GermanStemCellNetwork

5th Annual Conference

of the German Stem Cell Network (GSCN)
11 – 13 September 2017

Friedrich Schiller University Jena

www.gscn.org

Program & Abstracts

Supported by

Federal Ministry of Education and Research

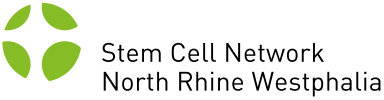
VDI Technologiezentrum

Collaboration with

fli Leibniz Institute on Aging – Fritz Lipmann Institute

MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE IN THE HELMHOLTZ ASSOCIATION

Kompetenznetzwerk Stammzellforschung NRW



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Friedrich Schiller University Jena

Organizer:
German Stem Cell Network (GSCN)
c/o Max Delbrück Center (MDC)
Robert-Rössle-Str. 10
13125 Berlin

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Welcome address

Dear Friends and Colleagues,

It is a great pleasure to welcome you to the **5th Annual Conference of the German Stem Cell Network (GSCN) in Jena,** which is hosted by the Leibniz Institute on Aging – Fritz Lipmann Institute e.V. (FLI). The conference will foster the interaction between researchers working on different types of stem cells, including pluripotent stem cells, stem cells during development and in adult organs as well as cancer stem cells. This year, we will have a particular focus on "stem cells and aging", and a slightly different format with some more plenary sessions. Two poster sessions, meet-the-expert-tables, an industry exhibition and two evening events will offer you many opportunities for exciting discussions, academic exchange and reconnecting with old friends and new colleagues.

Since promotion of junior scientists is a particular aim of the GSCN, most speakers are selected from the best abstracts that have been submitted. Again, awardees of the GSCN prizes will present their results during the Presidential Symposium. The program will be completed by invited keynote lectures to present the international points of reference for this fast growing field of research. We are happy that Elaine Fuchs (New York), Margaret Goodell (Texas), Heinrich Jasper (Novato), Jürgen Knoblich (Vienna) and Maike Sander (San Diego) will present their most recent results. We will conclude the conference with a Joined Session with the Leibniz Institute on Aging – Fritz Lipmann Institute e.V. (FLI) followed by a public outreach event in the stylish scenery of the "Volksbad" in Jena.

I hope you will enjoy this conference and wish you an interesting and enjoyable stay in Jena!

Best wishes,

Ulrich Martin (GSCN Acting president)

For the program committee

Daniel Besser (Berlin)
Ulrich Martin (Hannover)
Ana Martin-Villalba (Heidelberg)
Karl Lenhard Rudolph (Jena)
Julia von Maltzahn (Jena)
Claudia Waskow (Dresden)

sysmex

EmbryoS@fe / StemS@fe

SAFETY CONCEPTS

Working environment
(EN12469:2000; ISO 14644 CLASS 5)
dedicated to:

- human assisted reproduction techniques
- safe production and handling of iPS cells

www.sysmex.ch/safety-concepts

Table of Contents

Conference Information

Venue

Friedrich Schiller University Jena, Abbe Campus
Ernst-Abbe-Platz / Carl-Zeiss-Str. 3 | 07743 Jena

Date

Monday, 11 September to Wednesday, 13 September 2017

Registration

Regular fee:	*570 €*	Technical assistant fee:	*120 €*
Regular member fee:	*280 €*	Day ticket fee (onsite):	*200 €*
Student fee:	*235 €*	Day ticket member fee (onsite):	*160 €*
Student member fee:	*140 €*		

The registration fee includes the admission to all scientific sessions, poster and commercial exhibition, the get-together on 11 September and the networking evening on 12 September, lunch and coffee breaks, free internet access, conference documents including badge, final program and abstract book.

Internet

Internet access via Wireless LAN is free of charge. The login data is provided at the registration desk.

Poster exhibition

Posters will be displayed during the conference in two sessions on the ground floor. Authors are asked to be present at their poster during the poster session. You will find the number of your poster in this abstract volume. Posters in poster session I should be mounted on Monday, 10:00 – 17:00 h and removed latest on Tuesday at 14:00 h. Posters in poster session II should be mounted on Tuesday, 14:00 – 16:00 h and removed latest on Wednesday at 16:00 h.

Poster session I
Monday, 11 September 2017,
18:00 – 20:00 h

Even numbers will be presented 18:00 – 19:00 h and odd numbers 19:00 – 20:00 h.

- Stem cells in regenerative therapies (P001 – P028)
- Stem cells in disease modeling and drug development (P029 – P068)

Poster session II
Tuesday, 12 September 2017, 17:00 – 19:00 h

Even numbers will be presented 17:00 – 18:00 h and odd numbers 18:00 – 19:00 h.

- Stem cells in diseases: cancer stem cells (P069 – P076)
- Computational stem cell biology and systems biology (P077 – P084)
- Somatic stem cells and development (P085 – P103)
- Pluripotency and reprograming (P104 – P124)
- Hematopoietic stem cells (P125 – P142)

GSCN awards

Travel awards

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

- *Claudia Bruedigam,* QIMR Berghofer Medical Research Institute Brisbane, Australia
- *Christina Galonska,* Max Planck Institute for Molecular Genetics Berlin
- *Vishal Menon,* University of Freiburg
- *Waseem Nasr,* University Hospital Leipzig
- *Eszter Szabo,* Research Center for Natural Sciences – Hungarian Academy of Science, Budapest

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

eppendorf

Poster awards

There will be two poster awards for each poster session. The awardees will receive 500 €. Authors are asked to be present at the poster award ceremony, which will take place on Wednesday, 13 September 2017, 16.30 – 17.00 h in the lecture hall 2.

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

PEPROTECH
OUR SUPPORT, YOUR DISCOVERY

Scientific awards 2017

A top-class commission supported the GSCN in choosing this year's awardees. The three awardees will give a presentation in the Presidential Symposium on Tuesday, 12 September 2017, 14:30 – 16:45 h.

- **GSCN Young Investigator Award:**
  Francesco Neri, Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Jena
- **GSCN Female Scientist Award:**
  Elly Tanaka, Institute of Molecular Pathology (IMP), Vienna, Austria / Technical University Dresden
- **The GSCN Publication of the Year Award** (July 2016 to June 2017)
  goes to *Gray Camp* and *Barbara Treutlein* of the Max Planck Institute for Evolutionary Anthropology, Leipzig together with *Keisuke Sekine* and *Takanori Takebe,* Cincinnati Children's Hospital Medical Center (Ohio, United States). Their publication "Multilineage communication regulates human liver bud development from pluripotency" appeared in the journal Nature (Camp, JG et al., 2017, Nature 546, 533-538, doi:10.1038/nature22796).

Social events

Get-together

Monday, 11 September 2017
20:00 – 21:30 h

Mensa Ernst-Abbe-Platz
Ernst-Abbe-Platz 8
07743 Jena

All participants and exhibitors are invited to a **Get-together** with dinner buffet in the Mensa Ernst-Abbe-Platz.

Networking evening in the Volksbad

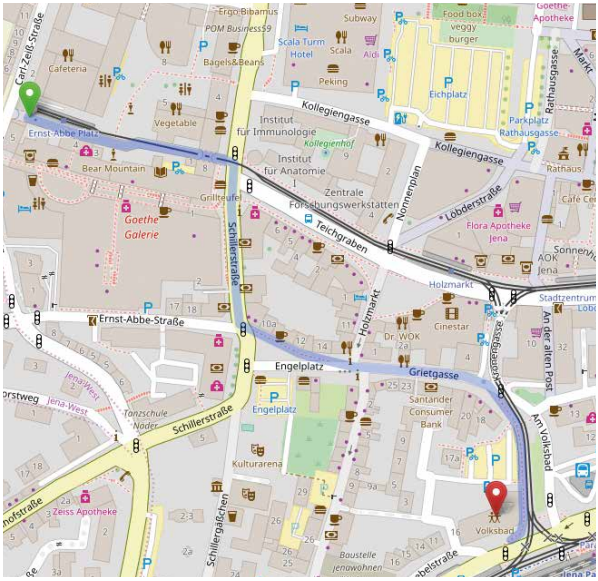
Tuesday, 12 September 2017
19:30 – 01:00 h

Volksbad
Knebelstraße 10 | 07743 Jena

Experience an extraordinary evening in the unique **Volksbad,** a 100 year old former bathing hall.

The networking evening with dinner buffet and DJ is included in the registration fee and open for all participants and exhibitors.

The Volksbad is located within walking distance to the Abbe Campus.



Public outreach event (in German)

Moderne Zelltherapien – Stammzellen bei Alterung, Krebserkrankungen & Organoiden

Podiumsdiskussion zur aktuellen Forschung mit Wissenschaftlern und Ärzten

Mittwoch, 13. September, 18:30 – 21 Uhr

Ort: **Volksbad**
Knebelstraße 10
07743 Jena

Programm: 18:30 Uhr: Einlass & Posterausstellung zu Stammzellen
19:00 Uhr: Podiumsdiskussion & Kurzfilme
20:30 Uhr: Ausklang mit Brezeln und Getränk
21:00 Uhr: Ende

Grundlagenforschung an Stammzellen bietet wichtige Erkenntnisse für die Entwicklung moderner Zelltherapien. Heute erhalten Sie Antworten auf Ihre Fragen:

- Wie laufen Alternsprozesse in Stammzellen ab?
- Warum nehmen Krankheiten mit steigendem Alter zu?
- Wie können Krankheitsverläufe z.B. bei Blutkrebs durch Stammzelltherapie verändert werden?
- Welche Forschungsmöglichkeiten bieten Organoide aus Stammzellen?
- Wie ist der Stand der Forschung?
- Welche Aussichten zeichnen sich für die Klinik und die Anwendung ab?

