in trypsin-EDTA solution, both for 15 to 30 min. minimum. We have developed a fast and easy method to isolate separate ES cell clones cultivated on feeders. The ES cell colonies are trypsinized on the 10cm-dish for one minute only to detach the clones from the plate thereby producing a mixture of single colonies. The trypsin is inactivated with medium and the intact colonies are then collected in culture medium instead of PBS in a very gentle, efficient and fast way. Since a suspension of single colonies is prepared it is easy to extract and transfer them to a 96-well plate with a mouth pipette. At the end every well can easily be checked for the presence of a single clone under the microscope. To generate single cell suspensions the isolated clones are trypsinized for 7 minutes and diluted 1:3 on the next day. With practice the whole procedure takes half the time of the classical way of picking clones and the incubation time under inappropriate conditions is reduced to a minimum. To address the problem of mixed clones after picking we will present data were we co-cultivated stainable and unstainable ES cells in the picking plate, isolated them with the method described and checked for mixed clones after picking with our new method.

Abstract No. P016

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 culturing them 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 (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, the signature of dKO ESCs is remarkably similar to an expression signature previously reported for diapause arrested pre-implantation embryos (Hamatani et al., 2004). In mice, reversible diapause of early embryos is observed in mothers still feeding a litter. Very low expression of networks such as DNA synthesis, cell division, metabolic activity but high activity of the IGF pathway is observed in both diapause embryos and Myc-dKO ESCs. In summary, our data identify c/N-Myc activity as a key element controlling the metabolic and proliferative machineries of naïve ESCs without affecting the pluripotency network. These data suggest that self-renewal can be separated into two biological processes: maintenance of pluripotency and cellular proliferation/metabolism of which only the latter is dependent on Myc activity. Moreover, our data raise the possibility that Myc activity controls the reversible arrest of diapause embryos, suggesting that Myc levels regulate the spectrum of overall cellular activity ranging form dormancy to high proliferative growth.

Abstract No. P017

Karyotypic abnormalities in human iPSCs: a result of LINE1 retrotransposition?

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Human pluripotent stem cells (hPSCs) are considered as favourite cell source for regenerative medicine. Recent findings indicate that potentially tumorigenic chromosomal abnormalities and mutations arise in hPSCs during their generation, expansion and differentiation. Such mutations could be induced by human non-LTR retrotransposons (LINE1, Alu, SVA). It has been reported that LINE1s are activated in hPSCs. Aim of this study is to investigate whether LINE1 mobilization may also affect the genomic integrity of hPSCs. Using a novel retrotransposition reporter assay, optimized for the use in hPSCs and for stable integration into the human AAVS1 safe harbour locus, we are investigating whether LINE1 activity may cause genetic aberrations in hPSCs. In this regard, LINE1 expression levels are assessed using immunoblottings, immunofluorescence staining and qRT-PCR. LINE1 mediated genomic destabilization and preferential integration sites are analysed by Array-CGH and high-throughput sequencing. Applying TALEN technology we have introduced LINE1 reporter vectors into the AAVS1 locus of hiPSCs with an efficiency of up to 1,25%. Single cell reporter hiPSC clones were established and further cultivated. Reporter expression and retrotransposition of the LINE1 reporter transgenes was demonstrated. However, qRT-PCR analyses revealed unexpectedly low levels of LINE1 reporter mRNA in the established clones and the number of LINE1 de novo insertions in hPSCs did not increase during long term cultivation. These two findings may point to an activation of intracellular cellular defense mechanisms. So far, no karyotypic abnormalities were found during approximately 50 passages after LINE1 reporter integration. Ongoing analyses will demonstrate whether LINE1 expression and retrotransposition can result in karyotypic abnormalities and whether specific cellular defense mechanisms become activated after LINE1-activation to maintain genomic integrity.

P018 – P038: Programing and reprograming

(posters will be displayed in the tent)

P018 Improving the specificity and delivery of CRISPR system
Egija Berga

P019 Programming and Isolation of Highly Pure Physiologically and Pharmacologically Functional Sinus-Nodal Bodies from Pluripotent Stem Cells
Robert David

P020 The StemCellFactory – Automated Production of Induced Pluripotent Stem Cells
Andreas Elanzew

P021 Cross talk between p53 and GSK3b in neuronal stem cells differentiation
Margarita Glazova

P022 Generation of iPSCs derived from PD study patient fibroblast lines using CytoTune®-iPS 2.0 Sendai virus in the Essential® feeder-free media system
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P023 Generation of human iPSCs with an improved safety profile by specific modulation of mitogen-activated protein kinase (MAPK) signalling pathways
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P026 Enabling Successful Reprogramming of Peripheral Blood Mononuclear cells to Induced Pluripotent Stem Cells with the CytoTune™ –iPS 2.0 Sendai Kit
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P027 Lipofectamine® 3000: A new transfection reagent for iPSC generation and stem cell genomic engineering
Peter Mark

P028 Direct conversion of human fibroblasts into Sox2-positive, expandable neural stem cells
Sandra Meyer

P029 Features of amnion derived multipotent stromal cells that may clear the path to pluripotency during experimentally induced reprogramming
Olena Pogozhykh

P030 Mitochondrial pattern in mRNA-derived iPS cells is distinct from that in their differentiated counterparts
Leili Rohani

P031 Reprogramming Triggers Mutagenic Endogenous L1, Alu and SVA retrotransposition in human induced pluripotent stem cells
Gerald Schumann

P032 Direct conversion of murine embryonic fibroblasts into hepatocytes
Guangqi Song

T37/P033 Mesenchymal stem cells derived from iPS cells from aged individuals acquire fetal characteristics
Lucas Spitzhorn

P034 Yamanaka-type reprogramming of Dicer-less fibroblasts results in trophoblast-like intermediates omitting the iPS cell-state
Marc Christian Thier

P035 Antibody-based tools and protocols for improving stem cell characterization workflows
Tim Wessel

P036 A nanoparticle-based gene delivery system for the safe virus-free application in gene and cell therapy
Nadine Wilhelm

P037 Generation of induced pluripotent stem cell lines for establishment of preclinical large animal models
Stephanie Wunderlich

T21/P038 Modeling and pharmacological rescue of ion channel diseases enabled by improved cardiac induction of human pluripotent stem cells in 2D and 3D formats
Boris Greber

Abstract No. P018

Improving the specificity and delivery of CRISPR system

Egija Berga [1,*], *Yujia Cai* [2], and *Jacob Giehm Mikkelsen* [2]

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Clustered regulatory interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein 9 system provides a robust and multiplexable genome editing tool, enabling researchers to precisely manipulate specific genomic elements, and facilitating the elucidation of target gene function in biology and diseases. Genome engineering in human induced pluripotent stem cells (hiPSCs) holds great promise for biomedical research and regenerative medicine. In this study we optimized and described dimeric dCas9-FokI fusion system for performing robust and highly specific genome editing in human cells. We propose an alternative methodology based on RNA-guided dCas9-FokI fusion that could offer considerable potential for gene targeting and genome editing applications with reduced off-targeting mediated mutations. The ability to use Cas9-FokI fusion to program sequence specific DNA cleavage defines a new class of genome engineering tools. Here, we have shown that this system efficiently can be used in mammalian cells to facilitate genome editing. However, several aspects of dCas9-FokI fusion system should be further improved to increase its efficiency and versatility.

Abstract No. P019

Programming and Isolation of Highly Pure Physiologically and Pharmacologically Functional Sinus-Nodal Bodies from Pluripotent Stem Cells

Robert David [1]

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Therapeutic approaches for ‘‘sick sinus syndrome’’ rely on electrical pacemakers, which lack hormone responsiveness and bear hazards such as infection and battery failure. These issues may be overcome via ‘‘biological pacemakers’’ derived from pluripotent stem cells (PSCs). Here, we show that forward programming of PSCs with the nodal cell inducer TBX3 plus an additional Myh6-promoter-based antibiotic selection leads to cardiomyocyte aggregates consisting of >80% physiologically and pharmacologically functional pacemaker cells. These induced sinoatrial bodies (iSABs) exhibited highly increased beating rates (300–400 bpm), coming close to those found in mouse hearts, and were able to robustly pace myocardium ex vivo. Our study introduces iSABs as highly pure, functional nodal tissue that is derived from PSCs and may be important for future cell therapies and drug testing in vitro.

Abstract No. P020

The StemCellFactory – Automated Production of Induced Pluripotent Stem Cells

Andreas Elanzew [1,*], *Daniel Langendoerfer* [2], *Michael Kulik* [3], *Ulrich Marx* [3], *Adam Malik* [4], *Mirko Trutnau* [5], *Werner Zang* [5], *Michael Peitz* [1], *Robert Schmitt* [3,4], *Martin Zenke* [6], *Simone Haupt* [2], and *Oliver Brüstle* [1,2]

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Human induced pluripotent stem cell (hiPSC) technology provides unique prospects for a large variety of biomedical applications. As a result, stem cell biomanufacturing is impacting on cell-based disease modeling, drug development and regenerative medicine. In particular, paralleled automated processes for reprogramming and expansion are required to generate hiPSC line collections from large patient cohorts. However, industrialization of stem cell technologies demands a broad set of expertise. We tackled these challenge by combining expertise in stem cell research and process automation to establish a large system integration for the automated production of patient-specific hiPSC lines (www.StemCellFactory.de). The StemCellFactory provides automation, standar¬dization and parallelization of all required cell culture steps, ranging from adult human dermal fibroblast (HF) expansion via feeder-free Sendai-based reprogramming to clonal selection and expansion of the obtained hiPSC. In a biological validation process, we could show that the StemCellFactory enables robust reprogramming of HF via the Sendai virus technology. We developed a fully automated E8-medium-based reprogramming protocol that delivers footprint-free primary hiPSC within 3 weeks and with state-of-the-art efficiencies (0.08% ± 0.007%). For subsequent automated clonal selection of primary hiPSC colonies a picking device (CellCelector, ALS) was integrated into the workflow of the StemCellFactory. HiPSC can be automatically detected, harvested and clonally propagated in 24-well plates. In order to obtain sufficient material for QC analysis and banking, expansion and splitting protocols were established for the automated maintenance of hiPSC. Automatically propagated hiPSC exhibit normal growth characteristics and pluripotency-associated marker expression profiles. FACS analysis showed sustained Tra-1-60 expression across 34 days (10 passages) of automated cultivation (automated 94.40% ± 4.3% vs. manual 96.20% ± 3.5%). The StemCellFactory is expected to advance medical research by providing large numbers of hiPSC lines for disease modeling and drug screening at industrial scale and quality.

Abstract No. P021

Cross talk between p53 and GSK3b in neuronal stem cells differentiation

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p53 and GSK3b are the known players in the regulation of embryonic stem cell self-renewal and differentiation. Among this, p53 positively regulates differentiation, while GSK3b mainly has an inhibitory effect. On the other hand, the functional connection between p53 and GSK3b was reported. The purpose of this study was to reveal how an alteration of p53 and/or GSK3b activity will affect on neural stem cells (NSC) differentiation. Our study was performed on NSC isolated from the hippocampus of new-born mice. During incubation of NSCs were treated with activator of p53 Nutlin-3 and/or inhibitor of GSK3b TCS2002. According to the results both Nutlin-3 and TCS2002 increased the number of neurons in the culture confirming that p53 and GSK3b have an opposite action. Interesting, Sox2 localization in the cells, which were treated with Nutlin-3, was detected mainly in the cytoplasm, while after inhibition of GSK3b it stayed in nuclei for the most part. Sox2 maintains stem cell self-renewal, but expression of Sox2 was also shown in some population of differentiating neurons, that proposes a role of Sox2 in neuronal differentiation (Penvy and Nicolis, 2010). That let us suggest that the of NSC differentiation is regulated by different mechanisms for p53 and GSK3b. Analysis of MAPK pathway, which controls neuronal differentiation, demonstrated activation of cRaf and ERK1/2 after Nutlin-3, but inhibition of GSK3b did not have any effect. The transcription factor CREB, positively regulated by ERK1/2 and negatively by GSK3b, was activated by Nutlin-3 and TSC2002 in both cases. Inhibition of GSK3b followed by activation of p53 potentiated activation of cRaf/ERK/CREB cascade. The obtained data proposed that p53 and GSK3b differentially regulate neural differentiation having transcriptional factor CREB as a common downstream target. This work was supported by grants from Russian Foundation for Fundamental Research (RFBR 13-04-01431-a).

Abstract No. P022

Generation of iPSCs derived from PD study patient fibroblast lines using CytoTune®-iPS 2.0 Sendai virus in the Essential® feeder-free media system

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The absence of cellular models for Parkinson's Disease (PD) represents a major bottleneck and unmet need in PD research. Patient-derived induced pluripotent stem cells (iPSCs) offer exciting potential in cell therapy and in vitro disease modeling. Efficient reprogramming of patient somatic cells to iPSCs in feeder-free conditions plays a key role in realizing this potential. Many reprogramming methods have been optimized for use with numerous cell lines, but lead to technical challenges for researchers in converting adult or disease somatic cells to iPSCs consistently and efficiently. The CytoTune®-iPS 2.0 Sendai Reprogramming Kit uses Sendai virus and polycistronic vectors to reprogram somatic cells into induced pluripotent stem cells (iPSCs) which provides a more robust reprogramming efficiency, lower cytotoxicity, and faster viral clearance to generate integration-free iPSCs in feeder-free conditions. In this study, fibroblasts from skin biopsies of two related Parkinson's disease (PD) study patients were reprogrammed in feeder-free conditions to iPSCs using Life Technologies CytoTune®-iPS 2.0 Sendai Reprogramming Kit. These iPSCs are transgene-free and karyotypically normal, express known pluripotency markers and are able to differentiate into embryoid bodies that present the three germ layer lineages: ectoderm, mesoderm, and endoderm. Gene expression analysis distinguishes these iPSCs from their parental fibroblasts and clusters them together with control Gibco® iPSCs and H9 ESCs. Given the efficiency, speed and ease of reprogramming of these adult, disease-related fibroblasts in feeder-free conditions, the CytoTune®-iPS 2.0 Sendai Reprogramming Kit can be applied to large scale reprogramming of multiple disease lines in an automated fashion to provide significant impact for researchers worldwide.

Abstract No. P023

Generation of human iPSCs with an improved safety profile by specific modulation of mitogen-activated protein kinase (MAPK) signalling pathways

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Human induced pluripotent stem cells (hiPSCs) offer new options for regenerative therapy in the field of personalized medicine. While the pluripotent state of stem cells is tightly regulated by a defined set of pluripotency factors (Oct4, Sox2, Nanog) and intracellular mitogen- and stress- activated-protein-kinase (MAPK) signalling pathways (ERK1/2; JNK; p38 MAPK) the functional role of these signalling cascades in the establishment of pluripotency in the human system is poorly understood on the molecular level. To gain a deeper insight into the activation pattern of MAPK signalling pathways during reprogramming, we generated human reporter fibroblasts carrying signalling responsive elements to monitor the functional activity of these MAPK pathways in the process of reprogramming. After characterization of these signalling pathways, we aim to use small molecule compounds to modulate those specific pathways which proved to be required for efficient reprogramming. Elucidating the functional significance of these signalling networks for reprogramming is important to discover new and more efficient reprogramming regimes then the protocols currently used and may be associated with an improved safety profile as compared to currently used integrative reprogramming methods.

Abstract No. P024

Directed differentiation of embryonic stem cells cells into Hematopoietic stem and progenitor cells

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The directed in vitro specification of pluripotent embryonic stem cells (ESCs) to form multipotent hematopoietic stem cells (HSCs) holds enormous promise to open new avenues for both the study and treatment of hematologic diseases. Despite major advances towards a better understanding of HSC development and a possible transfer of these insights to in vitro systems, to date there is no robust protocol for the specification of HSCs from ESCs. The major obstacles the field faces are the low number of HSCs generated by currently available protocols and the lack of a defined set of cell surface markers in order to safely isolate these cells. Recent studies highlight the homeobox transcription factor HOXB4 as a key regulator of HSC specification and maintenance, since ectopic expression of HOXB4 has been shown to enhance the self-renewal capacity of adult murine HSCs and enhance the hematopoietic specification of ESCs and IPSCs. We have developed ESC lines from a mouse with a knock-in of YFP into the HOXB4 locus and, following in vitro EB differentiation, found that YFP expression marked a sub-population of cells enriched for HSC/P characteristics. Based on their gene expression pattern and cell surface phenotype the development of these cells within the culture flask strongly resembles the embryonic development of HSPCs in the mouse and facilitates the comprehensive in vitro-analysis of the sequential steps of hematopoiesis. Functional colony-forming and transplantation assays highlight that potential HSPC cells seem to reside exclusively within the Hoxb4-expressing compartment of specified cells. Moreover, the prospective identification of specific novel markers could likewise enhance the isolation and specification of these cells. Hence, our in-vitro model represents a powerful platform for molecular characterization and functional testing of in vitro specified HSPCs expressing HOXB4.

Abstract No. P025

Realtime-based high throughput trisomy detection of iPS clones

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Induced pluripotent stem (iPS) cells represent a breakthrough in regenerative medicine by the possibility of individualized cellular therapies while minimizing the risk of immune rejection.
However, recent publications indicate that iPS cells display a high appearance of chromosomal aberrations leading to a higher risk of tumor development after transplantation. Determination of chromosomal duplications requires karyotyping of the iPS clones, a time consuming and expensive approach. Therefore, we designed a basic but efficient method using RT-PCR and quantitative realtime amplification. In this method specific single copy genes were utilized to quickly prescreen putative iPS clones for specific trisomies. Detection of the respective duplications was achieved by gDNA amplification with RT-PCR of a single copy gene fragment on the respective chromosome (TTC21B / Chr 6, Calcul / Chr 12 and ADAMTS1/ Chr 21). The product was utilized for qPCR with different oligonucleotides in the presence of positive and negative karyotyped controls. Interestingly, in our iPS generation with a small non-human primate (Callithrix jacchus), just trisomies of particular chromosomes occurred with different frequencies, similar to the human. Out of all compromised clones, duplications of chromosome 6 were found in nearly 81%, often additionally with duplication of Chromosome 12 (54%) and 21 (9%). qPCR detection results were compared with karyotyping and both methods showed the same results. Our realtime-based trisomy detection method is reducing time for karyotyping and cell culture and therefore costs for iPS production. Furthermore, it can be easily expanded to every desired chromosome region of interest.

Abstract No. P026

Enabling Successful Reprogramming of Peripheral Blood Mononuclear cells to Induced Pluripotent Stem Cells with the CytoTune™ –iPS 2.0 Sendai Kit

Roland Leathers [1,*], *Connie Lebakken* [1], *Reichling Laurie* [1], *Chad MacArthur* [1], *Jennifer Crean* [1], *Uma Lakshmipathy* [1], *Bonnie Hammer* [1], and *Tim Wessel* [1]

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Human induced pluripotent stem cells (iPSCs) derived from adult somatic cells hold great promise for disease modeling and may provide new cell sources for clinical therapies. Currently the majority of iPSCs are derived from donor fibroblast cells which are obtained by a skin biopsy and expanded prior to reprogramming. Utilizing a donor blood sample as a source for reprogramming is attractive as blood can be easily obtained from most patients and there are large banks of frozen peripheral blood mononuclear cell (PBMC) samples from patients available to researchers. Reprogramming from peripheral blood sources has been challenging due to cytotoxicity and low reprogramming efficiencies. Our goal was to enable the successful and consistent generation of iPSCs from frozen PBMCs utilizing our CytoTune™ iPS Sendai reprogramming tools. We optimized conditions throughout the workflow including culture conditions, timing of transduction and transduction methodology which led to increased reprogramming efficiencies from donor PBMCs. When these optimizations were used in conjunction with the CytoTune™ –iPS 2.0 Sendai Reprogramming Kit we observed reprogramming efficiencies of greater than 2% on MEF feeder layers in KSR-based iPSC medium and greater than 1% on Geltrex™ or Vitronectin substrates in Essential 8™ Medium. Together these improvements support the efficient and reproducible reprogramming of PBMCs and should provide a reliable tool to generate iPSCs from existing and future PBMC sources.

Abstract No. P027

Lipofectamine® 3000: A new transfection reagent for iPSC generation and stem cell genomic engineering

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Stem cells, specifically induced pluripotent stem cells (iPSCs), hold immense promise for the future of regenerative medicine and personalized therapeutic treatments for a myriad of diseases and conditions. However, the lack of advanced technologies has been hindering the current pace of research and discovery. One of the greatest challenges lies in the manipulation of stem cells. Lipofectamine® 3000, a new transfection reagent, has been developed for nucleic acid delivery to enable the use of new technologies for stem cell applications. In the present study, we first demonstrated that Lipofectamine® 3000 can be used to efficiently deliver the EPi5™ episomal reprogramming vectors to BJ skin fibroblasts for the generation of iPSCs. This method allows researchers to perform efficient in-situ reprogramming at lower cost, providing a great alternative over the standard electroporation techniques typically used for iPSC generation. Furthermore, it was discovered that Lipofectamine® 3000 can achieve optimal transfection efficiency of various sizes of plasmid DNA and low toxicity in both embryonic stem cells (ESCs) and iPSCs which have been traditionally proven to be hard to transfect. More importantly, manipulation of stem cells can be achieved utilizing TALENs and CRISPRs for genome engineering purposes. Transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPRs) allow for editing and engineering of DNA at specific loci. However, the effectiveness of these tools depends on the intrinsic properties of the locus of interest and efficient delivery. Lipofectamine® 3000 demonstrated efficient delivery of TALENs and CRISPRs into various iPSC clones, H9 human embryonic stem cells for targeted genome engineering. Taken together these advancements in delivery greatly improve downstream workflows, enable easier stem cell manipulation, and enhance site-specific insertion or deletion of transgenes in the cellular genome for the generation of knock-in or knock-out cell models and transgenic small animal models.

Abstract No. P028

Direct conversion of human fibroblasts into Sox2-positive, expandable neural stem cells

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Cellular reprogramming provides an attractive cellular source for numerous biomedical applications including cell replacement therapy, disease modeling and tissue engineering. Recent studies demonstrated that terminally differentiated cells can be reprogrammed not only into induced pluripotent cells (iPSCs) but also into different types of somatic stem cells by defined factors, thereby circumventing progression through the pluripotent state. Recently, we could demonstrate the direct conversion of murine fibroblasts into induced neural stem cells (iNSCs) by the curtailed overexpression of Oct4 while constitutively activating Sox2, Klf4 and c-Myc. The adaptation of this conversion protocol to the transdifferentiation of human fibroblast cells remained unclear. Here, we show efficient induction of neural stem cell properties in human fibroblasts by timely restricted expression of Oct4, Sox2, Klf4, as well as c-Myc. Leukemia inhibitory factor (hLIF) and small molecules inhibiting transforming growth factor β (TGF-β) as well as glycogen synthase kinase 3 β (GSK-3β) were used to stabilize freshly converted iNSCs. By applying this protocol, Sox2-positive neuroepithelial colonies appeared after 17 days of induction. Those colonies cut be isolated and stable cell lines established by monoclonal expansion. Thus far, these cell lines could be expanded for more than 10 passages and uniformly display morphological and molecular features of NSCs, such as the expression of Nestin and Sox2. The iNSC lines could be differentiated into neurons and astrocytes as judged by staining against Tuj1 and GFAP, respectively. In conclusion, we report a protocol for the direct conversion of human fibroblasts into stably expandable neural stem cells that might provide a robust basis for biomedical applications such as cell replacement and tissue engineering.

Abstract No. P029

Features of amnion derived multipotent stromal cells that may clear the path to pluripotency during experimentally induced reprogramming

Olena Pogozhykh [1,*], *Denys Pogozhykh* [1], *Rainer Blasczyk* [1], and *Thomas Mueller* [1]

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In experimentally induced reprogramming of cells, invasive methods lead to insertional mutations or chromosomal aberrations and immunological rejection of the generated target cells. Therefore cell types with low reprogramming barriers, e.g. low DNA methylation and basal activity of pluripotency marker genes are of foremost interest for Regenerative Medicine. While multipotent stromal cells (MSC) from bone marrow, adipose tissue and cord blood are well characterized, multipotent features of placental MSC populations remain controversial. During our experimental reprogramming (Wiedemann et al., 2012), cells from extraembryonic endoderm forming the placental amnion displayed extreme features of proliferation (> d120), metabolic activity comparable to immortalized controls (3T3 line) and high plasticity. To explain these features we generated primary cell cultures and investigated postnatal amnion membranes from the human and common marmoset monkey and found reduced overall DNA methylation especially of POU5F1 promoter in coincidence with basal expression of pluripotency genes like OCT4A and NANOG. Surprisingly, next to the expression of mesenchymal markers like CD105, CD90, CD73, VCAM, ALCAM and CD44 cells from the membranes displayed activity of early proliferation and germ line associated markers like SALL4, GPR125, STELLA, PGP9.5, MAGEA4 and VASA. Hypothesis: Certain cell populations in the epiblast/extraembryonic mesoderm seem to undergo similar epigenetic resetting known so far just from the inner cell mass at critical stages to increase developmental potential.

Wiedemann A. et al. Induced pluripotent stem cells generated from adult bone marrow derived cells of the non-human primate (Callithrix jacchus) using a novel quad-cistronic and excisable lentiviral vector. Cellular Reprogramming, 2012; 14(6):485-96

Abstract No. P030

Mitochondrial pattern in mRNA-derived iPS cells is distinct from that in their differentiated counterparts

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Objectives: Aging in somatic cells is accompanied by mitochondrial dysfunction causing multiple cellular dysfunctions. Reprogramming can reverse some aspects of aging in somatic cells of old individual. However, there is currently conflicting data regarding the ability of reprogramming to reset all aspects of aging. We therefore wanted to confirm the mitochondrial modification during reprogramming of somatic cells to mRNA-iPS cells. Material and Methods: Human IMR90 fibroblasts were transfected with mRNA including Oct4, Sox2, Klf4 and c-Myc for generation of mRNA-iPS cells. Pluripotency characterization of mRNA-iPS colonies was confirmed using teratoma assay, Karyotype analysis, immunostaining and microarray expression analysis. To assess ROS production and mitochondrial membrane potential (MMP), the mRNA-iPS cells and their donor fibroblasts were treated with H2DCFDA and membrane-sensitive dye JC-1. The results were analysed by flow cytometry. Evaluation of mitochondrial morphology and localization were performed using the Mitotracker deep red. Results: mRNA-iPS cells showed typical pluripotency markers and differentiation into all three germ layers. Regarding the gene expression profiles, they remarkably showed the same patterns as human embryonic stem cells. Flow cytometry data revealed lower ROS production in mRNA-iPS cells compare to their donor fibroblasts. mRNA-iPS cells showed less mitochondrial membrane potential in contrast to their parental fibroblasts. The morphology of mitochondria in mRNA-iPS cells pointed out less elongated mitochondria compared to their donor fibroblasts. Conclusion: The mRNA-iPS colonies developed in our lab showed pluripotency markers. Considering mitochondrial function, our mRNA-iPS cells revealed decreased ROS production and MMP indicating less active mitochondria in those pluripotent cells. Furthermore, more under developed mitochondria in mRNA-iPS cells shows that mRNA reprogramming might be able to rejuvenate the cells to a more youthful state. This is the first report of such changes in mRNA-iPS cells, yet more comprehensive testing of such iPS cells and their potential aging signature should be conducted.

Abstract No. P031

Reprogramming Triggers Mutagenic Endogenous L1, Alu and SVA retrotransposition in human induced pluripotent stem cells

Gerald Schumann [1,*], *Jung-Klawitter Sabine* [1], *Fuchs Nina* [2], *Kyle Upton* [3], *Martin Munoz-Lopez* [4], *Ruchi Shukla* [3], *Jichang Wang* [2], *Marta Garcia-Canadas* [4], *Daniel Gerhardt* [5], *Attila Sebe* [1], *Ivana Grabundzija* [2], *Sylvia Merkert* [6], *Anett Witthuhn* [6], *Alexandra Haase* [6], *Johannes Löwer* [1], *Ernst Wolvetang* [7], *Ulrich Martin* [6], *Zoltán Ivics* [1], *Zsuzsanna Izsvák* [2], *Jose Garcia-Perez* [4], and *Geoffrey Faulkner* [5]

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The use of human induced pluripotent stem cells (hiPSCs) holds great therapeutic promise for regenerative medicine. hiPSCs present unlimited capacity for proliferation, differentiate into all cell types of the germ layers, and represent a source of autologous cells compatible with the immune system. However, reprogramming and subsequent cultivation of hiPSCs in vitro can induce genetic and epigenetic abnormalities that can result in tumorigenic iPSCs. Genomic mutations may undermine their use in regenerative medicine, and it is unclear if hiPSCs-derived cells are safe for administration. Activation of the human endogenous mobile retrotransposons LINE-1 (Long Interspersed Element 1, L1), Alu and SVA can generate such mutations shown to be the cause of genetic disorders and tumor diseases. We investigated if these mutagenic endogenous retrotransposons mobilize in hiPSCs, contribute to their genomic destabilization and affect host gene expression. Our analysis of eight hiPSC lines and their parental cells uncovered that reprogramming reduced methylation of the L1 promoter significantly, induced L1 mRNA transcription levels by 3 to 2000-fold relative to parental cells resulting in excessive quantities of the L1 protein machinery in hiPSCs. To investigate if the observed L1 activation led to L1-mediated mobilization of endogenous transposable elements, we applied a novel high-throughput protocol named retrotransposon capture-sequencing (RC-seq). We identified, mapped and validated a multitude of individual L1, Alu and SVA mobilization events that occurred during reprogramming into hiPSCs and their subsequent cultivation. We demonstrate that mutagenic mobilization of endogenous retrotransposons occurs also in the genomes of human embryonic stem cells during their cultivation. 50% of all de novo retrotransposition events occurred in protein-coding genes, including genes playing roles in oncogenesis, development or signal transduction. We show that intronic L1 de novo insertions can perturb host gene expression in hiPSCs with unknown consequences in differentiated cells, questioning biosafety of hiPSCs and their derivatives.

Abstract No. P032

Direct conversion of murine embryonic fibroblasts into hepatocytes

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Hepatocyte transplantation is limited by shortage of donor organ. Therefore, we aimed to generate a source for donor organ independent cells for the treatment of liver diseases. Here, we show the generation of induced hepatocyte-like (iHep) cells from mouse embryonic fibroblasts (MEFs) by ectopic expression of hepatic transcription factors. Specifically, we transduced MEFs with a polycystronic lentiviral vector encoding human FOXA3, GATA4, HNF1α and HNF4α (4TF). Transduced MEFs acquired epithelial morphology and rapidly converted into iHep cells within two weeks. The hepatocyte phenotype was confirmed by transducing iHeps with a reporter lentivral vector, which expresses dTomato under the transcriptional control of the albumin promoter. Quantitative real time PCR analyses and microarray results revealed that the iHep cells had high expression of typical hepatocyte genes, whereas typical fibroblast genes were suppressed. Moreover, the iHep cells exhibited similar functional characteristics to hepatocytes such as glycogen storage, lipid accumulation, uptake and release of indocyanine green, albumin secretion, urea synthesis, and activity of Cytochrom P450 1A1, 3A4. Taken together, our findings suggest that forced expression of FOXA3, GATA4, HNF1α and HNF4α (4TF) in MEFs allows rapid generation of iHep cells that may serve as a desired cell source for the treatment of liver diseases.

Please find abstract P033 under 'Selected presentations' T37/P033

Abstract No. P034

Yamanaka-type reprogramming of Dicer-less fibroblasts results in trophoblast-like intermediates omitting the iPS cell-state

Marc Christian Thier [1,*] , *Caroline Kubaczka* [2], *Thileepan Sekaran* [3], *Hubert Schorle* [2], *Andreas Trumpp* [1], and *Frank Edenhofer* [4]

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Numerous studies have highlighted the role of microRNAs for the maintenance, proliferation or differentiation of various cell types. MicroRNAs have also been shown to play a role in reprogramming of somatic cells to induced pluripotent stem (iPS) cells. The overexpression of specific microRNAs can indeed enhance iPS cell generation and even substitute for the Yamanaka reprogramming factors Oct4, Sox2, Klf4, and c-Myc. However, the role of microRNAs during the reprogramming process remains largely unclear. To address this question, we derived mouse embryonic fibroblasts (MEFs) carrying a conditional loxP-modified Dicer allele. Upon Cre-mediated Dicer deletion, these cells are deprived of almost all functional microRNAs. Dicer-floxed MEFs were transduced with a doxycycline (dox)-inducible, polycistronic reprogramming cassette, treated with Cre and subsequently reprogrammed. Intriguingly, Dicer-null fibroblasts failed to produce iPS cells using this protocol (Kim et al., PLoS ONE (2012)). Unexpectedly, upon sustained transgene activity a dicer-deficient, epithelial and clonal cell population could be derived. In the presence of dox, these cells are viable, highly proliferative and undergo partial demethylation of the early trophoblast marker Elf5, maintaining promoter methylation of the ESC-associated factor Nanog. Subsequent dox-withdrawal results in a time-dependent up-regulation of additional trophoblast markers such as Cdx2, Mash2, Hand1, Tfap2c and Pl1 and is accompanied by a block in proliferation and increased cell-death. Expression profiling using microarray analysis confirms clustering of these cells with wild type trophoblast stem cells and their progeny. Intriguingly, reintroduction of full-length Dicer converts Dicer-deficient pre-iPS cells into stable and transgene independent iPS cells. These data indicate for the first time that Yamanaka-type reprogramming of Dicer-deficient fibroblasts results in a self-renewing, highly proliferative cell population with trophoblast differentiation potential. Our study demonstrates the importance of micro-RNAs for the reprogramming process, emphasizing their role in stabilizing the pluripotent niche as well as their importance for proliferation and cell viability.

Abstract No. P035

Antibody-based tools and protocols for improving stem cell characterization workflows

Tim Wessel [1,*], *Deborah Tieberg* [1], *Joanna Asprer* [1], *Michael Hancock* [1], *Shawn Honeyager* [1], *Tony Goossens* [1], and *Anne Chen* [1]

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Stem cell biology constitutes one of the fastest growing areas in the life sciences. Accordingly, there is strong demand for improving the characterization tools and protocols available to stem cell researchers. We report here the development of a series of antibody-based tool sets and protocols that facilitate detection of important cellular markers of pluripotent stem cells and the differentiated cell types that can be derived from them (e.g., three germ layers, neural stem cells, cardiomyocytes). First, optimized immunocytochemistry reagent sets were identified by screening panels of validated primary antibodies against established stem cell markers and matching them up with appropriate dye-conjugated secondary antibodies and optimized fixative, permeabilization, blocking, and wash buffer systems. We demonstrate how these reagent sets can be applied to simplify traditional fixed-cell immunocytochemistry workflows and enable more information per sample via multiplex staining strategies that are compatible with a variety of imaging platforms. A second series of live-cell imaging reagents was generated to improve culture characterization and clone selection during cellular reprogramming workflows that are used to generate induced pluripotent stem cells. These tool sets are composed of dye-conjugated primary antibodies against select cell surface markers that are paired together with an imaging medium specifically designed to maximize fluorescence signal detection while maintaining cell health. We anticipate that this series of protocol and technology improvements will significantly augment the current characterization approaches available to stem cell researchers.

Abstract No. P036

A nanoparticle-based gene delivery system for the safe virus-free application in gene and cell therapy

Nadine Wilhelm [1,*], *Jennifer Look* [2], *Nadja Noske* [3], *Christine Günther* [3], *Margarida Serra* [4], *Paula Alves* [4], *Manuel Carrondo* [4], *Roberto Juan Rodriguez* [5], *Felipe Prosper* [5], *Klaus Langer* [2], *Hagen von Briesen* [1], and *Erwin Gorjup* [1]

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Mainly, cells for use in cell and gene therapy are modified by viral vectors, but these carry safety risks for patients. They can promote severe immune response or activate oncogenes due to the integration into the host genome. A safe but challenging alternative to viral vectors are synthetic nanoparticle-based systems for gene delivery. Therefore, a biocompatible, biodegradable and ligand-functionalized nanoparticular delivery system was developed, optimized and evaluated. These human serum albumin (HSA)-based nanoparticles (NP) were loaded with DNA-vectors encoding either a suicide gene for their use in cell and cancer therapy or transcription factors for the reprogramming of primary somatic cells to pluripotent stem cells (iPS). To promote cellular uptake of the NP, they were ligand-funcionalized with different peptides like RGD or TAT. After the transfection of difficult-to-transfect cells like adult stem cells and primary cells with the surface-modified DNA-loaded HSA-NP, the transfection efficiency as well as the cellular uptake, intracellular distribution and gene expression was evaluated in vitro. Flow cytometry analysis and confocal laser scanning microscopy (CLSM) studies revealed efficient binding and uptake of the RGD- and TAT-modified HSA-nanoparticles by human mesenchymal stem cells (hMSC) and BJ-fibroblasts. RGD-modified HSA-NP led to considerable gene expression of the reporter gene eGFP which could be increased by TAT-modification in 293T-cells and BJ-fibroblasts. Both applications, stem cell based cancer therapy as well as iPSC generation, have an enormous clinical impact. The use of non-viral nanoparticle based gene delivery systems will allow safer clinical application and opens up broad future possibilities and markets.

Abstract No. P037

Generation of induced pluripotent stem cell lines for establishment of preclinical large animal models

Stephanie Wunderlich [1,*] , *Alexandra Haase* [1], *Sylvia Merkert* [1], *Jennifer Beier* [1], and *Ulrich Martin* [1]

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The prerequisite of transferring in vitro developed regenerative therapies based on pluripotent stem cells (PSC) into clinics is the establishment of large animal models to analyze cell fate and behavior in vivo. For cardiac replacement studies sheep as well as pig models are existing and well-established for preclinical testings. Induced pluripotent stem cells may represent an ideal cell source for future regenerative therapies. In the last years the generation of ovine (oviPSC) and pig induced pluripotent stem cells (piPSC) has been reported. Using HIV-derived reprogramming vectors encoding for human OCT4, SOX2, NANOG, KLF4, and C-MYC, we were now able to generate oviPS-like cell clones from endothelia cells. Resulting oviPS-like cells show typical characteristics of pluripotent stem cells and could be maintained under standard human embryonic culture conditions. OviPS-like cells stain positively for pluripotency markers such as OCT4, SOX2 and NANOG and differentiate in vitro into derivatives of all three germlayers. However, characterisation of the OviPS-like cells revealed dependency of transgenic reprogramming factors as demonstrated by high expression of all exogenous factors and relative low expression of endogenous factors, which was similar to the recently generated porcine iPSC-like cells. Interestingly, our data indicate that despite persisting transgene expression, the generated oviPS-like cells can not only be cultured for a prolonged time, but differentiate into various cell types of the three germ layers. Nevertheless, persistent dependency on the transgenic reprogramming factors representing a common phenomenon in “reprogrammed” cells of pig and sheep indicate incomplete reprogramming. More research is clearly required to obtain further insight into basic requirements for pluripotency in both species and for development of more suitable culture and reprogramming techniques with the final aim to obtain true pluripotent stem cells from pig and sheep.

Please find abstract P038 under ‘Selected presentations’ T21/P038

P039 – P061: Stem cells in development

(posters will be displayed in the tent)

T26/P039 Tet-dependent processing of 5-methylcytosine protects DNA methylation canyons against hypermethylation
Achim Breiling

P040 Roles of Pbx TALE homeodomain proteins in establishing the identity of pancreatic endocrine progenitor cells
Corinna Cozzitorto

P041 "Mechanical" Signals and the Acto-Myosin Pattern During Tissue Regeneration
Claus Fütterer

P042 Modifying the sulfation pattern of chondroitin sulfate proteoglycans (CSPGs) and its role in neural stem cells during forebrain development
Denise Harrach

P043 The transcriptome of native mesenchymal stem cells: detoxification of reactive oxygen species and expression of new surface markers
Andreas Heider

P044 Mesenchymal Stem Cell-Secreted Factors Prevent p57kip2 Nuclear Translocation in Neural Stem/Progenitor Cells: Role in Oligodendroglial Fate Decision?
Janusz Joachim Jadasz

P045 MicroRNA 361 and microRNA 665 are required in osteogenic differentiation of embryonic stem cells
Dorota Kaniowska

P046 Serotonin signaling modulates postnatal neurogenesis
Konstantin Khodosevich

P047 Improving pancreatic differentiation of human pluripotent stem cells via microRNA and glucose modulation
Konrad R. Knittel

P048 Single cell analysis demonstrates proliferative heterogeneity among stem cells in vivo
Isabel Krämer

T15/P049 Angiogenesis controls neural stem cell expansion by regulating tissue oxygenation and HIF signaling
Christian Lange

P050 Murine and human in vitro hematopoietic differentiation reveals the influence of microRNAs on hematopoietic development
Steffi Liebhaber

P051 Differentiation of human induced pluripotent stem cells towards trophoblasts
Svitlana Malysheva

P052 Endodermal Progenitor cells derived from integration-free iPSCs as an in vitro model for dissecting endodermal cell fate decisions
Peggy Matz

P053 Neuroblastoma cells convey CD24 surface expression to non-transformed cells
Vishal Menon

P054 Conversion of primary and pluripotent stem cell-derived neuroepithelial stem cells into region-specific radial glia
Laura Ostermann

P055 The surface molecular biography of human neural differentiation
Jan Pruszak

P056 Functional Analysis of STAR-Family Proteins during Oligodendrogenesis in the rodent Forebrain
Sabrina Schröder

P057 Radiation effects on early embryonic development
Insa Schroeder

P058 Neural Differentiation of Embryonic Stem Cells (ESCs): Regulation by Notch1 via Chromatin Protein High Mobility Group A2 (HMGA2)
Ralf Schwanbeck

P059 Interactions between the meninges and the cortical neuroepithelium during mouse embryonic development
Richard Sturm

P060 T ! õăúNI·ÔÚ rŜúNI·ÔúŜŘ
Tungala Suresh

P061 Characterizing the role of Polycomb repressive complex 1 members in human neural differentiation
Ria Thomas

Please find abstract P039 under 'Selected presentations' T26/P039

Abstract No. P040

Roles of Pbx TALE homeodomain proteins in establishing the identity of pancreatic endocrine progenitor cells

Corinna Cozzitorto [1,*] , *Julia Kofent* [1], *Matthew Koss* [2], *Licia Selleri* [2], and *Francesca M. Spagnoli* [1]

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Signals from the surrounding mesenchyme and proper epithelial/mesenchymal cell interaction are important for pancreas development. However, the mechanisms by which the mesenchyme influences the pancreatic epithelial transcriptional cascade is poorly understood. The Pbx TALE homeodomain transcription factors are expressed in both the pancreatic epithelium and mesenchyme of the mouse embryo starting from E10.5. Previous study demonstrated that Pbx1 is essential for pancreas development and function. Mouse embryos lacking Pbx1 exhibit pancreatic hypoplasia and defects in cell differentiation prior to death *in utero* at E15.5. Moreover adult Pbx1 heterozygous mutant display islet malformation, impaired glucose tolerance and hypoinsulinemia. Here, we present preliminary evidences suggesting a distinct contribution of the Pbx proteins in pancreatic epithelium and mesenchyme during embryonic development. Using Nkx2.5-Cre mice we specifically ablate Pbx1 gene expression in the splenopancreatic mesenchyme. Homozygous Pbx1 deletion in Nkx2.5-expressing cells on a Pbx2 deficient background leads to a reduced number of insulin-producing cells at E14.5. On the other hand, using Pdx1-Cre mice, we delete Pbx1 in all the epithelial pancreatic progenitors. Pbx-epithelium deleted (*Pdx1-Cre; Pbx1flox/flox; Pbx2+/-*) mutant embryos show more dramatic phenotype, including defects in pancreatic gross morphology and morphogenesis, together with a reduced number of insulin-producing cells. Future experiments are aimed to define the Pbx-dependent regulatory network in the pancreatic mesenchyme. In the long period, this knowledge will be useful to define new and more efficient strategies to generate β-cells from stem or differentiated cells for a cell-based diabetes therapy.

Abstract No. P041

"Mechanical" Signals and the Acto-Myosin Pattern During Tissue Regeneration

Claus Fütterer [1]

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Tissue regeneration requires considerable large-scale reshaping and repositioning of single cells and cell assemblies. Hydra polyps are morphologically simple and show eternal life based on permanent homeostatic renewal of all cells (pluripotent stem cells). Its function and morphology is very similar to the gastric system of higher organisms including humans and may serve as a reasonable model. We investigated the role of physical forces, cell-morphology and the activity of the acto-myosin system for large scale reorganization. The results clearly show the relation between cellular morphology, mechanical waves and fluctuations as an assumed means of supra-cellular communication - prerequisite of correct re-shaping. Quantitative measurements of the acto-myosin system and of the intrinsic mechanical forces strongly support this hypothesis. The acto-myosin pattern, observed in vivo by epi-fluorescence scanning confocal microscopy, clearly displays self-organized super-cellular structures generating surprisingly strong forces in the micro-Newton range during regeneration. These forces - we also measured the dynamics quantitatively - are responsible for the successful reshaping process. Furthermore it is known that the Wnt/beta-catenin pathway is involved in mechano-transduction and serves also as a co-factor for gene expression of development genes. This could explain the strong link of mechanical cues for controlling genetic programs related to growth and regeneration.

Michael Krahe, Iris Wenzel, Kao-Nung Lin, Julia Fischer, Joseph Goldmann, Markus Kästner, Claus Fütterer, Fluctuations and differential contraction during regeneration of Hydra vulgaris tissue toroids, New Journal of Physics 15 (2013), 1-18.

J. Whitehead, D. Vignjevic, C. Fütterer, E. Beaurepaire, S. Robine, E. Farge, Mechanical factors activate beta-catenin-dependent oncogene expression in APC1638N/+ mouse colon. HFSP J. Volume 1, Issue 1 (2008), 286-294

Abstract No. P042

Modifying the sulfation pattern of chondroitin sulfate proteoglycans (CSPGs) and its role in neural stem cells during forebrain development

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Chondroitin sulfate proteoglycans (CSPGs) and their sulfation by chondroitinsulfotransferases (Chsts) appear to play a crucial role for the behaviour of neural stem cells (NSCs) in the embryonic neural stem cell niche during mouse forebrain development. It has been shown that the inhibition of the sulfation by sodium chlorate or the degradation of the CSPG glycosaminoglycans by chondroitinase ABC leads to less proliferation and altered cell fate decisions of the NSCs 1-3. The proliferation and differentiation of cortical neural stem cells from E13.5 mouse embryos upon forced expression of distinct Chst-EGFP constructs was examined by neurosphere forming/proliferation assay and differentiation assay in vitro. Furthermore, the overexpression experiments were performed by in utero electroporation at E12.5. The overexpression of distinct Chsts in the NSCs was functional as revealed by an increased signal for the complex sulfated CS-epitope detected by the monoclonal antibody 473HD. In the differentiation assay a significant increase in neurogenesis at the expense of gliogenesis was observed. In consistence with previous observations, the sulfation of the CSPGs plays a role in the commitment of the NSCs within the neural stem cell niche and could function as a possible communication platform between the NSCs and their extracellular surrounding in the neural stem cell niche.

Akita, K. et al. Expression of multiple chondroitin/dermatan sulfotransferases in the neurogenic regions of the embryonic and adult CNS implies that complex chondroitin sulfates have a role in neural stem cell maintenance. Stem Cells 26, 798-809 (2008)

Sirko, S., von Holst, A., Wizenmann, A., Gotz, M. & Faissner, A. Chondroitin sulfate glycosaminoglycans control proliferation, radial glia cell differentiation and neurogenesis in neural stem/progenitor cells. Development 134, 2727-2738 (2007)

Abstract No. P043

The transcriptome of native mesenchymal stem cells: detoxification of reactive oxygen species and expression of new surface markers

Andreas Heider [1,*], *Sabine Hecht* [1], *Michael Cross* [2], and *Rüdiger Alt* [3]

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The biology of cultured mesenchymal stromal cells (MStrC) has been characterized extensively in recent years, and both their immunomodulatory properties and their ability to differentiate into connective tissues clearly hold therapeutic potential. In contrast, relatively little is known about their precursors, physiological mesenchymal stem cells (MSC), other than the fact that their ability to engraft recipient mice and generate fresh stroma is lost rapidly following isolation. One of the factors currently limiting the characterisation of MSCs is the lack of specific markers for the prospective isolation of pure populations. We have employed a combination of surface markers (Sca1+ Lineage- CD45-) to isolate a small population of primary cells from murine bone marrow that is enriched for fibroblast colony forming units (CFU-f), and gives rise to MStrC with the potential for prolonged expansion and tri-lineage differentiation. We performed microarray analysis to compare the properties of SL45 MSC and MStrC, and used the own developed virtualArray software to extend this comparative analysis to over 80 different murine cell types, including three independent isolates of CFU-f enriched primary putative MSCs. This analysis confirmed that the primary MSC populations specifically express a number of genes implicated in the development, organisation and maintenance of mesenchymal tissues. Furthermore these cells express a large number of redox-active enzymes suggesting a role in neutralising oxidative stress in the immediate environment. Consistent with this, we find SL45 but not MStrC cells to be particularly effective at protecting primary haematopoitic stem and progenitor cells during ex-vivo culture. Finally, our analysis has identified a range of potentially novel surface markers in the SL45 population. Two of these (LepR and CD51) have recently been shown by others to enrich prospectively for a functional population of MSCs. Others are currently being evaluated.

Heider, Andreas, und Rüdiger Alt. „virtualArray: A R/bioconductor Package to Merge Raw Data from Different Microarray Platforms“. BMC Bioinformatics 14, Nr. 1 (2. März 2013): 75. doi:10.1186/1471-2105-14-75.

Heider, Andreas, Ralitza Danova-Alt, Dietmar Egger, Michael Cross, und Rüdiger Alt. „Murine and Human Very Small Embryonic-like Cells: A Perspective“. Cytometry Part A 83A, Nr. 1 (1. Januar 2013): 72–75. doi:10.1002/cyto.a.22229.

Abstract No. P044

Mesenchymal Stem Cell-Secreted Factors Prevent p57kip2 Nuclear Translocation in Neural Stem/Progenitor Cells: Role in Oligodendroglial Fate Decision?

Janusz Joachim Jadasz [1,*], *Julia Domke* [1], *Nina Kirschbaum* [1], *Lucas-Sebastian Spitzhorn* [2], *Richard Oreffo* [3], *James Adjaye* [2], and *Patrick Küry* [1]

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Question: The generation of new oligodendrocytes and the repair of myelin sheaths represent processes that are essential for enhancing the ability of the brain to repair itself. These so-called remyelination phases follow the damage caused by diseases like multiple sclerosis in the adult central nervous system (CNS). We identified the multifunctional p57kip2 protein as a negative regulator of myelinating glial cell differentiation and as an important intrinsic switch for glial fate decision in adult neural stem/progenitor cells (aNSPCs) (Jadasz *et al.*, 2012). Furthermore, others and we have also shown that external stimulation of aNSPCs with mesenchymal stem cell (MSC)-secreted factors enhances oligodendrogenesis by yet unknown mechanisms (Jadasz *et al.*, 2013). Unpublished data suggest that a translocation of the p57kip2 protein in oligodendroglial progenitor cells promotes differentiation (Göttle *et al.*, in revision). Here, we address the question whether the MSC-derived oligodendrogenic effect on aNSPCs involves a translocation mechanism of the glial fate regulator p57kip2.
Methods: To this end we analyzed the localization of the p57kip2 protein within aNSPCs after stimulation with MSC-conditioned medium (MSC-CM) from rat and human sources and in addition under astroglial differentiation stimuli. Moreover, we also analyzed the expression of prominent oligodendroglial and astroglial markers.
Results: The exogenous stimulation of aNSPCs with MSC-CM resulted in increased cytoplasmic localization of p57kip2 whereas after astroglial stimulation the percentage of nucleic p57kip2 localization was significantly enhanced. Interestingly human MSC-CM resulted in similar translocation effects and contributed to oligodendrogenesis of rat aNSPCs.
Conclusions: We thus conclude that MSC-secreted factors prevent p57kip2 nuclear translocation in aNSPCs and this event might be involved in the oligodendrogenic molecular mechanism induced by MSCs.

Jadasz, J. J., Rivera, F. J., Taubert, A., Kandasamy, M., Sandner, B., Weidner, N., Aktas, O., Hartung, H.-P., Aigner, L., and Küry, P. (2012). p57kip2 regulates glial fate decision in adult neural stem cells. Development, 139(18):3306–15.

Jadasz, J. J., Kremer, D., Göttle, P., Tzekova, N., Domke, J., Rivera, F. J., Adjaye, J., Hartung, H.-P., Aigner, L., and Küry, P. (2013). Mesenchymal stem cell conditioning promotes rat oligodendroglial cell maturation. PLoS One, 8(8):e71814.

Abstract No. P045

MicroRNA 361 and microRNA 665 are required in osteogenic differentiation of embryonic stem cells

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Osteoblast differentiation is a key step in proper skeletal development and acquisition of bone mass. Skeletal precursor cells give rise to either chondrocytes to form cartilage or osteoblasts to build bone. Cartilage and bone both develop from mesenchyme derived from the neural crest (NC), paraxial mesoderm, or lateral plate mesoderm. All of the major developmental signals including Wnt and Notch signaling, along with transcriptional factors such as Runx-2 and osterix, have been shown to regulate the differentiation and/or function of osteoblasts. However, the role of microRNAs to turn stem cells or progenitors into osteoblasts remains inefficient. We focused on identifying osteogenic lineage committing microRNAs, which are known molecular regulators of other developmental processes. In a screen using a special microRNA array, we found altered microRNA levels after induction of embryonic stem cells into osteoblasts. We have identified microRNAs – miR-361 and miR-665 – that showed differential expression. Gain-loss-analyses were coupled with assessment of calcification potential and expression of osteoblast markers to determine whether miR-665 and miR-361 promoted osteoblast differentiation. Furthermore, bioinformatic predictions of miR-361 and miR-665 targets were experimentally followed to identify their mRNA targets in osteogenesis. Overexpression and knockdown of miR-361 and miR-665 caused changes in calcium deposition, ALP activity and osteogenic marker gene expression. Moreover, miR-361 and miR-665 overexpression resulted in a reduction of mRNA levels of Prickle1/2 and Dishevelled1, respectively which are important downstream components of the Wnt pathway. In order to demonstrate that miR-665 absence and miR-361 presence have an influence on NC formation and mesenchymal specification, we performed RT-PCR analysis for Snai1, Slug, Sox9, Twist1, Pax3, Sox-10 and T-Bra, which respectively marks each cell population. Assuming that miR-361 and miR-665 play a functional role in modulating osteogenic differentiation of ESCs we are indicating new mechanisms involved in this complex regulatory scheme.

Abstract No. P046

Serotonin signaling modulates postnatal neurogenesis

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In the postnatal brain, there are only two neurogenic regions that generate new neurons, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone of the hippocampus. SVZ-derived neuroblasts migrate over long distances along the rostral migratory stream (RMS) into the olfactory bulb, where they differentiate into interneurons. Recently, using microarray analysis we identified many novel signaling molecules that are involved in proliferation of precursor cells, neuroblast migration and differentiation. Serotonin receptor 3A (5HT3A) was one of the ligand gated ion channels that we identified in the screen. We found that serotonergic cells appear in the SVZ and RMS around the birth of mice. Furthermore, serotonergic projections from these local cells and from the raphe nucleus follow the RMS guiding migrating neuroblasts. Knockout of Htr3a impairs whereas overexpressing enhances neuroblast migration. Passing of Ca2+ ions through 5HT3A receptors was necessary for modulation of neuroblast migration. Downstream of 5HT3A, calmodulin and CaMKs were involved in propagation of serotonin signaling cascade.

Abstract No. P047

Improving pancreatic differentiation of human pluripotent stem cells via microRNA and glucose modulation

Konrad R. Knittel [1,#*], *Diana Oelschlägel* [1,2,#], *Bernd Fischer* [1], and *Insa S. Schroeder* [3]

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[#] equal contribution
*Presenting author

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 efficacy and derivatives show 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 until today in vitro formation of fully functional beta cells is not possible. Therefore, we analyzed the expression profile of some microRNAs (e.g. miR-375, -124a, -335, -30d, -200a and -200c) during in vitro-differentiation of two different human ESC lines, WA09 (H9) and HUES-8 and the human iPSC line IMR90. We compare our data from the different lines to show that changes in the microRNA expression profiles are not cell line specific, but demonstrate a general mechanism during the differentiation. Furthermore, we compare 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 and more efficient formation of endocrine pancreatic progenitors expressing PDX1 and NGN3. In addition to microRNAs, nutrients like glucose are important for a successful pancreatic development. We show that in vitro differentiation of hESC under high glucose conditions in a specified timeframe also leads to an increased expression of PDX1 and the combination of both strategies results in a synergistic impact. Currently, these experiments are performed with the human iPSCs. In summary, miR or glucose modulation is a powerful tool to direct the developmental potential of pluripotent cells and can be used to generate pancreatic cells supporting the establishment of in vitro test systems for the analysis of diabetes mellitus.

Abstract No. P048

Single cell analysis demonstrates proliferative heterogeneity among stem cells in vivo

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Individual members of a stem cell population display heterogeneities when grown in cell culture. These heterogeneities involve gene expression levels and differentiation potential. The existence and relevance of similar heterogeneities in vivo is just starting to be explored, favoured by genetic and molecular tools that allow single-cell labelling and cell lineage analysis. Heterogeneities can reflect either different (and fixed) co-existing populations of stem cells, stochastic behaviours or a differential access to niche factors. Accordingly, analysis requires studies on the single cell level and ideally following the same cells over time.
The posterior lateral line of the teleost fish medaka serves as a model to investigate embryonic and post-embryonic stem cells in vivo and their underlying heterogeneity. Its sensory organs, the neuromasts, offer several advantages, namely structural simplicity (they consist of only three cell types), accessibility for imaging and experimental manipulation, and solid regenerative responses. During homeostatic maintenance, mantle cells lining the neuromasts are thought to self-renew and give rise to support cells that, in turn, produce the mechanosensory hair cells – which are regularly lost and replaced. Here, we follow a comprehensive study to analyse heterogeneities of cell cycle length and proliferation rates among individual mantle and support cells within the same neuromast. For that purpose, IdU / CldU double incorporation experiments were complemented by 4D imaging in vivo. Additionally, we used multicolour and inducible labelling of single mantle and support cells to follow lineage progression and correlate proliferation rates with differentiation potential.

Please find abstract P049 under 'Selected presentations' T15/P049

Abstract No. P050

Murine and human in vitro hematopoietic differentiation reveals the influence of microRNAs on hematopoietic development

Steffi Liebhaber [1,*], *Nico Lachmann* [1], *Mania Ackermann* [1], *Adele Mucci* [1], *Doreen Lüttge* [1], *Theresa Buchegger* [1], *Nils Pfaff* [1], and *Thomas Moritz* [1]

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*Presenting author

While microRNAs (miRNA) constitute important regulators of hematopoietic differentiation, their exact role during hematopoietic development remains ill-defined. Thus, analysing the miRNA profile of iPSC derived hematopoietic cells and comparing it to related *in vivo* populations would improve our understanding of hematopoietic differentiation. To this end we performed hematopoietic differentiation of murine pluripotent stem cells and analyzed the clonogenic potential of defined populations (CD41neg,low,high). Differential expression of CD41 marked clonogenic potential *in vitro* in various bone marrow-derived iPSC and ESC lines, similar to the pattern observed for their *in vivo*-derived counterparts. Of note, the highest yield in CD41+ cells was observed after seven days of EB formation in one of the miPSC lines, whereas clonogenic potential was highest after eight days for all pluripotent cell lines investigated. Next, we analyzed the expression profile of hematopoiesis-associated miRNAs-125b, -142-3p and -223 during *in vitro* differentiation of murine and human iPS cells. Here, expression patterns of miRNA revealed similarities to *in vivo* differentiated hematopoietic cells. When compared to human iPSCs, expression of miRNA-125b and miRNA-223 increased 10 to 1000-fold during early hematopoietic specification, with maximum expression in hiPSC-derived macrophages comparable to *in vivo* differentiation. In contrast, miRNA-142-3p expression decreased in the early phase of human hematopoietic specification of iPSC, but accelerated 500-fold upon terminal differentiation towards CD14+, CD163+ macrophages. As all investigated miRNAs showed highest expression in terminally differentiated hematopoietic cells, expression patterns suggest a role in the early endothelial (CD144+,CD34-,CD45-) versus hematopoietic (CD144-, CD34+, CD45+) specification. Taken together, our data demonstrate that murine and human hematopoietic differentiation of pluripotent stem cells represents a valuable tool to study hematopoietic development and may contribute to a better understanding of *in vivo* and *in vitro* specified embryonic as well as adult hematopoiesis.

Abstract No. P051

Differentiation of human induced pluripotent stem cells towards trophoblasts

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Trophoblast cells, surrounding blastocyst cavity, appear as the first event of cellular specification (differentiation) in the developing embryo. Primarily, these cells define the success of implantation, placenta formation and further pregnancy outcome. Therefore, cultured in vitro trophoblast cells can be applied for modeling of placental malformation and miscarriages, as well as studying of early developmental processes. Initially, trophoblast cells were isolated from placenta [1]. However, this methods lack reproducibility and yield heterogeneous cell types with limited differentiation potential. Recently BMP proteins were reported as powerful inductors of trophoblast differentiation from pluripotent stem cells (PSCs), although their effect is controversial [2]. Therefore, reliable and rationale protocols for human trophoblast cell generation are still to be established. In the current study we aim at the detailed characterization on trophoblasts and trophoblast subtypes after differentiation of human induced pluripotent stem cells (hiPSCs) through the formation of embryoid bodies using a fetal bovine serum-based protocol. During differentiation cells formed dome-like structures from which floating spheres were formed, presumably through fluid-pumping trophoectoderm, similar to that surrounding blastocysts. The obtained differentiated cells express typical markers of the early trophoblast lineage including Cdx2, Eomes, Hand1, and Gcm1, as well as ones typical for more differentiated trophoblast cells, such as human chorionic gonadotropin and cytokeratin 7. Immunofluorescent staining demonstrated the presence of cytokeratin 7 and 8 as well as IDO (indoleamine-2,3-dioxygenase) in differentiated trophoblast cells. Further characterization of trophoblast subtype formation and functional tests will be presented during the poster session.

Stromberg et al. Isolation of function human trophoblast cells and their partial characterization in primary cell culture. In Vitro 1978, 14, 631-8.

Bernardo et al. BRACHYURY and CDX2 mediate BMP-induced differentiation of human and mouse pluripotent stem cells into embryonic and extraembryonic lineages. Cell Stem Cell 2011, 9, 144-55.

Abstract No. P052

Endodermal Progenitor cells derived from integration-free iPSCs as an in vitro model for dissecting endodermal cell fate decisions

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Human embryonic stem cells (hESCs) have two fundamental characteristics. First is pluripotency, i.e. the ability to differentiate to all cell types of the three germ layers endoderm, ectoderm and mesoderm in vitro (formation of embryoid bodies) and in vivo (teratoma formation in immunodeficient mice). Second, hESCs have the capability to self-renew indefinitely. Embryonic stem cells express pluripotency associated markers such as OCT4, NANOG and SOX2 also the surface markers SSEA-4, TRA-l-60, TRA-1-81 and TRA-2-49 but not SSEA-1. Induced pluripotent stem cells (iPSCs) are embryonic-like cells and can be generated from somatic cells derived from individuals. We have generated episomal-derived and integration-free iPSCs (E-iPSCs) from human fetal foreskin fibroblast cells (HFF1). This viral-free method has the advantage over viral-based protocols because of the lack of integrations which otherwise leads to chromosomal re-arrangements of the host genome. Using the E-iPSC line we derived endodermal progenitor (EP). These LGR5 positive cells grow as organoids and express HNF4A, PDX1 and CDX2 which are key transcription factors specifying liver, pancreas and intestine. This expression pattern implies that these EPs may be multipotent. Further studies are planned involving the use of the E-iPSCs derived EPs to generate hepatocyte and pancreatic cells. These studies will enable uncovering the genes and associated pathways that specify a bi-potential EP to differentiate to either liver or pancreas. Additionally, these E-iPSCs and derived EPs provide unique resources for disease modeling, developmental studies, drug screening and toxicology studies

Abstract No. P053

Neuroblastoma cells convey CD24 surface expression to non-transformed cells

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*Presenting author

Neuroblastoma is the most frequent extracranial solid tumor in childhood with multifaceted and still poorly understood pathophysiology. Cell surface-mediated cross-talk of cells with their immediate neighbors is critical not only for stem cell development in tissue regeneration, but also in cancer progression. Using in vitro co-culture and flow cytometric cell tracking paradigms, we explored the reciprocal interactions of neuroblastoma cells with their non-transformed neural neighbors. We encountered a rapid and robust upregulation of CD24 (heat stable-antigen; small-cell lung carcinoma antigen) expression on human induced pluripotent stem cell (hiPSC)-derived neural stem cells after co-incubation with the human neuroblastoma SH-SY5Y cell line. A range of other surface antigens remained unaffected. Neuroblastoma-mediated CD24 upregulation was also detected in human embryonic stem cell (hESC)-derived neural crest, immortalized neural and fibroblast cell lines. This effect was observed in an identical manner with a second CD24-positive neuroblastoma line and appeared to be associated with neuroblastoma cells, specifically. These data suggest that modulation of surface molecule-expression patterns may contribute to the interaction of neuroblastoma cells with adjacent non-transformed neural (stem) cells.

Menon V, Thomas R, Ghale A, Reinhard C, Pruszak J. Flow cytometry protocols for surface and intracellular antigen analyses of neural cell types. J Vis Exp. 2014. Video Article.

Abstract No. P054

Conversion of primary and pluripotent stem cell-derived neuroepithelial stem cells into region-specific radial glia

Laura Ostermann [1,*], *Julia Ladewig* [1], *Franz-Josef Müller* [2], *Jignesh Tailor* [3], *Austin Smith* [3], *Philipp Koch* [1], and *Oliver Brüstle* [1]

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In recent years it has become possible to isolate and expand neural stem cells (NSCs) from different sources using growth factor-based protocols. A still open question is to what extent these diverse stem cell systems reflect physiological stem cell states observed in vivo. During nervous system development, early neuroepithelial stem (NES) cells with a highly polarized morphology and responsiveness to regionalizing morphogens give rise to radial glia (RG) cells, which generate region-specific neurons. Recently, stable neural cell populations reminiscent of NES cells have been obtained from pluripotent stem cells (lt-NES cells) and the fetal human hindbrain (hbNES cells). Here, we explore whether these cell populations, similar to their in vivo counterparts, can give rise to regionally specified RG-like cells. To that end we propagated lt-NES and hbNES cells temporarily in differentiating conditions. Upon re-initiation of growth factor treatment, these cells were found to enter a developmental stage reflecting major characteristics of RG cells. These RG-like NSCs could be expanded for at least 25 passages and expressed markers typically associated with RG cells, while NES cell markers were down-regulated. RG-like cells exhibited stable region-specific transcription factor expression with anterior, hindbrain- or spinal cord-derived RG-like cells maintaining their positional identity during multiple passages of in vitro proliferation and upon in vivo transplantation. Preservation of positional identity was robust and could not be overcome even by strong regionalizing factors such as retinoid acid. Along this line, RG-like cells generated region-specific neurons appropriate for their positional identity. Importantly, RG-like cells obtained from PSC-derived lt-NES cells and hbNES cells as well as primary human RG cells showed similar properties, suggesting that conversion of NES cells into RG-like cells recapitulates the developmental progression of early NES cells into radial glia cells observed in vivo.

Koch et al., PNAS 106(9):3225-30, 2009

Tailor et al., J Neurosci 33(30):12407-22, 2013

Abstract No. P055

The surface molecular biography of human neural differentiation

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*Presenting author

Stem cell in vitro differentiation systems can profit from enhanced insights into the microenvironmental cues governing cell proliferation and phenotype establishment. Growth factor signals, cell-matrix and cell-cell interactions converge onto surface molecule-mediated downstream pathways, the specifics of which remain poorly understood. Furthermore, characteristic combinations of surface molecules can serve to identify and isolate specific cellular subsets by fluorescence-activated cell sorting (FACS). Exploiting a range of complementary systems including human embryonic stem cell lines, long-term expandable neural lines derived from human induced pluripotent stem cells and neural cancer lines, our research program is aimed at a comprehensive and functional analysis of cluster of differentiation (CD) antigens expressed in human neural lineage differentiation. Multivariate analysis paired with unbiased clustering algorithm methods yielded novel CD marker panels to define and subsequently isolate and characterize distinct subpopulations present in human neural stem cell differentiation. Low- and high-throughput flow cytometric screens resulted in further refinement of neural cell surface marker signatures towards a lineage tree of human neuropoiesis. Functionally, cell tracking and co-incubation approaches served to elucidate the dynamics of surface molecule changes due to reciprocal interactions of individual cell types within the dish. Moreover, cell surface-mediated signalling pathways (Hippo/YAP) were found to play a role in regulating critical lineage decisions in human neural stem differentiation in a density-dependent manner. These data provide candidates for further study of cell-cell communication in neural stem cell development as well as novel surface antigen codes for enriching neural stem cells, neural crest and post-mitotic neuronal subpopulations for biomedical applications including neural transplantation and in vitro models of human disease.

Abstract No. P056

Functional Analysis of STAR-Family Proteins during Oligodendrogenesis in the rodent Forebrain

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Oligodendrocytes are essential for the myelination of axons in the central nervous system. During development oligodendrocyte precursor cells (OPCs) generate oligodendrocytes in a controlled and regulated process through the activity of diverse intrinsic and extrinsic factors. The members of the STAR-family proteins are among the intrinsic factors involved in oligodendrocyte differentiation. The STAR family (Signal transducer and Activator of RNA Metabolism) consists of the members Sam68 (Src associated during mitosis of 68 kDa), Slm-1 and Slm-2 (Sam-like mammalian protein-1, -2). Characteristic of these proteins is the structural RNA-binding domain GRP33/SAM68/GLD-1 (GSG), which is flanked by regulatory regions containing motifs for protein-protein interactions and residues that are posttranslationally modified to control the activity of the STAR family proteins (Sette 2010). Due to this GSG domain, STAR family proteins are able to regulate alternative splicing events, RNA-transport and, additionally engage via the interaction with SH3-domain-containing proteins in signal transduction processes. Using the gene trap technology Sam68 was identified as a Tenascin C-regulated target gene in neural stem cells (Moritz et al., StemCells 2008). In further studies Sam68 and Slm-1 were as well identified as promoters of neural stem cell (NSC) differentiation. Both proteins promote the differentiation of NSCs into a neuronal fate but also enhance oligodendrocyte maturation. However, the mechanism(s) how Sam68, Slm-1 and Slm-2 control oligodendrocyte development are still unresolved.
Therefore, we currently assess several Sam68 constructs, which contain mutations in the RNA-binding site or the nuclear localisation signal to characterize the relevant domains of Sam68 that control oligodendrocyte development by transfection of OPCs. Furthermore migration assays will increase our understanding of the complex signalling pathways and function of the STAR-family proteins during oligodendrogenesis.

Abstract No. P057

Radiation effects on early embryonic development

Insa Schroeder [1,*], *Sabine Luft* [1], *Onetsine Arrizabalaga* [1], *Alexander Helm* [1], *Marco Durante* [1,2], and *Sylvia Ritter* [1]

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*Presenting author

The use of diagnostic and/or therapeutic applications based on ionizing radiation (IR) steadily increases. However, these procedures as well as the exposure to environmental radiation pose a threat to the early embryo possibly leading to prenatal death, growth retardation, organ malformation, mental retardation or childhood cancer. Thus, a thorough risk assessment of radiation effects is mandatory in situations of inevitable or unintended exposure of the conceptus in utero. This is exceptionally important in the novel hadron cancer therapies using charged particles. This densely IR deposits the energy along its track leading to a more clustered and complex damage compared to sparsely IR. Hence, we chose the human embryonic stem cell line WA09 (H9) as a model system for early embryonic development to investigate cell cycle progression delay, apoptosis induction, chromosome aberrations, changes in pluripotency, variations in the expression of key genes in the stem cell signaling pathways, and differentiation behavior after densely IR exposure. First in vitro experiments were performed irradiating cells with charged particles and X-rays. Karyotype analyses by means of the mFISH technique revealed an increased percentage of cells with numerical or structural aberrations after carbon irradiation. A pronounced G2 block and apoptosis were detected by flow cytometry depending on the radiation dose and quality. An increase in the differentiation marker SSEA-1 was associated particularly with charged particle irradiation while at the same time cells surviving IR showed an impaired capacity to differentiate into the endodermal lineage that gives rise to organs such as lung, liver and pancreas. We conclude, that hES cells predominantly respond to IR by apoptosis. However, surviving irradiated cells maintain their differentiation capacity albeit with lower efficiency than unexposed cells. Both phenomena possibly explain the retardations mentioned above and conceivable spontaneous abortions.