Wissenschaftler und Ärzte aus Jena, Wien, Heidelberg und Dresden stellen den aktuellen Stand der Grundlagenforschung und den möglichen klinischen Einsatz moderner Zelltherapien vor. Kurze Filme veranschaulichen ihre Arbeit. Anschließend diskutieren die Experten Einsatzmöglichkeiten von Stammzellen bei Alternsprozessen und Erkrankungen und beantworten Fragen aus dem Publikum:

- *Prof. Dr. Florian Heidel,*
  Universitätsklinikum Jena & Leibniz-Institut für Alternsforschung – Fritz-Lipmann-Institut
- *Prof. Dr. Jürgen Knoblich,*
  Institut für Molekulare Biotechnologie, Wien
- *Prof. Dr. Andreas Trumpp,*
  Deutsche Krebsforschungszentrum, Heidelberg
- *Prof. Dr. Claudia Waskow,*
  TU Dresden

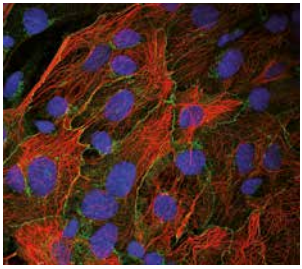


Photo: © MDC / Chen Chen

Moderation: *Dr. Stefanie Seltmann*

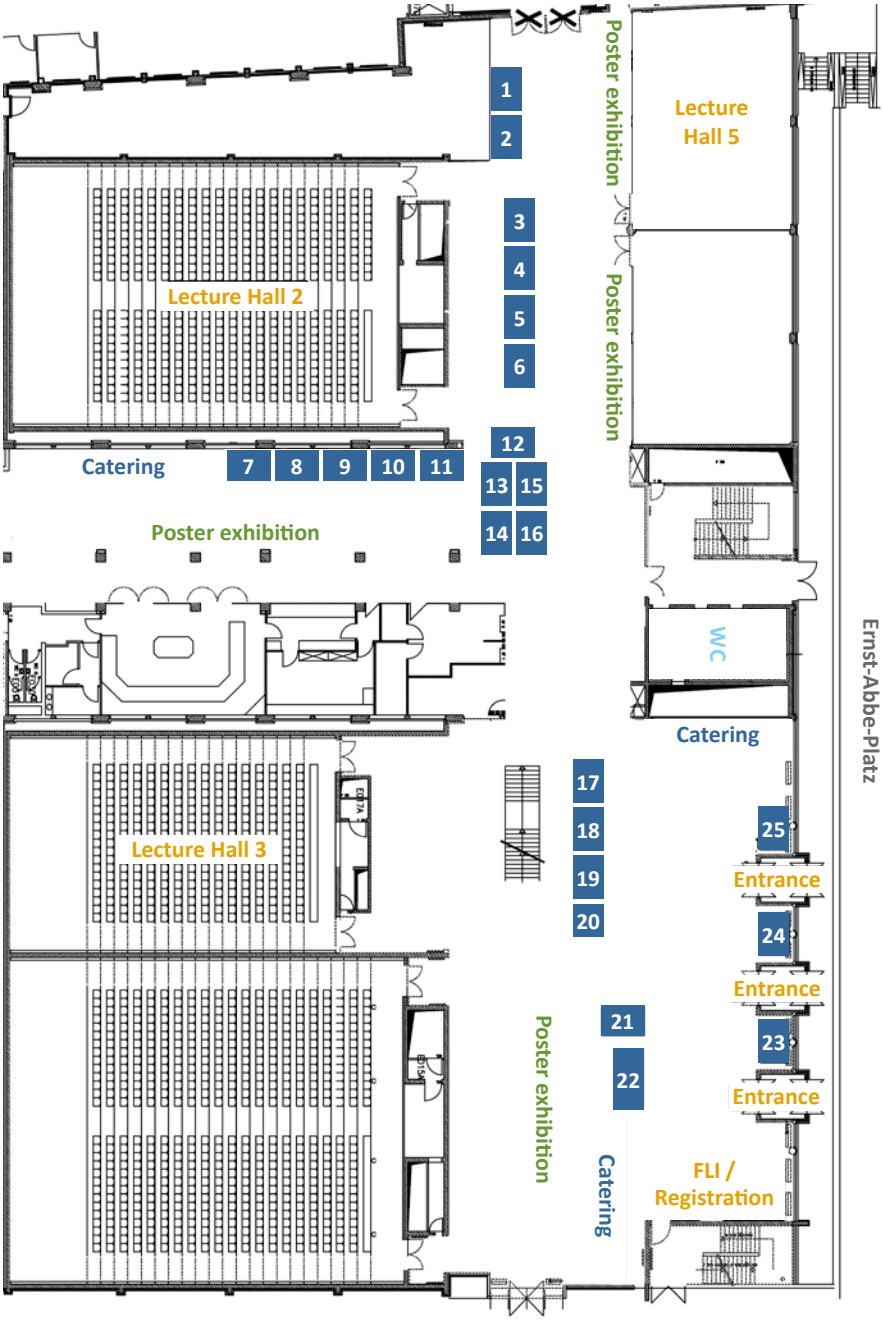
Floor plan

Sorted in alphabetical order

COMPANY	NUMBER
ALS Automated Lab Solutions	25
Becton Dickinson	21
BioLamina AB	6
Biological Industries	9
Bioquell UK	1
BioSpherix	23
BioTek Instruments	2
ChemoMetec A/S	10
Consarctic	16
Corning B.V. Life Sciences	4
Cygenia	20
Eppendorf	11
I&L Biosystems	22
La Vision BioTec	7
Lonza Cologne	3
Maco Pharma International	19
Miltenyi Biotec	13
neoFroxx	9
OLS OMNI Life Science	5
PELOBiotech	8
PeproTech	14
PromoCell	24
ReproCELL Europe	8
Sarstedt	15
STEMCELL Technologies	17
Takara Bio Europe, SAS	18
Thermo Fisher Scientific	12

Sorted by booth number

NUMBER	COMPANY
1	Bioquell UK
2	BioTek Instruments
3	Lonza Cologne
4	Corning B.V. Life Sciences
5	OLS OMNI Life Science
6	BioLamina AB
7	La Vision BioTec
8	PELOBiotech / ReproCELL Europe
9	Biological Industries / neoFroxx
10	ChemoMetec A/S
11	Eppendorf
12	Thermo Fisher Scientific
13	Miltenyi Biotec
14	PeproTech
15	Sarstedt
16	Consarctic
17	STEMCELL Technologies
18	Takara Bio Europe, SAS
19	Maco Pharma International
20	Cygenia
21	Becton Dickinson
22	I&L Biosystems
23	BioSpherix
24	PromoCell
25	ALS Automated Lab Solutions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Poster exhibition
Catering
FLI /
Registration

Program overview

MONDAY, 11 September 2017

	Lecture hall 2	Lecture hall 3	Lecture hall 5
10:00 – 11:00	Registration		
11:00 – 11:30	**Opening**		
11:30 – 12:15	**Keynote lecture I** *Elaine Fuchs*		
12:15 – 13:00	**Keynote lecture II** *Heinrich Jasper*		
13:00 – 14:30	Lunch break / Industry exhibition		
14:30 – 16:20	**Scientific working group session I**		
	Federico Calegari Pluripotency and reprogramming Somatic stem cells and development		
16:30 – 16:45	**Scientific working group discussions I**		
	Pluripotency and reprogramming		Somatic stem cells and development
16:30 – 17:00	Coffee break / Industry exhibition		
17:00 – 18:00		**GSCN Members Meeting**	
18:00 – 20:00	**Poster session I (P001 – P068)**		
18:00 – 19:00	posters with even numbers		
19:00 – 20:00	posters with uneven numbers		
20:00 – 21:30	**Informal GSCN get-together** with dinner buffet in the Mensa Ernst-Abbe-Platz		

TUESDAY, 12 September 2017

	Lecture hall 2	Lecture hall 3	Lecture hall 5
09:00 – 11:00	**Scientific working group session II**		
	Thomas Höfer Hematopoietic stem cells Computational stem cell biology Stem cells in diseases: cancer stem cells		
11:00 – 11:15	**Scientific working group discussions II**		
	Hematopoietic stem cells	Computational stem cell biology	Stem cells in diseases: cancer stem cells
11:00 – 11:30	Coffee break / Industry exhibition		
11:30 – 13:00	**Industry session: "Technologies from GSCN industry partners"**		
	PeproTech GmbH Thermo Fisher Scientific Eppendorf AG	Miltenyi Biotec GmbH Takara Bio Europe, SAS Biological Industries	BioSpherix, Ltd. Corning B.V. Life Sciences STEMCELL Technologies GmbH
13:00 – 14:30	Lunch break / Industry exhibition / Poster viewing		
13:15 – 14:15	**Meet-the-expert tables** in the 1st floor (limited to 10 participants each)		
	Seminar room 121 *Thomas Höfer*	Seminar room 122 *Thomas Braun*	Seminar room 131 *Claudia Waskow*

	Lecture hall 2	Lecture hall 3	Lecture hall 5
14:30 – 16:45	**Presidential Symposium**		
14:30 – 15:15	*Jürgen Knoblich*		
15:15 – 15:45	**Female Scientist Award** *Elly Tanaka*		
15:45 – 16:15	**Young Investigator Award** *Francesco Neri*		
16:15 – 16:45	**Publication of the Year Award** *Gray Camp*		
16:45 – 17:00	Coffee break / Industry exhibition		
17:00 – 19:00	**Poster session II (P069 – P142)**		
17:00 – 18:00	posters with even numbers		
18:00 – 19:00	posters with uneven numbers		
19:30 – 01:00	**GSCN networking evening** with dinner buffet and DJ at the Volksbad		

WEDNESDAY, 13 September 2017

	Lecture hall 2	Lecture hall 3	Lecture hall 5
09:00 – 11:00	**Scientific working group session III**		
	Bert Klebl Stem cells in regenerative therapies Stem cells in disease modeling and drug development		
11:00 – 11:15	**Scientific working group discussions III**		
	Stem cells in regenerative therapies		Stem cells in disease modeling and drug development
11:00 – 11:30	Coffee break / Industry exhibition		
11:30 – 13:00	**Concurrent strategic working group session**		
	Career development	Technologies in stem cell research	Clinical trials and regulatory affairs
13:00 – 14:30	Lunch break / Industry exhibition / Poster viewing		
13:15 – 14:15	**Meet-the-expert tables** in the 1st floor (limited to 10 participants each)		
	Seminar room 121 *Ralf Sanzenbacher*	Seminar room 122 *Andreas Bosio*	Seminar room 131 *Bert Klebl*
14:30 – 16:30	**Joined session with FLI**		
14:30 – 15:15	**Keynote lecture III** *Maike Sander*		
15:15 – 15:30	**FLI talk I** *Florian Heidel*		
15:30 – 16:15	**Keynote lecture IV** *Margaret Goodell*		
16:15 – 16:30	**FLI talk II** *Julia v. Maltzahn*		
16:30 – 17:00	**Poster award ceremony and closing remarks**		
	End of conference		
18:30 – 21:00	**GSCN public outreach event with panelists in the Volksbad (in German)** Moderne Zelltherapien – Stammzellen bei Alterung, Krebserkrankungen & Organoiden		

Opening | Evening events | Members meeting | FLI session
Keynote lectures | GSCN awardees
Scientific working group sessions
Poster sessions and Poster award ceremony
Strategic working group sessions | Meet-the-expert tables
Industry session

Program

MONDAY, 11 September

10:00 – 11:00	Registration
Lecture hall 2	**Opening**
11:00 – 11:10	Welcome to Friedrich Schiller University (FSU), Jena *Walter Rosenthal,* President FSU
11:10 – 11:20	Welcome to Jena and Thuringia *Wolfgang Tiefensee,* State Minister of Economy, Science and the Digital Society of Thuringia
11:20 – 11:25	Leibniz Institute on Aging – Fritz Lipmann Institute (FLI) *Daniela Barthel,* Administrative Director
11:25 – 11:30	GSCN Annual Conference 2017 *Ulrich Martin,* President GSCN
11:30 – 12:15	**Keynote lecture I** K1 – Stem cells in silence, action and cancer *Elaine Fuchs,* Howard Hughes Medical Institute, Rockefeller University, New York, USA (Chair: *Ana Martin-Villalba*)
12:15 – 13:00	**Keynote lecture II** K2 – Age-related stem cell dysfunction: causes and consequences *Heinrich Jasper,* Buck Institute, Novato, USA (Chair: *Hartmut Geiger*)
13:00 – 14:30	Lunch break / Industry exhibition
Lecture hall 2	**Scientific working group session I** (Chairs: *Micha Drukker* / *Thomas Braun*)
14:30 – 14:50	T01 – Control of mammalian neurogenesis *Federico Calegari,* Center for Regenerative Therapies / TU Dresden
14:50 – 15:35	**Pluripotency and reprogramming**
14:50 – 15:05	T02 – FACT is a reprogramming barrier in C. elegans and human cells *Baris Tursun,* Max Delbrück Center for Molecular Medicine (MDC), Berlin
15:05 – 15:20	T03 – A novel molecular mechanism of cell-fusion mediated reprogramming *Karthik Arumugam,* Centre for Genomic Regulation (CRG), Barcelona, Spain
15:20 – 15:35	T04 – Directing cell fates with artificially evolved transcription factors *Ralf Jauch,* Guangzhou Institutes of Biomedicine and Health, China
15:35 – 16:20	**Somatic stem cells and development**
15:35 – 15:50	T05 – Single-cell RNA sequencing reveals aging-induced quiescence in neural stem cells of the subventricular zone *Georgios Kalamakis,* German Cancer Research Center (DKFZ), Heidelberg

15:50 – 16:05 T06 – Vitamin A in stem cell aging
Simon Schwörer, Leibniz Institute on Aging / Fritz Lipmann Institute (FLI), Jena

16:05 – 16:20 T07/P – Regulation of epidermal stem cells by flightless I protein during wound repair
Nanxing Yang, University of South Australia, Adelaide

16:30 – 16:45 **Scientific working group discussions I**

Lecture hall 2 Pluripotency and reprogramming

Lecture hall 5 Somatic stem cells and development

16:30 – 18:00 Coffee break / Industry exhibition

Lecture hall 3

17:00 – 18:00 **GSCN General membership meeting**

18:00 – 20:00 **Poster session I** supported by Thermo Fisher Scientific
Stem cells in regenerative therapies (P001 – P028)
Stem cells in disease modeling and drug development (P029 – P068)
Even numbers: please present your poster from 18:00 – 19:00
Odd numbers: 19:00 – 20:00

20:00 – 21:30 **Informal GSCN get together for all participants**
with dinner buffet at the Mensa Ernst-Abbe-Platz

TUESDAY, 12 SEPTEMBER

Lecture hall 2 **Scientific working group session II**
(Chairs: *Andreas Trumpp / Claudia Waskow*)

09:00 – 09:20 T08 – Learning hematopoietic stem cell dynamics from fate mapping
Thomas Höfer, German Cancer Research Center (DKFZ), Heidelberg

09:20 – 09:50 **Hematopoietic stem cells**

09:20 – 09:35 T09 – Human haematopoietic stem cell differentiation follows a continuous Waddington-like landscape
Simon Haas, German Cancer Research Center (DKFZ) / HI-STEM, Heidelberg

09:35 – 09:50 T10 – Paraoxonase-2 and its effects on hematopoietic stem cell proliferation and differentiation
Lisa Spiecker, University Medical Center of the Johannes Gutenberg-University Mainz

09:50 – 10:20 **Computational stem cell biology**

09:50 – 10:05 T11 – A computational systems biology approach to identify niche determinants of stem cell identity
Srikanth Ravichandran, University of Luxembourg

10:05 – 10:20 T12 – Adult neural stem cells increase the rate of asymmetric divisions during aging
Lisa Bast, TU Munich, Helmholtz Zentrum Munich

10:20 – 10:50	**Stem cells in disease: cancer stem cells**
10:20 – 10:35	T13 – Myeloid leukemia stem cells depend on phospholipase C gamma 1 (Plcg1) signaling *Tina Schnöder,* University Hospital & Leibniz Institute on Aging, Jena
10:35 – 10:50	T14 – C/EBPβ-LIP regulates the let-7/Lin28 circuit to control cellular metabolism *Tobias Ackermann,* European Research Institute for the Biology of Ageing (ERIBA), Groningen, The Netherlands
11:00 – 11:15	**Scientific working group discussions II**
Lecture hall 2	Hematopoietic stem cells
Lecture hall 3	Computational stem cell biology
Lecture hall 5	Stem cells in disease: cancer stem cells
11:00 – 11:30	Coffee break / Industry exhibition
11:30 – 13:00	**Industry session: "Technologies from GSCN industry partners"**
Lecture hall 2	**Main supporter** (Chair: *Jan Lohmann*)
11:30 – 12:00	C1 – Tissue engineering of primary human in vitro models – influence of mechanical and biochemical stimulation on tissue functionality *Antje Appelt-Menzel,* University Hospital & Fraunhofer IGB Würzburg, representing PeproTech GmbH
12:00 – 12:30	C2 – Evolving media systems for modern stem cell applications *David Kuninger,* Thermo Fisher Scientific
12:30 – 13:00	C3 – Current challenges in stem cell production for regenerative medicine and drug development *Christof Knocke,* Eppendorf AG
Lecture hall 3	**Supporter** (Chair: *Benedikt Berninger*)
11:30 – 12:00	C4 – Novel technologies for the cultivation and quality control of iPSCs and their derivatives *Sebastian Knöbel,* Miltenyi Biotec GmbH
12:00 – 12:30	C5 – A novel maintenance medium extends the life-span and enables long term applications for both human primary hepatocytes and human pluripotent stem cell derived hepatocytes in conventional 2D cultures *Barbara Küppers-Munther,* Takara Bio Europe, SAS
12:30 – 13:00	C6 – Novel serum-free, xeno-free vitronectin-based culture system for hPSC *Suzanne Badoux,* Biological Industries
Lecture hall 5	**Supporter** (Chair: *Insa Schröder*)
11:30 – 12:00	C7 – Avoid irrelevant, non-reproducible data – the neglected variable: physiologic oxygen *Paul Michaels,* BioSpherix, Ltd.
12:00 – 12:30	C8 – Systems and solutions for culture and scale up of human pluripotent stem cells *Florent Bornes,* Corning B.V. Life Sciences