Abstract No. P058

Neural Differentiation of Embryonic Stem Cells (ESCs): Regulation by Notch1 via Chromatin Protein High Mobility Group A2 (HMGA2)

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Notch signaling is a crucial component of the early development involved in cell-lineage decisions, differentiation, self-renewal and apoptosis. Recently, we have shown that Notch1-mediated upregulation of the direct target gene Sox9 is essential for chondrogenesis (1) and neurogenesis (2). In the latter case, Sox9 functions as mediator of the Notch1 signaling critically involved in cell-lineage decisions between glia and neuronal cells, and in stem cell maintenance. In a screen for factors regulated by Notch1 during early differentiation, we identified a member of the High-Mobility-Group (HMG) A protein, the HMGA2. The HMGA proteins –highly abundant in undifferentiated cells– are non-histone chromatin factors involved in various gene regulatory processes. Recently, HMGA2 was shown to be involved also in neurogenesis and generation of neural stem cells (NSCs). Here, we investigated the role of HMGA2 in the regulation of neural differentiation upon Notch1 activation. ESCs carrying an inducible form of Notch1 were differentiated via the neuroectodermal lineage. Though not being a direct target of Notch1, HMGA2 was strongly upregulated by Notch1 activation (Sox9-independently), peaking around day 4. Simultaneous knock-down of HMGA2 using siRNA reduced the Notch1-induced Hmga2 peak within the first 4-5 days of differentiation, which resulted in a reduction of certain marker genes like Notch2 and Nestin, and an increase of Hes1 and Id3. However, after knock-down for about 5 days, the concentration of Hmga2 was even increased, probably caused by a negative feedback loop in the regulatory network compensating the down-regulation of HMGA2. FACS analysis at day 8 revealed that this increase led to a boosted generation of strongly Nestin-positive cells indicating an increased number of NSCs. Thus, the positive effect of Notch1 on NSC generation is most likely partially mediated by the upregulation of HMGA2, describing a novel regulatory mechanism of neural differentiation on chromatin protein level by Notch signaling.

(1) Martini S, Bernoth K, Main H, Camargo Ortega CD, Lendahl U, Just U, Schwanbeck R (2013) A critical role for Sox9 in Notch-induced astrogliogenesis and stem cell maintenance. Stem Cells, 31:741–751.

(2) Haller R, Schwanbeck R, Martini S, Bernoth K, Kramer J, Just U, Rohwedel J. (2012) Notch1 signaling regulates chondrogenic lineage determination through Sox9 activation. Cell Death Differ 19: 461–469.

Abstract No. P059

Interactions between the meninges and the cortical neuroepithelium during mouse embryonic development

Richard Sturm [1,*], and *Alexander von Holst* [1]

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The mammalian cortex integrates signals from the environment, processes them and transfers these signals to different parts of the brain that subsequently mediate an adequate behavioral outcome. Remarkably, the elaborate cortex originates from a single neural stem cell layer. Initially, these cortical neural stem cells (cNSCs) are called neuroepithelial cells (NECs) and undergo proliferative, self-renewing divisions that lead to a lateral expansion of the developing cortex. Around E12 in mice the NECs give rise to a new neural stem cell type, the radial glia cell (RG) that mainly undergoes asymmetric, neurogenic divisions. After another five days, neurogenesis ceases and the RGs give birth to astrocytes and later on oligodendrocytes. The meninges emerge shortly before neurogenesis starts. The meninges surrounding the developing cortex secrete retinoic acid (RA) towards the cortical ventricular zone. This triggers a switch from proliferative to neurogenic divisions of cNSCs. Therefore, the meninges represent an important signaling source that influences cNSC behavior. By using an in vitro co-culture assay, where meningeal cells and cNSCs are co-cultivated in a transwell system, we could show that meningeal secreted factors transiently increase neurogenesis and astrocytogenesis, while they decrease the responsiveness of cNSCs to FGF2. In a proteome array we identified some of the molecules that are exclusively present in the meningeal-cNSC co-culture supernatant, among them IGFBP-2 and Endostatin. IGFBP-2 enhances astrogliogenesis independent of IGF signaling, and decreases the responsiveness of cNSCs to FGF2 when either IGF1 or IGF2 are present in the medium. Endostatin reduces the proliferation of cNSCs For the first time, this work provides an overview of the meningeal secretome and characterizes the influence of some of the identified factors on cNSC behavior.

Abstract No. P060

This abstract was retracted by the author after the conference

Tungala Suresh [1]

[1]MDC Berlin

The author apologizes for any inconvenience this process may have caused.

Abstract No. P061

Characterizing the role of Polycomb repressive complex 1 members in human neural differentiation

Ria Thomas [1,*], *Tsu-Yi Su* [1], *Christopher J. Hindley* [1], *Jason A. Davis* [1], and *Jan Pruszak* [1]

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Understanding the mechanism controlling pluripotency, multipotency and phenotype establishment during development is vital for the translation of human stem cell biology to biomedical and clinical scenario. Polycomb group (PcG) proteins are epigenetic modifiers capable of silencing gene expression by means of histone trimethylation and monoubiquitination. The polycomb repressive complex 1 (PRC1) members chromobox homologs – 2 (CBX2), CBX4 and CBX7 have been shown to be critical for controlling pluripotency and for regulating lineage-specific patterns of gene expression in development as well as in cancer. Their role in human neural differentiation remains poorly understood. Using embryonic and induced pluripotent stem cell-derived neural cells as well as human cancer cell lines, we generated a comprehensive profile of PRC1 members in four independent systems of human neural differentiation at the mRNA (qRT-PCR) and protein level (western blot, immunocytochemistry, flow cytometry). Further, candidates of interest were overexpressed and their effect on cell fate was analyzed at the surface marker expression and transcript level by flow cytometry and qRT-PCR, respectively. We found that CBX7 shows a maintained expression in cells of human neural lineage development including neural stem cells and post mitotic neurons, contrasting its key role in maintenance of pluripotency in murine embryonic stem cells and thus warranting further analysis into its context-specific function and regulation.

P062 – P072: Somatic stem cells

(posters will be displayed in the tent)

Abstract No. P062

The progeny of single adult neural Stem cells from the SEZ reveals insights into the mechanisms maintaining adult neurogenesis

Filippo Calzolari [1,2,*] , *Julia Schwausch* [1], *Emily Violette Baumgart* [1], *Fabian Theis* [1,3], *Magdalena Götz* [1,2], and *Jovica Ninkovic* [1,2,3]

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Adult neural stem cells (NSCs) in the mammalian subependymal zone (SEZ) are responsible for life-long, yet declining, generation of olfactory bulb (OB) neurons. Long-term self-renewal of individual NSC is generally assumed, but neither has it been shown nor have the mechanisms of neurogenesis decline at later stages been addressed at the clonal level. To address this issue we clonally traced the progeny of single adult NSCs of the SEZ under physiological conditions at 2 and 12 months of age, via genetic fate mapping using the "confetti" multicolor reporter and an inducible Cre expressed in adult NSCs (GLAST::CreERT2).

Our clonal data show that some NSCs repeatedly undergo division, producing progeny in multiple waves, which mostly expand at the transient-amplifying progenitor stage. Further, our data show that despite being able to divide surprisingly often, NSCs only rarely symmetrically self-renew, while their exhaustion seems to be a common occurrence at both young and old ages. Finally, clonally traced NSCs active in old mice seem to behave similarly to those active in young ones, thus suggesting that age-dependent decline in output from the SEZ may result from reduced numbers of active NSCs and/or impaired lineage progression steps downstream of NSC division.Thus the first observation of the progeny of single SEZ NSCs not only revises previous concepts on long-term self-renewal of NSCs but also provides insights into the mechanisms underlying age-related changes in adult neurogenesis.

Abstract No. P063

Culture bag systems allowing animal-serum free 3D-cultivation of adult human neural crest-derived stem cells for clinical applications

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Cellular therapies hold great promise for treatment of neurodegenerative diseases as well as complex craniofacial injuries such as those common after cancer therapy. In this regard, adult human neural crest-derived stem cells (NCSCs), which exhibit a broad differentiation capability, offer the possibility of autologous transplantation, avoiding the need of long-term immunosuppression in patients. Considering the severe contamination risks during in vitro expansion steps prior to transplantation, we established a cost-reducing and animal-serum free cultivation system for adult human NCSCs within cGMP-grade Afc-FEP bags using a human blood plasma (BP)-matrix.

After investigating the biocompatibility of the human BP-based fibrin matrix, we cultivated inferior turbinate neural crest-derived stem cells (ITSCs) within the BP-matrix. Here, ITSCs proliferated significantly faster compared to sphere culture conditions, while maintaining their stemness characteristics. Cultivated within cGMP-grade Afc-FEP bags, ITSCs grew three-dimensionally in the BP-matrix and exhibited no significant changes in morphology, proliferation rate and vitality compared to cells conventionally cultured within the BP-matrix. Bag-cultured ITSCs further showed unchanged expression profile, telomerase activity and genetic stability. We also observed unaffected differentiation of ITSCs after bag-culture into ectodermal and mesodermal cell types, including neuronal, osteogenic and adipogenic cells. In conclusion, the here described culture bag system using human blood plasma is suitable for NCSC-cultivation under clinical grade conditions and has a great potential for future medical applications.

Abstract No. P064

Characterization and comparison of human and ovine mesenchymal stem cells from three different niches

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Introduction:
Large bone defects are unlikely to regenerate due to the significant quantity of bone required. While the use of autologous bone graft is the gold standard in reconstructing of large bone defects, the surgical stress and the low quantity of extracted bone are limiting factors. MSCs cell-therapies have the potential to provide an effective alternative for bone regeneration due to their osteogenic potential. Sheeps are currently used as animal models in orthopedics and infection research. However, up to now, human rather than ovine MSC have been transplanted into sheep. Therefore, the current study aimed to compare human and ovine MSCs from three different niches, for use of oMSC in an orthopedics sheep model.
Methods:
Sources for oMSCs: adipose tissue, bone and fat marrow; hMSCs: adipose tissue, femur head, vertebral bodies. Characterization: surface-marker expression, differentiation potential and immunomodulatory capacity.
Results Both h- and oMSCs differentiated into adipo-chondro-osteogenic lineages. Both possessed osteogenic differentiation potential (Alizarin-Red-S/ALP). Both expressed surface markers: CD73, CD90, CD105. Both showed immunomodulatory capacity by suppressing lymphocytes proliferation.
Discussion:
Somatic stem cells are easy to harvest and represent no ethical concern and do not tend to form tumors. Here, we report for the first time a successful isolation, characterization and comparison of oMSCs and hMSCs from three different niches.
Taken together, MSCs based therapy in combination with extracellular matrix scaffolds and specific growth factors are therefore an attractive source for developing new solution in bone and tissues regeneration. MSCs from the large animal model offer a possibility for implants incorporation and interaction in bone regeneration both in vitro and in vivo studies. Moreover MSCs could provide a useful tool to investigate their contribution in the pathogenesis of different diseases like osteoporosis. Understanding MSCs biology during development will help to engineer complex tissues for regenerative medicine applications.

Abstract No. P065

Reconstruction of the cardiac stem cell niche for in vitro maintenance of cardiac stem cells

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We developed a strategy to obtain stable clonally derived stem cell lines from murine hearts by co-culture of triturated heart cells with embryonic stem cells and mitotically inactivated SNL76/7 fibroblasts secreting leukemia inhibitory factor (LIF). This mixture of cells and secreted factors creates an environment which mimics a cardiac stem cell niche allowing firstly, the embryonic stem cell-assisted survival during the initial phase of isolation, and secondly, the LIF-dependent continuous proliferation of cardiac stems cells in vitro. These cardiovascular progenitor cell lines remain diploid and phenotypically stable over more than 149 passages. While self-renewing these cells express the stemness genes Oct4, Sox2, Nanog, and Tert1, and at the same time, significant levels of the mesodermal and early myocardial transcription factor genes, Brachyury, Mesp1, Nkx2.5, Isl1, and Mef2C. In the absence of LIF stemness and mesodermal genes are down regulated and the terminally committed cells exclusively differentiate to vascular endothelial cells, smooth muscle cells, and cardiomyocytes. Differentiation to cardiomyocytes is enhanced in the presence of Bmp2, and LIF attenuated Bmp2-dependent cardiomyogenesis in a dominant negative manner. Finally inhibition of SPARC (secreted protein acidic and rich in cysteine), which is secreted by the cardiovascular progenitor cells, significantly inhibits in vitro cardiomyogenesis. When SPARC is added at the beginning of cardiomyogenesis, it induces the temporal upregulation of Brachyury and Nkx2.5.
These results demonstrate the feasibility of the isolation and indefinite in vitro maintenance of murine cardiovascular progenitor cells by a reconstructed cardiac stem cell niche, suggest SPARC as a new player in the regulation of cardiomyogenesis, and introduce a new model to study molecular and cellular aspects of early cardiomyogenesis and myocardial regeneration in cardiovascular progenitor cells.

Hoebaus et al. (2013). Cells Tissues and Organs. 197, 249-68.

Moumita Koley et al. (2013) Med. Chem. Commun., 2013, 4, 1189–1195.

Please find abstract P066 and P067 under 'Selected presentations' T14/P066 and T16/P067

Abstract No. P068

The effect of Wistar rats prenatal and postnatal cerebrospinal fluid on neural differentiation and proliferation of stromal mesenchymal stem cells derived from bone marrow

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Introduction: Fetal cerebrospinal fluid (CSF) contains many neurotrophic and growth factors. The CSF can modulate proliferation and differentiation of neural stem cells. Mesenchymal stem cells (MSCs) are pluripotential stem cells that not only can differentiate into several types of mesenchymal cells, but also can differentiate into non-mesenchymal cells, such as neural cells. In the present study, the effect of fetal CSF on proliferation and neural differentiation of bone marrow mesancymal stem cells (BMSCs) was investigated. Materials & Methods: The CSF was aspirated from the cisterna magna of Wistar rat fetuses from E17 and P1. CSF samples were centrifuged at 4,000 rpm at 4 °C for 10 min.The supernatant was stored at -40 °C until usage.Bone marrow samples were collected from adult NMRI mice(6–8 weeks)femur and tibia. BMSC were cultured in DMEM,15% FBS and appropriate penicillin and streptomycin. All cellswere incubated at 37 °Cwith 5% CO2. Following the second passage, cells were transferred into 24-well plates. After attachment, cells were exposed to CSF (E20 and P1) with concentrations of zero, 3, 7and10 % (v/v).Cell viability and proliferation were measured byMTT assay. Cell morphology of BMSCs was examined by changes in the neurite outgrowths after one week. Results: Inverse microscopic examination of BMSC revealed neuron like cells in cell cultures treated with prenatal and postnatal (E20 and P1) CSF compare to control group which could concluded as a differentiation effects of e-CSF.MTT assay showed proliferation of BMSCs. Viability and cell proliferation of BMSCs cultured in CSF-supplemented medium with 10% concentration from E20 and P1 CSF were significantly elevated relative to the control group. Conclusion :CSF neurotrophic factors can support proliferation and neuronal differentiation of BMSCs.. Thus we conclude here that regulation of CSF-born growth factor may be involved in some neurodegenerativedisease

Keywords: Prenatal and Postnatal CSF , BMSCs, Neural Differentiation, Proliferation

Abstract No. P069

Expression pattern of neural cell adhesion molecule in mesenchymal stromal cell proliferation and differentiation

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Mesenchymal stromal cells (MSCs) are attractive candidates for cellular therapy and tissue engineering. Detailed molecular characterization of human MSCs is important in the context of cell therapy to guarantee the stability of transplanted cells and patient safety. Neural cell adhesion molecule (NCAM, CD56) is a transmembrane glycoprotein modulating cell-cell and cell-matrix interactions. It is highly expressed in the developing nervous system, but occurs also in other tissues; especially in tissues of mesodermal origin. NCAM is often looked on as a marker of neural lineage commitment and, thus, NCAM expression on multipotent cells has been considered undesirable. On the other hand, several studies have shown that lack of NCAM expression reduces the differentiation potential of MSCs in animal models. Human bone marrow-derived MSCs are considered to be devoid of NCAM expression, but more rigorous characterization of the presence of this molecule is needed. We have observed that human bone marrow-derived MSCs may sometimes express NCAM. However, there is little - if any - polysialic acid, a frequent modification of NCAM, expressed on these cells. It is possible that NCAM expressing MSCs are capable of multilineage differentiation, and if so, NCAM might not be a direct sign of neural lineage commitment. Our study extensively utilizes mRNA detection, flow cytometry analyses, and immunofluorescence staining to determine the cell surface characteristics of human bone marrow-derived MSCs.

Abstract No. P070

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 to differentiate is tightly controlled by epigenetic modifications. We aim at understanding the crucial role of epigenetic modification in cell fate determination of satellite cells. We have established a method to isolate a large and pure population of muscle stem cells using a transgenic Pax7 reporter mouse line combined with FACS sorting. These cells can be efficiently transduced with recombinant lentiviruses. We performed a multi-parametric high-throughput, lentiviral RNAi screen to identify chromatin modifiers that regulate muscle stem cell self-renewal and differentiation. We identified novel 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 might determine skeletal muscle stem cell fate. We are in particular interested in understanding the role of the nucleosome remodeling and histone deacetylase (NuRD) complex as a key determinant of differentiation of adult muscle stem cells. In a more general context, the studies described here will shed fundamental insights into the mechanisms of skeletal muscle development and cell fate specification.

Abstract No. P071

Signaling via Toll-like Receptors in Neural Stem Cells and Astroglioma Stem Cells

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In Drosophila melanogaster, Toll proteins are transmembrane receptors regulating the establishment of dorso-ventral polarity and are further involved in recognizing the invasion of pathogens. them to key players in innate immunity. In mammalia, Toll-like receptors (TLRs) are involved in nonspecific immune responses via recognition of Gram-positive and Gram-negative bacteria, DNA and RNA viruses, fungi and protozoa. In addition to such pathogen-associated molecular patterns (PAMPs), TLRs can also be activated by endogenous ligands, the so called danger-/damage-associated molecular patterns (DAMPs). At the molecular level, the engagement of TLRs via DAMPs or PAMPs can activate the transcription factors NF-kappaB as well as the interferon regulatory transcription factor 3 (IRF3), depending on the ligand and the recruited adaptor proteins. Remarkably, NF-kappaB is known to induce proliferation and differentiation of neural stem cells (NSCs) and glioma stem cells (GSCs), whereas IRF3 exhibit anti-proliferative properties. Here, we investigated the signaling pathways activated by TLR4- and TLR3 stimulation in NSCs and U251 and U373 cell lines cultivated under NSC-conditions. We show that crucial members of TLR3 and TLR4-signaling complex are expressed in NSCs and GSCs and that their activation leads to both IRF3- and NF-kappaB-mediated signaling. We present evidences that all ligands tested in NSCs and GSCs showed respective but specific dynamic and kinetic patterns of activation for both transcription factors. We further demonstrate that this ligand-specific dynamic and kinetic balance between IRF3 and NF-kappaB activation has tremendous impact on proliferation, differentiation and migration of NSCs and GSCs.

Abstract No. P072

Mathematical Modeling of Neural Stem Cell Dynamics in the Adult Hippocampus

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The dentate gyrus of the hippocampus harbours a niche of stem cells, capable of generating new neurons throughout adulthood. Although multiple studies have been conducted in the past to identify qualitative stem cell features such as multipotency or the age-related decline of the stem cell pool, a quantitative understanding of the dynamics of adult neurogenesis is still missing. This lack of quantification is mainly due to sparse data and diverse labelling approaches used by different studies in order to observe neural stem cells. Accordingly, different hypotheses about their dynamics have been formulated.

The two landmark studies on neural stem cell dynamcis were conducted by Bonaguidi et al. (2011) and Encinas et al. (2011). While the former performed clonal analysis and concluded that stem cells can get activated multiple times from their quiescence in order to produce offspring, Encinas carried out population level analysis and reasoned that neural stem cells get activated only once, enter a series of asymmetric divisions and vanish by differentiating into astrocytes.

In this study, we investigate both hypotheses by formulating them as mathematical models of ordinary differential equations. Moreover, we perform a quantification of stem cell dynamics by estimating model parameters from the data published in both studies and our own data. We find that the Bonaguidi model explains a wider range of data sets than the one of Encinas, provided that it subscribes to Encinas' theory of stem cell depletion. Additionally, we make experimentally testable predictions in order to differentiate between the two models.

Our study shows that mathematical modelling contributes to a deeper understanding of adult neural stem cells by providing a unified framework to test multiple hypotheses about cellular dynamics. Moreover, the quantification of the presented study can be used by the stem cell community in order to re-evaluate already performed experiments.

Encinas, J. M. & Enikolopov, G. et al. 2011 Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. Cell Stem Cell, 8, 566-579.

Bonaguidi, M. A., Wheeler, M. A., Shapiro, J. S., Stadel, R. P., Sun, G. J., li Ming, G. & Song, H. 2011 In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. Cell, 145, 1142-1155.

P073 – P097: Hematopoietic stem cells

(posters will be displayed in front of room K 1+2)

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P093 c-Myc expression in hematopoietic stem and progenitor cells is driven by a novel enhancer region 1.7Mb downstream of the coding region
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P097 Understanding the marrow niche: advanced 3D-model system allows functional analysis of the interaction with human hematopoietic progenitor cells
Patrick Wuchter

Abstract No. P073

Sfrp2 regulates homeostasis of hematopoietic stem cells under stress conditions

Franziska Bock [1,*] , *Christina Schreck* [1], *Rouzanna Istvanffy* [1], *Sandra Grziwok* [1], *Christian Peschel* [1], and *Robert Oostendorp* [1]

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In previous studies of stromal cells which maintain hematopoietic stem cells (HSCs) in culture, we found strong expression of Sfrp1 and Sfrp2. We already described that Sfrp1 is involved in the regulation of HSC self-renewal in vivo. Here, we explore the role of Sfrp2. Sfrp1 and 2 are supposedly redundant molecules which act as inhibitors of canonical Wnt signaling. In studying Sfrp2-/- mice we did not find major changes in function or distribution of early mesenchymal (OBC, MSC) or of hematopoietic populations. The exception being changes in CD34- CD150+ LSK cells (which contain the bulk of HSC) with increased myeloid progenitor potential and more BrdU incorporation. This result suggests that without Sfrp2 (an in the presence of BrdU) a subtle activation of HSCs might occur. Since activation is important for regenerative responses during stress, we studied situation of hematopoietic stress. Indeed, we found in 5-FU treated mice and in 12-16 month-old mice a strongly increased population of CD34- CD150+ LSK cells. To find out whether these increases were caused by alterations in niche function, we transplanted wild-type HSC into Sfrp2-/- mice. These experiments showed no changes in engraftment of 1° recipients, but an increase in donor LSK cells in the marrow. Engraftment of sorted donor LSKs in 2° and 3° showed a progressive decrease in LSK engraftment, indicative of a slow exhaustion of HSC activity after initial activation in 1° recipients. HSC activation in 1° recipients was exemplified by increased Cyclin D1 expression and canonical Wnt signaling and activation of Nfatc1 and Cebpa. Thus, our results show that niche Sfrp2 is required to limit HSC activation during hematopoietic stress.

Abstract No. P074

Gene transfer of cytidine deaminase (CDD) and multidrug resistance gene 1 (MDR1) protects murine hematopoietic cells in vitro from anti-cancer therapy

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Genetic modification of hematopoietic cells with chemotherapy resistance genes (CTX-R), referred to as myeloprotective gene therapy, can reduce the myelotoxicity of anti-cancer chemotherapeutic agents. Given that anti-cancer therapy generally is applied as a combination of different agents, simultaneous delivery of multiple CTX-R genes represents a logical strategy. To proof this concept in the context of antileukemic therapy we have evaluated the combined expression of cytidine deaminase (CDD) and multidrug resistance gene 1 (MDR1) in hematopoietic cells by lentiviral gene transfer to obtain protection against cytosine arabinoside (Ara-C) as well as daunorubicin (DNR), the two drugs most relevant for the treatment of acute leukemias. Lentiviral vectors expressing (i) human CDD-cDNA (SFFV.CDD), (ii) human codon-optimized MDR1-cDNA (SFFV.MDR1), or (iii) both CTX-R genes in combination (SFFV.CDD.2A.MDR1) were utilized. Initially, murine 32D cells were transduced and subsequently treated with Ara-C and/or DNR. SFFV.MDR1 transduced cells were completely protected from 125 nM DNR (non-transduced cells died at 25-50 nM) while susceptible to Ara-C, whereas SFFV.CDD gene-modified cells were protected from Ara-C (≥2000 nM; non-transduced cells affected from 500 nM onwards) but lacked DNR resistance. Importantly, cells co-transduced with SFFV.MDR1 and SFFV.CDD were protected against combined Ara-C/DNR treatment (≥2000/200 nM). This is similar in SFFV.CDD.2A.MDR1 gene-modified cells although Ara-C resistance was reduced 2-fold in comparison to co-transduced cells. Studies with primary lin- cells revealed protection of SFFV.CDD transduced clonogenic cells up to 600 nM Ara-C with control cells susceptible from 100 nM onwards and SFFV.MDR1 transduced lin- cells demonstrated successful colony formation up to 60 nM DNR (control affected from 30 nM onwards). More important, SFFV.CDD.2A.MDR1 transduced lin- cells were protected from combined Ara-C/DNR (100/50nM) treatment. Taken together, these data clearly demonstrate protection of murine hematopoietic cells against Ara-C and DNR by transgenic expression of CDD and MDR1, an observation potentially relevant for antileukemic therapy.

Abstract No. P075

Transcriptome-wide profiling and post-transcriptional analysis of hematopoietic stem/progenitor cell differentiation toward myeloid commitment

Nina Cabezas-Wallscheid [1,*], *Daniel Klimmeck* [1], *Alejandro Reyes* [2], *Lisa von Paleske* [1], *Simon Renders* [1], *Jenny Hansson* [2], *Jeroen Krijgsveld* [2], *Wolfgang Huber* [2], and *Andreas Trumpp* [1]

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Hematopoietic stem cells possess life-long self-renewal activity and generate multipotent progenitors that differentiate into lineage-committed and subsequently mature cells. We present a transcriptome analysis of ex vivo isolated mouse multipotent hematopoietic stem/progenitor cells (LinnegSca-1+c-Kit+) and myeloid committed precursors (LinnegSca-1negc-Kit+). Our data display dynamic transcriptional networks and identify a stem/progenitor gene expression pattern, which is characterized by cell adhesion and immune response components including kallikrein-related proteases. We identify 498 expressed lncRNAs, which are potential regulators of multipotency or lineage commitment. By integrating these transcriptome data with our recently reported proteome data, we found evidence for post-transcriptional regulation of processes including metabolism and response to oxidative stress. Finally, our study identifies a high number of genes with transcript isoform regulation upon lineage commitment. This in-depth molecular analysis outlines the enormous complexity of expressed coding and non-coding RNAs and posttranscriptional regulation during the early differentiation steps of HSCs toward the myeloid lineage.

Abstract No. P076

Transient modulation of gene expression in human hematopoietic stem and progenitor cells using novel RNA molecules

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Modulation of gene expression in hematopoietic stem and progenitor cells (HSPCs) is a useful tool for understanding HSPC biology or to expand these cells in vitro for therapeutic approaches. Most of the successful studies in this field utilize stable gene modification such as viral vectors that are still controversial in clinical settings. Therefore, our study aims at the identification of new ways to transiently modify HSPC gene expression at the post-transcriptional level by using novel and established RNA-based molecules. First, we tested different methods to deliver these molecules into stem cells. The delivery of siRNAs targeting surface markers with standard transfection methods such as lipofection or cationic polymers did not lead to a knockdown at the protein level although we observed more than 90% transfection efficiency of a fluorescently labeled control siRNA. This indicates an efficient internalization of the siRNA but insufficient release from the endosomal compartment. Accordingly, electroporation resulted in an efficient, siRNA-mediated protein knockdown. For transient overexpression of target proteins we used novel mRNA molecules with modified 5´- and 3´-UTRs and human codon optimized sequences. Electroporation of mRNA encoding GFP resulted in fast, efficient and persistent protein expression for up to seven days. The use of mRNAs encoding functional, HSPC-relevant proteins such as transcription factors and anti-apoptotic proteins showed also fast and efficient although shorter overexpression of the respective protein. Taken together, our data offer new opportunities for DNA-free gene modulation in HSPCs.

Abstract No. P077

Increased Cdc42 activity levels determine alterations in 3-dimensional epigenetic mark distribution in hematopoietic stem cells upon aging

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Hematopoietic stem cell (HSC) aging is characterized by a loss of polar distribution (epigenetic polarity) and a decrease in the level of acetylation on histone 4 on lysine 16 (AcH4K16). Treatment with CASIN, a Cdc42-activity inhibitor that can functionally rejuvenate aged HSCs, re-establishes epigenetic polarity and increases level of AcH4K16. Based on these observations, we hypothesize that alterations in the distribution and/or level of specific epigenetic marks (AcH4K16 first but also AcH4K12, K8, K5, AcH3K27, 1meH3K4, 3meH3K4, 3meH3K27, 3meH3K9, 3meH3K36) are linked to Cdc42 activity level and influence the 3D-epigenetic organization in HSCs upon chronological aging. Therefore we investigated the distribution of several histone marks (AcH4K16 first but also AcH4K12, K8, K5, AcH3K27, 1meH3K4, 3meH3K4, 3meH3K27, 3meH3K9, 3meH3K36) relative to the HSC nuclear architecture. Particularly, we assay whether, in addition to AcH4K16, other epigenetic marks are also altered in distribution and/or level upon HSC aging and if CASIN treatment impacts on their level and/or distribution. Finally we measure if in vitro treatment of purified HSCs with butyrate, a known class I and II histone deacetylase inhibitor, can improve function of aged HSCs by increasing levels of AcH4K16. Results demonstrate that aged HSCs show apolar distribution and decreased level of AcH4K12, similarly to AcH4K16. Distribution of 2meH3K9 is also altered upon aging of HSCs. In vitro treatment of aged HSCs with butyrate increases levels of overall acetylation on histone 4 but does not affect epigenetic polarity of AcH4K12 and of AcH4K16 and, most importantly, function of aged HSCs. This implies that AcH4K12 and AcH4K16 distribution is marking some part of euchromatin that is either different or differentially distributed upon HSC aging and that is specifically targeted by the rejuvenation induced by CASIN treatment.

Abstract No. P078

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. Homozygous *Fubp1* gene trap embryos, which lack FUBP1 activity, die *in utero* at day E15.5 and exhibit an anemic phenotype. The total fetal liver cell count and the number of long-term repopulating HSCs (LT-HSCs) are severely reduced in the homozygous embryos. Competitive transplantation experiments with E15.5-derived fetal liver cells or lentivirally transduced adult LT-HSCs revealed a significantly lower engraftment in primary transplants and no engraftment in secondary transplants of FUBP1-deficient cells. These data confirm that FUBP1 is a central player in the regulation of fetal and adult HSC self-renewal. To find out how FUBP1 controls the self-renewal capacity of LT-HSCs, we investigated the transcriptional network regulated by FUBP1 with *Affimetrix* gene profiling arrays and quantitative real-time PCR. Previous data already identified FUBP1 as a regulator of the proto-oncogene *c-myc* and the tumor suppressor gene *p21* in other cell types[1,2]. First results confirm these data in adult LT-HSCs and imply that FUBP1 additionally, amongst others, represses the expression of the pro-apoptotic gene *Noxa.* In accordance with the gene expression analyses, single cell tracking of adult *Fubp1* knockdown LT-HSCs demonstrated significantly prolonged generation times and increased apoptosis rates. *Ex vivo* culturing of these cells confirmed a drastic expansion defect as a consequence of FUBP1 inactivation. We are also interested in the upstream signaling of FUBP1 in LT-HSCs and try to identify HSC-relevant transcription factors (TFs) involved in the transcriptional regulation of the *Fubp1* gene. ChIP experiments using murine bone marrow mononuclear cells already demonstrated the binding of TAL1/SCL within the *Fubp1* promoter region. The ongoing studies will further our understanding of how FUBP1 is implicated in the regulatory network that controls the development and maintenance of the hematopoietic system.

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Abstract No. P079

Hematopoietic Lineage Specification and Asymmetric Cell Division

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Hematopoietic stem cells (HSCs) contain lifelong potentials to self-renew and to create progenitors of all mature blood cells. Apparently HSC-niches as well as asymmetric cell divisions control HSC homeostasis. In our previous studies we have linked the process of asymmetric cell division of human hematopoietic stem and progenitor cells (HSPCs) with the expression kinetics of the stem cell surrogate antigen Prominin-1/CD133 (1). More recently, we characterised subpopulations of human HPSCs by their CD133 surface expression and gained evidence that CD133(+) multipotent progenitors (MPPs) create CD133(+) lympho-myeloid daughter cells containing neutrophil potentials and CD133(low) erythro-myeloid daughter cells containing basophil and eosinophil potentials (2). By using the novel HC7 monoclonal anti-CD133 antibody we observed asymmetric HC7 epitope distribution on a proportion of fixed HSPCs which all showed symmetric distribution of AC133 epitopes (epitopes of the classical anti-CD133 antibody). Moreover we are able to track asymmetric cell divisions with labelled anti-HC7 antiobodies in living cells. To test whether binding of the HC7 antibody affects biological features of living CD133(+) HSPCs, we performed different functional assays and neither detected any effect of the HC7 binding on cellular polarization, cell migration, adhesion nor on in vitro/in vivo differentiation processes. Upon studying the cell division pattern in different CD133(+)CD34(+) progenitors, i.e. in MPPs, LMPPs and GMPs, we now show for the first time that with respect to the distribution of the HC7 epitopes most MPPs divide asymmetrically while most MPPs and GMPs divide symmetrically. In our ongoing study, we perform paired daughter cell experiments and analyse the functional outcome of asymmetric versus symmetric MPP divisions at the single cell level. Altogether, our data qualify the new HC7 antibody as a useful tool to study early HSPC fate decision processes. Furthermore we learned that under conventional culture conditions asymmetric cell divisions rather are lineage instructive than self-renewing.

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Abstract No. P080

Bone marrow failure and aging of hematopoietic stem cells — a model suggestion

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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 etiology and the molecular mechanism of FA as well as the association with aging of the hematopoietic system. We advocate the view that accompanying mathematical models foster the systematic understanding of disease and aging mechanisms and will be helpful tools to assist in experimental design. We developed various strategies to mathematically model progressive bone marrow failure on a cellular level. In particular, we use a single-cell 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 increased proliferation as a potential process that 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 mathematical models. Based on these findings, we are able to simulate different scenarios of activated HSC populations. Our models give suggestions how the progression of FA is systemically linked to the normal aging process in HSCs.
We demonstrate the general ability of our mathematical 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. P081

3D-Genomic Organization in HSC Ageing

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Interphase chromosomes are non-randomly positioned inside eukaryotic nuclei, occupying distinct territories. Many studies have shown that chromosome positioning depends on gene density and size of chromosomes. Gene-rich chromosomes frequently occupy the interior region of the interphase nucleus and gene-poor chromosomes are located at the nuclear periphery. Similarly, transcriptionally active genes tend to be closer to the interior than do inactive genes. Both gene activity and higher-order chromatin structure are regulated by specific histone modifications, such as acetylation and methylation of specific lysine residues on N-terminal histone tails which are fundamental to the formation of eu- and heterochromatin. We previously showed that Cdc42 regulates the level of epigenetic modifications in long-term hematopoietic stem cells (LT-HSC) as well as changes in intranuclear polarity of H4K16a upon aging. This could imply that young and aged HSCs have different nuclear architecture. Hence our hypothesis is that the spatial organization of chromosomes is distinct in young HSCs and undergoes reorganization/redistribution upon aging. Novel nuclear fluorescent in situ hybridization (FISH) protocols for three-dimensionally preserved nuclei were established to be able to determine the genomic structure of HSCs, and fluorescence localization/colocalization and distances between chromosomes were measured by Volocity 3D Image Analysis Software. Our preliminary data suggest that distribution of chromosomes is also non-radom in HSCs. Based on this we are interested to investigate the localization of chromosomes within the nucleus of young and aged HSCs and co-localization with H4K16ac to further analyze whether there is a connection between chromosome distribution and HSCs function. Moreover we would like to see the role of Cdc42 in reorganization of genome upon aging, anticipating that information on changes of chromosome organization over time will be instrumental to investigate stem cell aging and aging-associated diseases.

Abstract No. P082

Inflammation-driven fast-track differentiation of HSCs into the megakaryocytic lineage

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The continuous production of mature blood and immune cells is regulated by a small number of multipotent hematopoietic stem cells (HSCs). Among these mature blood cells are megakaryocytes (Mks); large, polyploid cells releasing blood platelets into the circulation. According to the current paradigm of hematopoiesis, HSCs generate a series of progenitor cells that undergo several consecutive commitment steps until they produce Mk-lineage restricted progenitors. Even though expression of Mk transcripts in HSCs has been reported, the functional role of such “Mk lineage priming“ is not well understood. Here, we investigated the role of Mk lineage priming in HSCs during homeostasis and inflammation. Single-cell transcriptomics revealed that the phenotypic HSC compartment contains HSCs expressing Mk transcripts in a stochastic fashion as well as a smaller subpopulation of HSCs exhibiting a coordinated Mk transcript expression program, whereas the overall single-cell transcriptomes of these populations were indistinguishable. Single-cell ex vivo lineage tracking and in vivo transplantation assays demonstrated that the switch from a stochastic to a coordinated Mk transcript expression program is associated with commitment of multipotent HSC towards the Mk lineage. These newly identified “Mk-committed HSCs” show ex vivo and in vivo Mk potential that is superior to so far identified Mk progenitor populations and most likely derive directly from multipotent HSCs. Quantitative proteomics, ribosome profiling and flow cytometry analysis revealed that “Mk-committed HSCs” are maintained in a quiescent state, and suppress translation of Mk transcripts during homeostasis. In contrast, acute inflammatory signaling triggers an efficient translation of Mk transcripts accompanied by a fast maturation program. Thus, our data reveal a highly efficient mechanism of inflammation-driven Mk maturation from HSCs, mediating a rapid platelet recovery after inflammation-induced platelet loss.

Abstract No. P083

Comparison of different cytokine conditions reveals Resveratrol as a new molecule for ex vivo cultivation of CB-derived HSC

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Human cord blood (CB)-derived hematopoietic stem cells (HSC) are of great interest as a source of HSC transplantations or for gene therapeutic applications. However, as the number of CB-HSC from a single donation is low and gene therapy relies on the ex vivo modification of HSC, protocols for ex vivo cultures and expansion are of particular importance. Current protocols are based on the use of various cytokine combinations, including insulin-like growth factor binding protein-2 (IGFBP2) and Angiopoietin-like proteins or combinations with so called “small molecules” such as Stemregenin-1. The aim of our project was to compare different CB-HSC expansion strategies side-by-side in vitro and in a serial transplantation model into NOD/SCID/IL2rg-/- (NSG) immunodeficient mice. We further identified Resveratrol, a naturally occurring polyphenol, as a new alternative small molecule in the combination with cytokines to facilitate serum-free ex vivo expansion of human CB-HSC. Resveratrol led to higher levels of CD34+/CD133+ double positive cells after 9 days ex vivo culture (13%±2) compared to a commonly used standard cytokine condition including SCF, THPO, Flt3-L and Il-6 (Std; 8%±2) and amplified the total numbers of CD34+/CD133+ most efficient (3.5-fold). This was higher compared to Stemregenin-1 (13%±2 and 2.8-fold expansion of CD34+/CD133+ HSC) and the recently established serum-free medium including IGFBP2 and Angiopoietin-like 5 (STAI3, 8%±2 and 1.6-fold expansion). Serial transplantation studies further confirmed the action of Resveratrol and led to robust multilineage engraftment in primary and secondary NSG recipients. Herein, Resveratrol-cultivated cells engrafted in secondary recipients (defined as >0.5% human CD45 in bone marrow) in 44% of mice (4 out of 9), whereas only 22% of secondary mice receiving Std- and STAI3- cultivated cells (2/9) repopulated. Interestingly, secondary recipients receiving Stemregenin-1 cultivated cells displayed no chimerism (0/5). Our study established Resveratrol as a new molecule for improving CB-HSC ex vivo culture.

Abstract No. P084

CD105 is a surface marker for receptor-targeted gene transfer into human long-term repopulating hematopoietic stem cells

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Hematopoietic stem cells (HSCs) are an important target cell population for gene therapy since they can reconstitute the entire hematopoietic system. HSC-enriched cell populations can be recognized based on cell surface marker expression, such as CD34, which is broadly expressed on immature and partially differentiated cells. In mice, co-expression of CD34 and CD105 was previously shown to be relatively more specific for the most immature, long-term repopulating HSCs. Here, we evaluated if CD105, which is expressed on 30-80% of CD34+ cells, is a marker also for human long-term repopulating HSCs. Therefore, we tracked the mature progeny of CD34+ cells transduced with the CD105-targeted lentiviral vector CD105-LV in xenotolerant mice. Transduction was blocked with soluble CD105 protein confirming specificity. Importantly, CD105-LV transduced human CD34+ cells engrafted in NOD-scid IL2Rγ-/- mice with up to 20% reporter gene-positive cells detected long-term in all human hematopoietic lineages in bone marrow, spleen and blood. In addition, competitive repopulation experiments in mice showed a superior engraftment of CD105-LV transduced CD34+ cells in bone marrow and spleen compared to cells transduced with a conventional non-targeted lentiviral vector. Thus, human CD34+/CD105+ cells are enriched for early HSCs with high repopulating capacity. Targeting this cell population with CD105-LV offers a novel gene transfer strategy to reach high engraftment rates of transduced cells and highlights the applicability of receptor-targeted vectors to trace cell subsets offering an alternative to prospective isolation by surface markers.

Abstract No. P085

PRC2 inhibition counteracts the culture-associated loss of engraftment potential of human cord blood-derived hematopoietic stem/progenitor cells

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Cord blood hematopoietic stem cells (CB-HSCs) are an outstanding source for hematopoietic transplantation approaches. However, the amount of cells per donor is limited and expansion of CB-HSCs in culture is accompanied by a loss of engraftment potential. Thus, in order to analyze the molecular mechanisms leading to this impaired potential we profiled global and local epigenotypes during the expansion of human CB hematopoietic stem/progenitor cells (HPSCs). Human CB-derived CD34+ cells were cultured in serum-free medium together with SCF, TPO, FGF, with or without Igfbp2 and Angptl5 (STF/STFIA cocktails). As compared to the STF cocktail, the STFIA cocktail maintains in vivo repopulation capacity of cultured CD34+ cells. Upon expansion, CD34+ cells genome-wide remodel their epigenotype and depending on the cytokine cocktail, cells show different H3K4me3 and H3K27me3 levels. Expanding cells without Igfbp2 and Angptl5 leads to higher global H3K27me3 levels. ChIPseq analyses reveal a cytokine cocktail-dependent redistribution of H3K27me3 profiles. Inhibition of the PRC2 component EZH2 reduces H3K27me3 levels and counteracts the culture-associated loss of NOD scid gamma (NSG) engraftment potential. Collectively, our data reveal chromatin dynamics that underlie the culture-associated loss of engraftment potential. We identify PRC2 component EZH2 as being involved in the loss of engraftment potential during the in vitro expansion of HPSCs.

Abstract No. P086

Characterization of hematopoietic progenitor cells generated with human induced pluripotent stem cells in a teratoma-based model.

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Objective
Induced pluripotent stem cells (iPSC) have become an interesting target for regenerative science. In perspective, autologous patient iPSC can be corrected by retroviral vectors and suitable clones can be differentiated into hematopoietic stem cells (HSC). So far, in vitro differentiation protocols show the successful development of hematopoietic cells of different lineages, but cells with HSC features confer no long term engraftment in mice. However, in studies using iPSCs for teratoma formation, the isolation of transplantable HSCs was achieved. In this study we are employing teratoma formation as an in vivo model for hematopoietic differentiation and characterize early hematopoiesis by identifying cell types involved in this process.
Methods
CD34+ derived hiPSC were injected into the flanks of NSG mice. After teratoma formation, tissue samples were examined by immunolabelling of cell type specific surface markers. Determination was done by Flow Cytometry (FC) and Immunohistochemistry (IHC). For localization studies (IHC), tissue samples were fixed with formaldehyde, paraffin-embedded and dehydrated.
Results
The injection of hiPSC into immunodeficient mice gave rise to teratomas in 8-13 weeks. H&E staining and FC revealed diverse cell composition of teratomal tissue. IHC analysis confirmed the presence of tissue from all three germ layers. Vascular cell types identified by hCD31 and hCD34 antibodies were mostly localized around luminal structures. Double positive cells for CD90 and CD34 appeared in cell clusters near mesodermal descendants, representing cells in early differentiation stages which harbor the potential to develop into hematopoietic progenitor cells. Positive immunostaining for hCD45 indicates a low abundance of hematopoietic cells distributed throughout the teratoma.
Conclusion
We have confirmed the reproducible development of human cells from the hematopoietic lineage in this teratoma-based model, making this approach suitable for hematopoietic stem cell research. Characterization of hemogenic cell populations will contribute to enlighten the complex differentiation process of hiPSC derived HSCs.

Abstract No. P087

IFNα stimulation of hematopoietic stem cells and the bone marrow stem cell niche

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The bone marrow niche regulates stem cell function, influences stem cell fate and is also responsible for the engagement of specific programs in response to stress, such as infection or inflammation. The primary response to infection involves synthesis of immune-modulatory interferon cytokines, such as interferon (IFN). We, and others, have clearly shown that in contrast to the anti-proliferative effect of IFNα on HSCs in vitro, IFNα induces cell cycle entry of even the most dormant population of HSCs in vivo. Given the contrasting outcome of IFNα exposure in vitro and in vivo, we hypothesize that niche cells and molecular maintenance signals from the niche are required for HSC activation. Here, we interrogate the role of the niche in the switch of HSCs into cell cycle by IFNα and the direct and indirect effect of IFNα on the niche. We can clearly show that direct interferon signaling on the niche side is not required for HSC activation, however components of the niche respond to both IFNα directly and to HSC activation alone. This data illustrates a novel interplay between HSCs and the bone marrow niche during inflammation.

Abstract No. P088

Running against the dogma: 2D culture conditions may be advantageous for mimicking the hematopoietic stem cell niche

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During the last decades, intensive research of bone marrow stem cell niches allowed a detailed characterization of cell types involved in hematopoietic stem cell (HSC) regulation, but we still need to define the interactions with distinct niche cells, especially in humans. Besides, the number of attempts to switch from 2D to 3D is likewise increasing as 3D conditions are thought to reflect the in vivo situation more accurately than culturing cells in monolayers. Accordingly, we established a model by using Perfecta3D®Plates for the co-culture of CD34+ hematopoietic stem and progenitor cells (HSPC) with mesenchymal stromal cells (MSC) which are known to have important regulatory functions in the niche.
In this simplified model, cells are maintained in hanging drops without any contact to a stiff plastic surface. When seeded in a ratio of 5000 MSC to 500 HSPC, MSC readily formed a compact spheroid of around 200 µm in diameter which was surrounded by HSPC. Within the newly formed spheroids no HSPC were found indicating strong homotypic interactions of MSC. Extracellular matrix molecules like fibronectin or osteopontin and HSC-regulating factors like SCF were expressed inside the spheroids. Immunofluorescence staining of cryosections revealed a disruption of the MSC core and higher adhesive potential of HSPC with time as confirmed by electron microscopy. This effect was accompanied by an expansion of the HSPC. However, further analysis showed that this 3D model was not superior to the 2D co-culture in a flat-bottom 96-well plate in terms of HSPC proliferation. Hence, the question arises whether the MSC need a solid ground for their HSPC-supportive function and whether scaffold-based models that allow MSC adhesion thus increasing their cell surface are more suitable for HSPC expansion? Do the HSPC need direct cell-cell contact to the MSC? Are 2D conditions in this case even better than 3D conditions?

Abstract No. P089

Niche Wnt5a is required for maintenance of HSCs in vivo

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Hematopoietic stem cells (HSCs) reside in the bone marrow niche which regulates their self-renewal, proliferation and trafficking. We previously identified Wnt5a as a secreted factor that maintained 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 how Wnt5a-deficiency affects the niche affects and its ability to maintain HSC in young mice. The BM niche in Wnt5a-deficient mice show increased number of CD31+ endothelial cells. The number of mature and immature mesenchymal cells was not different, but multipotent stromal cells (MSCs) showed increased proliferation and calcification upon differentiation. To assess the possible effect of Wnt5a deficiency on self-renewal capacity of HSCs in vivo, wild-type (WT) HSCs were transplanted in Wnt5a-deficient recipients. In primary recipients, engraftment was similar to that in WT recipients. Unexpectedly, however, HSCs from the Wnt5a-deficient recipients completely failed to engraft in 2° recipients, indicating that no self-renewal had taken place in the 1° recipients. When Lineage- Sca-1+ Kit+ (LSK) cells were sorted from 1° recipients, we observed aberrations in Wnt signaling: decreased expression of β-catenin and increased Ca2+-dependent Camk2. In addition, expression of the non-canonical Wnt mediator Cdc42 was decreased and it was expressed in an apolar manner. In experiments designed to study homing of LSKs from 1° recipients, we found that although the percentage of viable cells was the unchanged, homing of LSKs from Wnt5a-deficient 1° recipients was decreased by 50%.
In conclusion, our experiments show that Wnt5a-deficient mice show hypervascularisation and fail to support engraftment of self-renewing HSCs. This is associated with increased non-canonical calcium-dependent Wnt signaling and decreased catenin-dependent signaling. Thus, our results show that Wnt5a is required to maintain self-renewing HSC during regenerative stress.

Abstract No. P090

Role of septins in HSC aging

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Aging functionally impairs hematopoietic stem cells (HSCs). The underlying molecular mechanisms are poorly understood. The small Rho GTPase Cdc42 has been shown to be involved in HSC aging. It switches between an active and an inactive state, thereby regulating actin and tubulin organization as well as cell polarity in distinct cell types. Cdc42 activity is increased in aged compared to young HSCs. This is associated with the apolar distribution of polarity proteins and correlates with a decrease in stem cell function. Polarity is organized and maintained by cytoskeletal proteins. Septins, a family of GTP-binding proteins, form filaments and act as scaffolds or diffusion barriers that segregate membrane areas into discrete domains. In yeast, Cdc42 was shown to act upstream of septins via effector proteins called borgs. Moreover, in cell lines, ectopic expression of active Cdc42 causes a loss of septin filament assembly, probably by inhibiting the interaction of borgs with septins.
Based on this current scientific knowledge, we hypothesize that septins play a role downstream of Cdc42 in the establishment and/or maintenance of polarity in LT-HSCs, which gets lost upon aging and that borgs might be the possible link between Cdc42 and septins in this polarization pathway. Single cell immunofluorescence analysis revealed that selected septins present themselves with a polar distribution in HSCs and that this polarity is regulated by the activity of Cdc42. Protein expression levels of septins were significantly higher in long-term HSCs (LT-HSCs) compared to more committed progenitors, implying a stem cell-specific role of septins. Distinct septins showed aging-associated changes in expression and/or polar distribution. As in yeast, distinct septins co-localize with borgs within HSCs. Additional experiment are underway to define septin/borg interactions in HSCs mechanistically. Elucidating mechanism of cytoskeletal remodeling upon aging will help to improve our understanding of aging-associated hematopoietic dysfunction and disease.

Abstract No. P091

Ectopic HOXB4 expression expands the pool of hemogenic endothelium-initiating progenitor cells during pluripotent stem cell differentiation

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Generation of hematopoietic stem cells (HSCs) from pluripotent stem cells, in vitro, holds great promise for future somatic gene and cell therapy. So far, HSCs capable of long-term multilineage reconstitution in mice have only been obtained when the homeodomain transcription factor HOXB4 was ectopically expressed during pluripotent stem cell differentiation(1,2). However, the primary "target" cell of HOXB4 during hematopoietic development, in vitro, is not yet known. Its identification is a prerequisite for unambiguously identifying the molecular circuits driving HSC development. To pin down this cell, we retrovirally expressed HOXB4 or a Tamoxifen-inducible HOXB4-ERT2 fusion protein in different reporter and knock-out ESC-lines. For these experiments, ESCs were differentiated for 6 days as embryoid bodies (EBs), dissociated and subsequently cocultured on OP9 stroma cells. Use of a Runx1(-/-) ESC-line ("iRunx1"; kindly provided by G. Lacaud, Manchester) containing a Doxycycline-inducible Runx1 coding sequence uncovered that HOXB4 acts during formation of the hemogenic endothelium (HE) from which HSCs arise. Without Runx1 induction, hematopoietic development was arrested at the HE-stage. Under these conditions, ectopic HOXB4 expression mediated an approximately 30-fold increase in the number of circular endothelial sheets being Flk1+VE-Cadherin+Tie2+. Those were formed by progenitors first detectable in day 5 EBs. Notably, HOXB4 increased the frequency of those progenitors from approximately 1:400 (ctrl) up to 1:14 (HOXB4) indicating that it expands upstream HE-progenitors. In concordance with this observation, the amounts of Sox17 and Lmo2 transcripts were significantly increased (5-fold and 10-fold, respectively) in HOXB4 HE-cultures. After additional Runx1 induction, the endothelial sheet structures underwent Endothelial-to-Hematopoietic Transition (EHT), upregulated transcription of Gfi1, Gfi1b and Pu.1 and initiated surface expression of the pan-hematopoietic marker CD45, thus proving their identity as real HE. In summary, our results strongly suggest that HOXB4 first acts by increasing the pool of HE-forming progenitor cells during mouse pluripotent cell differentiation.

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Abstract No. P092

Cytokine-regulated GADD45G induces differentiation and lineage selection in hematopoietic stem cells

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Long-term repopulating hematopoietic stem cells (LT-HSCs) regenerate the blood system throughout life. Therefore, the balance between self-renewal and differentiation in LT-HSCs must be strictly controlled to prevent either organ exhaustion or excessive expansion leading to malignancies. In recent years much effort was put into the identification of genes involved in HSC self-renewal, but only little is known about the immediate factors that switch the HSC self-renewal program into differentiation. Hematopoietic cytokines can promote differentiation in LT-HSCs extrinsically, however, the molecular mechanisms which govern differentiation induction in LT-HSCs remain obscure.

We identified the tumor suppressor Growth Arrest and DNA-Damage-inducible 45 gamma (GADD45G) as an instructor of murine LT-HSC differentiation under the control of differentiation-promoting cytokine receptor signalling. GADD45G immediately induces and accelerates differentiation in LT-HSCs, and overrides the self-renewal program by specifically activating MAP3K4-mediated MAPK p38. Inhibition of p38 activation lead to a reversion of the GADD45G induced enhanced differentiation. Conversely, the absence of GADD45G enhances the self-renewal potential of LT-HSCs in Gadd45g knock-out mice. Long-term time-lapse microscopy-based cell tracking of single LT-HSCs and their progeny revealed that, once GADD45G is expressed, the development of LT-HSCs into lineage-committed progeny occurred very fast within 36 h, and uncovered a selective lineage choice with a severe reduction in megakaryocytic-erythroid cells. Here we report an unrecognized role of GADD45G as a central molecular linker of extrinsic cytokine differentiation and lineage choice control in hematopoiesis. Knowing this mechanism may help to rationally approach stem cell maintenance / expansion for future therapeutic regenerative applications.

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Abstract No. P093

c-Myc expression in hematopoietic stem and progenitor cells is driven by a novel enhancer region 1.7Mb downstream of the coding region

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The c-Myc transcription factor is a central regulator of cellular proliferation, growth, metabolism and differentiation in many cell types including stem cells. Although it is known that *c-myc* expression is tightly controlled and can drive transformation if de-regulated, the mechanisms of its transcriptional regulation remain elusive. Here, we identified a cluster of enhancer-associated chromatin marks 1.7 Mb downstream of the mouse *c-myc* gene, present only in hematopoietic tissues. A *LacZ* reporter gene inserted next to this cluster showed specific expression in hematopoietic stem and progenitor cells (HSPCs). Mice homozygous for a deletion of this enhancer region presented with almost no myeloid and B cells, while HSPCs and megakaryocytes accumulated in the bone marrow, thereby closely mimicking the phenotype of mice in which the *c-myc* gene was conditionally deleted using Mx-Cre (1). Deletion of this enhancer region led to a dramatic reduction of *c-myc* expression in HSPCs. Importantly, compound heterozygous mice carrying one enhancer deletion allele and one *c-myc* null allele (deletion of the coding region) displayed a phenotype highly similar to the conditional knockout. Altogether, these data provide genetic evidence that this enhancer region directly controls, *in cis*, *c-myc* expression in HSPCs. Analysis of enhancer-associated H3K27ac marks by ChIP revealed the presence of individual modules within this enhancer which contribute differently to *c-myc* expression in either HSPCs or granulocytes. Strikingly, the enhancer region is highly conserved in human and focally amplified in a number of AML patients, suggesting that it may be a critical component driving high c-MYC expression in human leukemias. In summary, we identified a distant hematopoietic-specific enhancer region for *c-myc* and provide genetic data for its critical function as a key regulatory region in normal hematopoiesis and likely leukemia.

(1) Wilson et al. (2004). c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. Genes Dev 18(22): 2747-2763.

Abstract No. P094

Stress-driven exit of HSCs from dormancy drives premature aging of the hematopoietic system

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A hallmark of aging is the accumulation of DNA damage in hematopoietic stem cells (HSCs) and this is thought to drive age-related attrition of the hematopoietic system. In line with this hypothesis, accelerated aging disorders are frequently associated with defective DNA repair, such as the bone marrow failure (BMF) syndrome Fanconi anemia (FA). It has been shown that long-term quiescence is a feature of the most functionally potent long-term (LT)-HSCs and we hypothesize that this state of cell cycle dormancy protects the genome from the replication-induced DNA damage that drives functional decline. HSCs can be forced to enter cell cycle in vivo in response to stimuli that mimic physiologic stress such as infection or blood loss. Under these conditions of hematopoietic stress, we could observe that wild type (WT) murine LT-HSCs demonstrate a dramatic induction of de novo DNA damage, correlating with altered mitochondrial metabolism. Thus, upon LT-HSC exit from quiescence, mitochondria showed a shift towards energy production via oxidative phosphorylation and a concurrent increase in reactive oxygen species (ROS) production. DNA damage was rescued by overexpression of ectopic ROS scavenging enzymes, confirming this link. The FA DNA repair pathway is critical for resolving this stress-induced DNA damage, since FA knockout mice demonstrated extremely high levels of DNA damage compared to WT in response to stress stimuli, as well as a rapid depletion of functional LT-HSC resulting in eventual BMF. Although serial exposure to stress agonists did not provoke full BMF in WT mice, functional HSC numbers were reduced to >10% of age matched controls and a strong myeloid differentiation bias was evident, consistent with a premature aged phenotype. In summary, stress-induced proliferation can drive accelerated aging of LT-HSC and a functional FA pathway is critical for preserving genomic and functional integrity in the face of such attrition.

Abstract No. P095

Dysregulation of hematopoietic stem cell homeostasis by interleukin-1b in NADPH oxidase 2-deficient mice

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Inflammatory processes can influence hematopoietic stem cell (HSC) homeostasis by altering stem cell self-renewal and differentiation. We studied HSC homeostasis in NADPH oxidase 2 (NOX2)-deficient mice and found that the function of HSCs/hematopoietic progenitor cells (HPCs) is impaired. In competitive repopulation experiments HSCs from NOX2-deficient mice showed reduced long-term multilineage engraftment compared to HSCs from wild type animals and were outcompeted by wild type cells in co-transplantation experiments. The HPC population in NOX2-deficient mice was enlarged at the expense of quiescent HSCs. This was associated with increased extramedullary hematopoiesis in NOX2-deficient mice. Similar changes to the HSC/HPC pool were observed in human chronic granulomatous disease patients suffering from the lack of functional NOX2. We found that numerous inflammatory cytokines and chemokines are dysregulated in NOX2-deficient mice. Amongst them, interleukin-1b was able to induce HSC activation, expansion of the HPC pool and extramedullary hematopoiesis. Treatment of the NOX2-deficient mice with the interleukin-1 receptor antagonist anakinra reduced the HPC cell frequency in the bone marrow and spleen. The exhaustion of HSCs in NOX2-deficient mice might thus be counteracted by interference with the interleukin-1b signaling pathway, emphasizing the role of this inflammatory cytokine on HSC/HPC homeostasis.

Abstract No. P096

Paraoxonase-2 controls hematopoietic stem cell differentiation through redox signalling

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Intracellular levels of reactive oxygen species (ROS) play important roles in regulating numerous signaling events such as gene expression, cell fate decisions and differentiation, including haematopoietic stem cell differentiation, under both physiologic and pathologic conditions. Redox disturbances are caused and exploited by many tumors, including leukemias and thus represent a potential therapeutic target. We hypothesized that paraoxonase-2 (PON2) is relevant to this concept because this enzyme counteracts ROS generation, prevents apoptosis and was found up-regulated in many tumors. In leukemias, high PON2 levels corresponded to front-line therapy failure in pediatric ALL and imatinib resistance in CML patients. In vitro, PON2 knock-down induced apoptosis of K562 CML cells and increased sensitivity to chemotherapeutics (e.g. imatinib). These findings and the known impact of redox signaling on quiescence, apoptosis, differentiation and self-renewal of hematopoietic stem cells (HSCs) prompted us to analyze the role of PON2 in HSC biology and differentiation in PON2-/- mice. These mice showed severe alterations of the hematopoietic stem cell compartment, i.e. a significant increase in the LSK fraction, particularly LT-HSCs, ST-HSCs and MPPs, especially in aged animals. Changes in these fractions also translated into significant alterations in peripheral blood cell counts. Deficiency of anti-oxidative PON2 enhanced ROS levels in all fractions of the LSK population, especially in MPPs. These data suggest a prominent role for PON2 in redox control during hematopoietic stem cell differentiation. In support, we demonstrate that a gradual increase in ROS during differentiation from HSCs to MPPs and further to CMP is accompanied by corresponding changes in PON2 expression. Bone marrow transplantation studies imply that PON2 controls ROS and early differentiation of stem cells by direct cell effects and not through the niche. Collectively, these studies propose PON2 as crucial redox control enzyme in hematopoietic stem cells and potential target in anti-leukemia therapies.

Abstract No. P097

Understanding the marrow niche: advanced 3D-model system allows functional analysis of the interaction with human hematopoietic progenitor cells

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We previously demonstrated that “stemness” of human hematopoietic progenitor cells (HPC) was maintained in a co-culture setting with a monolayer of human mesenchymal stromal cells (MSC). To simulate and monitor the marrow microenvironment of the HPC niche more precisely we have established a 3D co-culture system based on a proprietary 3D-KITChip.

The KITChip was developed by the Karlsruhe Institute of Technology (KIT) and represents a unique microchip with defined microwell cavities for 3D cell cultures. Sample acquisition was approved by the local Ethics Committee. MSC were derived from human bone marrow of healthy voluntary donors and HPC were isolated from umbilical cord blood. Cells were inoculated into the KITChip, which was subsequently mounted into a microbioreactor allowing precise control of medium flow and oxygen saturation. After 1 to 5 days of co-culture, the two cell populations were analyzed by immunostaining, RT2-PCR and colony formation assay. MSC formed a complex 3D mesh in the microcavities of the KITChip. We have demonstrated that HPC were distributed three-dimensionally inside this MSC mesh and could be kept viable in this environment for more than 14 days. A defined proportion of CD34+ HPC adhered to the MSC in the microcavities and built up direct cellular connections to the surrounding MSC. By means of RT2-PCR, we could demonstrate that HPC were maintained in the 3D-environment more efficiently than compared to conventional co-culture with MSC monolayer. This was confirmed by Western blotting after the isolation of both cell populations from the chip.This novel model system allows analysis of the major determinants of the niche and the impact of a 3D microenvironment on vital stem cell functions. Early HPC were maintained more efficiently and showed a superior plasticity potential when cultured in the 3D-KITChip as compared to conventional 2D co-culture systems.

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P098 – P129: Stem cells in regenerative therapies

(posters will be displayed in the tent)

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P112 A single-tube real-time PCR assay for mycoplasma detection as a routine quality control of advanced therapy medicinal products (ATMP)
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P113 The use of mesenchymal stem cells for multiple sclerosis patients: 10-years experience of preclinical and clinical studies
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P114 Generation of Clara cells from murine pluripotent stem cells -
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P116 Government Investment in Public Research Institutions and Generating New Business in Private Firms: The Case of the Regenerative Medicine Industry
Naoko Kishi

P117 Activation of Bone Marrow- Derived Very Small Embryonic-Like Stem Cells by Acute Tissue Injury and their Regenerative Potential in vivo
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P119 Intrastriatal transplantation of adult human neural crest-derived stem cells improves functional outcome in rat model of Parkinson's disease
Janine Müller

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Ortwin Naujok

Abstract No. P098

Mesenchymal Stem/Progenitor Cell Therapy is an Alternative to Autologous Chondrocyte Implantation for Articular Cartilage Regeneration.

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Hyaline articular cartilage (AC) is a tissue with a very limited ability to self-regeneration. Accordingly, and based on recent results from our group using adults mesenchymal stem cells (MSCs), the objective of this study is to investigate and identify the most appropriate cell source and cell type for chondrogenic differentiation which will permit the tissular and functional regeneration of the hyaline AC. Since bone marrow (BM)-derived MSCs (BMMSCs) are an excellent cell source for cartilage regeneration, our aim is to implant autologous BMMSCs using a novel combined biomaterial. Cells from rabbit marrow were plated and cultured as passage 0. Groups were: 1) MSCs cultured at passage 1, and 2) MSCs pre-differentiated in vitro to chondrogenesis. One million of autologous MSCs were seeded by vacuum on a biphasic-collagen membrane, and incubated for 1h. At the time of transplantation, a full thickness osteochondral defect was created in the femur, and the defects were filled with the MSCs/biphasic-collagen composites. In controls, defects were let empty. At 24 weeks the defect area in the control group decreased but still remained. In the group 2, the defects were covered with whitish tissue but the margins were still distinct. In the group 1, the peripheral lesion of the defect appeared to integrate into the surrounding native cartilage. Histologically, at 24 weeks the defects in the control and group 2 were filled with fibrous tissue with no or poor cartilage matrix formation, respectively. However, with BMMSCs the cartilage defects were healed and cartilage matrix was well developed. We have demonstrated the optimal culture conditions using BMMSCs for in vivo chondrogenesis implantation, and that this novel osteochondral biphasic-scaffold is safe and easy to use.
Supported by FIS PI13/00666, P07-CVI-2781, PAIDI BIO-217, and Instituto de Salud Carlos III (Red de Terapia Celular (RD06/0010/0014), and CIBER-BBN). Spain.

Andrades JA, et al. 2012. Induction of superficial zone protein (SZP)/lubricin/PRG 4 in muscle-derived mesenchymal stem/progenitor cells by transforming growth factor-β1 and bone morphogenetic protein-7. Arthritis Research and Therapy, 14(2): 72-77.

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Abstract No. P099

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

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Human induced pluripotent stem cells (hiPSCs) pose reliable and effective cell sources to generate differentiated functional brain cells and have the advantage to be independent of postnatal brain tissue biopsies including their variations and limitations during in vitro culture.

The aim of this study is the direct differentiation of hiPSCs into human brain astrocytes and endothelial cells. In particular, we want to develop a 3D in vitro co-culture model, which closely simulates the in vivo situation and can be used as a tool in preclinical research such as in drug transport or infection studies. We are able to maintain undifferentiated hiPSCs under feeder free conditions shown by positive FACS analyses for the pluripotency markers Nanog, Oct 3/4 and Sox-2 and alkaline phosphatase assay. Furthermore, differentiation protocols are adapted to generate brain endothelial cells as well as human astrocytes from iPSCs mimicking the in vivo embryogenesis. Specific hiPSCs differentiation into functional endothelial cells and astrocytes are performed as described recently (Lippmann et al. 2012, Yan et al. 2013). BBB endothelial cells were characterized by detection of CD31, ZO-1 and specific transporter molecules like glucose transporter 1 as well as tight junction proteins like claudin 5. Neural progenitor cells were spontaneously differentiated into brain astrocytes by treatment with 1 % FCS for 21 days, enrichment was performed by CNTF treatment and under hypoxic conditions. The cells were characterized by immunohistological stainings against Nestin, GFAP and S100ß as well as by PCR analyses using cell-specific primers for the same gene targets. Finally, it is our aim to combine both cell types in a co-culture setup. Therefore, we use a 3D scaffold consisting of collagen and the typical basal membrane proteins as well as a dynamic flow reactor system to simulate the bloodstream.

Lippmann et al. 2012

Yan et al. 2013

Abstract No. P100

U-DERIVED STEM/PROGENITOR CELLS: A NON-INVASIVE CELL SOURCE FOR REGENERATIVE PURPOSES.

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Backround:
Urine (U)-derived cells, originally discovered in 1972, have recently been introduced as a non-invasive source of stem cells (USCs) for regenerative purposes.

Rationale:
We tested whether animal serum-free procedures established previously to select and expand human mesenchymal stem/progenitor cells (MSPCs) from various organs are sufficient to propagate USCs from urine sediment.

Methods:
Single emiction sediments were produced by centrifugation and cultured after re-suspension under previously optimized MSPC culture conditions at 10% oxygen with pooled human platelet lysate (pHPL) fully replacing fetal bovine serum. USC phenotype was documented by microscopy and flow cytometry. Clonogenicity, multipotent mesodermal lineage differentiation and wound repair potential were analyzed using standard protocols. Immune modulatory capacity was assessed in polyclonal peripheral blood mononuclear cell proliferation assays testing the impact of USCs as compared to mesenchymal stem/progenitor cells (MSPC) on T cell proliferation.

Results:
USC outgrowth was observed in 4/6 donors. Despite a mixed epithelial/mesenchymal appearance in culture, flow cytometry revealed a uniform greater than 95% CD73/90/105 and lower than 2% CD45/34/14/19/HLA-DR reactivity matching common MSPC criteria. USC displayed pluripotency-associated molecules SSEA-4, Tra-1/60 and Tra-1/81. Colony formation, three-lineage mesenchymal differentiation, wound repair potential as well as immune modulatory capacity were less efficient than in MSPC controls.

Conclusion:
USCs can be selectively propagated with a solid outgrowth rate that compared favorably to most cord blood MSPC retrieval efficiencies. Lifelong availability of the starting material qualifies USCs as an alternative and also non-invasive, highly accessible cell source. USCs may represent a valuable cell type to establish in particular autologous regenerative strategies and individualized advanced therapy medicinal products.

Abstract No. P101

Improvement of liver function by mesenchymal stem cells after extended liver resection

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Malignant liver tumors often require extended liver resection, rendering the residual liver to solely take over metabolic capacity until the liver mass is regenerated. These high metabolic and regenerative requirements often result in liver dysfunction and failure. Because of their anti-inflammatory as well as pro-regenerative features, mesenchymal stem cells (MSC) could be a feasible new therapeutic approach to prevent post-hepatectomy liver failure (PHLF) by supporting remnant liver parenchyma regeneration.
Extended liver resection (90%) was performed in F344 Fischer rats deficient in dipeptidyl peptidase type IV (CD26) by a natural mutation. This model allows for detection of CD26 in transplanted wildtype stem cells by histochemical staining. Before transplantation, MSC were pre-differentiated into hepatocytic cells in vitro and their effect in vivo was analysed by evaluation of liver-related blood values (INR (international normalized ratio of prothrombin time), bilirubin, AST and ALT) and liver histology (HE staining and SUDAN III staining to evaluate the metabolic overload as verified by lipid depositions). Apoptotis and proliferation were investigated by the TUNEL assay and Ki67-staining, respectively.
After liver resection, blood values of INR, bilirubin, AST and ALT increased, all indicating the functional impairment of and hepatocyte damage in the remnant liver. Yet, they were significantly improved by the application of MSC. Furthermore, the increase in metabolic overload as represented by hepatocyte lipid accumulation indicating lipid metabolism imbalance was attenuated by MSC treatment. In addition, the increase in apoptosis and the impairment of hepatocyte proliferation after extended resection were blunted showing the improvement of the regenerative capacity of the remnant liver parenchyma by MSC treatment.
In conclusion, MSC treatment after extended liver resection is a new therapeutic option to prevent PHLF due to metabolic overload, thus restoring liver parenchymal function and promoting liver regeneration.

Abstract No. P102

A reliable and efficient protocol for human pluripotent stem cell differentiation into the definitive endoderm based on dispersed single cells

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The definitive endoderm (DE) formation is the intial step to differentiate pluripotent stem cells (PSCs) into endoderm-derived organs. Differentiation of primed PSCs is usually initiated from colonies, a suboptimal starting material due to different colony sizes, colony numbers and their potentially altered sensitivity to media supplements. This study developed a reliable and highly efficient protocol for DE differentiation of human PSCs from dispersed single cells. These DE cells could be differentiated into the pancreatic lineage.
Three hESC lines and one hiPSCs line were passaged in a defined number of single cells and differentiated 24h later. Multiple DE inducing protocols were tested by combining various concentrations of Wnt3a, CHIR-99021, LY-294002 and ActivinA for four days. Subsequent differentiation into the pancreatic lineage was performed with FGF10, retinoic acid, Dorsomorphine and SB-431542. The different steps were analyzed by flow cytometry, IF and qPCR.
Combined CHIR-99021 plus ActivinA for 24h and subsequently ActivinA alone resulted in the most efficient DE formation for all hPSC lines. Under this condition additional PI3K inhibition had no beneficial effect. The expression of DE-marker genes was significantly increased compared to a reference protocol or random differentiation. Quantification of DE cells, by IF and flow cytometry, revealed efficiencies of >70% (HUES4, HES3, hCBiPS2) or >80% (HUES8), a >2-fold increase compared to the reference protocol (~33-40%). Additionally, the highest proliferation rates were observed under this condition. These DE-cells differentiated into PDX1-positive pancreatic progenitors (~40%, d10) and NGN3-positive endocrine precursor cells (~10%, d14) with an expression profile of marker genes similar to the pancreatic in vivo development.
This protocol was able to differentiate all hPSC lines highly efficient into the DE. Only low initial cell numbers and reduced concentrations of growth factors were required without growth limitations. Additionally, these cells were able to differentiate into the pancreatic lineage.