12:30 – 13:00 C9 – Highly efficient single-cell human pluripotent stem cell cloning and robust cardiomyocyte differentiation
Katharina Debowski, STEMCELL Technologies GmbH

13:00 – 14:30 Lunch break / Industry exhibition / Poster viewing supported by STEMCELL Technologies GmbH

13:15 – 14:15 **Meet-the-expert tables**
(in the 1st floor, please register at registration desk, limited to 10 participants)

Seminar room 121 *Thomas Höfer,* DKFZ Heidelberg (Bioinformatic tools in stem cell research)

Seminar room 122 *Thomas Braun,* MPI Bad Nauheim (Stem cell approaches in heart and lung development)

Seminar room 131 *Claudia Waskow,* TU Dresden (Hematopoietic stem cells)

Lecture hall 2 **Presidential Symposium**
(Chair: U*lrich Martin*)

14:30 – 15:15 PS1 – Cerebral organoids: modelling human brain development and tumorigenesis in stem cell derived 3D culture
Jürgen Knoblich, IMBA, Vienna, Austria

15:15 – 15:45 Female Scientist Award 2017
PS2 – Unraveling the cellular and molecular basis of limb regeneration
Elly Tanaka, Institute of Molecular Pathology (IMP), Vienna, Austria / Technical University Dresden

15:45 – 16:15 Young Investigator Award 2017
PS3 – DNA methylation and demethylation dynamics in stem cells
Francesco Neri, Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Jena

16:15 – 16:45 Publication of the Year 2017 Award
PS4 – Multilineage communication regulates human liver bud development from pluripotency
J. Gray Camp, Max Planck Institute for Evolutionary Anthropology, Leipzig

16:45 – 17:00 Industry exhibition

17:00 – 19:00 **Poster session II** supported by Miltenyi Biotech GmbH
Stem cells in diseases: cancer stem cells (P069 – P076)
Computational stem cell biology and systems biology (P077 – P084)
Somatic stem cells and development (P085 – P103)
Pluripotency and reprograming (P104 – P124)
Hematopoietic stem cells (P125 – P142)

Even numbers: please present your poster from 17:00 – 18:00
Odd numbers: 18:00 – 19:00

19:30 – 01:00 **GSCN networking evening for all participants**
with dinner buffet and DJ at the Volksbad

WEDNESDAY, 13 SEPTEMBER

Lecture hall 2	**Scientific working group session III** supported by Takara Bio Europe, SAS (Chairs: *Bert Klebl, Richard Schäfer*)
09:00 – 09:20	T15 – Stem cell based assays as the next generation phenotypic drug screens *Bert Klebl,* Lead Discovery Center (LDC), Dortmund
09:20 – 10:05	**Stem cells in regenerative therapies**
09:20 – 09:35	T16 – Human iPSC-derived phagocytes as a cellular therapeutic to treat bacterial infections *Nico Lachmann,* REBIRTH / Hannover Medical School (MHH)
09:35 – 09:50	T17 – HMGB1 accelerates regeneration of multiple tissues by transitioning stem cells to G(Alert) *Ana Isabel Espirito Santo,* University of Oxford, UK
09:50 – 10:05	T18 – Metabolic targeting by mesenchymal stem cells in a mouse model of diet-induced NASH *Bruno Christ,* University Hospital Leipzig
10:05 – 10:50	**Stem cells in disease modeling and drug development**
10:05 – 10:20	T19 – Genetically encoded calcium indicators for drug screening in human engineered heart tissues *Umber Saleem,* University Medical Center Hamburg-Eppendorf (UKE)
10:20 – 10:35	T20 – Establishment of murine 3D bronchoalveolar lung organoids from adult somatic stem cells for organ development and disease modeling *Ana Ivonne Vazquez Armendariz,* Universities Giessen & Marburg Lung Center (UGMLC)
10:35 – 10:50	T21 – A xenotransplantation approach using human pluripotent stem cell-derived pancreatic organoids *Meike Hohwieler,* University Medical Center Ulm
11:00 – 11:15	**Scientific working group discussions III**
Lecture hall 2	Stem cells in regenerative therapies
Lecture hall 5	Stem cells in disease modeling and drug development
11:00 – 11:30	Coffee break / Industry exhibition
11:30 – 13:00	**Concurrent strategic working group session**
Lecture hall 2	**Career development** (Chairs: *Hartmut Geiger, Insa Schröder*)
11:30 – 12:15	S1 – Options and opportunities for scientists in science communication *Tobias Maier,* National Institute for Science Communication (NaWik), Karlsruhe
12:15 – 13:00	S2 – Tips and tools for efficient science communication in the public and private sector *Stefanie Seltmann,* Pfizer Deutschland GmbH, Berlin
Lecture hall 3	**Technologies in stem cell research** (Chairs: *Andreas Bosio, Michael Cross*)
11:30 – 12:00	S3 – Reconstructing human neurogenesis using single-cell transcriptomics *Barbara Treutlein,* Max Planck Institute for Evolutionary Anthropology, Leipzig

12:00 – 12:30 S4 – Multimodal single cell genomics
Sascha Sauer, Max Delbrück Center Berlin

12:30 – 13:00 S5 – tba
tbd

Lecture hall 5 **Clinical trials and regulatory affairs**
(Chairs: *Zoltan Ivics, Ralf Sanzenbacher, Torsten Tonn*)

11:30 – 12:00 S6 – Development of (stem) cell-based medicinal products: Regulatory aspects
Ralf Sanzenbacher, Paul Ehrlich Institute, Langen

12:00 – 12:30 S7 – Development of (stem) cell-based medicinal products: Turning research into GMP
Martin Hildebrandt, TUMCells Interdisciplinary Center for Cellular Therapies, Munich

12:30 – 13:00 S8 – Development of (stem) cell-based medicinal products: Clinical considerations for cell-based therapies
Mohamed Abou-El-Enein, Berlin-Brandenburg Center for Regenerative Therapies (BCRT)

13:00 – 14:30 Lunch break / Industry exhibition / Poster viewing

13:15 – 14:15 **Meet-the-expert tables**
(in the 1st floor, please register at registration desk, limited to 10 participants)

Seminar room 121 *Ralf Sanzenbacher,* PEI, Langen (ATMPs and GMP)

Seminar room 122 *Andreas Bosio,* Miltenyi, Bergisch Gladbach (Technologies and private sector)

Seminar room 131 *Bert Klebl,* LDC, Dortmund (Disease modeling and translation)

Lecture hall 2 **Joined session with FLI**

14:30 – 15:15 **Keynote lecture III**
K3 – Deconstructing pancreatic development and disease with human pluripotent stem cells
Maike Sander, University of San Diego, California, USA
(Chair: *Julia v. Maltzahn*)

15:15 – 15:30 FLI01 – Maintenance of Jak-inhibitor treated MPN depends on cold shock protein Ybx1
Florian Heidel, University Hospital Jena

15:30 – 16:15 **Keynote lecture IV**
K4 – DNA methylation: from stem cell differentiation to chromation organisation
Margaret Goodell, Baylor College of Medicine, Texas, USA
(Chair: *Claudia Waskow*)

16:15 – 16:30 FLI02 – Functional relevance of Klotho for maintenance and regeneration of skeletal muscle
Julia v. Maltzahn, Leibniz Institute on Aging / Fritz Lipmann Institute (FLI), Jena

Lecture hall 2 **Closing ceremony**

16:30 – 16:45 Poster award ceremony and announcement of industry quiz winners
Daniel Besser, GSCN

16:45 – 17:00 **GSCN Outlook 2018**
Daniel Besser, GSCN

Speaker abstracts

5th Annual Conference
German Stem Cell Network
11 – 13 September 2017

Keynote lectures: K1 – K4

K1 Stem cells in silence, action and cancer
Elaine Fuchs

K2 Age-related stem cell dysfunction: causes and consequences
Heinrich Jasper

K3 Deconstructing pancreatic development and disease with human pluripotent stem cells
Maike Sander

K4 DNA methylation: from stem cell differentiation to chromatin organisation
Margaret Goodell

Abstract No. K1

Stem cells in silence, action and cancer

Elaine Fuchs

Laboratory of Mammalian Cell Biology and Development; Howard Hughes Medical Institute, The Rockefeller University, New York, USA

Adult tissues require stem cells to replenish cells during normal turnover (homeostasis) and in response to injury. How stem cells balance self-renewal and differentiation is of fundamental importance to our understanding of normal tissue maintenance and wound repair. Moreover, increasing evidence suggests that the regulatory circuitry governing this balancing act is at the roots of aging as well as some types of cancers. The skin is an excellent model system to understand how stem cells function in normal tissue generation, why they reduce their activity in aging and how they accelerate it uncontrollably in cancer. Using skin as our paradigm, we've identified a niche of stem cells located within the hair follicle. In normal homeostasis, these stem cells become activated to fuel cyclical bouts of hair growth. Upon injury, these cells become activated to migrate upward and repair the skin epidermis. Upon acquiring oncogenic mutations, these cells give rise to squamous cell carcinomas (SCCs), which affect many epithelial tissues, making them among the most common and life-threatening cancers world-wide. We've been dissecting how extrinsic signaling to stem cells triggers a cascade of changes in chromatin and transcriptional re-modeling that governs the activation, polarization and migration of stem cells during tissue development, homeostasis, hair cycling and wound repair. We've also been exploring what instructs activated stem cells to return to quiescence and stop making tissue when wounds are repaired, why aging skin slows the process of tissue regeneration and how cancer stem cells avoid these brakes on tissue growth. Our findings have provided us with new insights into our understanding of stem cell dynamics, and in so doing have begun to unveil new avenues for therapeutics in skin regeneration, aging and cancer.

Abstract No. K2

Age-related stem cell dysfunction: causes and consequences

Heinrich Jasper

Buck Institute for Research on Aging, Novato and Genentech Inc., South San Francisco, CA, USA & Leibniz Institute for Aging Research, Jena, Germany

Proliferation of stem cells has to be carefully controlled to maintain long-term regenerative capacity of high-turnover tissues while preventing cancer. We study barrier epithelia in Drosophila and mice as genetically accessible models in which to explore stem cell function. Our work focuses on the regulation of intestinal stem cell (ISC) and tracheal basal cell (BC) proliferation by stress and growth factor signaling. ISCs over-proliferate in aging flies, limiting lifespan. In recent work, we have explored some of the underlying causes of this hyper-proliferative phenotype, and have established a role for age-related immunosenescence and associated commensal dysbiosis in this breakdown of homeostasis. Our findings deepen our understanding of the regulation of proliferative homeostasis in aging barrier epithelia, and suggest potentially conserved mechanisms by which proliferative homeostasis can be preserved in the long term, extending lifespan. I will present recent findings on the regulation of ISC and BC maintenance, on the control of proteostasis in stem cells, and on the impact of this control on age-related tissue dysfunction.

Abstract No. K3

Deconstructing pancreatic development and disease with human pluripotent stem cells

Maike Sander

Departments of Pediatrics and Cellular & Molecular Medicine, Pediatric Diabetes Research Center, Sanford Consortium for Regenerative Medicine; University of California-San Diego, La Jolla, USA

The goal of research in my laboratory is to understand the molecular mechanisms that underlie the ability of multipotent stem/progenitor cells to produce the different cell types of the pancreas. We investigate this question in the context of normal pancreatic development, pancreas regeneration, and pathogenesis, employing genetic mouse models as well as a human pluripotent stem cell (hPSC)-based *in vitro* differentiation system. A major recent direction of my laboratory has been to define the dynamic changes in gene expression, transcription factor occupancy, and chromatin that occur during pancreatic differentiation through the use of next generation sequencing-based assays. Combined with molecular, cell-based, and genetic approaches in both mice and hPSCs, these assays have provided insight into fundamental mechanisms of cell fate determination in the context of normal development, regeneration, and pancreatic disease. In recent work, we have utilized genome-wide chromatin maps of pancreatic beta cells and their precursors to identify diabetes-risk associated genetic variants with potential relevance in beta cell development and function. We have employed CRISPR-Cas9 gene editing to ascertain enhancer-target gene relationships and to identify mechanisms by which diabetes-risk variants cause disease.

Abstract No. K4

DNA methylation: from stem cell differentiation to chromatin organization

Margaret Goodell

Baylor College of Medicine, Houston, Texas, USA

Hematopoietic stem cells (HSCs) sustain blood production through decades of life due to their capacity to both self-renewal and differentiate. Numerous genetic factors can influence the capacity of individual HSCs to regenerate the blood, with epigenetic regulators recently emerging as particularly important over time. Loss of DNA methyltransferase 3A (DNMT3A) can promote HSC self-renewal in the mouse, and mutations in DNMT3A are associated with clonal advantage in aging humans. We now show that loss of DNMT3A can lead to HSC immortalization with indefinite expansion capacity but no overt malignant transformation. Immortal HSCs exhibit marked changes of DNA methylation associated with highly conserved genes that are master regulators of tissue function. Regulation of DNA methylation, potential interactions with other epigenetic regulators, and implications for chromatin organization will be discussed.

Presidential Symposium lectures: PS1 – PS4

PS1 Cerebral organoids: modelling human brain development and tumorigenesis in stem cell derived 3D culture
Jürgen A. Knoblich

PS2 Unraveling the cellular and molecular basis of limb regeneration
Elly Tanaka

PS3 DNA methylation and demethylation dynamics in stem cells
Francesco Neri

PS4 Multilineage communication regulates human liver bud development from pluripotency
J. Gray Camp

Abstract No. PS1

Cerebral organoids: modelling human brain development and tumorigenesis in stem cell derived 3D culture

Jürgen A. Knoblich

IMBA - Institute of Molecular Biotechnology of the Austrian Academy of Science Vienna, Austria

The human brain is unique in size and complexity, but also the source of some of the most devastating human diseases. While many of these disorders 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. We have used 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. More recently, we have been able to generate organoid based models for human brain cancer and demonstrated their feasibility for drug testing. 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. PS2: Female Scientist Award

Unraveling the cellular and molecular basis of limb regeneration

Elly Tanaka

Research Institute of Molecular Pathology (IMP) Vienna, Austria

The phenomenon of limb and tail regeneration has fascinated biologists since Spallanzani's discoveries in 1768. For many years this complex process that involves many tissues responding to injury was difficult to decipher. Recently we have developed a number of molecular tools for the axolotl that have allowed us to identify the stem cells that undertake limb regeneration and the molecules that control them. In particular the connective tissue fibroblasts play an important role in templating regeneration. We show that these cells enter the blastema and reprogram to a multipotent limb bud progenitor-type cell. This process is stimulated by a number of conserved and non-conserved factors present in the wound epidermis. We have also recently sequenced and assembled the giant genome of the axolotl which will allow us to understand how the genome is re-awakened during regeneration.

Abstract No. PS3: Junior Investigator Award

DNA methylation and demethylation dynamics in stem cells

Francesco Neri

Epigenetics group, Leibniz Institute on Aging – Fritz Lipmann Institute (FLI) Jena

DNA methylation is a heritable epigenetic modification required for development, which causes transcriptional repression. In mouse embryonic stem cells (ESCs), developmental gene promoters are hypomethylated, but maintained repressed by Polycomb Repressive Complex 2 (PRC2). DNA methylation increases around implantation to remain stable in fully differentiated cells. Many of the developmental genes that undergo promoter methylation during cell differentiation are bivalent (target of PRC2) in stem cells. We discovered that PRC2 itself inhibits the establishment of DNA methylation on its targets. We showed that PRC2 recruits Dnmt3L and Ten Eleven Translocation 1 (Tet1) to maintain hypomethylated the promoter of bivalent developmental genes through a passive and active mechanism respectively. Active DNA demethylation is mediated by Tet proteins, that oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC). We developed a genome-wide approach, methylation-assisted bisulfite sequencing (MAB-seq), to map 5fC/5caC at single-base resolution and measure their abundance in stem cells. Thanks to the high sensitivity of the MAB-seq technique we could detect the enrichment of 5fC/5caC on the promoters of actively transcribed genes revealing an unexpected active process of methylation/demethylation on these genes. While the role of promoter DNA methylation is well understood, the molecular function of intragenic DNA methylation has been unknown even though deregulation of this epigenetic feature has been associated with several diseases. We showed that the Dnmt3b-dependent intragenic DNA methylation protects the gene body from RNA Polymerase II (RNA Pol II) spurious entry and cryptic transcription initiations.

Our results elucidate the functional role of the intragenic DNA methylation, and the existence of a RNA Pol II-triggered epigenetic crosstalk involving SetD2, H3K36me3, Dnmt3b and DNA methylation, to ensure gene transcription initiation fidelity. This security feedback is probably lost during cancer development, where global intragenic hypomethylation frequently occurs.