Abstract No. P103

Enrichment and Molecular Characterization of Epithelial Stem Cells of the Human Cornea

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One of the most common causes for blindness worldwide is the loss of a functional cornea, due to defective regeneration. Regeneration and maintenance of the human corneal epithelium is ensured by limbus-resident epithelial stem cells (LESCs). Hence, LESCs are of key relevance for clinical application to rebuild the cornea after substantial damage, such as burns or infections, or for the treatment of degenerative processes caused by genetic defects leading to LESC-deficiency (LSCD). In clinical routine, stem cell-containing limbus biopsies are commonly used as a source for generating cornea-grafts, in vitro. Long-term engraftment after transplantation is assumed to correlate with their frequency within the graft. However, because their identity is ill-defined, their quantitation in an individual transplant is currently not feasible, making it impossible to predict the success of transplantation and also impedes the systematic improvement of graft generation, in vitro. The aim of this project is the enrichment and characterization of LESCs to facilitate their prospective identification and purification for cell-based therapies. Because tissue stem cells commonly reside in a subpopulation of slowly cycling cells, we have developed a novel doxycycline-inducible lentiviral expression vector allowing for pulse-chase labeling of cells with histone H2B-GFP protein-fusion. Limbus tissue explants were successfully transduced and labeled by a transient, 24 hour pulse of H2B-GFP expression followed by a 7 day chase, in vitro. This allowed us to detect label-retaining cells within tissue sections, in situ, which concomitantly express proteins known to be associated with LESC identity, such as Pax6, ABCB5 or ABCG2. Based on this information, defined cell fractions will be isolated by flow cytometry, tested for their capability to generate stratified cornea epithelium layers in vitro and used for molecular profiling.

Abstract No. P104

Beneficial effects of bone marrow-derived mesenchymal stem cells transplantation in a non-Immune model of demyelination

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Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination and axonal loss throughout the central nervous system. Most of the studies have been conducted to evaluate the efficacy of mesenchymal stem cell (MSCs) utilized immune models such as experimental autoimmune encephalomyelitis (EAE). However, with this experimental setting, it is not clear whether MSCs exert the functional improvement via an indirect consequence of MSCs-mediated immunomodulation or via direct replacement of lost cells, paracrine actions, and/or enhancement of endogenous repair. This study is the first to report the capability of bone marrow-derived MSCs (BM-MSCs) to migrate, engraft, and improve demyelination in non-immune cuprizone model of MS. Ultrastructural analysis conducted in this study revealed that the observed histological improvement was due to both reduced demyelination and enhancement of remyelination. However, the detected remyelination was not graft-derived as no differentiation of transplanted cells towards oligodentroglial phenotype was detected. These results suggest that the therapeutic potential of BM-MSCs for MS is not only dependent of their immunosupressive and immunomodulatory nature, but also on their ability to induce neuroprotection and enhance the endogenous repair. Proving and dissecting the mode of action of BM-MSCs in non-immune model of MS should enrich our knowledge on how these cells exert their beneficial effects and eventually help us to enhance and maintain efficacious and sustainable cell therapy for MS.

Abstract No. P105

Optimization of kidney decellularization for stem cell based renal tissue engineering

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Chronic kidney disease is an increasing public health problem. Today, lifelong dialysis or kidney transplantation are the only available treatment options. The shortage of donor organs on one hand and the loss of quality of life by dialysis or immunosuppressive drugs on the other hand require the identification of alternative therapies. Possible approaches include the development of novel drugs, cell transplantation and whole organ tissue engineering. Here suitable in vitro models are needed to examine these options.

One approach to set up such a model is the generation of scaffolds by kidney decellularization and recellularization using human induced pluripotent stem cell derived renal progenitor cells. Here the native composition, ultrastructure and 3D architecture of decellularization-derived extracellular matrix (ECM) may provide a microenvironment that supports attachment, proliferation and guidance of differentiation to stem cells.

Methods for tissue decellularization utilize a combination of physical and chemical treatments for cell removal. Every agent and method will alter ECM composition and structure to some degree. Since the quality of the matrix is crucial for recellularization, careful optimization of the applied method for a specific tissue is necessary.

The aim of this study was to identify a protocol that preserves the kidney ECM as good as possible while efficiently removing all cellular components.

Several decellularization strategies using whole organ perfusion of rat or immersion of porcine kidney tissue samples with the detergents sodium dodecyl sulfate and sodium deoxycholate at different temperatures were examined. The yielded matrix scaffolds were compared by their histology, protein composition and efficiency of cell removal.

A good preservation of tubular and glomerular structures was observed with all methods. Differences in removal of cellular components, measured by remaining DNA content, were detected depending on the temperature and detergent applied. These factors also showed an influence on the protein composition of the ECM.

Abstract No. P106

Intermittent but not continuous parathyroid hormone-related protein exposure enhances mesenchymal stromal cell chondrogenesis via cAMP/PKA signaling

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Phenotype instability and pre-mature hypertrophy are major obstacles for the use of human mesenchymal stromal cells (MSC) for cartilage regeneration. Aim of this study was to investigate whether intermittent supplementation of parathyroid hormone-related protein (PTHrP), as opposed to constant treatment, can beneficially influence MSC chondrogenesis and to explore molecular mechanisms below catabolic and anabolic responses. For this purpose, human MSC were subjected to chondrogenic induction in high-density pellet culture for 6 weeks and received PTHrP(1-34), forskolin, dbcAMP or PTHrP(7-34) either constantly or via 6 hour pulses (3-times weekly). Deposition of proteoglycans, collagen type II, and collagen type X, gene expression and alkaline phosphatase (ALP) activity were assessed at different time points.

While constant application of PTHrP(1-34) resulted in a suppression of MSC chondrogenesis, pulsed application significantly increased COL2A1 expression and the collagen type II, proteoglycan and DNA content of pellets after 6 weeks. COL10A1 expression and collagen type X deposition was little affected but IHH mRNA levels and ALP activity were significantly down-regulated by intermittent PTHrP. Stimulation of cAMP/PKA signaling by forskolin reproduced major effects of both treatment modes, whereas application of the N-terminally truncated PTHrP(7-34), capable to activate PKC- but not cAMP/PKA-signaling, was ineffective.

In conclusion, intermittent PTHrP exposure stimulated chondrogenesis and simultaneously reduced undesired endochondral differentiation of MSC. cAMP/PKA was the major signaling pathway triggering the opposing effects of both treatment modes, indicating that not the activated pathway but signal timing might be the decisive variable. Intermittent application of PTHrP thus represents a simple and novel means to improve chondrogenesis of MSC and may thus be considered as a supporting clinical treatment mode for MSC-based cartilage defect regeneration.

Abstract No. P107

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

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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 the cystic fibrosis transmembrane conductance regulator (CFTR) gene, but the most common is the ΔF508 mutation, leading to a misfolding and therefore 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 to treat the disease, depending on the affected organ. 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 cells (hESCs) and induced pluipotent stem cells (iPSCs) are utilized for differentiation approaches. Cells are monitored for their capacity to generate definitive endoderm and hepatic endoderm by flow cytometric analysis. Maturation of hepatoblasts into cholangiocytes is achieved by the addition of various growth factors as well as bile salts and splitting onto collagen 1 coated plates. CFTR expression is monitored via qRT-PCR and western blot and can be directly observed by application of hESCs and hiPSCs carrying a CFTR Tomato reporter construct.

Results:

The generation of definitive endoderm with hESCs as well as hiPSCs is with up to 85% highly efficient. Endodermal cells further differentiate into hepatoblasts and early cholangiocytes, detectable by the expression of AFP, CK19, Sox9 and CK7. Subsequently cholangiocytes are enriched and further maturated to express CFTR.

Conclusion:

hPSC derived cholangiocytes might represent a suitable basis for disease modeling and drug screening approaches of liver associated diseases e.g. identification of new modulators and correctors for the treatment of cystic fibrosis.

Abstract No. P108

Smooth Muscle Potential of Mesenchymal Stem Cells in the Presence of TGF-β1, PDGF-AB and Ascorbic Acid after Expansion in Various GMP Compliant Media

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More than 10% of the adult population suffer from stress urinary incontinence (SUI): the involuntary loss of urine. Many patients exhibit degeneration of the urethral sphincter smooth and striated muscle. We investigated the smooth muscle myogenic differentiation potential of human bone marrow mesenchymal stem cells (MSC) for treatment of SUI. MSC were expanded under various GMP-compliant conditions (in DMEM media containing human plasma or serum +/- platelet lysate), and then differentiated into smooth muscle cells (SMC) using myogenic differentiation media (mDM) containing TGF-β1, PDGF-AB and ascorbic acid. MSC expanded in media containing only plasma died at passage 1. MSC pre-expanded in either human plasma or serum + platelet lysate and then cultured in mDM for 1 week expressed similar and significantly higher levels of contractile SMC-specific genes (αSMA, transgelin, calponin, and SM-MHC) vs. MSC in control medium (mCM) or MSC pre-expanded in media containing only human serum prior to mDM. Using MSC pre-expanded in human plasma + platelet lysate prior to myogenic differentiation showed that contractile SMC genes significantly increased after 1-2 weeks vs. MSC in mCM, decreased at week 3, and peaked again at week 4, suggesting possible phenotypic switching after week 2. Protein levels of αSMA, transgelin, calponin, and SM-MHC following myogenic differentiation also increased vs. mCM (immunofluorescence). The distribution of these proteins was similar to primary human bladder SMC. Osteogenic or adipogenic differentiation did not occur in the presence of myogenic media. Hence optimal and specific myogenic differentiation of MSC towards smooth muscle cells can be achieved by expansion of MSC in GMP media containing human plasma + platelet lysate, followed by 1-2 weeks of differentiation using TGF-β1, PDGF-AB and ascorbic acid. Near future experiments will determine the efficacy of these differentiated MSC in animals and if regeneration of the urethral sphincter smooth muscle occurs.

Abstract No. P109

Graft-vs-Host-Disease after Cord Blood Transplantation can be treated with Umbilical Cord Mesenchymal Stromal Cells in a syn- and allogeneic setting

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Graft-versus-Host-Disease (GvHD) is a major complication after hematopoietic stem cell transplantations (HSCT). Mesenchymal stromal cells (MSCs) exhibit immune-modulating features to suppress GvHD. The ability of umbilical cord-derived MSCs (UC-MSCs) to control GvHD-like reactions was investigated in vitro using mixed-lymphocyte reactions (MLRs) and in vivo using NSG mice.
Firstly, MLRs were adapted to establish robust immune reactions between umbilical cord blood-derived mononuclear cells (UCB-MNCs) from immune-incompatible donors comparable to those seen with peripheral blood mononuclear cells (PBMCs). Effects of varying doses of UC-MSCs and bone marrow-derived MSCs (BM-MSCs) on 3H-thymidine incorporation were compared. Secondly, a GvHD-like reaction was induced in NSG mice with UCB-MNCs. Then mice were treated with UC-MSCs against GvHD. Both allogeneic and syngeneic combinations of UCB-MNCs and UC-MSCs were used. Mice were monitored over 54 days for weight, GvHD symptoms, by flow cytometry and blood counts as well as final histology.
Robust MLR reactions using UCB-MNCs were achieved by cytokine stimulation. Both BM-MSC and UC-MSC had similar effects on the MLR reaction. The effects ranged from suppression to activation of the reaction in a donor/batch dependent manner. The in vivo results suggest 1.75•107 UCB-MNCs as sufficient to induce a modest GvHD-like reaction. UC-MSCs were given in different doses (5•104 and 1•106) on d0 or d14 following HSCT. The administration of 5•104 syngeneic MSC at d0 led to a significant higher survival rate compared to controls. Flow cytometric analysis revealed a significant decrease in human CD8+ cells and CD45 chimerism after administration of 1•106 MSC, with a higher decrease in a syngeneic setting. Qualitative histological analyses confirmed a reduction of GvHD in skin and intestine after treatment.
We have established a model system of GvHD using UCB-MNCs and an in vitro assay to test for immune-compatibility and -suppression. The in vivo results confirm a suppression of GvHD by UC-MSC.

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Abstract No. P110

Cytokine-directed differentiation of hepatic hPSC-derivatives

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Human pluripotent stem cells (hPSCs) hold great promise in regenerative medicine. Hepatic derivatives of hPSCs might eventually serve as transplants for metabolic or acute liver diseases and are considered as a valuable tool for research on disease models and drug screening. So far, the generation of hepatic derivatives exhibiting full metabolic capabilities in a sufficiently homogenous population for cell transplantation purposes is not yet well established and needs further attention.
In our study, we aimed for an efficient protocol that is applicable to specify hPSCs into an endodermal progenitor lineage prior to further terminal differentiation into hepatic cells. We evaluated a combined cytokine– and small molecule–based protocol activating the WNT pathway by the GSK3β inhibitor CHIR99021 for an improved definitive endoderm differentiation. Subsequently, we evaluated the inhibition of WNT signalling by sFRP-5 with respect to an enhanced differentiation towards a foregut endoderm cell population. We analysed the effect on WNT target gene expression by an hPSC reporter cell line. The activation and subsequent inactivation of the WNT pathway was investigated by quantitative Western Blots of the active (non-phosphorylated) β-catenin levels and the phosphorylated GSK3β. The endodermal cells' differentiation status was determined by immunocytochemistry and qRT-PCR for the definitive endodermal markers SOX17 and FOXA2 and for the foregut endodermal markers GATA4 and AFP. The maturation and metabolic function of terminally differentiated hepatic cells was analysed by qRT-PCR for the hepatic markers ALB, AFP, HNF4 and TTR as well as by Albumin ELISA and Cytochrome P450-1A1 activity assay.
In conclusion, activation of the WNT pathway by the small molecule CHIR99021 supported the generation of a homogenous endodermal cell population. Subsequent inhibition of the WNT pathway led to an improved foregut endoderm differentiation that allows an enhanced maturation and metabolic functionality of the hepatic cells obtained in the terminal differentiation step.

Abstract No. P111

Optimizing conditions to differentiate human induced pluripotent stem cells into mesenchymal stem/progenitor cells

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Human mesenchymal stem/progenitor cells (MSPCs) bear a profound 'natural' immune modulatory potential. Restricted proliferation capacity of MSPCs limits both clinical applicability and mechanistic studies that are urgently needed to better understand their mode of action. We induced two pluripotent stem cell (iPSC) lines from healthy parental MSPCs derived from human bone marrow (BM) and umbilical cord blood (UCB) to optimize conditions for generating virtually unlimited amounts of MSPCs and study mechanisms of differentiation and function.

MSPCs were established as published under completely animal serum-free conditions. After primary large scale culture, purity and identity of the cells were characterized by clonogenicity (CFU-F), flow cytometry and three-lineage differentiation 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. After an initial MEF feeder culture in DMEM/KOSR, iPSCs were transferred to Matrigel/TeSR conditions at deescalating oxygen concentration to generate MEF-free cultures. Differentiation along mesenchymal lineage was initiated in conditioned medium derived from the parental or third party MSPC lines (MCM; at 5 vs. 20% O2). Clonogenicity and phenotype of iPSC-derived compared to parental MSPCs were determined. Immune modulatory potential of iPSCs and their maturated MSPC progeny as a prototypic function was compared to parental MSPCs as positive controls and mature ECFCs as negative controls (in case of UCB-MSPCs related MHC identical). Under oxygen-optimized feeder-free conditions iPSCs efficiently differentiated into CD73+/CD90+/CD29+ MSPC-like cells. MSPC clonogenicity was established to be most efficient under reduced oxygen at 1 MSPC/cm2.

This strategy builds the basis to study mechanisms of mesenchymal specification and function.

Abstract No. P112

A single-tube real-time PCR assay for mycoplasma detection as a routine quality control of advanced therapy medicinal products (ATMP)

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GMP-compliant manufacturing of cell-based products requires testing for absence of Mollicutes species including Mycoplasma, Ureaplasma, Acholeplasma and Spiroplasma according to the European Pharmacopeia (EP). We developed a single-tube real-time PCR assay including an internal control (IC) for rapid and sensitive detection of all Mollicutes species stipulated by the EP.

Primers and a TaqMan probe (FAM labeled) were deduced from 16S rDNA sequence alignment of 18 Mollicutes species. In addition we designed a synthetic IC-DNA molecule with binding sites for the Mollicutes primers but not for the Mollicutes TaqMan probe, detected by an IC-specific TaqMan probe (VIC labeled). The analytical sensitivity (genomes/ml) of the assay was determined on dilution series of DNA from 12 Mollicutes strains followed by Probit analysis. Specificity was proven using DNA from other bacteria. The required 10 CFU/ml detection limit of the PCR assay was validated using 9 Mollicutes species spiked into mesenchymal stromal cells as a matrix. The IC-DNA was added to the cell culture samples prior DNA extraction.

Analytical sensitivities of the PCR assay were in the range of 405 to 2,431 genomes/ml for 11 of the 12 tested mollicute DNA samples. The lowest sensitivity was found for U. urealyticum (19,239 genomes/ml). The detection limit of 10 CFU/ml was demonstrated for all 9 Mollicutes species. Mollicutes-specificity of the PCR assay was proven by negative results for DNA samples from three different ubiquitous bacteria. Simultaneous detection of the IC enabled monitoring of the entire process including DNA extraction and PCR amplification. Our single-tube real-time PCR assay with internal process control enables sensitive and specific detection of Mollicutes contaminants. The assay can be performed directly on cell culture samples simply by adding the IC-DNA followed by DNA extraction and real-time PCR. The PCR is suitable for routine quality control of cell therapeutics, including ATMPs.

Abstract No. P113

The use of mesenchymal stem cells for multiple sclerosis patients: 10-years experience of preclinical and clinical studies

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Mesenchymal stem cells are non-hematopoietic stem cells residues in different tissue of the body such as bone marrow, adipose, cord-blood and others. The classical role of MSC is to support the hematopoeisis process and to give rise to cells from the mesodermal lineage. In the last decade these cells were found to hold immunomodulatory and neuroprotective features. In diseases such as Multiple Sclerosis that have two phases of diseases progression, inflammation and neurodegeneration, the use of MSC might be useful in term of diseases amelioration and progression. Therefore, a lot of efforts were done to perform preclinical studies with MSC in different animal models of MS. These studies resulted in encouraging results in terms of disease control and amelioration. The mechanisms of action suggested were via immunomodualtion and neuroprotection. Followed by large numbers of preclinical studies, few small clinical studies were conducted and positive results regarding safety and feasibility and even efficacy were reported. Recently, several centers around the world started to prepare for larger and more controlled studies with MSC for MS while determining the best dose and administration method.

Abstract No. P114

Generation of Clara cells from murine pluripotent stem cells -

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Airway epithelial cell production in vitro offers new options to treat airway diseases, including genetic disorders like cystic fibrosis. Pluripotent stem cells (PSCs) (embryonic (ESCs) or induced pluripotent stem cells (iPSCs)) represent a suitable exogenous cell source for cell replacement strategies. Aiming at the long-term restoration of functional airway epithelium, epithelial progenitor/ stem cells will be required, e.g. Clara cells. Clara cells are able to regenerate the airway epithelium following injury. With the aim to establish a mouse model of long-term airway epithelial regeneration, we aimed at the in vitro generation of Clara cells from murine PSCs. Using iPSCs established from two different Clara cell reporter mouse strains enabled identification of generated Clara cells.

iPSCs from CCSP-rtTA2s-M2/GFP-tetO7-lacZ mice as well as ESCs were differentiated towards Clara cells using a serum-free monolayer (ML) protocol. The medium was supplemented with dexamethasone, 8-Bromo-cAMP and isobutylmethylxanthine (DCI), with or without keratinocyte growth factor (KGF). Specific marker expression was measured by qRT-PCR. iPSC-derived lacZpos Clara cells were visualized via X-gal staining and were further analyzed by electron microscopy. Pre-differentiated iPSCs were injected under the kidney capsule of immunodeficient mice and analyzed two weeks later. Furthermore, we established additional iPSC clones from CCSP-2A/YFP-2A/iCre knock-in mice.

We have identified the factor combination DCI as an important inducer of the Clara cell marker CCSP in differentiation cultures of murine PSCs. The CCSP-driven expression of lacZ enabled the monitoring of iPSC-derived Clara cells and the confirmation of the Clara cell phenotype in isolated lacZpos areas by enhanced CCSP mRNA expression and a Clara cell typical ultrastructure. Moreover, the iPSC-derived lacZpos cells formed epithelial-like structures in vivo with similarities to lacZpos airways of the Clara cell reporter mice. The recently established iPSC clones from CCSP-2A/YFP-2A/iCre knock-in mice were already successfully differentiated into YFPpos cells using the DCI supplemented ML protocol.

Abstract No. P115

PREDICTING AND OPTIMIZING THE IMMUNE MODULATION POTENTIAL OF HUMAN MESENCHYMAL STEM/PROGENITOR CELLS.

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Controlled clinical applicability of mesenchymal stem/progenitor cells (MSPCs) as potent immune modulators is hampered by a profound variation in their immune modulatory capacity due to donor-dependence and organ-of-origin-variability as well as possible freeze/thaw-related functional impairment. We tested two different MSPC types in polyclonal lymphocyte proliferation cultures to develop an appropriate potency assay predicting efficiency of the advanced therapy medicinal product.

MSPCs from five independent human bone marrow (BM) and white adipose tissue (WAT) donors were expanded under humanized culture conditions with pooled human platelet lysate (pHPL) replacing fetal bovine serum. We analyzed their potency to inhibit PHA- vs. CD3/CD28-driven polyclonal T cell responses, thus determining donor variance & organ/source variation. Based on clinical practice, MSPCs were used immediately after thawing or after an approximately 72h 'rescue culture'. A supplementary safety measure was introduced by lethal irradiation to minimize the risk of unintended MSPC differentiation when transplanted for immune response control.

MSPC phenotype/identity and viability was confirmed by microscopy, three-lineage differentiation and flow cytometry showing greater than 95% CD73/90/105 and lower than 2% CD45/34/14/19/HLA-DR reactivity. All MSPCs displayed immune modulatory potential in particular at 1:3 and 1:10 modulator:responder ratio with a profound degree of variability. Pooling multiple donor-derived MSPCs partly compensates variability at the expense of efficiency of the most potent donors. Contrary to published results, we did not observe a generally higher immune modulatory capacity in either organ-derived MSPCs. Impaired immune modulation immediately after thawing was donor-variable and could be corrected by 'rescue culture'. Irradiation did not hamper MSPC functionality.

The profound variability in immune modulatory efficiency urgently demands predictive potency assays for autologous MSPC therapies and/or selection of potent pre-tested donors or donor-pools for off-the-shelf allogeneic immunotherapy. MSPC irradiation adds an additional safety measure of particular advantage to allogeneic MSPC therapies.

Abstract No. P116

Government Investment in Public Research Institutions and Generating New Business in Private Firms: The Case of the Regenerative Medicine Industry

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The objective of this research is to show that government investment in public research institution contributes to generating new business in private firms with case studies in the regenerative medicine industry.

In developed countries, financial deficit causes the reduction of government investment in public research institutions including high education and demands performance based system even for the basic research. The trend may lead to weaken national technological capability in the long run. This research shows that their most advanced knowledge promotes for private firms to generate new business particularly in the dawn of industry. The case studies of private firms in Japan, which generate new business for cell therapy, show that new entrants at the dawn of the regenerative medicine industry have organizational members connecting strongly to public research institutions. They recognized their business opportunities from researchers' information in the institutions. Also, some firms collaborated to develop new product with the researchers, and others were outsourced to manufacture products based on researchers' knowledge.

Some economic literature suggest that there is a possibility that government investment in R&D have less impact on private firms' business performance than expected, because existing firms have already made overinvestment in R&D. However, this research suggests that the investment has an important impact. Particularly in case of the dawn of industry, public research institutions do basic research, from which private firms are uncertain to gain profit, and function to provide them with the most advanced knowledge.

Therefore, the case studies show that government investment in public research institutions lead for private firms to generate new business, and it promotes to grow the new industry particularly at the dawn. Base on the findings, the result suggests that government should maintain the amount of investment in them regardless of fiscal situation.

Abstract No. P117

Activation of Bone Marrow- Derived Very Small Embryonic-Like Stem Cells by Acute Tissue Injury and their Regenerative Potential in vivo

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Very small embryonic-like stem cells (VSELs) were identified as rare quiescent population of nonhematopoietic cells in adult murine and human tissues including bone marrow (BM). BM-derived VSELs are mobilized into peripheral blood (PB) in tissue injury. Moreover, these primitive stem cells can participate in heart repair after injection into infarcted myocardium by enhancing tissue perfusion and angiogenesis.

In this study, we examined if acute tissue injury may stimulate both proliferation of quiescent VSELs in BM and their mobilization into PB. The other goal was to investigate regenerative potential of VSELs injected into ischemic tissues.

Thus, 4-6 week old C57BL/6 mice underwent a hind limb ischemia (LI) by permanent proximal femoral artery occlusion. Mice were administrated with BrdU and scarified at 2, 7, 14 and 28 days following LI. The presence of proliferating (BrdU+): VSELs (Sca-1+/Lin-/CD45-), endothelial progenitor cells (EPCs; Flk-1+/Sca-1+/Lin-/CD45-/dim) and hematopoietic stem/progenitor cells (HSPCs; Sca-1+/Lin-/CD45+) in PB and BM was evaluated by flow cytometry and ImageStream system. The expression of genes related to the presence of VSELs and EPCs was examined by real-time PCR. Moreover, we examined the change in expression of angiogenesis-related proteins. Then, eGFP VSELs sorted from ischemic and non-ischemic mice were injected at 2d following LI into injured tissues. At 2, 7, 14 and 28days post transplantation, blood flow were measured by Laser Doppler System. Paraffin-embedded ischemic tissue sections were analyzed for eGFP and PCNA co-expression.

We found that the number of proliferating VSELs and EPCs was significantly increased in BM of ischemic mice at 7d post injury. Elevated number of BrdU+ VSELs was accompanied with change in expression of genes guiding their proliferation. Moreover, VSELs injected into ischemic tissues enhance tissue perfusion. The data indicates vast impact of acute injury on activation of VSELs proliferation in vivo and possibility of their application in regenerative medicine.

Abstract No. P118

integration-free iPSCs as a tool for modeling hepatogenesis in vitro

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Induced pluripotent stem cells (iPSCs) are embryonic-like cells and can be generated from somatic cells by the over-expression of OCT4 and SOX2 in combination with either KLF4 and c-MYC or NANOG and LIN28. Like ES cells iPSCs self-renew and are pluripotent, thus making them an idea source for studying human gastrulation in vitro. We have generated episomal-derived and integration-free iPSCs (E-iPSCs) from human fetal foreskin fibroblast cells (HFF1). E-iPSCs were fully characterized and their transcriptomes are more similar to that of hESCs (R2 = 0.9363) in comparison to viral-derived HFF-iPSCs (R2 = 0.8176). We used the E-iPSC line to model hepatogenesis in vitro. The differentiation of hepatocyte-like cells (HLCs) involves a three-step process, from the undifferentiated E-iPSC to definitive endoderm (DE), to hepatic endoderm (HE) and ultimately HLCs. The HLCs were fully characterized biochemically, i.e. glycogen storage, ICG uptake and release, UREA production, and CYP3A4 activity. Ultra-structure analysis by electron microscopy revealed the presence of lipid and glycogen storage, tight junctions and bile canaliculi- all typical features of hepatocytes. Furthermore, the transcriptome of undifferentiated E-iPSC, DE, HE and HLCs were compared to that of fetal liver and primary human hepatocytes (PHH). K-means clustering identified 100 clusters which include developmental stage-specific groups of genes, e.g. OCT4 expression at the undifferentiated stage, SOX17 marking DE stage, AFP and ALB at the HLC stage. The NOTCH and HIPPO pathways were over represented in HLC, fetal liver and PHH.

In summary, we have generated episomal-derived iPSCs (E-iPSCs) and demonstrated that they are pluripotent both in vitro and in vivo. These E-iPSCs are able to mimic hepatogenesis and represent a tool for studying human gastrulation at the molecular and cellular levels in vitro.

Abstract No. P119

Intrastriatal transplantation of adult human neural crest-derived stem cells improves functional outcome in rat model of Parkinson's disease

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Being one of the most severe neurodegenerative diseases, Parkinson's disease (PD) is particularly associated with dysfunctions of the motor system as well as non-motor symptoms such as depression and dementia. On account of the limitations regarding current pharmacological treatment strategies, cell replacement therapy aims at restoring DA neurons lost during the disease rather than solely fighting its symptoms. Here, stem cells residing within the adult human body remain as a promising tool, since they exhibit a remarkably low tumorigenicity and also allow autologous cell transplantation.
In this regard, we investigated the potential of adult neural crest-derived stem cells isolated from the inferior turbinate (ITSCs) of the human nose to regenerate the Parkinsonian phenotype in a 6-OHDA rat model. ITSCs efficiently gave rise to functional neurons in vitro, while additional directed dopaminergic differentiation resulted in 100% Pitx3-positive dopaminergic progenitors, which further differentiated into TH+ neurons in vitro. Transplantation of undifferentiated or SHH/FGF8-pretreated ITSCs into a unilaterally lesioned 6-OHDA rat PD model resulted in robust restoration of rotational behavior. Accordingly, we observed significant recovery of endogenous DA neurons within the Substantia nigra (SN) and their projections within the Striatum. Notably, ITSCs extensively migrated towards the posterior direction followed by their integration into the midbrain and near the lesioned SN. We further observed differentiation of ITSCs into TH-positive neurons within the Locus coeruleus. In summary, we demonstrate here for the first time the capability of adult human ITSCs to functionally recover a PD rat model.

Abstract No. P120

Anterior-posterior patterning of definitive endoderm derived from human embryonic stem cells by retinoic acid and Wnt/beta-catenin signaling

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The in vitro differentiation of pluripotent stem cells (PSCs) into the definitive endoderm (DE) is nowadays robustly performed. The next differentiation step requires anterior-posterior (A-P) patterning of the endoderm into the broad primitive gut tube domains foregut and hindgut. Subsequently specific domains are patterned, which give later rise to the organ primordia. The activities of Wnt/FGFs and BMPs in the posterior half and all-trans-retinoic acid (ATRA), TGF-β-ligands, Wnt- and BMP-inhibitors in the anterior half of the endoderm sheet are thought to be responsible for A-P patterning. However, it is currently unclear how these complex interactions can be translated into a differentiation protocol for PSCs.
Two PSCs lines were differentiated into DE-like cells applying a single cell based protocol. Next, the effects of Wnt/beta-catenin-, TGF-β-, ATRA-, and FGF2-signaling were tested by various combinations of ligands and inhibitors. Differentiated cells were analyzed by qPCR and immunofluorescence.
The treatment of DE-cells with 5µM CHIR-99021, 25ng/ml BMP4, 100nM ATRA and 100ng/ml bFGF for 48h resulted in a midgut/hindgut population positive for CDX2. Additionally, these cells expressed a specific combination of HOXC5, HOXC6, and HOXB8 indicating their midgut/hindgut identity. If Wnt- and BMP4-signalling were chemically suppressed, ATRA could induce and posteriorize foregut cells in a concentration-dependent manner. Specifically 0.5/1/2µM ATRA resulted in a cell population that highly expressed HNF6, HNF1B, and FOXA2 on the gene and protein level typical for foregut cells of the foregut/midgut boundary. The induced gene expression of MNX1, SHH, HOXC5 and HOXA3 was also detected whereas the anterior foregut endoderm markers TBX1 and HEX1 were suppressed.
This study shows that the foregut/hindgut identity is controlled by ATRA- and Wnt/beta-catenin signaling. The treatment of DE-cells with 0.5-2µM ATRA resulted in a cell population reminiscent of the posterior foregut; a domain which gives rise to liver and pancreas primordia during further human development.

Abstract No. P121

The challenges of surface receptor engineering on MSCs

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Mesenchymal stem cells (MSC) show great potential for the development of alternative (cell based) therapies for various disorders including neurodegenerative diseases. In clinical settings, systemic delivery of MSCs has several advantages and circumvents serious problems compared to site-directed transplantation. However, MSC homing and migration towards injured tissues is not yet satisfying after intravasal administration and need to be improved to enhance functional recovery. Thus, this study aimed to improve adhesion and migration of MSCs by engineering their surface receptor expression.

Various vectors were designed for expression of integrin alpha 4 ([ITGA4] part of very late antigen 4 [VLA4], receptor for vascular cellular adhesion molecule 1 [VCAM-1]) and C-C-Motif Chemokine Receptor 2 (CCR2, receptor for monocyte chemotactic protein-1). Lentiviral constructs were driven by either CMV-, EF-1alpha or UbC promoter. Additional vectors contained a T7 promoter for RNA polymerase dependent in vitro synthesis of mRNA. Capping (anti-reverse cap analog [ARCA], Cap-0, Cap-1) and poly-A tailing of the in vitro transcribed mRNA were performed for stabilization and effective translation after transfection. GFP-containing vectors served as controls.

All control vectors showed efficient expression of GFP. Of note, however, only MSCs infected with UbC driven surface receptor constructs expressed the intended transgene. After mRNA transfection, on the other hand, detectable expression was achieved only from physiologically capped constructs, but not ARCA. Even more interesting, this difference was much less pronounced for GFP. Taken together, these data indicate that transgenic surface receptor expression depends on promoter activity in the target cell, but also correct mRNA and nascent protein processing.

Abstract No. P122

Problems of cartilage and bone regeneration using mesenchymal stem cell

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A major problem in the treatment of diseases of cartilage is the fact that it cannot regenerate, because the avascular tissue, chondrocytes have a weak proliferation capability. Chondrocytes and cartilage that occurs by differentiation of MSCs are structurally inferior to the primary tissue of cartilage. Their poor clinical benefit is based mainly on immunomodulatory role.

There are many attempts to rebuild cartilage. Mesenchymal stem cells are extensively applied since 1987 in animal models, first in rabbits and goats, and today more frequently in horses with acute injuries of tendons and ligaments. There is little effectiveness in the differentiation of chondrocytes that can be used clinically to repair or regeneration of cartilage, particularly articular.

Cartilage tissue prepared in the laboratory does not have the orientation of the collagen type II. In general, the problem of all methods for regenerating cartilage is that its result is fibrous cartilage, and even when in the initial period collagen type II dominates, after 6-12 months dominates collagen type I. Newly formed cartilage often is not properly integrated into an existing cartilage, and abruption can happen.

There are attempts of MSCs differentiation into osteoblasts that can be clinically used together with scaffold, i.e. carriers for bridging large bone defects. Bad sides in these studies were poorly accepted and limited differentiation in vivo, the ability to differentiate into unwanted cell lines, malignant transformation, the results of clinical studies have not been convincing enough, it is unclear which type is best to apply, it is unclear which method of application is best to apply, aging stem cells and very expensive equipment.

Lanza R, ed. Essentials of Stem Cell Biology. 2nd ed. Elsevier Academic Press, 2009.

Bongso A, Lee EH. Stem Cells: From Bench to Bedside (Second Edition). World Scientific Publications Co, 2010.

Abstract No. P123

hiPSC derived endothelial cell types from scalable cultures for biofunctionalization and tissue engineering

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Applications like full endothelialisation of gas exchange membranes in extracorporal membrane oxygenation (ECMO) devices for improved hematocompatibility, cell therapy of pulmonary hypertrophy or tissue engineering require large numbers of (patient-specific) endothelial cells (ECs). The isolation of ECs from peripheral blood or explanted vessels is well established however especially cells from older individuals show a limited proliferation capacity. Patient specific ECs from pluripotent stem cells (hiPSCs) might be an alternative suitable cell source. The opportunity to generate large amounts of undifferentiated hiPSC in defined media under scalable conditions [1] allows for the generation of cell numbers in dimensions which are suitable for envisioned applications. By differentiation of these well monitored cell populations a virtually unlimited number of (autologous) ECs may become available for disease modelling, tissue engineering approaches and biofunctionalization of ECMO devices.

The growth factors BMP4 and VEGFA as well as modulation of the WNT pathway were utilized for the differentiation of the scalable suspension cultures to endothelial cell types [2]. Differentiation approaches resulted in up to 31% of CD144 positive (VEcadherin) and 10% CD144 and CD31 double positive cells on day 14 of differentiation.