Abstract No. PS4

Multilineage communication regulates human liver bud development from pluripotency

J. Gray Camp[1,], Keisuke Sekine[2], Takanori Takebe[2,3], and Barbara Treutlein[1,4]*

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[2]Department of Regenerative Medicine; Yokohama City University Graduate School of Medicine, Kanazawa-ku, Yokohama, Japan
[3]Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Ohio, USA
[4]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
*Presenting author

Conventional two-dimensional differentiation from pluripotency fails to recapitulate cell interactions occurring during organogenesis. Three-dimensional organoids generate complex organ-like tissues; however, it is unclear how heterotypic interactions affect lineage identity. Here we use single-cell RNA sequencing to reconstruct hepatocyte-like lineage progression from pluripotency in two-dimensional culture. We then derive three-dimensional liver bud organoids by reconstituting hepatic, stromal, and endothelial interactions, and deconstruct heterogeneity during liver bud development. We find that liver bud hepatoblasts diverge from the two-dimensional lineage, and express epithelial migration signatures characteristic of organ budding. We benchmark three-dimensional liver buds against fetal and adult human liver single-cell RNA sequencing data, and find a striking correspondence between the three-dimensional liver bud and fetal liver cells. We use a receptor-ligand pairing analysis and a high-throughput inhibitor assay to interrogate signaling in liver buds, and show that vascular endothelial growth factor (VEGF) crosstalk potentiates endothelial network formation and hepatoblast differentiation. Our molecular dissection reveals interlineage communication regulating organoid development, and illuminates previously inaccessible aspects of human liver development.

FLI lectures: FLI1 – FLI2

FLI1 Maintenance of Jak-inhibitor treated MPN depends on cold shock protein Ybx1
Florian Heidel

FLI2 Functional relevance of Klotho for maintenance and regeneration of skeletal muscle
Julia von Maltzahn

Abstract No. FLI1

Maintenance of Jak-inhibitor treated MPN depends on cold shock protein Ybx1

Florian Heidel

Universitätsklinikum Jena & Leibniz Institute on Aging/FLI, Jena

Development of Janus-kinase inhibitors has revolutionized the therapeutic landscape for the treatment of myeloproliferative neoplasms (MPN). While Jak-inhibitors effectively reduce the inflammatory phenotype and constitutional symptoms, they do not affect disease burden or presence of the mutated clone to a major extent. Here, we identify cold shock protein Ybx1 as downstream effector of mutated Janus kinase. We provide the first evidence that inactivation of Ybx1 sensitizes Jak2V617F mutated MPN, but not normal hematopoietic cells, to Jak-inhibitor induced apoptosis. Jak-dependent signaling nodes and pro- and anti-apoptotic proteins were significantly de-regulated upon Ybx1 inactivation, identifying the apoptotic machinery as a tractable downstream regulator of therapeutic efficacy. Critically, Ybx1 inactivation caused regression of Jak2V617F allelic burden and disease penetrance of murine and human cells in vivo. These data indicate a novel treatment strategy to alter disease course in Jak2V617F mutated MPN via targeting Ybx1 and regulators of cell survival in combination with Jak-inhibition.

Abstract No. FLI2

Functional relevance of Klotho for maintenance and regeneration of skeletal muscle

*Hellen Ahrens, Judith Hüttemeister, Manuel Schmidt, and Julia von Maltzahn**

Leibniz Institute on Aging/Fritz Lipmann Institute (FLI), Jena
*Presenting author

Skeletal muscle has diverse functions in the organism and a remarkable ability to adapt to physiological demands such as growth, training and injury. Furthermore, it is one of the organs with the highest ability to regenerate. During aging a decrease in muscle mass and function such as force generation occurs, this phenomenon is also called sarcopenia. Additionally, the regenerative capacity declines during aging.

The klotho hypomorphic mouse is a common model for accelerated ageing. Beginning at an age of three weeks, these mice develop premature ageing phenotypes including a progressive decline in muscle mass and grip strength and a severely reduced life span. Here, we investigated the function of klotho in skeletal muscle maintenance and address the question which form of klotho (secreted or membrane-bound) is responsible for the muscle-specific phenotype. Klotho hypomorphic mice demonstrate several characteristics of sarcopenia including reduced myofiber diameters and a depletion of the muscle stem cell (satellite cell) pool. To investigate the regeneration process, we injured the tibialis anterior muscles of klotho hypomorphic mice with cardiotoxin. These mice showed regeneration deficits, which were more pronounced with increasing age. Muscle stem cells contribute essentially to muscle regeneration. Therefore, we analyzed the functionality of muscle stem cells on isolated myofibers where they are still in their endogenous niche, but are cultured independently of systemic influences. This ex vivo analysis demonstrated a perturbed function of muscle stem cells from klotho hypomorphic mice whereas in vitro assays with isolated myoblasts showed no influence of klotho expression on the differentiation potential. These results support the hypothesis that the soluble klotho –possibly secreted by the muscle stem cell niche- rather than the membrane-bound form of klotho influences the regeneration potential in skeletal muscle.

Selected presentations

5th Annual Conference
German Stem Cell Network
11 – 13 September 2017

Selected presentations: T01 – T21

(the PXXX number indicates an accompanying poster presentation)

T01 Control of mammalian neurogenesis
Federico Calegari

T02 FACT is a reprogramming barrier in C. elegans and human cells
Baris Tursun

T03 A novel molecular mechanism of cell-fusion mediated reprogramming
Karthik Arumugam

T04 Directing cell fates with artificially evolved transcription factors
Ralf Jauch

T05 Single-cell RNA sequencing reveals aging-induced quiescence in neural stem cells of the subventricular zone
Georgios Kalamakis

T06 Vitamin A in stem cell aging
Simon Schwörer

T07/P103 Regulation of epidermal stem cells by Flightless I protein during wound repair
Nanxing Yang

T08 Learning hematopoietic stem cell dynamics from fate mapping
Thomas Höfer

T09 Human haematopoietic stem cell differentiation follows a continuous Waddington-like landscape
Simon Haas

T10 Paraoxonase-2 and its effects on hematopoietic stem cell proliferation and differentiation
Lisa Spiecker

T11 A computational systems biology approach to identify niche determinants of stem cell identity
Srikanth Ravichandran

T12 Adult neural stem cells increase the rate of asymmetric divisions during aging
Lisa Bast

T13 Myeloid leukemia stem cells depend on phospholipase C gamma 1 (Plcg1) signaling
Tina M. Schnoeder

T14/P069 C/EBPβ-LIP regulates the let-7/Lin28 circuit to control cellular metabolism
Tobias Ackermann

T15 Stem cell based assays as the next generation phenotypic drug screens?
Bert Klebl

T16 Human iPSC-derived phagocytes as a cellular therapeutic to treat bacterial infections
Nico Lachmann

T17 HMGB1 accelerates regeneration of multiple tissues by transitioning stem cells to G_{Alert}
Ana Isabel Espirito Santo

T18 Metabolic targeting by mesenchymal stem cells in a mouse model of diet-induced NASH
Bruno Christ

T19 Genetically encoded calcium indicators for drug screening in human engineered heart tissues
Umber Saleem

T20/P065 Establishment of murine 3D bronchoalveolar lung organoids from adult somatic stem cells for organ development and disease modeling
Ana Ivonne Vazquez Armendariz

T21 A xenotransplantation approach using human pluripotent stem cell-derived pancreatic organoids
Meike Hohwieler

Abstract No. T01

Control of mammalian neurogenesis

Federico Calegari

Proliferation of Mammalian Neural Stem Cells; Center for Regenerative Therapies Dresden (CRTD) / TU Dresden

My laboratory has shown that the length of the G1 phase of the cell cycle of somatic stem cells controls their differentiation. This led us to promote the expansion of neural stem cells during development and adulthood thus increasing the number of neurons generated in the mammalian brain. This finding was important to reveal a new role of progenitor subtypes in establishing brain size and shape during development and evolution and the role of adult neurogenesis in cognitive function. Current work addresses the role of novel genes involved in corticogenesis including long non-coding RNAs and epigenetic marks. Our ultimate ambition is to understand and manipulate endogenous neurogenesis to improve brain function.

Abstract No. T02

FACT is a reprogramming barrier in C. elegans and human cells

Ena Kolundzic[1], Sebastian Diecke[2], Scott Lacadie[1], and Baris Tursun[1,]*

[1]Berlin Institute for Medical Systems Biology (BIMSB); Max Delbrück Center (MDC) for Molecular Medicine in the Helmholtz Association, Berlin
[2]Stem Cell Facility; Max Delbrück Center (MDC)
*Presenting author

Direct reprogramming of cellular identities by mis-expressing transcription factors is limited in different tissue contexts. We previously identified the histone chaperone LIN-53 as a reprogramming barrier in the nematode C. elegans. Depletion of LIN-53 allows direct conversion of germ cells to specific neuron types (Tursun et al., Science 2011). Analogously, a recent study showed that the mammalian LIN-53/CAF-1p48-containing histone chaperone CAF-1 is a barrier for cellular reprogramming in mouse cells (Cheloufi et al., Nature 2015) suggesting that more barrier factors might be conserved among C. elegans and higher organisms. Therefore, we conducted genetic screens using C. elegans and found that the histone chaperone FACT (FAcilitates Chromatin Transcription) is a novel reprogramming barrier. FACT protects different tissues from being directly reprogrammed into neuron-like cells in C. elegans. Moreover, we demonstrate that FACT depletion also enhances the reprogramming of human fibroblasts. Additionally, we identified a new FACT subunit in C. elegans, which restricts reprogramming of germ cells into neurons. FACT is predominantly known as a positive effector that regulates gene expression by facilitating transcription. Nevertheless, FACT can also act as a repressor in certain instances such as at the mating locus in yeast. We performed transcriptome analysis as well as chromatin profiling using ATAC-seq and found that loss of FACT results in transcriptional de-repression at specific genomic loci. Furthermore, FACT depletion-mediated reprogramming requires the presence of the histone variant H2A.Z and Histone H3 phosphorylation by an Aurora Kinase. Hence, aberrant genomic H2A.Z and H3ph localization might facilitate gene activation leading to enhanced reprogramming. Our findings demonstrate the versatility of C. elegans as a discovery tool for identifying and understanding the role of epigenetic regulators in the context of safeguarding cellular identities.

Abstract No. T03

A novel molecular mechanism of cell-fusion mediated reprogramming

Karthik Arumugam[1,] , William Shin[2], Valentina Schiavone[3], Neus Romo[3], Xiaochuan Tu[4], Andrea Califano[2], and Maria Pia Cosma[3]*

[1]Centre For Genomic Regulation (CRG), Barcelona, Spain
[2]Department of Systems Biology; Columbia University, New York, USA
[3]Gene Regulation, Stem Cells and Cancer; Centre For Genomic Regulation, Barcelona, Spain
[4]Engineering and Life Sciences Faculty; HAN University of Applied Sciences, Arnhem, Netherlands
*Presenting author

Cell fusion-mediated reprogramming is a physiological process where a somatic cell fuses with a stem cell to form a hybrid and the dominant stem cell genome can reprogram the somatic genome to pluripotency. Tcf3 is a transcriptional repressor of Wnt canonical pathway and deletion of Tcf3 in mouse embryonic stem cells (mESCs) strongly enhances reprogramming efficiency after fusion. To investigate the molecular mechanism of somatic cell reprogramming, we fused murine Tcf3-/- mESCs with human B-lymphocytes and isolated heterokaryons for RNA-sequencing at different time points after fusion. We used a computational reverse-engineering approach to identify transcription factor regulatory networks that drive the early events during reprogramming of the somatic human B cell. This method measures the activity of the transcription factor by computing the differential expression of its targets. This allows for an unbiased prediction of transcription factors as key drivers or “Master Regulators”, whose activities are most causally related to global changes in gene expression. This approach led us to a surprising discovery that the human B cell genome within the heterokaryon was reprogrammed to a hematopoietic stem cell (HSC) like state within 5 days after fusion. Importantly, we identified a sequential activation of two novel transcription factor regulatory networks in the human B-lymphocytes, namely “early” and “late” that are associated with this reprogramming process. A correlation analysis with a human HSC dataset showed that these early and late transcription networks were highly similar to the lineage-committed hematopoietic progenitors and the hematopoietic stem progenitors respectively. In conclusion, we have obtained novel insights into regulatory mechanisms that could reprogram human B-lymphocytes into human hematopoietic stem progenitors. Coincidentally, we have also discovered novel regulatory mechanisms that could control human hematopoietic stem cell maintenance and renewal.

Abstract No. T04

Directing cell fates with artificially evolved transcription factors

Ralf Jauch, Yanpu Chen, Vikas Malik, Veeramohan Veerapandian, Xiaoxiao Yang, Dennis Zimmer, and Ole Ackermann*

Guangzhou Institutes of Biomedicine and Health, China
*Presenting author

My laboratory studies how reprogramming transcription factors (TFs) recognize DNA to 'read' the regulatory information encoded in mammalian genomes and direct cell fate decisions. We use structural modeling and quantitative biochemical assays to study TF dimerization on regulatory DNA. Further, we use genomics techniques (ChIPseq, RNAseq and MNase-seq) to contrast the dynamic binding and gene regulation of paralogous TFs and engineered factors to decipher the 'enhancer codes' regulating gene expression programs. We specifically ask how combinations of lineage specifying TFs (including SOX, OCT, PAX and FOX family proteins) work together to guide cell fate conversions in a step-wise manner. By combining the insights from biochemical and genomic studies, we are able to select functionally critical structural elements of key TFs and modify them using a directed evolution strategy. This method includes pooled library screens, cell selection based on phenotypic read-outs and amplicon sequencing. This way, we generate artificially evolved TFs (eTFs) that program cell fates faster, more efficiently and in a more controlled fashion outperforming their wild-type counterparts. I will discuss examples leading to the identification of novel eTFs substantially enhancing cellular reprogramming.

Abstract No. T05

Single-cell RNA sequencing reveals aging-induced quiescence in neural stem cells of the subventricular zone

Georgios Kalamakis, Sheng Zhao, Janina Kupke, Enric Llorens Bobadilla, Jan Bolz, and Ana Martin-Villalba*

Molecular Neurobiology; German Cancer Research Center (DKFZ), Heidelberg
*Presenting author

The adult rodent brain consists of two predominant neurogenic niches, the subgranular zone of the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. Adult neurogenesis is regulated by a plethora of signals that are not only multidimensional – as they involve multiple cues¬– but they also vary dynamically with age. In the adult SVZ, neurogenesis is known to decline with aging however the age-dependent mechanisms remain largely unexplored. Here, we performed single-cell RNA sequencing of neural stem cells from the ageing brain to unravel age-associated changes. We identify that old neural stem cells are mostly in a quiescent state and we set off to activate them in order to increase the generation adult-born neurons, which would counteract age-related behavioral decline.

Abstract No. T06

Vitamin A in stem cell aging

Simon Schwörer[*]*, Ute Köber, Kristina Tramm, Elias Amro, George Garside, Friedrich Becker, Stefan Tümpel, and Karl Lenhard Rudolph*

[1]Leibniz Institute on Aging/Fritz Lipmann Institute (FLI), Jena
*Presenting author

Retinoic acid is a metabolite and the active form of Vitamin A. As a ligand, it binds to retinoic acid receptors which interact with regulatory regions triggering specific genetic programs, including Hox genes and other important factors for embryonic development. Recently, it has been demonstrated that Vitamin A also regulates the function of hematopoietic stem cells. However, whether Vitamin A also controls other adult stem cells has not yet been addressed. Moreover, despite observations of an altered availability in aging humans and rodents, the role of Vitamin A in the context of aging remains elusive. In our previous work we demonstrated that the aberrant re-activation of Hox gene expression contributes to the aging-associated functional decline in muscle stem cells (MuSCs), the cells responsible for skeletal muscle regeneration. Interestingly, we observed an upregulation of almost all components of Vitamin A/retinoic acid signaling and metabolism in MuSCs from aged compared to young mice. Therefore, we hypothesize that altered retinoic acid signaling contributes to a dysregulated Hox expression profile in aged MuSCs and thus, to a decline in stem cell function. Upon exposure of young MuSCs to all-trans retinoic acid (ATRA), Hox genes and other retinoic acid target genes are rapidly induced. In addition, ATRA-treated MuSCs show elevated expression of $p16^{Ink4a}$ and a delayed entry into the cell cycle. To investigate how alterations in Vitamin A availability during aging affect stem cells and tissue homeostasis *in vivo*, we established a cohort of mice receiving either a Vitamin A deficient diet or Vitamin A supplementation for >20 months. Analysis of different stem cell compartments of these mice reveals tissue-specific beneficial and adverse effects of Vitamin A, indicating that its intake needs to be well-balanced throughout life.

Schwörer, S., Becker, F., Feller, C., Baig, A. H., Köber, U., Henze, H., … Rudolph, K. L. (2016). Epigenetic stress responses induce muscle stem-cell ageing by Hoxa9 developmental signals. Nature, 540(7633), 428–432.

Cabezas-Wallscheid, N., Buettner, F., Sommerkamp, P., Klimmeck, D., Ladel, L., Thalheimer, F. B., … Trumpp, A. (2017). Vitamin A-Retinoic Acid Signaling Regulates Hematopoietic Stem Cell Dormancy. Cell, 169 (5), 807–823.