FACS-sorted CD31 positive iPSC derivatives will be characterized in detail with respect to their molecular phenotype, proliferative capacity and functionality. In addition, the generation of transgenic hiPSC reporter lines, which express a fluorescence reporter / antibiotic resistance under the control of EC specific promotors (VEcadherin or CD31) for monitoring of differentiation and selection/purification of resulting cell types is in progress.

Resulting patient- (and lung disease-) specific iPSC-derived ECs will represent a novel cell source for disease modelling or biofunctionalization of gas exchange membranes as well as for vascularisation of tissue engineered constructs. In addition, TALEN-based gene correction in iPSCs might enable novel concepts of ex vivo gene therapy for respiratory diseases.

[1] Olmer, R., et al., Suspension culture of human pluripotent stem cells in controlled, stirred bioreactors. Tissue Eng Part C Methods, 2012. 18(10): p. 772-84

[2] Orlova, V.V., et al., Functionality of endothelial cells and pericytes from human pluripotent stem cells demonstrated in cultured vascular plexus and zebrafish xenografts. Arterioscler Thromb Vasc Biol, 2014. 34(1): p. 177-86.

Abstract No. P124

The combination of biomechanical forces and optimized substrate elasticity induces intermediate markers of myogenic differentiation in human BmMSCs

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Stress urinary incontinence results from dysfunction of the urethral sphincter. Current treatment methods do not address associated sphincter degeneration. The usage of mesenchymal stroma cells (MSC) might be a promising strategy. Our goal (within the KFO273) was to investigate the effects of biomechanical forces on the differentiation of human bone marrow-derived MSCs (BmMSCs) towards smooth muscle cells (SMCs).

Human bmMSCs from patients undergoing hip replacement procedures (n=7) were expanded in DMEM containing pooled plasma and platelet lysate, then seeded at passages 2-5 at a density of 5000 cells/1cm2 at “day 0” onto a collagen type I sheet (80mg/ml). Its substrate elasticity (on macro- and nanoscale) was optimized by sheet composition and compression to support BmMSC differentiation towards SMC. Adhering BmMSCs were biomechanically treated (n=13) at day 5 and 6 by uniaxial dynamic stretch (1Hz, 2.5 and 5%, 1 hour) using an ElectroForce 5210 system (Bose). Cells were assessed for cell numbers and cell shape by Calcein staining (quantification via ImageJ), and for SMC markers by qRT-PCR and immunofluorescence.

BmMSCs demonstrated no differences in their cell numbers when comparing biomechanically treated and non-treated control BmMSCs, or when comparing days 5 and 6 only within the control group or the biomechanically treated group. Compared to controls, the biomechanically treated cells demonstrated at day 5 a significant shape elongation (p lower than 0.001), which decreased at day 6 but remained significantly higher than those of controls. qRT-PCR of BmMSCs revealed a significant increase in alpha-actin (at day 6; p lower than 0.05), transgelin (at day 6; p lower than 0.05), and calponin expression (at day 5; p lower than 0.05) under 5% stretch. Protein content was confirmed on day 7 by immunofluorescence under stretch.

Biomechanical stretch altered BmMSC shape. Together with optimized substrate elasticity, it induced early and intermediate myogenic differentiation gene expression and protein levels without the use of differentiation-inducing substrates or growth factors.

Abstract No. P125

Cardiac Point-Of-Care Stem Cell Therapy (C-POCST): Preliminary results of a new method.

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Ischemic cardiomyopathy is a sort of dilatative cardiomyopathy (DC) that often progress to heart failure and dead. The current pharmacological and mechanical treatments are not optimal and heart transplantation is not always readily accessible. Intracoronary infusion of stem cells (SCs) in heart diseases has been proved to be safe and effective.

A clinical study approved by the local ethical commit was performed including 10 patients with DC and an ejection fraction (EF) below 30% with or without coronary heart disease. The method used is an on-site intracardiac infusion of autologous bone marrow-derived mononuclear stem cells (BM- MNCs) that allows the clinician to harvest and infuse maximal concentration of them without cell modification within a 30 minutes time frame.

The patients were infused with 10cc of BM-MNCs concentrate and 5cc wash of BM-derived plasma in each, right and left coronary artery. Some of them receive additional PTCA. The mean EF improvement was between 5 and 12% in a 3 months follow-up without significant side effects

C-POCST is a new and effective method. This is a small and heterogeneous group, however with homogenous positive effect on cardiac performance. A large clinical study approved by the local ethical commit is ongoing.

Seth S, Bhargava B, Narang R, Ray R, Mohanty S, Gulati G, et al. The ABCD (Autologous Bone Marrow Cells in Dilated Cardiomyopathy) trial a long-term follow-up study. J Am Coll Cardiol. 2010 Apr 13;55(15):1643–4.

Mathiasen AB, et al. Rationale and design of the first randomized, double-blind, placebo-controlled trial of intramyocardial injection of autologous bone-marrow derived Mesenchymal Stromal Cells. Am Heart J. 2012 Sep;164(3):285–91.

Abstract No. P126

Myogenic differentiation of human mesenchymal stromal cells interferes with the interaction with laminin isoforms

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Introduction:

Mesenchymal stromal cells (MSC) can differentiate into various cell lineages and are therefore a promising source for cell-based tissue regeneration. For differentiation into the myogenic lineage there is still a need for the development of a robust method including the evaluation of effects of extracellular matrix proteins such as laminins (LM). In the present study we analyzed the interaction of different LM isoforms with myogenic differentiated MSC.

Methods:

Human MSC were isolated from bone marrow aspirates. For differentiation into the myogenic lineage MSC were cultured in media containing ascorbic acid, PDGF-AB and TGF-β1. Human bladder smooth muscle cells and sphincter sections from pig were used as controls. The expression pattern of LM isoforms was analyzed by WB, IFL and RT-PCR. Functionally, the influence of LM isoforms on MSC was studied by proliferation and adhesion assays and by single cell force measurements.

Results:

IFL staining of sphincter sections revealed the expression of the LM α2, α4 and α5 chains. Undifferentiated and myogenic differentiated MSC mainly showed, similar to smooth muscle cells, the expression of LM-411 and LM-511. LM-211 was hardly expressed. Proliferation of MSC decreased in the presence of different LM isoforms. The MSC adhered to all LM isoforms tested, but with different adhesive strengths. LM-511 was the strongest adhesive substrate for smooth muscle cells and undifferentiated MSC. Myogenic differentiation, however, drastically diminished the binding of MSC to LM-511. LM-211 was only a weak adhesive substrate for smooth muscle cells and MSC, and upon myogenic differentiation the adhesive capacity of LM-211 was further diminished.

Discussion:

Although LM-211 is the major isoform in myogenic tissues, MSC that underwent myogenic differentiation showed a weaker interaction with LM-211 versus undifferentiated MSC. Whether the decrease in MSC proliferation by LM-211 favors the differentiation of these cells has to be shown in future work.

Abstract No. P127

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

Malte Sgodda [1,*], *Oliver Papp* [1], *Jeannine Hoepfner* [1], *Mandy Kleinsorge* [1], *Susanne Alfken* [1], and *Tobias Cantz* [1]

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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 a two-stage protocol to differentiate hESCs into hepatic cells. The first stage of this protocol is a cytokine-directed monolayer differentiation including four different steps 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.

In the first stage, we were using a cytokine- and small molecule- based protocol modulating the WNT pathway in a way to mimic the natural development to differentiate hESCs into hepatic cells. The obtained cells were aggregated in an organoid-like structure and maturated in a suspension cell culture system. After this final maturation step these organoid-like structures could be dissociated and were plated on a collagen matrix. Quantitative real time PCR for progenitor (FOXA2, SOX17, AFP) and mature (HNF4a, ABCC2, TTR) cell markers were performed to analyse the efficiency of this maturation process. Moreover, secretion of albumin and urea into the supernatant and metabolic assays (Cyp1A1, Cyp3A4) were measured 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 dissociate these organoid-like structures and to harvest the matured hepatic cells and the properties of these cells in drug screening and transplantation studies needs to be revealed in further experiments.

Abstract No. P128

Generation of respiratory epithelial cells from human pluripotent stem cells – new therapeutic approach for (genetic) lung diseases

Saskia Ulrich [1,*], *Sandra Weinreich* [1], *Ralf Haller* [1], *Sandra Menke* [1], *Ruth Olmer* [1], and *Ulrich Martin* [1]

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*Presenting author

The in vitro production of respiratory epithelial (progenitor) cells from human pluripotent stem cells (hPSCs) offers promising new options for the treatment of respiratory diseases. Importantly, efficient technologies for targeted gene correction, based on e.g. zinc finger nucleases (ZFNs) or transcription activator like effector nucleases (TALENs), may facilitate the hPSC-based treatment of genetic lung diseases like cystic fibrosis (CF) and surfactant deficiencies. A prerequisite for such approaches is an efficient and robust differentiation strategy for the in vitro generation of the desired respiratory epithelial cell types.

We therefore aim at the differentiation of human embryonic (hESCs) as well as human induced pluripotent stem cells (hiPSCs) into respiratory epithelial cell types. To evaluate the earliest respiratory differentiation steps, we make use of the human (h)ESC reporter cell line hES3 NKX2.1-GFP (kindly provided by the lab of A. Elefanty) expressing eGFP under the endogenous promoter of NK2 homeobox 1 transcription factor (NKX2.1), known as the earliest marker in lung development. With our current serum-free monolayer-based differentiation strategy we were able to generate highly enriched FOXA2+/SOX17+ and CXCR4+/C-KIT+ definitive endoderm. Via subsequent anteriorization, FOXA2+/SOX2+ anterior foregut endoderm was induced, which then gave rise to NKX2.1-eGFP+ cells. Coexpression of NKX2.1-eGFP with the endodermal marker FOXA2 as well as qRT-PCR analysis of the purified NKX2.1-eGFP+ population indicated a respiratory phenotype of the NKX2.1-eGFP+ cells, most likely excluding relation of the NKX2.1 expression to a neuronal or thyroidal cell fate.

In summary, targeted generation of NKX2.1+ respiratory epithelial progenitor cells could be demonstrated. Future work will focus on further optimization of the differentiation strategy and maturation of the cells with regard to cell replacement therapies as well as for disease modeling, drug screening and toxicity tests in vitro.

Abstract No. P129

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

Sandra Weinreich [1,*], *Saskia Ulrich* [1], *Ralf Haller* [1], *Ruth Olmer* [1], and *Ulrich Martin* [1]

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*Presenting author

One promising therapeutic option to cure hereditary pulmonary diseases like cystic fibrosis might be a cell replacement therapy comprising the generation of patient specific autologous induced pluripotent stem cells (iPSCs), followed by the correction of the genetic mutation, differentiation into the needed airway cell type and replacement of the endogenous cells. For long term restoration, most likely airway progenitor or stem cells like basal cells or submucosal gland duct stem cells will be required. A prerequisite is the development of an efficient and robust protocol for the generation of the desired airway stem cells from human iPSCs (hiPSCs). The transcription factor NK2 homeobox1 (NKX2.1) is expressed in lung epithelial progenitor cells which can give rise to airway stem cells. Thus, NKX2.1 represents a suitable marker for optimizing differentiation protocols. The aim of the present study was the generation of a hiPSC reporter line targeting the NKX2.1 locus. The hHSC_F1285_T-iPS2 line was used for the transfection with the NKX2.1 targeting vector (kind gift of Andrew G. Elefanty), which consists of two homology arms for homologous recombination flanking an eGFP coding sequence and a floxed antibiotic selection cassette. One correctly targeted clone out of 191 neomycin resistant clones was identified by PCR analysis. Southern blot analysis using an eGFP probe verified that the vector had integrated correctly into one of the two NKX2.1 alleles without any further integration sites. Differentiation of the identified clone with our established protocol resulted in up to 67 % eGFP expressing cells, occurring on day 11 of differentiation. The established hiPSC NKX2.1 reporter line represents an optimal tool for the improvement of protocols for the pulmonary differentiation of hiPSCs.

P130 – P161: Stem cells in disease modeling and drug development

(posters will be displayed in the tent)

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Katharina Cullmann

P131 Generation of human spastin-deficient iPS cells and neurons to model hereditary spastic paraplegia in vitro
Kristina Dobrindt

P132 Defining crucial time windows of sensitivity during early neurodevelopment
Nadine Dreser

P133 Improved correction of patient-specific A1AT deficiency iPSC applying double nicking with Cas9_D10A nickase
Reto Eggenschwiler

P134 Degenerative changes in iPSC-derived neurons from individuals with a mutation in the microtubule-associated protein TAU
Marc Ehrlich

P135 Generation of CFTR iPS reporter cell lines using TALEN-based transgene integration for the isolation of human CFTR expressing epithelial cells
Lena Engels

P136 Patient-specific iPS cells as a tool to study Metachromatic leukodystrophy
Kim Lina Erwes

P137 Extracellular vesicle-mediated spread of α-synuclein in the pathogenesis of multiple system atrophy
Julia Fischer

P138 Derivation of retinal neurons and (retinal) pigmented epithelial cells from JNCL patients' iPSCs
Cristina Golfieri

P139 Establishment of mouse neuroepithelial cell lines as a tool for Parkinson's disease modelling
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P140 Generation of disease-specific iPSCs and development of transgenic reporter cell lines for cystic fibrosis disease modelling and drug screening
Madline Götz

P141 Comparative assessment of different types of hSC- derived cardiomyocytes for predictive electrophysiological safety screening
Elke Guenther

P142 Generation of human embryonic stem cell-derived sensory neurons to assess peripheral neurotoxicity
Lisa Hoelting

P143 Generation of exocrine pancreatic cells from plucked human hair derived induced pluripotent stem cell
Meike Hohwieler

P144 Intrinsically active and pacemaker neurons in pluripotent stem cell-derived neuronal populations
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P145 Generation of cortical neurons and glial cells from human induced pluripotent stem cells for the development of phenotypic assays
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P146 Neural derivatives can be obtained from patient-specific B cells for the analysis of psychiatric disease
Matthias Jung

P147 Intracellular Ca2+-release induces nuclear ataxin-3 positive inclusions in Machado-Joseph disease-specific neurons
Johannes Jungverdorben

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P150 Modelling catecholaminergic polymorphic ventricular tachycardia using patient-specific iPSC-derived engineered heart tissues.
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P151 Generation and footprintless gene correction of Cystic Fibrosis-patient derived iPS cells
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P152 Axonal pathology in patient-derived neurons harboring SPG11 mutations: An iPSC model for spatacsin-linked Hereditary Spastic Paraplegia
Himanshu K. Mishra

P153 IPSC-derived hematopoietic cells recapitulate the GM-CSF dependent functional defects of hereditary Pulmonary Alveolar Proteinosis in a murine model
Adele Mucci

P154 Development of an iPSC-based model for Angelman syndrome
Anika Neureiter

P155 Induced pluripotent stem cell models of schizophrenia: a review of published studies
János M. Réthelyi

P156 Generation of a stable and efficient in vitro model to study neuroinflammation in Parkinson´s Disease
Annika Sommer

P157 Maintenance of genomic imprinting in iPSCs derived from Angelman syndrome patient cells
Jana Stanurova

P158 Stem cell transplantation in immunodeficient mice for the detection of engraftment and cell differentiation: useful system for embryotoxicity testing
Maria Stecklum

P159 Inhibition of Notch signaling increases photoreceptor genesis in mESC-derived retina organoids
Manuela Völkner

P160 Efficient and scalable GMP-grade culture media system for rapid cardiomyocyte differentiation of pluripotent stem cells in human disease research
Tim Wessel

P161 Visualization of intestinal stem cells using medaka fish
Narges Aghaallaei

Abstract No. P130

Differentiation and characterization of in vitro-platelets from murine embryonic stem cells

Katharina Cullmann[1,*], *Niels Heinz*[1], *Saskia Kohlscheen*[1], *Lisette Latorre*[1], *Franziska Schenk*[1], and *Ute Modlich*[1]

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Platelets are natural carriers that store coagulation factors and inflammatory modulators and release them from their granules. Due to this natural characteristic they are potential tools for protein delivery. Platelets can be differentiated from ES cells in culture, however, recovered platelet numbers are low. The aim of this study was the optimized in vitro production of functional platelets from murine embryonic stem cells (CCE cells). Murine ESCs were differentiated into the hematopoietic lineage via embryoid body (EB) formation. After EB dissociation, cells were co-cultivated on OP-9 feeder cells, a murine bone marrow stroma cell line known to support the production of platelets in vitro. Medium containing Thrombopoetin (Thpo) further increased differentiation into the megakaryocytic lineage. Cyto-morphological analysis identified megakaryocytes (MK) after 5 days of co-cultivation. Pro-platelet-like structures were detected within the culture at day 6. Flow cytometric analysis of (pro)-platelets released into the cell culture supernatant verified expression of the surface markers CD41 and CD42d by day 6 of co-cultivation and lasted until day 20. Ultrastructure analysis by electron microscopy of in vitro platelets (day 16) identified morphological structures unique for platelets such as granula and the open canalicular system (OCN). Functional assays of in vitro platelets were performed by activation with thrombin and subsequent analyses of CD62P membrane expression which is released from platelet α-granules upon activation. To increase platelet production and release within the culture we genetically modify mES cells to express NF-E2 and GATA1, two transcription factors known to force terminal MK differentiation and platelet release. To avoid off target expression during differentiation we make use of the tet-inducible all-in-one lentiviral vector system that allows controlled transcription by doxocyclin application.

Abstract No. P131

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

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Hereditary spastic paraplegia (HSP) is a relatively rare, heterogeneous group of genetic disorders with progressive spasticity in the lower limbs caused primarily by axonal degeneration of corticospinal motor neurons. The most frequent type of autosomal dominant paraplegia, spastic paraplegia 4 (SPG4), is found in about 40% of all HSP cases and caused by mutations in the SPAST gene, which codes for the microtubule severing enzyme spastin. Here we report the generation of a SPG4 in vitro model based on patient-specific induced pluripotent stem cell (iPSC)-derived cortical cultures. To this end, fibroblasts of family members carrying identical heterozygous SPAST nonsense mutations were reprogrammed yielding several fully validated SPG4 iPSC lines. In order to assess potential HSP-specific phenotypic changes in neurons, iPSCs were differentiated into cortical cultures comprising >80% glutamatergic pyramidal neurons, which express the layer V and VI markers CTIP2 and TBR1, respectively. Neuronal cultures expressed more spastin compared to iPSCs. However, spastin levels in both SPG4 iPSCs and neurons were reduced by approximately 50% compared to controls. We focused on the identification of early neuronal HSP-related phenotypes and observed that one-week-old HSP neurons exhibit a 40% decrease in neurite length as early as 24 hours post plating. In addition, within only five days of neuronal maturation, axonal swellings, a hallmark of HSP, could be detected at a frequency of 1.7 per mm axon length in SPG4 neurons. The diameter of the swellings varied between 1 and 7 µm. They were positive for the axonal marker tau1 and acetylated tubulin. Since these phenotypic changes develop within a time period of only a few days, they should provide a useful basis for the development of standardized assays for studying the pathogenesis of HSP and for identifying therapeutic compounds counteracting neuronal degeneration associated with this disease.

Abstract No. P132

Defining crucial time windows of sensitivity during early neurodevelopment

Nadine Dreser [1,*], *Nina Balmer* [1], *Stefanie Klima* [1], *Tanja Waldmann* [1], *Johannes Meisig* [2], *Nils Blüthgen* [2], and *Marcel Leist* [1]

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In the field of neurodevelopmental toxicity there is an urgent need for appropriate in vitro tests. We established a human embryonic stem cell based test system. However, the major challenge of such a system is that gene expression patterns change already in untreated control conditions during differentiation/ over time. Therefore we hypothesize that not only concentration and duration of treatment matter but also the time window. We used microarray analysis to get (i) a closer insight into the underlying processes of neurodevelopment and (ii) to investigate drug effects for different treatment scenarios.
Using the HDAC inhibitors VPA and TSA as well characterized DNT compounds, we treated the cells for 6h/4d/6d. We found that developmentally regulated genes and drug regulated genes overlapped up to 90% at late time points but showed only a small overlap at the early time point. Furthermore, the cells treated at later time points did not show the capacity to form neural tube like rosettes anymore. This may allow distinguishing between cell biological and developmental toxic effects.
To narrow down the window of sensitivity, cells were treated with TSA for different time periods and at different time points of differentiation. Changes in marker gene expression as well as the capacity of cells to form rosettes were used as an endpoint. We found that the time period of d1-d4 is crucial for the cells to form rosettes and to develop as in control conditions. To model the processes taking place during this crucial time window and to investigate how and where toxins could interfere, gene expression of undisturbed early differentiation was modeled and analyzed in a tight time course.

Abstract No. P133

Improved correction of patient-specific A1AT deficiency iPSC applying double nicking with Cas9_D10A nickase

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Alpha 1-antitrypsin (A1AT) is secreted by hepatocytes and serves as the major serum proteinase inhibitor. A congenital point mutation results in a destabilized form (Z-A1AT), which polymerizes and accumulates in producer cells. Affected homozygous individuals (ZZ) have a 90% reduction of A1AT serum levels, which leads to chronic obstructive pulmonary disease and to liver cirrhosis caused by aggregating misfolded proteins.
Here, we aimed to correct the Z-A1AT point mutation by CRISPR/Cas9 based genomic precision engineering in induced pluripotent stem cells (iPSC) generated from a ZZ patient with chronic liver disease. First, we evaluated the gene targeting potency of A1AT-specific gRNAs with Cas9_D10A nickase in an in vitro reporter assay. We used two opposite strand gRNAs for introduction of a double nick with 5' overhang and different amounts of donor plasmid with homologous flanking arms. This protocol yielded up to 1% homology directed repair (HDR) gene targeting efficiency in our reporter cells. Subsequently, we compared the gene targeting accuracy of Cas9 nuclease and Cas9 nickase in ZZ-iPSC by transfection of a piggyBac-flanked puromycinΔtk-selectable correction donor. Applying multiplex PCR we found that most of the Cas9 nuclease targeted clones had off-target or erroneous on-target integrations, while all of the nickase targeted clones showed correct monoallelic integration in the A1AT locus. Moreover, when we used the double nick technique with two gRNAs, we found that the donor had correctly integrated on both alleles in 40% of all clones. We then transfected these biallelic targeted clones with piggyBac transposase for excision of the selection cassette and subjected the cells to FIAU for counter-selection.
Taken together, the introduction of two opposite strand nicks together with an appropriate donor allows precise CRISPR/Cas9-based genome engineering in patient-specific iPSC. Using this technology, we have successfully targeted the A1AT locus for genetic correction of severe A1AT deficiency.

Abstract No. P134

Degenerative changes in iPSC-derived neurons from individuals with a mutation in the microtubule-associated protein TAU

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Frontotemporal dementia (FTD) is the second most common cause of neurodegenerative dementia, accounting for about 20 per cent of all pre-senile dementias. Mutations in the microtubule-associated protein tau (MAPT) have been linked to a familial form of FTD and result in accumulation of phosphorylated tau in neurons and glia. However, the underlying aetiology of the disease is still unknown due to the lack of available human neurons from patients with FTD. A human *in vitro* model based on patient-specific neurons could overcome these limitations and provide a powerful tool for studying the pathogenesis of the disease.
In this study, we investigated induced pluripotent stem cell (iPSC) derived neurons from two individuals with a MAPT gene mutation and compared them to iPSC-derived neurons from two healthy control individuals. We first applied an *in vitro* differentiation protocol to generate neural progenitor cells which were limitless expandable and efficiently differentiated to midbrain-like dopaminergic neurons expressing TUJ1, MAP2, TH and FoxA2 with comparable differentiation capabilities amongst all cell lines. Immunocytochemical analysis of these neurons exhibited a significant increase of hyperphosphorylated tau in MAP2-positive neurons from FTD-patients as well as an impaired neurite outgrowth compared to neurons from healthy individuals, revealing disease-related phenotypes. Further analysis of tau expression on protein level showed a significant increase of tau fragments compared to full-length tau in disease-affected neurons. In contrast, we did not observe differences in full-length tau expression on RNA levels. Finally, the application of the neurotoxin rotenone resulted in a decreased cell viability of FTD-neurons accompanied by a significant increase of TH and cleaved CASPASE3 double-positive cells indicating increased oxidative stress in FTD-iPSC-derived neurons. Therefore, our patient-specific iPSC-based model provides a suitable platform to shed light on the underlying disease mechanisms of FTD and might be a valuable tool for drug screening assays.

Abstract No. P135

Generation of CFTR iPS reporter cell lines using TALEN-based transgene integration for the isolation of human CFTR expressing epithelial cells

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Cystic Fibrosis (CF) is the most common autosomal recessive disorder in western countries, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, a chloride channel expressed primarily at secretory epithelia in the airways, intestine, liver and other tissues. Pluripotent stem cell (PSC)-derived CFTR-expressing epithelial may represent excellent cell sources for CF disease modelling, drug screening and ultimately cellular therapies. Despite significant progress in targeted differentiation of airway and bile duct epithelia, efficient generation of mature CFTR-expressing iPSC derivatives will require further optimization that is complicated by the lack of reliable anti-CFTR antibodies.

Thus, we focussed on the generation of knock-in reporter cell lines monitoring the expression of the CFTR gene during differentiation of PSCs. CFTR specific transcription activator-like effector nucleases (TALENs) and an appropriate donor construct were designed, both to be used in our efficient targeting protocol for the integration of an antibiotic resistance linked to a dTomato fluorescence protein into the exon 1 of the endogenous CFTR locus by homologous recombination.

We were able to generate several human iPS as well as ES cell clones carrying the reporter cassette. PCR analyses revealed correct heterozygous or homozygous targeting of several clones obviously without any influence on the cellular characteristics of the undifferentiated PSC clones. First differentiation experiments aiming at bile duct specification revealed CFTR-dTomato+ cells appearing on day 17 of differentiation. Further optimization of the differentiation protocol as well as the verification of the CFTR specificity of the dTomato-positive cells is currently ongoing. The establishment of our transgenic CFTR reporter PSC lines will facilitate the development of robust and reliable differentiation and selection protocols for CFTR expressing epithelial cells as important prerequisite for CF disease modelling and high throughput drug screening.

Abstract No. P136

Patient-specific iPS cells as a tool to study Metachromatic leukodystrophy

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The lysosomal storage disorder Metachromatic leukodystrophy (MLD) is caused by a functional deficiency of the metabolic enzyme arylsulfatase A (ARSA). ARSA is involved in the lysosomal degradation of the membrane lipid 3-O-sulfogalactosyl ceramide (sulfatide). In MLD patients' brains sulfatide is not degraded and accumulates mainly in oligodendrocytes. This leads to demyelination and severe neurological symptoms. Yet, the underlying cellular pathomechanisms remain largely enigmatic. This may be due to the fact that current animal models do not fully recapitulate MLD-associated symptoms. In this study, fibroblasts from four MLD-patients with distinct mutations in the ARSA gene were successfully reprogrammed into induced pluripotent stem cells (iPSC) using non-integrating Sendai viral vectors. Subsequently, fully validated MLD-iPSC as well as healthy control iPSC clones were converted into radial glia-like neural stem cells (RGL-NSC), which readily differentiate into all major neural lineages upon growth factor withdrawal. ARSA enzyme activity assays confirmed the functional enzyme deficiency in patient-specific cells. Upon differentiation along the oligodendrocyte lineage sulfatide deposits were specifically found in association with O4-positive immature oligodendrocytes. However, prominent differences between patient and control oligodendroglial cells were not discernable in this short-time in vitro culture system. In order to ensure long-term maturation of oligodendrocytes in a disease-relevant microenvironment, we generated a novel MLD mouse model, which combines ARSA-deficiency with congenital myelin-deficiency. Histological analyses revealed pronounced sulfatide deposits particularly within the myelin-deficient white matter tracts. First transplantation studies into neonatal recipients indicate that RGL-NSCs survive for at least twelve weeks. Grafted cells were able to differentiate into MBP-positive oligodendrocytes in large parts of the corpus callosum. We expect this xenograft model to provide insights into oligodendroglial dysfunction in the context of ARSA-deficiency.

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Abstract No. P137

Extracellular vesicle-mediated spread of α-synuclein in the pathogenesis of multiple system atrophy

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Intercellular communication and cargo delivery (e.g. proteins and nucleic acids) via small extracellular vesicles (EVs) under physiological and pathophysiological conditions has gained much attention throughout recent years. For the central nervous system it has been proposed that EV-mediated trafficking might contribute to the cellular spread of pathological agents, eventually culminating in severe neurodegeneration. Here, we address the question whether EV-mediated spread of α-synuclein (SNCA) might contribute to the pathogenesis of Multiple System Atrophy (MSA), a spontaneous neurodegenerative disorder characterized by neuronal and oligodendroglial cytoplasmic SNCA inclusions. To this end, we initially generated hiPSCs from MSA patients and further differentiated these cells into a radial glia-like neural stem cell population (RGL-NSCs). RGL-NSCs readily give rise to neurons, astrocytes, and, most importantly, oligodendrocytes. RT-PCR and immunocytochemical analyses revealed that SNCA is strongly expressed by neurons in our culture system. In contrast, oligodendrocytes (OLs) do not express the SNCA gene. However, they exhibit strong SNCA immunoreactivity in mixed neural cultures differentiated from RGL-NSCs, suggesting a yet unknown uptake-mechanism of SNCA by oligodendrocytes. Upon employing Western-blot analyses of neuronal cell culture supernatants we detected SNCA and CD63, a tetraspanin typically used as EV marker protein. We next harvested EVs from neuronal cell culture supernatants. Biochemical and biophysical characterization demonstrated that SNCA was present in vesicles with diameters of 120-170 nm. These data imply that SNCA being expressed in neurons is secreted as an EV cargo protein and taken up by oligodendrocytes via EV trafficking. Interestingly, pathological aggregation of SNCA can be induced in both neurons and oligodendroytes via inhibition of mitochondrial or proteasomal function. We expect this iPSC-based culture model to provide novel insights into the pathomechanisms underlying MSA-associated SNCA spread and aggregation.

Abstract No. P138

Derivation of retinal neurons and (retinal) pigmented epithelial cells from JNCL patients' iPSCs

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Introduction: Neuronal Ceroid Lipofuscinosis (NCL) is a group of progressive neurodegenerative lysosomal storage disorders. The most common form is the juvenile one (JNCL or Batten disease), caused by a mutated version of CLN3 gene. JNCL leads to retina degeneration, followed by CNS neurodegeneration and premature death. Interestingly, whereas in JNCL patients retina is affected first, retinal defects are not severe and appear only later in JNCL mouse models. Therefore, human cell based-models might enable new insight into pathomechanisms and therapy development.
Methods: JNCL hiPSC have been generated from fibroblasts of two JNCL patients and one healthy donor carrier for the common mutation[1]. Taking advantage of protocols established in the last years[2], we investigated JNCL hiPSCs potential to differentiate into neuronal lineage, retina and (retinal) pigmented epithelium.
Results: Here we show that, following dissociation to single cells, JNCL hiPSCs efficiently reaggregated when plated onto low adhesion 96-wells plates. During the following days aggregates became more compact, acquired some patterning (translucent/brighter vs darker areas) and formed potential optic vesicle(OV)-like epithelial evaginations. OV-like structures excised and cultured in retina maturation conditions continued to grow, developed pigmentation and expressed Rax eye-field transcription factor. After one month in culture we observed formation of neural retina (including ganglion cells [Brn3+] and photoreceptor precursor [Crx+]) and pigmented epithelial structures (potentially RPE or ciliary epithelium).
Conclusions: We conclude that JNCL hiPSCs can be used to obtain JNCL patients'-specific neural retina and pigmented epithelium samples. It has been already shown that JNCL hiPSCs-derived neurons display progressive storage material accumulation and organelle abnormalities typical of JNCL disease[1]. Because of its early retinal disease onset, progressive neurodegeneration and potential involvement of different retinal cell types, we hypothesize that hiPSCs-derived retinal cells will be a great tool to model JNCL disease, study retinal cell specific pathomechanisms and possibly discover/validate therapies.

[1] Lojewski, X. et al. Human iPSC models of neuronal ceroid lipofuscinosis capture distinct effects of TPP1 and CLN3 mutations on the endocytic pathway. Hum Mol Genet (2013).

[2] Nakano, T. et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell stem cell 10, 771-785 (2012).

Abstract No. P139

Establishment of mouse neuroepithelial cell lines as a tool for Parkinson's disease modelling

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide. The pathology of PD is characterized by a selective loss of dopaminergic (DA) neurons in the substantia nigra pars compacta, responsible of the motor symptoms of the disease. So far, there is no suitable PD mouse model, therefore there is an unmet need for more suitable methods for drugs screening that allow the "easy" identification of neuro-protective drugs. Furthermore, the production of dopaminergic neurons in culture is inefficient and the establishment of protocols and cell lines capable of differentiate into dopaminergic neurons is essential for their use as an ,*in vitro* and *in vivo* model for PD.
In the present work we have established a new cell culture model of mouse neuroepithelial stem cells (mNESCs). We investigated the possibility of obtaining mNESCs using different starting materials, such as mouse epiblast stem cells (mEpiSCs), mouse embryonic stem cells (mESCs) and mouse embryonic tissue. Our cell culture system is based on the addition of chemically defined small molecules, including modulators of developmental signalling pathways, including Wnt and Sonic hedgehog signalling. The derived cultures are suitable for *in vitro* propagation as well as multilineage differentiation. Furthermore we demonstrate that they are able to efficiently differentiate into midbrain dopaminergic neurons, constituting a valuable tool for transplantation and *in vitro* disease modelling.

Abstract No. P140

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 that overexpress mutant CFTR variants have been used for disease modelling and to screen compound libraries. In fact, CFTR protein-modulators have been identified and are currently under clinical investigation, 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: lung, liver, pancreas and intestine. To address these unmet needs we focus on the generation of induced pluripotent stem cell (iPSC) lines from patients with the most common (F508del) trafficking mutation, including known intragenic haplotypes with mild, moderate and severe phenotypes. Therefore CF-iPSCs were reprogrammed from CD34+ cells isolated from small volumes (20 ml) of non-mobilized peripheral blood. The resulting CF-iPSCs are currently characterised in detail. Moreover, a halide sensitive yellow fluorescent protein (YFP) was introduced into the safe harbour locus AAVS1 of wild-type human iPSCs via transcription activator-like effector nuclease (TALEN) induced homologous recombination to enable monitoring of CFTR function in an iPSC-based high-throughput screening assay. The established reporter cell lines showed robust transgene expression without obvious negative effects on cell viability, phenotype and culture characteristics for up to 28 passages. Furthermore, the transgenic cells differentiated into all three germlayers, an important prerequisite for the differentiation towards CFTR-expressing epithelial cells. Hence, the stable integration of this halide reporter into a safe harbour locus of CF-patient-specific iPSCs should enable disease modelling of F508del-based CF with regard to the individual genetic context and the implementation of high-throughput screenings for novel correctors and potentiators of CFTR trafficking mutations.

Galietta LJ, Haggie PM, Verkman AS. Green fluorescent protein-based halide indicators with improved chloride and iodide affinities. FEBS letters. 2001;499(3):220-4. Epub 2001/06/26

Abstract No. P141

Comparative assessment of different types of hSC- derived cardiomyocytes for predictive electrophysiological safety screening

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The need for new strategies in preclinical compound testing is under intense debate in the pharmaceutical industry. In this respect, cardiomyocytes generated from human stem cells (hSCs) are regarded as a promising source to develop meaningful in vitro test models of adverse cardiovascular effects, including electrophysiological safety screening. However, it still has to be shown that human stem-cell based approaches result in more predictive data then currently available methods. Our study is the first to compare the electrophysiological phenotype and pharmacological profile of different types of hSCs by means of microelectrode array (MEA) recordings and to relate it to existing electrophysiological preclinical cardiac safety models.

Commercially available human embryonic or induced pluripotent stem-cell derived cardiomyocytes (hiPSCs, hESCs) from three providers were electrophysiologically validated against ten compounds with different modes of action. Spontaneous field action potentials (fAP) were recorded from the cells directly seeded on the recording electrodes of 6-well MEAs.

Compound effects comprised changes of the initial phase of the fAP (Na+ component), fAP duration as well as changes of the spontaneous beating frequency and regularity. For most of the reference compounds, all cell types investigated expressed the same alterations in the parameters analyzed in response to the compounds, but with different sensitivities. Comparison of our results with literature data from other preclinical cardiac safety models revealed in most but not all cases a good pharmacological correlation for all cell types tested.

Based on these findings, we conclude that hSC-derived cardiomyocytes in principle are a promising cell source for electrophysiological cardiac safety assays but still need to be improved towards the expression of a mature electrophysiological phenotype to avoid false-negative or -positive responses.

Abstract No. P142

Generation of human embryonic stem cell-derived sensory neurons to assess peripheral neurotoxicity

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Human embryonic stem cell (hESC) technology provides a tool to recapitulate relevant aspects of early neurodevelopment, such as proliferation, differentiation, migration, axonogenesis and synaptogenesis. For the central nervous system, even small disruptions in these processes may result in a severe impairment of normal function and lead to neuropathogenesis and developmental disabilities. At present, little is known about how the developing peripheral nervous system (PNS) is affected by toxicants.

We intended to establish a human neurogenesis model based on hESCs to assess peripheral neurotoxicity by using neurite growth and calcium signaling as functional endpoints.

Based on the combination of small molecule inhibitors that induced the differentiation of hESCs into sensory neurons [1], we generated a population of peripheral neuronal progenitor cells within 8 days of differentiation. After this stage, cells were cryopreserved. Freshly thawed cells developed neurites and formed a dense neurite network, which was quantified by live cell imaging. Moreover, hESC-derived sensory neurons showed strong calcium signaling upon depolarization.

Quantification of neurite growth and of calcium responses on single cell level were optimized as endpoints to assess peripheral neurotoxicity. In this system we identified compounds with several biological activities that had an inhibiting as well as an accelerating effect on neurite growth.

Our data indicates that we have established a model for human sensory neuron development, which is capable to detect potential developmental peripheral neurotoxic compounds.

Chambers, S.M. et. al., Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. Nat Biotechnol, 2012. 30(7): p. 715-20.

Abstract No. P143

Generation of exocrine pancreatic cells from plucked human hair derived induced pluripotent stem cells

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Human induced pluripotent stem (iPS) cells can be generated from easily accessible tissues such as skin or plucked hair. 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 iPS cells towards the pancreatic lineage remains inefficient and variable. Moreover, current protocols are mostly aiming to generate endocrine cells while exocrine differentiation protocols remain elusive. Herein, we report the generation of human keratinocyte-derived iPS cells from healthy donors. We successfully applied a novel and stepwise pancreatic differentiation protocol aiming to generate exocrine pancreatic cells. With high efficiency we produce Pdx1- and Nkx6.1-expressing pancreatic progenitor cells that can be further differentiated into amylase-positive cells. This set-up will provide the basis for future studies dissecting the pathophysiology of exocrine pancreatic disease in a dish but patient specific context.

Abstract No. P144

Intrinsically active and pacemaker neurons in pluripotent stem cell-derived neuronal populations

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Autonomous network activities rely on neurons, which are able to be spontaneously active even in conditions where fast synaptic communication has been silenced. These neurons have therefore been termed intrinsically active neurons (IAN). IAN can be found in nearly all CNS regions during the development and in the adult brain. Thus, IAN represent key neuronal elements which spontaneously induce activities in other neurons and might regulate different types of orchestrated activities in neuronal assemblies. Neurons generated from pluripotent stem cells self-organize into functional neuronal assemblies in vitro generating synchronous network activities. Intriguingly, pluripotent stem cells derived (PSC)-neuronal assemblies develop spontaneous activities that are independent of external stimulation, suggesting the presence of so-far undetected intrinsically active neurons (IAN). Here, by using mouse embryonic stem cells, we provide evidence for the existence of IAN in PSC-neuronal networks based on extracellular multi-electrode array- as well intracellular patch-clamp-recordings. IAN remain active after pharmacological inhibition of fast synaptic communication and possess intrinsic mechanisms required for autonomous neuronal activity. PSC-derived IAN are functionally integrated in PSC-neuronal populations, contribute to synchronous network bursting, and exhibit pacemaker properties. The intrinsic activity and pacemaker properties of the herein identified neuronal sub-population might be particularly relevant for interventions involving transplantation of neural tissues. IAN might be a key element in the regulation of the functional activity of the grafted as well as the pre-existing host neuronal networks.

Illes S, Jakab M, Beyer F, Gelfert R, Couillard-Despres S, Schnitzler A, Ritter M, Aigner L. Intrinsically active and pacemaker neurons in pluripotent stem cell-derived neuronal populations. Stem Cell Reports. 2014 Feb 20;2(3):323-36.

Abstract No. P145

Generation of cortical neurons and glial cells from human induced pluripotent stem cells for the development of phenotypic assays

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Successful transfer of disease treatments from bench to bedside suffers often from poor translation of results obtained in commonly used rodent in vitro cellular models or in vivo animal models. The human induced pluripotent stem cell (hiPSC)-technology offers the possibility to build an ethically acceptable human-based drug discovery platform for target identification, compound profiling and in vitro disease modelling in specialized human cell types.

This study demonstrates the differentiation of hiPSC lines to cortical neurons and glial cells. Molecular and functional characterization studies carried out during several stages of the differentiation process employing RT-qPCR and immunofluorescence staining demonstrated an increase in expression of typical cortical markers (e.g. FOXG1, TBR1, CTIP2) as well as glial markers (e.g. GFAP, S100ß). In addition neurotransmitter response studies demonstrated the activity of glutamatergic receptors in the differentiated cortical neurons.

This in vitro system will be used for phenotypic assays to model neurodegenerative disorders.

Acknowledgements

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Disclosures

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Abstract No. P146

Neural derivatives can be obtained from patient-specific B cells for the analysis of psychiatric diseases

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Objectives: Psychiatric diseases such as schizophrenia are multifactorial disorders caused by the dysfunction of neural cells. Currently, the molecular mechanisms regulating healthy and diseased development and maturation of neurons and glia cells are poorly understood. We recently applied genome-wide association studies (GWAS) to identify copy number variation (CNVs) and/or single nucleotide polymorphisms (SNPs), which are associated with onset, progression, and treatment of psychiatric disorders. The production of patient-specific induced pluripotent stem (iPS) cells and their neural differentiation provides a potent tool to analyze the impact of DNA variations.

Materials and Methods: B lymphoblastoid cell lines (B LCLs) obtained from healthy donors were treated with non-viral reprogramming vectors. Efficiency of vector delivery was analyzed by flow cytometry. The mRNA and protein expression of pluripotency marker genes was analyzed. Further, endogenous induction of reprogramming factors was studied. After induction of pluripotency factors, cells were differentiated into neural progenitors. The mRNA and protein expression of neuron- and glia-associated marker genes was verified.

Results and Conclusion: The application of the non-viral reprogramming vectors induced pluripotency-associated marker genes including Oct4, Sox2, Nanog, Lin28, Klf4, and c-Myc. Their expression was stable for several passages on the mRNA and on the protein level. The endogenous expression of pluripotency factors was verified. The transfection procedure revealed about 20% transfected cells at day 3. The induction of pluripotency factors enabled the induction of neural cell fates. Pax6 and Sox2 protein were verified during early neural differentiation suggesting the generation of neural progenitors. NeuN, TUBB3, GFAP, and O4 protein expression showed the differentiation of neural progenitors. The induction of GABA-positive cells suggests the conversion into neural derivatives suitable to for the analysis of psychiatric diseases.

In conclusion, conversion of patient-specific B cells into neural derivatives provides a powerful tool to study psychiatric disorders within disease-specific differentiation models.

Abstract No. P147

Intracellular Ca2+-release induces nuclear ataxin-3 positive inclusions in Machado-Joseph disease-specific neurons

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Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 is the most frequent form of inherited spinocerebellar ataxias worldwide. Expansion of a polyQ repeat increases the aggregation propensity of ataxin-3 and thereby causes the generation of ataxin-3-positive inclusions. Previous studies employing MJD iPSC-derived neurons showed that excitation by glutamate or NMDA activates calpain-mediated cleavage of ataxin-3, which initiates the subsequent generation of SDS-insoluble ataxin-3 microaggregates (Koch et al., 2011). However, this approach requires long-term differentiation of iPSC-derived neurons across 4-6 weeks and yields only microaggregates invisible in conventional microscopy. Here we set out to establish an iPSC-based assay for ataxin-3 aggregate formation, which is amenable to high content imaging and which bypasses lengthy neuronal differentiation protocols unsuitable for high throughput analysis. Reasoning that calpain-mediated cleavage depends strongly on intracellular calcium levels, we investigated the early expressed purinergic receptor family for cytosolic calcium rise induction and found that ATP-stimulation of P2Y receptors enables 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. Yet, both glutamate and ATP-mediated aggregation rely on receptors types with largely variable spatio-temporal distribution patterns. To develop a more general and maturation-independent stimulation paradigm, we explored direct modulation of intracellular calcium homeostasis via activation of the ubiquitously present ryanodine receptors as well as inhibition of sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) as alternative routes to induce aggregate formation. Both stimulation paradigms resulted in ataxin-3 NIIs after only 8 days of differentiation with SERCA inhibition yielding up to 80% MJD neurons carrying ataxin-3-positive aggregates. Our data suggest that direct dysregulation of intracellular calcium homeostasis offers a time-saving opportunity to model pathological protein aggregation in MJD patient-derived neurons.

Abstract No. P148

Disturbed neurodevelopment in a hESC-based in vitro system and its epigenetic changes

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There is an urgent need for new test strategies in testing developmental neurotoxicity (DNT). Therefore in vitro systems based on hESC are a promising tool in the field of DNT. Here we introduce a system to investigate cellular memory on an epigenetic level. We differentiate hESC for 6 days towards neuroectodermal precursor cells (NEPs) and treat them for different time periods with the histone deacetylase (HDAC) inhibitor trichostatin A (TSA). There we could observe that the primary mode of action, a global increase in histone acetylation, was increased quickly and returned to baseline after 48h. Instead, histone H3 lysine methylation of the neurodevelopmental regulators PAX6 and OTX2 was affected and remained persistent. These alterations correlated with neurodevelopmental defects and with changes in PAX6 expression, even with a drug washout after 3 days. We hypothesised that drug exposures altering only acetylation lead to reversible transcriptome changes (indicating MoA) and altered methylation leads to irreversible developmental disturbances. Data from pulse-chase experiments corroborated this assumption. Short drug treatment triggered reversible transcriptome changes; longer exposure disrupted neurodevelopment. The disturbed differentiation was reflected by an altered transcriptome pattern. The changes were similar after a 4 days washout. We conclude that transcriptome data after prolonged treatment of differentiating cells mainly reflects the altered developmental stage of the model system and not the drug MoA.

Abstract No. P149

TDP-43 pathology and cellular phenotypes in iPSC-derived cortical neurons of patients with sporadic ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder affecting cortical as well as spinal motor neurons. The characteristic neuropathological feature found in post mortem tissue samples are cytoplasmic TDP-43 inclusions in sporadic ALS (sALS). Since animal models cannot be used for studying sporadic disorders, the recently discovered advantage of reprogramming somatic cells into induced pluripotent stem cells (iPSC) brought a new opportunity of revealing disease mechanisms of sALS.
We successfully differentiated iPSC from sALS and controls into cortical neurons (CN) of layer V according to a previously published protocol (Shi et al., 2012). Punctual cytoplasmic staining and insoluble, truncated TDP-43 was observed in cortical stem cells (CSC) indicating presence of TDP-43 pathology-like alterations in neural progenitor cells. Longer differentiation into CN showed a deterioration of the TDP-43 aggregation. Additionally increases in endogenous stress markers are present in CN of sALS. Moreover a decrease in neurite length and branching points is detected. The histone-acetyl-transferase inhibitor anacardic acid that recently was shown to rescue neurite length defects in lower motor neurons of an iPSC-model of familial ALS (Egawa et al., 2012), was also able to restore the neurite length but not the branching of our CN of sALS. Here we show that iPSC are an adequate model to study sporadic neurodegenerative disorders and in addition are a suitable platform for drug screenings.

Shi, Y., Kirwan, P. and Livesey, F.J. (2012a) Directed differentiation of humanpluripotent stem cells to cerebral cortex neurons and neural networks. Nat. Protoc., 7, 1836–1846.

Egawa, N., Kitaoka, S., Tsukita, K., Naitoh, M., Takahashi, K., Yamamoto, T., Adachi, F., Kondo, T., Okita, K., Asaka, I. et al. (2012) Drug screening for ALS using patient-specific induced pluripotent stem cells. Sci. Transl. Med., 4, 145ra104.

Abstract No. P150

Modelling catecholaminergic polymorphic ventricular tachycardia using patient-specific iPSC-derived engineered heart tissues.

David Letuffe-Brenière [1,*], *Sebastian Schaaf* [1], *Kaja Breckwoldt* [1], *Christiane Neuber* [1], *Ingra Vollert* [1], *Anika Benzin* [1], *Tessa Werner* [1], *Thomas Schulze* [1], *Thomas Eschenhagen* [1], and *Arne Hansen* [1]

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Background and objectives:
iPSCs were proven to efficiently model Mendelian diseases in vitro. catecholaminergic polymorphic ventricular tachycardia (CPVT) is a disease involving a mutation in the ryanodine receptor 2 (RyR2), provoking life threatening arrhythmias. The disease was already successfully modelled in patient-specific iPSC-derived cardiomyocytes, however studies performed in single cells ignore the complexity of cell/cell interaction. In this study, we used fibrin matrix based engineered heart tissue (EHT) to provide multicellular complexity level to patient-specific iPSC-derived cardiomyocytes and to confirm a typical CPVT phenotype.
Method:
iPSCs from two different CPVT patients and one healthy individual were cultured in mouse embryonic fibroblast conditioned medium. Differentiation was performed according to a custom protocol based on embryoid bodies formation and sequential growth factors inductions.
Cardiomyocytes were then dissociated and embedded in a fibrin matrix between two flexible silicone posts. Contractility was then assessed by video recording and a custom programmed figure recognition software. The EHTs were then exposed to a variety of ion channel agonists, antagonists and different calcium concentrations.
Results:
Baseline contractility recordings showed a relaxation prolongation in CPVT EHTs compared to control EHTs. Disease-specific EHTs also presented early after depolarisations when incubated for over 7 hours in a high calcium containing medium, in contrast to control muscles. Those prolongations and arrhythmias could however not be rescued by classical RYR2 stabilising drugs like JTV-519, flecainide or dantrolene. Exposure to sodium, potassium and calcium channel agonists and antagonists displayed expected effects on frequency, relaxation and contraction times.
Conclusion and perspective:
Our data suggest that EHTs are a good model to include the complexity of cell/cell interaction of a heart tissue. However, the findings should be further confirmed by additional EHTs from CPVT patients and control individuals to increase statistical relevance.

Abstract No. P151

Generation and footprintless gene correction of Cystic Fibrosis-patient derived iPS cells

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Cystic Fibrosis (CF) is a monogenetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Current therapies for CF still deal with treating the symptoms, but the availability of human induced pluripotent stem cells (iPSCs) with their almost unlimited potential for proliferation and differentiation and the ability to genetically modify iPSCs offers novel opportunities for the development of CF-patient-specific regenerative therapies, in vitro disease modelling or the establishment of drug screening assays. A powerful method to manipulate the genome and correct genetic defects in iPSCs represents the gene targeting by homologous-recombination via customized designer nucleases. Thereby, the omission of genetic selection cassettes and the application of single-stranded oligodeoxynucleotides (ssODN) would maintain the original CFTR gene structure and influences of patients' specific genomic background.

For the generation of patient-specific iPSCs, endothelial cells from the peripheral blood of CF-patients were reprogrammed. The CF-iPSCs morphologically resemble human ESCs, express pluripotency markers and analysis of CFTR expression from the mutated locus of CF-iPSCs during differentiation revealed mRNA expression on a similar level to wild-type iPSCs and also the presence of immature CFTR protein. For gene targeting approaches we established an efficient designer-nuclease based protocol in human pluripotent stem cells for footprintless gene editing(ref1) which served as the basis for the targeting of the F508del-mutation in our CF-iPSCs. CFTR targeting using specific TALENs and an appropriate ssODN carrying the 3 missing base pairs CTT was performed and subsequent wild-type-specific PCR screening revealed correct targeted single cell clones which are currently characterized more in detail.

The generation and footprintless genetic correction of CF-patient specific iPSCs using the TALEN technology and the differentiation of corrected CF-iPSCs towards secretory epithelial cells will allow for the investigation of the clinical heterogeneity of the disease which might ultimately contribute to the development of novel individualized therapies.

Merkert, S. et al., (2014). Efficient designer nuclease-based homologous recombination enables direct PCR screening for footprint less targeted human pluripotent stem cell clones. Stem Cell Reports 2, 107-118.

Abstract No. P152

Axonal pathology in patient-derived neurons harboring SPG11 mutations: An iPSC model for spatacsin-linked Hereditary Spastic Paraplegia

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Hereditary spastic paraplegias (HSPs) are a heterogeneous group of inherited motor neuron diseases characterized by progressive spasticity and weakness of the lower limbs. They are classified genetically as autosomal dominant, autosomal recessive and X-linked HSP. Mutations in the Spastic Paraplegia Gene11 (SPG11), encoding spatacsin, cause the most frequent form of autosomal recessive HSP. Partly due to lack of a relevant disease model, the underlying molecular mechanisms have not been studied in detail. To overcome this limitation we, for the first time, generated induced pluripotent stem cells (iPSCs) from two SPG11 patients, having heterozygous nonsense and/or splice site mutations, and two age matched controls. We differentiated these iPSCs into forebrain neurons and investigated the neuronal pathology associated with the disease. SPG11 patients' derived neurons exhibited severely impaired outgrowth and branching of axonal processes, implicating a compromised neuritic complexity compared to controls. Gene expression analysis further revealed down regulation of specific motor, synaptic and microtubule associated genes. A reduced expression of acetylated tubulin in the neuronal cells was indicative of the axonal instability, which was further corroborated by ultra structural analysis of these cells showing pathological accumulation of membranous bodies within axonal processes. Finally, time lapse assays performed in SPG11 patients' derived neurons highlighted a reduction in the anterograde vesicle trafficking indicative of impaired axonal transport. Altogether, our SPG11-iPSC model provides the first evidence that mutations in SPG11 have a detrimental effect on the homeostasis of neuronal cells and more importantly disturb the critical balance of transport activity in SPG11 patients' neurons. Furthermore, our human model offers an ideal platform to define new targets to intervene the course of this progressing motor neuron disease.

Stevanin, G., Santorelli, F.M., Azzedine, H., Coutinho, P., Chomilier, J., Denora, P.S., Martin, E. et al. (2007) Mutations in SPG11, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum. Nat. Genet., 39, 366–372

Hehr, U., Bauer, P., Winner, B., Schule, R., Olmez, A., Koehler, W., Uyanik, G. et al. (2007) Long-term course and mutational spectrum of spatacsin-linked spastic paraplegia. Ann. Neurol., 62, 656–665

Abstract No. P153

IPSC-derived hematopoietic cells recapitulate the GM-CSF dependent functional defects of hereditary Pulmonary Alveolar Proteinosis in a murine model

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Following the discovery of induced pluripotent stem cells in 2006 the interest has been focused increasingly on expanding their applicability into different areas such as disease modeling. The use of disease modeling not only allows for the discovery of mechanisms underlying a given disease but also enables the evaluation of alternative treatment options. Hereditary pulmonary alveolar proteinosis (herPAP) is an extremely rare lung disease resulting from the inability of alveolar macrophages to clear the alveolar spaces from surfactant material. This defect is due to mutations within the genes encoding the α- or β-chain of the GM-CSF receptor, CSF2RA or CSF2RB, respectively. Thus far only a symptomatic treatment is available consisting of intensive antibiotic therapy and repetitive whole lung lavage. Thus, we here applied iPSC technology to model herPAP in a relevant murine disease model for Csf2rb-deficiency (Csf2rb-/- mice).
iPSCs were generated from lin- bone marrow cells of Csf2rb-/- mice utilizing lentiviral overexpression of the standard Yamanaka-factors OSKM. The resulting PAP-iPSCs displayed all major pluripotency criteria and could be differentiated into CD41+ hematopoietic progenitor cells (HPC). In contrast to control iPSC-HPCs, PAP-iPSC-HPCs failed to form colonies in a clonogenic assay supplemented with GM-CSF only. CD41+-HPCs could also be further differentiated into macrophage-like (Mϕ) cells expressing the classical surface markers of bone marrow-derived macrophages. When PAP-iPSC-Mϕ were subjected to GM-CSF-dependent functional assays, such as phosphorylation of the transcription factor STAT5, PAP-iPSC-Mϕ, contrary to control iPSC-Mϕ, recapitulated the deficiency in GM-CSF signaling typical of herPAP.
In summary, we generated murine Csf2rb-deficient iPSC lines, which upon hematopoietic differentiation recapitulated GM-CSF dependent functional defects characteristic of PAP.

Abstract No. P154

Development of an iPSC-based model for Angelman syndrome

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The Angelman syndrome is a rare neurodevelopmental disorder caused by lack of the E3 ubiquitin ligase UBE3A in neurons. Clinically, the disease is characterized by seizures, frequent laughter, microcephaly, movement disturbances, hyperactivity, developmental delay, and an absence of speech and language. In neurons of healthy individuals, the *UBE3A* gene is expressed only from the maternal chromosome due to silencing of the paternal copy during neuronal development. Silencing is caused by allele-specific expression of an antisense, long non-coding RNA, which overlaps the entire paternal *UBE3A* gene. In neurons of Angelman syndrome patients, the maternal *UBE3A* locus is defective thus resulting in the complete absence of the UBE3A enzyme. Because primary neurons from the brains of patients are inaccessible, we are developing patient-specific neurons from induced pluripotent stem cells (iPSCs) derived from an AS-patient carrying a defined 3bp deletion in the maternal copy of the *UBE3A* gene (AS_3bp-iPSCs). Fibroblasts from a skin biopsy of this patient and of a healthy control person were reprogrammed by SIN-lentiviral expression of the Yamanaka-factors. The vector contains FRT-sites at the deleted U3 position of the SIN-LTR allowing for excision of the reprogramming vector by FLP recombinase. Pluripotency of obtained iPSCs was evaluated by determining alkaline phosphatase activity, surface expression of SSEA4, TRA1-60 and TRA1-81, expression of endogenous, pluripotency-associated genes by qRT-PCR and immunocytochemically. Functional verification was performed by embryoid body formation with proof of the presence of all three germ layers. Southern blot based integration analysis revealed that two of five analyzed AS_3bp-iPSC clones contained a single lentiviral integration. Those were selected for Flp-based excision of the vector and directed differentiation towards dopaminergic neurons. In depth characterization of the obtained neurons will reveal whether key properties are altered compared to those derived from healthy controls. Results of this ongoing work will be presented.

Abstract No. P155

Induced pluripotent stem cell models of schizophrenia: a review of published studies

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The advent of induced pluripotent stem cell (IPSC) technology raised expectations of applying this method for the research of schizophrenia, a severe, debilitating neuropsychiatric disorder. IPSC based disease modeling offered the formerly unavailable means for investigating patient-specific live neural tissue by differentiating pluripotent cells into neurons in vitro. After the publication of the first dozen of studies in this area it is worth taking a closer look at the results as a whole. By reviewing the literature we ask what IPSC based disease modeling has added to our understanding about schizophrenia and what the future directions suggested by these findings are.

The published studies proved the feasibility of the method and demonstrated differences between patient-derived cell lines and healthy controls in various cellular phenotypes, including transcriptomic alterations, neural connectivity, neurite outgrowth, electrophysiological maturation, neural precursor migration, mitochondrial function, and response to antipsychotic medication. During this year three fundamental papers showed increased rate of LINE1 retrotranspositions in 22Q11DS derived neurons, aberrant cytoskeletal dynamics in cell lines carrying 15q11.2 microdeletion, and the applicability of nuclease-based genome editing methods for studying and correcting the effects of mutations in *DISC1*, a schizophrenia candidate gene.

Using targeted differentiation protocols to investigate specific neuronal populations, such as forebrain GABAergic interneurons, moreover combined single-cell transcriptomics and electrophysiology will help in decreasing the heterogeneity of results. For the same reason specific genetic and clinical subgroups of patients should be included in studies. The scalability of this approach remains limited due to cost and labor intensity. It is also unclear at his point what the primary application of schizophrenia IPSC models should be: disease mechanisms, delineating disease subgroups within schizophrenia, or drug screening? In summary, despite methodological limitations and heterogeneity, IPSC based disease modeling is emerging as a promising approach in the armamentarium of schizophrenia research.

Wen, Z., Nguyen, H.N., Guo, Z., Lalli, M.A., Wang, X., Su, Y., et al., 2014. Synaptic dysregulation in a human iPS cell model of mental disorders. Nature.

Wright, R., Rethelyi, J.M., Gage, F.H., 2014. Enhancing induced pluripotent stem cell models of schizophrenia. JAMA psychiatry 71, 334-335.

Abstract No. P156

Generation of a stable and efficient in vitro model to study neuroinflammation in Parkinson´s Disease

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The main hallmark of Parkinson's disease (PD) is the degeneration of dopaminergic (DA) neurons in the substantia nigra (SN) pars compacta and of nerve terminals in the striatum, resulting in motor symptoms. Reprogramming of human somatic cells into human induced pluripotent stem cells (hiPSC) allows the generation of patient-specific hiPSC. These can subsequently be differentiated into DA neurons, but to date the challenge is a limited quantity of pure midbrain DA-neurons. To overcome this roadblock, a recently published protocol by Doi and colleagues (Doi et al., 2014) suggested the differentiation of hiPSC by a combination of dual SMAD inhibition, floor plate induction, and an additional sorting step using Corin, a serine protease thought to be expressed in floor plate cells. The aim of our study was to optimize this protocol for studying neuroinflammation in PD.

Control hiPSC and hESC lines were differentiated according to the published protocol using two dual SMAD inhibitors, as well as the floor plate inducers SHH, PMA, FGF8 and GSK3b inhibition, and sorted for Corin-positive cells after 12 days of differentiation. The sorting and replating procedures were optimized aiming a pure population of DA neurons and less death cells.

Eventually, this optimized protocol will be used to differentiate control and patient hiPSC into DA neurons, thus generating an in vitro model suitable for studying different approaches in PD. One interesting approach will be to assess the inflammatory pathways of peripheral immune cells on the degeneration of DA neurons in PD by coculturing DA neurons with immune cells.

Abstract No. P157

Maintenance of genomic imprinting in iPSCs derived from Angelman syndrome patient cells

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Genomic imprinting is an epigenetic phenomenon resulting in parent-of-origin dependent gene expression. The hallmark of imprinted genes is a differentially methylated region (DMR) which acquires DNA methylation in one of the parental germlines. This differential DNA methylation is stably maintained throughout development and thought to be stable during reprogramming.

We generated and characterised iPSCs of a patient with Angelman syndrome and a healthy control person. 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 absence of funtional UBE3A protein in the brain. Imprinted expression of UBE3A is controlled by the DMR PWS-SRO, whose 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.

Several iPSC clones were positively tested for expression of various pluripotency genes using alkaline phosphatase staining, immunohistochemistry, FACS analysis and qRT-PCR. Quality testing included Southern blotting to assess the number of vector integration sites, karyotyping and testing for cellular identity.

Analysis of methylation imprint stability was performed using next generation amplicon bisulfite sequencing on the Roche 454 GS Junior system. We investigated the gametic DMRs of the six best studied imprinted gene loci: for Angelman and Beckwith-Wiedemann syndrome, IGF2/H19, RB1, GNAS and DLK1/MEG3. We observed a stable level of differential methylation at the PWS-SRO locus but hypermethylation at RB1 and DLK1/MEG3. In-depth analysis of the remaining three loci is ongoing and will be presented. Our preliminary data indicate that stability of imprint methylation during reprogramming is variable and depends on the gene locus.

Abstract No. P158

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

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Objective

Potential therapeutic applications of stem cells require pharmacodynamic and safety investigations. Therefore, we developed a method using Luc-transfected stem cells to monitor their engraftment, distribution and differentiation in vivo. In further experiments we defined and standardized a method for teratoma forming by stem cells. Due to their character these teratomas could be a useful surrogate model for the evaluation of drugs with embryotoxic potential.

Methods

Transfected 7AC5/FF (7AC5/Luc) murine embryonic stem cells were injected subcutaneously (s.c.), intrahepatically (i.hep.), intrasplenically (i.splen.) and intravenously (i.v.) into immunodeficient mice and luciferase signal was determined at different points in time. 7AC5/Luc and derived teratomas were analysed concerning their pluripotency and germ layer marker expression on RNA and protein level. As 7AC5 stem cells developed teratomas after transplantation, we standardized this process to evaluate embryotoxic potential of drugs (non, weak, strong embryotoxic).

Results

After transplantation of 7AC5/Luc into mice increasing luciferase signals were measured over time. For s.c., i.hep. and i.splen. transplantation signals only at the application site could be observed. After i.v. application an enrichment of cells could be detected in the lung and in the bones. Histological analysis revealed teratoma growth at the these sites. Expression profile of teratomas showed markers of the three germ layers and lower levels of pluripotency markers. 5-FU treatment reduced significant teratoma growth and weight. Expression profile of pluripotency and differentiation markers was changed in teratomas under 5-FU treatment, but all three germ layer structures developed.

Conclusion

Using bioluminescence imaging, the kinetic of teratoma growth of transplanted cells by different application routes can be followed in vivo. Cytotoxic activity of reference drugs could be determined in vivo by the reduced bioluminescence signal, teratoma growth and weight. Changes in expression profile not allow a distinct classification of reference drugs with different embryotoxic potential.

Abstract No. P159

Inhibition of Notch signaling increases photoreceptor genesis in mESC-derived retina organoids

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The differentiation of pluripotent stem cells into retinal cell types opens up new possibilities for studies on retinal development, degeneration and regeneration. Here, we modified recently developed mouse embryonic stem cell (mESC) three-dimensional differentiation approaches, to make the protocol independent of a transgenic eyefield reporter, and investigated the efficiency of mESC derived retinogenesis at all steps of the protocol. Further, we investigated the effect of Notch signaling inhibition on retina organoid-genesis.
We achieved efficient eyefield induction (82 ± 12 SD % of aggregates, N=7), as well as patterning into RPE and neural retina domains. Further, upon trisecting aggregates into three evenly sized pieces, the majority (87 ±3 SD % of aggregates, N=4) generated big, stratified retinal tissue, reminiscent of early postnatal retina in vivo. Each aggregate retina had between 0.41 to 1.9 mm (1.4 mm ± 0.4 SD) photoreceptor layer circumferential length. Inhibition of Notch signaling by DAPT treatment prevented the differentiation of retinal bipolar cells and Müller glia, reduced progenitors and organoid culture time-dependently increased Crx+ photoreceptors.
Our results suggest that our novel approach makes the generation of mESC derived, stratified retina organoids simpler and independent of an eyefield transgenic fluorescent reporter. Further, differential responses of retina organoids to Notch inhibition at different culture times suggests that progenitors in retinal organoids pass through different intrinsically regulated competence states.

Abstract No. P160

Efficient and scalable GMP-grade culture media system for rapid cardiomyocyte differentiation of pluripotent stem cells in human disease research

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Simple and robust derivation of spontaneously contracting cardiomyocytes derived from human pluripotent stem cells (hPSCs) would provide a valuable source of cells for basic research into cardiac biology and mechanisms of heart disease as well as applied studies in pharmacological drug discovery and toxicity screening. Currently a number of protocols exist for inducing embryoid bodies (EB) suspension or monolayer cultures of hPSC to differentiate into cardiomyocyte (Iglesias-Garcia et al 2013; Priori et al 2013). These cardiomyocyte differentiation protocols have led to varying results and differing purity levels of cardiomyocytes. To enable consistent differentiation of hPSCs, we developed a simplified cardiomyocyte differentiation media system, consisting of three ready-to-use components. This easy to use cardiac differentiation system is designed for monolayer hPSC affording flexible culture formats and a scalable workflow enabling generation of large numbers of consistent, spontaneously active cardiomyocytes.
Multiple parameters were evaluated during differentiation media testing and our results demonstrate significant influences arise from the quality and confluence level of the PSC prior to induction and with the incubation period in different media.
Our findings suggest the complete differentiation media system could serve as a standardized culture system for generating large numbers of consistent, spontaneously active cardiomyocytes in research studies. Further investigations will be performed to assess cardiomyocyte differentiation on different hPSC lines as well as verifying gene expression profiles, cardiac markers, and sensitivity to pharmacological agents.

Abstract No. P161

Visualization of intestinal stem cells using medaka fish

Narges Aghaallaei [1,*], *Franziska Gruhl* [2], *Venera Weinhardt* [1], and *Joachim Wittbrodt* [1]

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*Presenting author

Intestine is a beautiful system to study stem cell biology, as it is one of the most rapidly regenerated tissues in the body. Adult intestinal stem cells are critical for maintaining the function and repair of gastrointestinal tissue. On the other hand intestinal stem cells are the most subpopulation of the malignant cells in the intestinal- and colorectal cancer, one of the most frequently developing cancer types worldwide.
It would be very helpful to establish new model systems and techniques that can uncover stem cells behavior in their natural environment in healthy organ as well as in tumor. Due to the optical transparency of larvae, the Japanese killifish medaka (Oryzias latipes) is an attractive model system for studying gastrointestinal diseases and cancer research by intravital imaging. Medaka has a primitive form of the digestive tract. The intestine transits directly from esophagus and is regionally segmented into a small intestine and colon-like region. Although, the medaka intestine has a simple architecture and lacks well-defined crypts, the morphological and molecular fingerprints indicate that there is an analogy to mammalian intestine. Based on the proliferation assays and expression survey of stem cell markers such as Wnt target genes sox9b and lgr genes family, we can characterize the proliferation niche in embryonic and adult digestive tract in medaka. To monitor the stem cells homeostasis in real-time, we developed a transgenic reporter line for the sox9b gene (sox9b:gfp). Additionally this transgenic model will allow us to in vivo study the role of intestinal stem cells in cancer development. Long-term intravital imaging of this reporter in combination with a cancer-inducible line can facilitate tracking the intestinal stem cells in the processes that underlie cancer initiation and progression.

P162 – P178: Stem cells in disease: Cancer stem cells

(posters will be displayed in front of room K 1+2)

P162 Injury accelerates brain tumor initiation
Si Chen

P163 Isolation and characterization of chemo-resistant cells derived from HT29 and HCT116 human colorectal cell lines: A selection of cancer stem cells?
Flaria el Khoury

P164 Exploring the PDAC-subtype-associated microenvironment in PDX models and patients
Elisa Espinet Hernandez

P165 Single-cell Lineage Analysis of Melanoma (Stem-) cells and Tumor Progression in an Intact Organism
Eva Hasel

P166 miRNA profiles in hybrid cells derived from breast cancer cells and cells with stem cell properties differ from that of parental cells
Benjamin Heikens

P167 Isolation of cancer stem cells in Methotrexate resistant side population T cell Leukemia cell line
Jayapragasam Madhumathi

T05/P168 Shp2 Signaling is Essential to the Suppression of Senescence in Mammary Gland Cancer Stem Cells in Mice
Linxiang Lan

P169 Quantification of cell fusion events between breast cancer cells and breast epithelial cells exhibiting stem cell characteristics
Marieke Mohr

P170 Cancer stem cell-targeted viral vectors
Alexander Muik

P171 Modeling Polyclonal Dynamics in Glioblastoma
Roman Reinartz

P172 Detection of Self Renewal Genes NANOG and STAT3 Expression in Cancer Bladder cells.
Shereen Shawky

P173 SFB 873 – Maintenance and Differentiation of Stem Cells in Development and Disease.
SFB 873 – Consortium

P174 Analysis of Stemness-Factor Heterogeneity by using High Content Imaging of Primary Glioblastoma Cells
Andreas Till

P175 Evaluation of stem cell properties in ovarian carcinoma cells using different spheres assays
Hui Wang

P176 Transcriptomics approaches and epigenetic profiling of neurally-differentiating stem cells to characterize developmental toxicants
Tanja Waldmann

P177 Application of primary glioblastoma (stem) cells for identification of novel therapy options
Anja Wieland

T08/P178 CD151+ cells drive tumor-initiation, hierarchical growth and proliferation-associated signaling in patient-derived models of serous ovarian cancer
Franziska M. Zickgraf

Abstract No. P162

Injury accelerates brain tumor initiation

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Malignant gliomas are the most commonly occurring tumors in the adult central nervous system (CNS) and are essentially incurable. The etiology of primary gliomas is largely unknown. However, infection and injury of the brain have been associated with higher risk for gliomas. Similarly, chronic inflammation has been shown to be causative for other types of tumors such as in the gut or liver. Neural stem cells are thought to be the cell of origin of gliomas in which oncogenic mutations first occur and accumulate to develop to a full-blown malignancy. Here, we hypothesize that injury to the CNS might increase the risk of tumor formation of genetically pre-disposed mice. One type of injury, transient global ischemia, induces proliferation of neural stem cells as well as pronounced inflammation and ROS production, all factors that are known to be involved in tumor initiation. By combining the glioma model Tlx CreERT2 Ptenfl/fl p53fl/fl, in which the tumor suppressors Pten and p53 are deleted specifically in the neural stem cell compartment marked by Tlx, and an injury model of transient global ischemia we find accelerated tumor initiation in the brain after injury. Furthermore, our preliminary data show that the survival time of Pten and p53 deleted mice with injury is lower compared to non-injured mice. To gain further insight into the underlying molecular mechanisms of injury-induced tumor initiation RNA sequencing and whole genome bisulphate sequencing are being performed.

Abstract No. P163

Isolation and characterization of chemo-resistant cells derived from HT29 and HCT116 human colorectal cell lines: A selection of cancer stem cells?

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Colorectal Cancer (CRC) is the third most common cancer worldwide. A growing understanding of the tumor mass defines it as a heterogeneous model, driven by a functional sub-population of self-renewing, multipotent cells, recognized as Cancer Stem Cells (CSC). Epithelial cancers, including CRC, are initiated by a small fraction of CSC. These cells are resistant to cancer treatments, and capable of metastasis, partly because of genetic and epigenetic alterations. In CRC, there is a set of anti-cancer agents such as oxaliplatin, irinotecan or docetaxel. However, drug resistance, mainly due to CSC, is a limitation to these therapies. We were initially interested in assessing the presence of CSC within the general population of two human colorectal cancer cell lines HT29 and HCT116, based on potential CSC surface markers. We showed, by RT-PCR, the expression of several marker or inducing CSC genes (EpCAM, Oct4…) in both cell lines, while other markers are present either in HT29 or HCT116 cells. We validated these results by western blot, flow cytometry and/or immunocytochemistry experiments. We pursued this study by considering two important CSC markers: CD24 and CD44. We tested the sphere-forming ability of the cells in Serum-Free Medium and thus obtained an enhanced expression of CD24 in HCT116 spheroids. Finally, we selected cells resistant to docetaxel (HT29/DocR and HCT116/DocR) or oxaliplatin (HT29/OxR) from the parental cells. The phenotypic characteristics (morphology, growth under anchorage-independent conditions) of the resistant cells were compared with the corresponding parental cells. The effects of chemo-resistance on the expression of CSC markers show an over-expression of integrin beta-1, a protein involved in cell adhesion and motility, in the resistant cells, compared to the parental ones. Taken together, our results confirm and extend previous observations upon the possibility to identify potential CSC from established tumor cell lines, and select sub-populations resistant to chemotherapeutic drugs.

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Abstract No. P164

Exploring the PDAC-subtype-associated microenvironment in PDX models and patients

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease with dismal prognosis. Despite extensive research and the discovery of several promising drug candidates, little progress in PDAC treatment has been reported in the last years. Two facts can be behind these disappointing results. In one hand, three distinct molecular subtypes that show differences in drug response in vitro have been described. This highlights the importance of considering PDAC as a heterogeneous disease and point to the stratification of patients as a possible way to improve treatment response. An additional reason behind the limited efficacy of PDAC treatment might be the tumor microenvironment. PDAC is the solid tumor with the highest stromal content, accounting for up to 90% of the tumor mass. The PDAC microenvironment is known to affect tumorigenesis and may impair drug delivery. Thus, rendering the PDAC microenvironment as an appealing therapeutic target.
We have developed a novel workflow to efficiently generate patient-derived orthotopic xenografts (PDX) and serum-free cell cultures from primary resected PDAC tumors. The established primary cell lines comprise the three described PDAC subtypes and, when re-injected into immunodeficient mice, generate xenografts with high pathological similarity to the original patient tumor, including a prominent stromal presence. To explore the differences in the microenvironment associated to the individual PDAC subtypes we have now generated gene expression profiles for the stroma of a number of xenografts from our PDX model representing all three subtypes.
Besides, the generation of RNA sequencing data from several sub-populations isolated from fresh primary human PDAC tumors may reveal interesting interactions between the different tumor compartments.
We believe that these approaches will shed some light on how different stromal expression patterns are interconnected with different epithelial expression profiles and vice versa, and how this information can be ultimately exploited for patient stratification and therapy.

Abstract No. P165

Single-cell Lineage Analysis of Melanoma (Stem-) cells and Tumor Progression in an Intact Organism

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The combination of novel genetic labeling techniques and strong cancer models open new possibilities to study tumor origin, the evolution of heterogeneity within tumors and the concept of cancer stem cells in vivo. There is a great variance in growth pattern of cancers originating from different tissues. Melanoma for instance is a cancer that evolves to higher malignancy fast and shows great heterogeneity and therefore is an ideal model to study cancer progression to its full extend. A simple and strong transgenic melanoma model, developed by Manfred Schartl et al., is based on the over-expression of only one oncogene, the EGF-Receptor variant Xmrk in the Japanese rice fish Medaka (Oryzias latipes). Tumors in this model appear in various histo- and patho types as it is the case in human melanoma (1). In this project we plan to combine pigment cell specific expression of Xmrk induced by transactivators with Cre-lox based nuclear fluorescent labeling as used in the “nucbow” approach
(2).Fish cancer models have several advantages compared to other vertebrate system, most striking being the possibility for repeated live imaging of tumors. For optimal imaging conditions all transgenic fish lines that will be used will be generated de novo in a pigment free Medaka background (QuiHs) that does not show any auto-fluorescence.
By this approach it will be possible to visualize, influence and compare dynamics in pigment cell tumor tissues in living animals to answer a variety of questions concerning the tumorigenic potential of specific cell types, the existence of melanoma stem cells and factors that influence behaviors of cells in tumor tissue.

(1) Schartl et al., The Journal of investigative dermatology, 2010

(2) Livet et al., Neuron, 2014

Abstract No. P166

miRNA profiles in hybrid cells derived from breast cancer cells and cells with stem cell properties differ from that of parental cells

Benjamin Heikens [1,*], *Silvia Keil* [1], *Robert Weingarten* [1], *Bernd Niggemann* [1], *Bernd Denecke* [2], *Kurt S. Zänker* [1], and *Thomas Dittmar* [1]

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Cell fusion occurs in many processes, including cancer. The resulting hybrid cells could exhibit novel properties. The hybrid cell lines M13HS-2 and M13HS-8 were obtained in vitro from fusion events between M13SV1-EGFP-Neo cell line exhibiting stem cell characteristics and HS578T-Hyg breast cancer cell line. Earlier studies demonstrated altered MMP1 and ERα expression in hybrid cells compared to parental cells, which might be caused by altered miRNA expression profiles. To verify for this assumption the expression levels of various miRNAs supposed to regulate MMP1 and ERα were investigated.

Microarray analysis showed different miRNA expression profiles in hybrid cells compared to parental cells. MiRNA data were validated by qPCR and a luciferase-reporter assay.

Western Blot studies showed that M13SV1-EGFP-Neo cells were ERα positive, but lack MMP1 expression. By contrast, HS578T-Hyg, M13HS-2, and M13HS-8 cells lack ERα expression, but are positive for MMP1.

For MMP1 this correlates well to high expression levels of MMP1-regulating miRNAs (miR-133a, miR-221-3p, miR-222-3p, miR-365-3p, miR-373-5p) M13SV1-EGFP-Neo cells, but low expression in the other cells. This suggest an impact of these miRNAs on MMP1 protein expression. Interestingly, qPCR revealed high expression of supposed ERα-regulating miRNAs (miR-221-3p, miR-222-3p, miR-373-5p) in M13SV1 and lower expression of these miRNAs in HS578T-Hyg, M13HS-2, and M13HS-8 suggesting that these miRNAs are not involved in the regulation of ERα expression.

In summary, the miRNA expression profiles differ among all tested cell lines indicating that cell fusion-evolved hybrid cells exhibit not only a unique gene expression, but also a unique miRNA expression profile.

Abstract No. P167

Isolation of cancer stem cells in Methotrexate resistant side population T cell Leukemia cell line

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With the emerging evidence for the existence of cancer stem cells (CSC's) that show chemotherapy resistant side population, there is a need to characterize and target these cells to prevent recurrence of disease. Methotrexate is a well known drug used for cancer therapy. But there is an increasing concern over the resistance developed to the drugs by cancer cells which become more difficult to manage after therapy. We isolated methotrexate resistant cells from human acute lymphoblastic T cell leukemic cell line, MOLT-4 and characterized the cells using FACS analysis for cancer stem cell markers CD34, CD90, CD123 and CD117. The gene expression analysis was done for transcription factors and signaling pathways involved in cancer stem cells. The results showed that methotrexate resistant cells had upregulation of cancer stem cell markers. This indicates that drug treatment enhances the cancer stem cell population which could be attributed to their chemo- resistance and hence there needs to be an alternative therapeutic strategy to circumvent the chemo-resistance in cancer.

Please find abstract P168 under 'Selected presentations' T05/P168

Abstract No. P169

Quantification of cell fusion events between breast cancer cells and breast epithelial cells exhibiting stem cell characteristics

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Hybrids of cancer and normal cells can arise from spontaneous cell-cell fusion processes in vitro and in vivo. Recent data suggest that hybrids of cancer cells and BMDCs or even progenitor or stem cells could harbour a more aggressive phenotype compared to parental cells. Beside genetic alterations cell-cell fusion can contribute to the adoption of properties provided by both merging cells and lead to an altered behaviour and cell shape. Cell fusion in cancer development could thereby reveal an often underestimated process promoting tumor progression.

While an influence of the chronic inflammatory microenvironment as cause for cell-cell fusion and cancer progression is increasingly being recognized, less is known about forces particularly triggering fusion between breast cancer and normal cells. How and which components could affect fusion and thereby disease outcome remains an important question and need reliable tools to investigate.

Quantification of fusion processes in vitro could provide a novel method to identify cancer promoting components. In the present study cell-cell fusion was measured by using a cre/loxP system. Various human breast cancer cell lines stable transfected with a double fluorescence reporter vector were co-cultured with a breast epithelial cell line exhibiting stem cell characteristics stably expressing the cre recombinase. Several stimuli were tested and revealed partly different alterations in fusogenic behaviour of cell lines, e.g. increased fusion activity could be observed while adding EGF or TNFα. Interestingly, hypoxia and TNFα stimulation together intensified the impact on spontaneous cell fusion of two cancer cell lines, whereas fusion capacity of others was not more than partly increased.

The present study establishes an efficient tool for the quantification of cell-cell fusion processes in vitro using the cre/loxP system and highlights a new opportunity to identify a potential link between components priming and triggering cell-cell fusion and therefore carcinogenesis.

Abstract No. P170

Cancer stem cell-targeted viral vectors

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Over the last years a number of cell surface markers have been proven useful for the detection and isolation of cancer stem cells (CSCs). Among these are CD133, CD44, CD24 and EpCAM. We have developed a strategy by which viral vectors can be engineered to use a cell surface marker of choice for cell entry. Among others, oncolytic measles virus targeted to CD133 (MV-CD133) and adeno-associated viral vectors targeted to EpCAM (EpCAM-AAV) have been generated as novel therapeutic and/or diagnostic tools.

MV-CD133 selectively infects and kills CD133+ tumor cells and shows an unexpectedly potent oncolytic activity against glioma, hepatocellular carcinoma and colon cancer in vitro and in vivo when compared to unmodified virus (Bach et al., 2013). Interestingly, enhanced tumor cell killing by CD133-scFv contact has been excluded as causative, suggesting infection and elimination of a distinct CD133+ cell population that is highly relevant for tumor growth. To enhance its anti-tumoral activity further, MV-CD133 was armed with the suicide gene SuperCD, a modified cytosine deaminase prodrug-convertase, or modified with genes from wild-type MV strains allowing infection and killing of tumor cells with intact anti-viral defense. First data indicate that both CD133-targeted viruses display an increased anti-tumoral activity without increasing their overall toxicity.

EpCAM-AAV is a replication-deficient vector that delivers genes exclusively into EpCAM+ cells. Assessing EpCam-AAV as a diagnostic tool for the detection of EpCAM+ circulating tumor cells in complex human whole blood, we spiked human blood with different amounts of EpCAM+ MDA-MB-453 human breast cancer cells. Remarkably, even in blood samples spiked with as few as 100 EpCAM+ tumor cells, most of these (>90%) were successfully detected by EpCAM-AAV. Most importantly, EpCAM-AAV did not transduce any cell in tumor-cell-free samples.

Abstract No. P171

Modeling Polyclonal Dynamics in Glioblastoma

Roman Reinartz [1,*], *Sied Kebir* [1], *Daniel J Silver* [2], *Shanshan Wang* [3], *Axel Hillmer* [4], *Daniel Trageser* [1], *Fatima Kreusch* [5], *Anja Wieland* [1], *Tong Zheng* [3], *Marius Küpper* [1], *Andreas Till* [1], *Laurel Rauschenbach* [1], *Timothy M Shepherd* [3], *Martin Glas* [1], *Andrea Staratschek-Jox* [5], *Sven Cichon* [4], *Anthony Yachnis* [6], *David W Pincus* [2], *Brent A Reynolds* [2], *Oliver Brüstle* [1], *Dennis A Steindler* [2], and *Björn Scheffler* [1]

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Most malignant types of solid cancer share the diagnostic feature of cellular heterogeneity and great efforts have been directed towards describing the polyclonal architecture of tumors. However, so far, the functional consequences of intra-tumor polyclonality remain largely unknown. Here we present how co-existing clonal subpopulations of human glioblastoma can be separated for vital analysis. Distinct patterns of copy number alterations, gene expression, tumorigenicity, cellular plasticity, and drug sensitivity can be dissected and investigated ex vivo. By application of experimentally selected compounds we learned that specific clonal populations can be targeted in vitro. Furthermore, the post-treatment dynamics of intra-tumor cellular architectures can precisely be predicted in orthotopic xenograft models as well. In perspective, these data present a first step for developing assay systems that enable prospective investigation of endogenous drug resistance before recurrent tumor growth occurs in a patient. These results additionally place single clone analysis as most relevant tool for investigation of tumor cell heterogeneity revealing insights into the functional ramifications of glioblastoma malignancy.

Abstract No. P172

Detection of Self Renewal Genes NANOG and STAT3 Expression in Cancer Bladder cells.

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Background: The recently identified bladder cancer stem cells are considered to be mediators of resistance to current therapies and therefore represent strong candidate biological targets. To characterize bladder cancer stem cells (CSCs) at the molecular level, the expression of Oncoprotiens that have been implicated in the self-renewal of adult or embryonic stem cells, is detected. These Oncoprotiens include Nanog and STAT3. Objectives: The aim of this study was to detect the presence of cancer stem cells of bladder cancer by detection of the two genes, Stat3 and Nanog in cultured bladder cancer cells. Subjects and Methods: The current study was performed on tumor biopsies obtained from 30 recently diagnosed subjects with transitional cell carcinoma of the urinary bladder. Tumor tissue was disrupted and cultured in serum free, medium stem cell- selective media. Cancer stem cells were positively selected through serial passaging. Flowcytometric characterization of isolated tumor cells (CSCs) was done. The expression of stem cell marker genes Nanog and STAT3 was detected by real time RT-PCR. The genes expression levels were compaired to their level of expression in 15 normal bladder tissue biopsies. CSCs were then injected in nude mice with subsequent assessment for tumor growth. Results: Statistical comparison for the expression levels of Nanog and STAT3 between the bladder carcinoma patients cultured tissue and the controls tissue showed a highly statistical significant difference, P value lower than 0.001 for Nanog and P value = 0.003 for STAT3.Three weeks after injection of CSCs into nude mice, a bladder tumor formed.Conclusion: Current clinical judgment in bladder cancer relies primarily on pathological stage and grade. Identification of new molecular markers would allow for improved risk stratification so that we may better use risk-adapted therapies. CSCs have been proposed to play a critical role in metastatic progression and resistance to commonly used cancer treatment.

Abstract No. P173

SFB 873 – Maintenance and Differentiation of Stem Cells in Development and Disease

SFB 873 – Consortium[1]

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The basic principles controlling stem cell self-renewal and differentiation are strikingly conserved during evolution, while at the same time regulatory pathways can differ between various stem cell systems in the same organism and between homologous stem cell niches in different organisms. Since the circuits controlling stem cell function in highly complex mammalian systems are often difficult to study and frequently show significant molecular redundancy, our consortium also takes advantage of simpler model systems to illuminate the cellular and molecular mechanisms governing stem cell function.

The long-term goal of the Collaborative Research Center SFB873 is to define the regulatory principles underlying the balance between maintenance, expansion and differentiation of stem cells in diverse systems on a mechanistic level.

This question is tackled by studying intrinsic and extrinsic control of stem cell behavior in various tissues, such as blood, nervous system, or gut in a wide range of model systems including *Arabidopsis*, *Hydra*, *Drosophila*, medaka, *Xenopus*, as well as mouse and human. In addition to analyses of normal stem cell function, we focus on diseases such as cancer, since they not only can serve as steppingstones for future translational research, but also represent important models for stem cell dysregulation. To this end we apply an integrated quantitative experimental approach ranging from biophysics with single cell resolution, to live cell imaging and genetics and genomics to describe stem cell regulation at the systems level.

Taken together, the challenge and motivation for the SFB873 is to bridge the gap between cell-based and *in vivo* approaches in experimentally amenable reference organisms and highly complex mammalian systems.

Abstract No. P174

Analysis of Stemness-Factor Heterogeneity by using High Content Imaging of Primary Glioblastoma Cells

Andreas Till [1,*] , *Anja Wieland* [1], *Roman Reinartz* [1], *Niklas Schäfer* [1], *Sied Kebir* [1], *Franziska Lorbeer* [1], *Sabine Normann* [1], *Mihaela Keller* [1], *Heike Höfer* [1], *Satya Samal Swarup* [2], *Ashar Ahmad* [2], *Joao Dinis* [2], *Matthias Simon* [3], *Holger Fröhlich* [2], *Oliver Brüstle* [1], *Martin Glas* [1], and *Björn Scheffler* [1]

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It is commonly accepted that the degree of cancer cell heterogeneity shows a negative correlation with the prognosis in many types of cancer. Given the potential role of tumor cells with stem like properties for progression of the malignancy, a particular interest has been developed in the heterogeneous nature of stemness-factor expression and activity. There is, however, no grading system available that would allow a reliable classification of cellular heterogeneity. We hypothesize that assessment of heterogeneity will be essential for establishing and fine-tuning of clinical strategies for individualized patient care. This study aims to establish a methodology for quick assessment of cellular features that could directly be applied for correlation to a clinical degree. We use a >600 sample-sized library of vital, primary patient-derived glioblastoma cell specimens in a high content imaging and quantitative image analysis approach. Automated image-based read out parameters will be acquired for >1,000 individual cells per sample and will include cell size, shape, morphology, nucleus organization, and investigation of a portfolio of characteristic protein markers with a particular emphasis on stemness-associated factors (CD133, CD44, CD15, Nestin, SOX2…). In a first step, the degree of intra-tumor heterogeneity vs. inter-individual heterogeneity is assessed based on the availability of several cellular samples from tumor core vs. periphery and primary disease vs. disease recurrence per patient. In a second step, these data are used to determine a potential correlation of image data with the respective patient's characteristics (age, gender, etc.) and the respective cancer's molecular fingerprints (gene expression pattern, DNA methylation, SNP profiles). In a final step, we aim to establish a classification algorithm correlating these biological parameters with the clinically relevant data (overall survival, progression-free survival, etc.). A set of exemplary data for this approach will be presented.

Residual tumor cells are unique cellular targets in glioblastoma. Glas M, Rath BH, Simon M, Reinartz R, et al., and Brüstle O, Scheffler B. Ann Neurol. 2010 Aug;68(2):264-9.

c-Met signaling induces a reprogramming network and supports the glioblastoma stem-like phenotype. Li Y, Li A, Glas M, et al., and , Scheffler B, Laterra J. Proc Natl Acad Sci U S A. 2011 Jun 14

Abstract No. P175

Evaluation of stem cell properties in ovarian carcinoma cells using different spheres assays

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Introduction: Ovarian cancers are suggested to harbor a heterogenous mixture of cells including a subpopulation of so-called "cancer stem cells" (CSCs). A commonly used method to assess CSC potential in vitro is the spheres assay in which cells are plated under non-adherent culture conditions in serum-free medium supplemented with growth factors. Here, we compare different types of reported multi to a single cell-based spheres assay.
Methods: A lentiviral reporter vector in which red fluorescent protein (RFP) is driven under the control of the SOX2 regulatory region is used to isolate putative ovarian CSCs in human serous ovarian carcinoma OVCAR3 cells. RFP+ and RFP- cells sorted by FACS are cultured using limiting dilution from 1000 cells to 1 cell per well in 96-well plates in previously reported spheres media (MGEM, DMEM/F12 with supplements, or DMEM/F12 with supplements and 1% methylcellulose) or in a single cell-based assay.
Results: Sphere formation is similar in MEGM versus DMEM/F12 cultures however the numbers of emerging spheres are highly dependent on cell density in multi cell-based assays. When plated at lower density (100 cells/well), multi and single cell-based spheres assays yield comparable results. Immobilization by addition of 1% methylcellulose makes sphere formation independent of cell density by limiting mechanical artifacts. Recognition of an active SOX2 regulatory region can select for OVCAR-3 cells with sphere initiation capacity. 36% RFP+ vs. 17% RFP- cells formed spheres in multi versus 27% RFP+ vs. 13% RFP- cells in single cell-based primary spheres assays. Only spheres derived from RFP+ but not RFP- cells were able to generate secondary spheres in replating assays.
Conclusion: Our results indicate that single cell-based assays provide more accurate and reproducible results than multi cell-based assays which can be highly influenced by the density of plated cells unless methylcellulose is added to the cultures to immobilize cells.

Abstract No. P176

Transcriptomics approaches and epigenetic profiling of neurally-differentiating stem cells to characterize developmental toxicants

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Transcriptome analysis is a powerful tool to study gene expression changes in cultured cells. We explored the use of this technology for the prediction of drug effects during human development. By using neurally-differentiating human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC), we studied the time-dependence of the transcriptome response concerning duration of exposure to toxicants and maturity of the culture system under investigation. In a next step, we investigated the design principles of concentration-dependent transcriptome deviations. The methylation pattern of histone H3 was identified as a predictor of the persistence of the developmental neurotoxic (DNT) effect. Finally, we examined discrimination of different classes of toxicants in the test system under conditions of blind testing. We found that short exposures to compounds yielded information on potential pathways of toxicity (mode of action), while long exposure mainly described phenotypic alterations of the cells exposed to the toxicants (developmental path). These latter phenotypic changes were suitable for compound classification and prediction. Moreover, they allowed a relative potency ranking. The studies showed that it will be beneficial in the future to group transcriptome alterations of individual genes into superordinate biological processes, in order to condense the information and to facilitate the interpretation of studies as well as the visualization of the results.

Balmer NV, (2014) From transient transcriptome responses to disturbed neurodevelopment: role of histone acetylation and methylation as epigenetic switch between reversible and irreversible drug effects. Arch Toxicol 88, 1451-1468

Balmer NV, (2012). Epigenetic changes and disturbed neural development in a human embryonic stem cell-based model relating to the fetal valproate syndrome. Hum Mol Genet 21, 4104-4114

Abstract No. P177

Application of primary glioblastoma (stem) cells for identification of novel therapy options

Anja Wieland [1,*] , *Sabine Gogolok* [1], *Laurèl Rauschenbach* [1], *Daniel Trageser* [1], *Roman Reinartz* [1], *Andreas Till* [1], *Matthias Simon* [2], *Oliver Brüstle* [1], *Martin Glas*[1], and *Björn Scheffler*[1]

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Despite combined and optimized surgical, radio- and chemotherapeutic measures in patients suffering from glioblastoma (GBM), recurrence of disease occurs always, leading to median overall survival of 14.6 months. Stem-like cancer cells (CSCs) may drive the progression of disease and they may facilitate resistance to standard therapy -- presenting with new cellular targets for anticancer therapy.

We have hence collected patient-derived primary GBM cells (pGBMs) containing CSCs from more than 300 tissue samples of 130 GBM patients. The cells can be maintained under adherent culture conditions enabling the onward, controlled study of human disease-typic traits for prolonged periods of time ex situ. Our comprehensive library of pGBMs is currently used as a discovery platform for the identification of novel therapeutic options. One approach includes the screening and validation of 160 synthetic and natural toxic substances. Considering the cellular and inter-individual heterogeneity of GBM, a portfolio of short-term expanded CSC-enriched human primary GBM cells (pGBMs, n=21), common glioma lines (n=5), and non-cancer human control cells (n=3) was applied. Pharmacodynamic analysis, study of cell cycle progression, proliferation, apoptosis, cell migration, and on the frequency of stem-like GBM cells were performed in vitro. In addition orthotopic xenotransplantation was used to confirm anticancer effects in vivo. Mechanism of action analysis revealed a pleiotropic impact of a most promising candidate compound on intracellular signaling cascades including the WNT/CTNNB1-, NOTCH-, mTOR-, and NFKB-pathways. Intriguingly, an associated genetic biomarker predicts a relevant synergistic activity with the current clinical standard medication in GBM. Our studies thus highlight the applicability of pGBMs for early drug development — presenting with a novel lead compound that is now developed to a clinical scale.

Supported by the Lichtenberg Program of the VW Foundation

Please find abstract P178 under ‘Selected presentations’ T08/P178

P179 – P187: Computational stem cell biology

(posters will be displayed in front of room K 1+2)

Abstract No. P179

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

Christoph Baldow [1,*], *Lars Thielecke* [1], *Sebastian Gerdes* [1], *Ingo Röder* [1], and *Ingmar Glauche* [1]

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Although a wide range of molecular mechanisms has been identified that are closely connected to cellular aging, it is rarely understood how such intra-cellular aging processes translate on the phenotypic level of the overall tissue. 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. In particular, we apply machine-learning approaches to identify measures for the reliable classification of clearly distinguishable scenarios, such as the early distinction between normal and potentially pathological developments. We report on our results to which extent these measures are suitable to prospectively predict 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 developments in these patients our identified measures and the resulting categorization can aggregate time course data and provide estimates for the risk of atypical clonal developments and predict the manifestation of leukemia.

Abstract No. P180

Cooperativity and allostery in protein-DNA recognition: implications for stem cell pluripotency and cell fate transitions

Vlad Cojocaru [1,*], *Felipe Merino* [1], *Hans Robert Schöler* [1], *Benjamin Bouvier* [2], *Calista Ng* [3], *Veeramohan Veerapandian* [4], and *Ralf Jauch* [4]

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OCT4 belongs to the POU family of transcription factors which have two independent DNA binding domains, a POU specific (POUS) and a homeodomain (POUHD) connected through a flexible linker. OCT4 lies in the core transcriptional network of pluripotent cells where it binds cooperatively with SOX2 to a composite motif in which their individual binding sites are juxtaposed ("Canonical"). From molecular simulations, we found that the presence of SOX2 modifies the dynamics of both domains of OCT4 despite the lack of direct interaction between SOX2 and POUHD. From free energy calculations we found that, in the absence of SOX2, POUHD has a higher affinity for DNA then POUS. Remarkably, SOX2 inverts the affinity balance between POUS and POUHD and influences the unbinding profiles of both. Therefore, we demonstrate that both direct and allosteric interactions modulate the OCT4-SOX2 cooperative binding to DNA.

Upon commitment of pluripotent cells to primitive endoderm, OCT4 redistributes to bind cooperatively with SOX17 to a distinct composite motif ("Compressed") which is lacking one base pair between the individual binding sites. From molecular simulations we revealed the structural basis for the OCT4-SOX17 interaction and elucidated the SOX-dependent motif preference of OCT4. Based on these findings, we designed a SOX2 double mutant that we predicted and confirmed experimentally to bind cooperatively with OCT4 to the Compressed motif. Furthermore, we found a strong correlation between the estimated and measured relative cooperative binding free energies for twelve wild-type and mutant OCT4-SOX-DNA complexes.

Taken together, these results demonstrate that both cooperativity and allostery modulate the DNA recognition by transcription factors, triggering alternative, context-dependent gene regulatory programs that ultimately lead to cell fate conversions. Moreover, we argue that in silico prediction and design of cooperativity and allostery in protein-DNA recognition is attainable and applicable to alter transcriptional circuitries and engineer cell fate transitions.

Merino F, Ng CKL, Veerapandian V, Schöler HR, Jauch R, Cojocaru V (2014). Structural basis for the SOX-dependent genomic redistribution of OCT4 in stem cell differentiation. Structure, In press

Jerabek S, Merino F, Schöler HR, Cojocaru V (2014). OCT4: dynamic DNA binding pioneers stem cell pluripotency. Biochimica Biophysica Acta 1839(3):138-54

Abstract No. P181

Investigation of microRNA involvement in neural development via quantitative integration of theoretical target prediction data

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MicroRNAs (miRNAs) are short non-coding RNA molecules shown to play an important role in the regulation of other RNA species including protein-coding transcripts, and have a demonstrated role in developmental programs, both in maintenance of and exit from pluripotency. Computational analyses have shown the potential for multiple, different miRNA target sequences to exist in a single mRNA transcript, as well as the targeting of a single miRNA to multiple mRNAs. The result is a complex regulatory network, wherein single miRNA molecules can effect changes in state through downregulation of multiple targets, while also harbouring the potential to reveal co-regulatory networks among mRNAs, mediated by common miRNA binding. To guide experimental investigations, various algorithms have been developed to predict miRNA and mRNA interactions, and many studies examining miRNA involvement in biological processes will employ one or more of these algorithms for guidance, often in some non-systematic way.

We present a quantified method of combining established prediction algorithm databases to a final database of theoretical human miRNA-mRNA interactions, using calculated receiver operating characteristic curves to compare weighting strategies including accuracy and likelihoods. The improvement of the final matrix over constituent individual algorithms was evaluated using a defined scoring set of validated interactions, revealing improved metrics including true positive rate. This database is applied to a combinatoric analysis of target transcripts, to identify miRNA candidates for in vitro study, as well as potentially co-regulated subsets as part of, or independently of characterized pathways.

Abstract No. P182

A bioinformatic analysis of the niche defines connective tissue growth factor as an extrinsic regulator of hematopoietic stem cell cycling activity

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Hematopoietic stem cell (HSC) fate decisions are regulated by the 'niche.' Precise interaction networks between these two entities are poorly understood. To understand the interactions during culture stress, gene expression analysis of HSC-supportive UG26-1B6 stromal cells was performed in contact with lineage- Sca-1+ Kit+ (LSK) cells. Clustering of microarray results of sorted stromal cells showed that the presence of LSK cells promotes bone and tissue remodeling. Candidate gene prioritization identified connective tissue growth factor (Ctgf) as a possible niche-secreted HSC regulator. Increased expression of Ctgf in co-cultures of LSK cells and stromal cells was confirmed using qPCR and ELISA. Ctgf has been shown to promote lymphopoiesis and the homing of leukemic cells. But, the role of Ctgf in the maintenance of normal HSCs has not been explored.

To find out how Ctgf affects intracellular signaling in LSK cells we performed co-cultures of LSK cells Ctgf deficient stroma (shCtgf). These experiments showed that Ctgf strongly affects Tgfb, Akt, and Wnt signaling. In LSK cells from shCtgf co-cultures a strong activation of Smad2/3 was found, with a concomitant stabilization of p27 (Cdkn1b), and decreased expression of Cyclin D1, suggesting a decreased G0/G1 transition. Indeed, LSK cells isolated on day after co-culture on shCtgf stroma confirmed this hypothesis. To study whether these changes in signaling events affect HSC maintenance, we transplanted co-cultured LSK cells into recipients. This experiment showed that HSC from shCtgf co-cultures show normal short-term engraftment, but a severely diminished long-term engraftment. In particular, engraftment of the myeloid lineage was dininished, with concomittant decreased engraftment of donor-derived myeloid progenitors and LSK cells.

Our study represents the first systematic characterization of how the niche regulates the initial stages stem cell cell cycle recruitment under stress conditions and shows that rapid upregulation of Ctgf under these conditions is required for HSC maintenance.

Abstract No. P183

Mathematical Modelling of Ageing-related Changes in the Polarity of Haematopoietic Stem Cell Divisions

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Although the differences between young and old haematopoiesis have been thoroughly studied on the cellular level, there is still a gap when it comes to causative interpretation of the underlying molecular mechanisms. Recent findings demonstrated that a significant proportion of haematopoietic stem cell (HSC) divisions in mice show a remarkable polarity with respect to the segregation of the small RhoGTPase Cdc42. It was demonstrated that elevated activity of Cdc42 in aged HSCs is causally linked to HSC ageing and correlates with a loss of polarity during HSC division. Furthermore, an inverse correlation of the activity distribution of Cdc42 with the level and nuclear localization of histone H4 lysine 16 acetylation (AcH4K16) was identified. Interestingly, a pharmacological inhibition of Cdc42 led to a rejuvenation of the age-associated HSC phenotype.

We established a subcellular model accounting for the concentrations of Cdc42 and AcH4K16. We demonstrate that a feedback coupling is sufficient to generate a bistable system behaviour and describe the inverse correlation of Cdc42 activity and AcH4K16 distribution. Introducing a cellular division process, which distributes the acetylation marks onto the daughter cells, we come up with an in silico model that describes the correlation between cell polarity and Cdc42 expression. We further explore how such feedback mechanisms are potentially altered during animal ageing and how this impacts on the frequency of asymmetric cell division.

Based on our mathematical model we demonstrate that an increase in concentration of Cdc42 can be inversely coupled to a decrease in acetylated H4K16 by an inhibitory feedback regulation. We use the model to speculate about age-associated changes that alter Cdc42 concentrations and influence the ratio of asymmetric to symmetric cell divisions.

Florian et al., Cdc42 Activity Regulates Hematopoietic Stem Cell Aging and Rejuvenation, Cell Stem Cell 10 (4 May 2012), 520-530.

Abstract No. P184

An extended gene expression landscape for the characterization and quality control of in vitro differentiated cells

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Cellular reprogramming and in vitro differentiation is increasingly performed to create cells of various types for disease modelling or regenerative medicine with a high demand on specificity of the products. Hence an essential step in the reprogramming process is the quality control of differentiated cells, comparing their molecular state to that of in vivo cells of the desired cell type with high precision. In order to avoid ambiguities in the cell state classification arising from heterogeneous protocols or experimental bias, special tools have to be developed allowing to remove noise from quality related signals. One possibility to tackle this task is the use of large scale gene expression data, utilizing the vast amount of publicly available microarray data as reference for comparison purposes.

Based on a previously published dataset (Lukk et al., 2010) of 5372 samples from 369 different tissues or cell lines, we created an extended gene expression landscape complementing the low dimensional principal components space (Lukk et al., 2010) with a tissue specific residual space. In vitro differentiated cells are then mapped to these two spaces in order to determine their similarity to the various tissues or cell lines.

We show that through the use of appropriate reference datasets and robust mapping algorithms, the extended expression landscape can be used to analyze data across microarray platforms. We apply the developed method to various in vitro differentiated cells, such as mesenchymal stromal cells and cardiomyocytes, detecting similarities and differences to the corresponding in vivo cell types. The mapping approach confirms the proper differentiation of mesenchymal stromal cells, derived from induced pluripotent stem cells, and is able to detect differences between cardiomyocytes that were differentiated and purified by different protocols. The method can thus be used as a quality control and optimization criterion for differentiation protocols.

Lukk et al. A global map of human gene expression. Nat Biotech, 28:322-324 (2010).

Abstract No. P185

MCAweb: an interactive graphical tool for Multiresolution Correlation Analysis in single-cell data

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Multiresolution Correlation Analysis (MCA) is a method for the hypothesis-free data exploration and visualization of local correlations in low-dimensional expression data, e.g. as emerging from qPCR or FACS. Using MCA it is possible to investigate the robustness of pairwise correlations with respect to perturbation or noise, graphically identify outliers that might skew correlation estimates, and identify factors that contribute to differential regulation motifs between pairs of covariates. Most importantly, one can visually identify subpopulations that yield different correlation networks, and which thus may imply a functional difference in the interactions between measured variables.

We demonstrate that MCA facilitates the identification of differentially regulated subpopulations in simulated data from a small gene regulatory network, followed by application to previously published single-cell qPCR data from mouse embryonic stem cells. We show that MCA recovers previously identified subpopulations, provides additional insight into the underlying correlation structure, reveals potentially spurious compartmentalizations, and provides insight into novel subpopulations.

We extend our work by developing MCAweb, an interactive, web-based user interface for MCA. Using MCAweb, it is possible to perform MCA analysis on gene expression data in a web browser. MCAweb facilitates subpopulation detection, pairwise correlation robustness analysis, and outlier identification in a fully interactive tool integrating MCA, scatter plots, histograms and a visualization of the correlation network. MCAweb also ranks factors according to their ability to induce the presence of robust regions of local correlation. This greatly simplifies analyses in high-dimensional data sets, and can be used e.g. to automatically identify factors that may exhibit a regulatory function. Our web tool is linked to an R-based computational framework that is also separately available for offline MCA analysis.

Feigelman, J., Theis, F.J., and Marr, C. (2014). MCA: Multiresolution Correlation Analysis, a graphical tool for subpopulation identification in single-cell gene expression data. BMC Bioinformatics 15, 240.

Please find abstract P186 under 'Selected presentations' T13/P186

Abstract No. P187

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 an *in vitro* culture 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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