Abstract No. T07/P103

Regulation of epidermal stem cells by flightless I protein during wound repair

Nanxing Yang[1,], Xanthe Strudwick[1], Zlatko Kopecki[1], Claudine Bonder[2], and Allison Cowin[1]*

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[2]Centre for Cancer Biology; University of South Australia
*Presenting author

Epidermal stem cells (EpSCs) reside in niches of interfollicular and follicular epidermis. These cells proliferate and migrate out of their niches during re-epithelialization of wound healing which requires the remodeling of the actin cytoskeleton. Initially identified as an essential actin-remodeling protein and a negative regulator of wound healing, Flightless I (Flii) protein has also been found to be an important regulator during hair follicle homeostasis and a positive regulator of digital tissue regeneration. Here, we investigated the mechanisms behind Flii involvement in EpSCs during wound repair. Using an incisional wound healing model in genetic Flii mice (Flii knockdown: Flii+/-, wild type: WT, Flii transgenic: FliiTg/Tg) our current study assessed the effect of differential Flii levels on the quiescent and proliferative property of EpSCs during different stages of wound repair. Flii was found to be highly expressed by purified CD34, K15 and Integrin a6 positive EpSCs as well as native follicular EpSCs in the skin. Interestingly, differential Flii levels did not change the number of epithelial stem cells in unwounded mice epidermis. Noticeably, reduced Flii resulted in decreased expression of Lrig1, a positive regulator of stem cell quiescence, in the EpSCs adjacent to the wound. Consistently, K14 expression which marks the population of undifferentiated cells in the epidermis was also reduced in Flii+/- EpSCs regions. Additional proliferative PCNA and EGFR1 positive were identified in EpSCs zones adjacent to the wound in Flii+/- mice compared to WT and FliiTg/Tg counterparts. The nuclearization of β-catenin and expression of proteins involved in Wnt/b-catenin signalling including Axin2, Lgr6 & Flap2 in the EpSCs were found to be shifted towards Wnt activation in response to reduced Flii. Together, our data suggests that Flii may participate in epidermal stem cell activation during wound repair which hold promise for design of novel therapies for improved wound healing outcomes.

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Abstract No. T08

Learning hematopoietic stem cell dynamics from fate mapping

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Fundamental questions of stem cell biology concern dynamics, and learning about dynamics from snapshot measurements is challenging. The fate mapping of stem cells, originally designed to identify stem cell progeny, has recently emerged as a tool to dissect stem cell dynamics in vivo. Applying mathematical reasoning to data on the propagation of fate mapping label and on the clonal composition of labeled cells uncovers rich information on the dynamics and topology of lineage differentiation pathways. I will discuss how these mathematical approaches, applied to novel experimental tools for the non-invasive fate mapping of hematopoietic stem cells, yield insight into the functioning of physiological hematopoiesis. Our findings support the currently contested model of tree-like hematopoietic lineage differentiation and challenge prevalent views on clonality and maintenance of HSC in the bone marrow.

Abstract No. T09

Human haematopoietic stem cell differentiation follows a continuous Waddington-like landscape

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Blood formation is believed to occur through step-wise progression of haematopoietic stem cells (HSCs) following a tree-like hierarchy of oligo-, bi- and unipotent progenitors. However, this model is based on the analysis of predefined flow-sorted cell populations. Here, we mapped human bone marrow hematopoiesis by quantitatively integrating flow cytometric, transcriptomic and functional lineage fate data at the single-cell level to quantitatively map early differentiation of human HSCs towards lineage commitment. We found that individual HSCs do not pass through discrete intermediate progenitor cell stages. In contrast, HSC lineage commitment occurs in a gradual manner best described by a continuous Waddington landscape with initially flat but progressively deepening valleys. Our data determine a detailed model of developmental trajectories within this landscape and demonstrates that distinct gene expression modules operate in a combinatorial manner to control stemness, early lineage priming and the subsequent progression into all major branches of haematopoiesis. These results establish the concept of a developmental continuum, which can replace the 'differentiation tree' as a comprehensive model of hematopoiesis.

Human haematopoietic stem cell lineage commitment is a continuous process. Velten L, Haas SF*, Raffel S*, ... Trumpp A*, Essers MA*, Steinmetz LM*. Nat Cell Biol. 2017 * equal contribution*

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Abstract No. T10

Paraoxonase-2 and its effects on hematopoietic stem cell proliferation and differentiation

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The enzyme paraoxonase-2 (PON2) locates to mitochondria and the endoplasmic reticulum, where it plays a central role in the control of ROS generation. Redox disturbances represent a potential therapeutic target, because many tumors, including leukemias, cause and exploit them. This is in line with the association of PON2 levels with imatinib resistance in CML patients and response to front-line therapies in pediatric ALL. These results in combination with the established influence of redox-signaling on quiescence, apoptosis, differentiation and self-renewal of HSCs led us to investigate the role of PON2 in the hematopoietic system.

First, analysis in PON2-/- mice showed severe alterations of the HSC compartment, i.e. a significant increase in the LSK fraction as well as at the level of LT-, ST-HSCs and MPPs. Further investigations revealed higher percentage of LSK cells in G0 phase of cell cycle and decreased apoptotic rates in old PON2-/- mice. In line with the anti-oxidative function of the enzyme we observed enhanced superoxide levels in whole bone marrow as well as enhanced ROS levels in all fractions of the LSK population, which correlates with the expression level of PON2 in WT mice. Transplantation of old PON2-/- bone marrow cells (BMCs) resulted in increased numbers of LT-, ST-HSCs and MPPs, that prompted us to more in-depth analysis. Reciprocal bone marrow transplantation assays revealed that PON2 functions through intrinsic cell signaling rather than the niche. Competitive bone marrow transplantation assays exposed significant advantages of PON2-/- BMCs in multi-lineage reconstitution compared to WT BMCs. To shed some light on, we treated PON2-/- mice with NAC to uncover whether the observed effects are ROS-dependent. We detected that antioxidant-treatment alters the effects caused by increased ROS levels due to PON2 deficiency.

Collectively, these studies propose PON2 as crucial redox control enzyme in HSCs and potential target in anti-leukemia therapies.

Abstract No. T11

A computational systems biology approach to identify niche determinants of stem cell identity

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Stem cells are under constant regulatory influence of their cellular microenvironment or niche resulting in the robust maintenance of their specific phenotypes. Despite advances in identification of several key niche cells and secreted factors, it is still not straightforward to comprehend the overall niche effect that shapes the stem cell state due to the dynamic nature of the niche and large number of non-linear stem cell-niche interactions. Such multifactorial complexity of stem cell-niche interactions is a major roadblock in identifying regulatory mechanisms that determine the functional state of the stem cells. Some of these limitations can be overcome by considering that stem cells robustly maintain their functional state due to a constant niche effect by integrating its cues via signaling pathways. Accordingly, a constant niche effect should induce sustained activation/inhibition of specific stem cell signaling pathways in all stem cells within heterogeneous populations exhibiting the same phenotype (niche determinants). This view of stem cell-niche interactions shifts the focus of the problem towards the constant niche effect on key stem cell signaling pathways instead of accounting for niche composition and its interaction with the stem cell explicitly. Based on this rationale, we propose a probabilistic computational method for the identification of niche determinants. The method relies on single cell gene expression data and publicly available interactome of signaling pathways, and employs an algorithm based on Markov chains to infer the signaling intermediates with high probability of signal transduction from the niche. Application of the proposed method to the single cell expression data of quiescent and active neural stem cells from young and old mice enabled identification of key niche induced signaling intermediates that are likely to be constantly active/inactive in a majority of cells in the respective cellular subpopulations. Further, novel computational predictions of niche determinants were experimentally validated.

Abstract No. T12

Adult neural stem cells increase the rate of asymmetric divisions during aging

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Neurogenesis, the process of generating neurons from neural stem and progenitor cells, continues throughout life in certain mammalian brain regions. However, it has been shown that neural stem cells have only a limited capacity to self-renew, and that the number of neurons being generated in the murine brain declines with age. How exactly neurogenesis changes during aging is unknown. To study the cellular mechanisms involved, we set up a model of adult neurogenesis and estimated unknown parameters by fitting 46 and 21 clonal observations using confetti reporters from young and old adult mice, respectively. Assuming four different division strategies for each dividing cell type, we compared 64 different models via the Bayesian Information Criterion. In accordance with previous data, the best models exhibit strong TAP self renewal, rapid NB differentiation and mainly asymmetric stem cell divisions. Most importantly, we identify an increase of asymmetric stem cell divisions at the expense of symmetric stem cell differentiation divisions with age. Our model is able to explain existing longitudinal population data and suggests a particular strategy of NSC homeostasis that allows insights into the aging of a stem cell compartment.

Abstract No. T13

Myeloid leukemia stem cells depend on phospholipase C gamma 1 (Plcg1) signaling

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The signaling molecule phospholipase C gamma 1 (Plcg1) is highly expressed in a subtype of acute myeloid leukemia (AML) characterized by the balanced translocation t(8:21). Transformation of hematopoietic cells with the resulting fusion oncogene RUNX1-ETO (AML1-ETO) leads to induction of Plcg1 expression. We performed ChIP-sequencing analysis in Kasumi-1 cells harboring the RUNX1-ETO oncogene. Here, binding of the fusion oncogene RUNX1-ETO – but not wildtype RUNX1 – could be detected at the promoter region of Plcg1. Genetic inactivation of RUNX1-ETO by RNAi abrogated RUNX1-ETO binding and decreased Plcg1 expression. Consistently, knockdown of Plcg1 in RUNX1-ETO transformed murine stem- and progenitor cells affected both, proliferation and re-plating capacity in vitro.

In order to assess for the functional impact of Plcg1 on RUNX1-ETO driven myeloid leukemia stem cells (LSC) in vivo, we generated a conditional knockout mouse model for Plcg1 with exons 3-5 being flanked with loxP sites. Conditional deletion of Plcg1 in AML1-ETO9a/KRAS-GFP (AE9a/KRAS) transformed cells abrogated colony formation in vitro and leukemia formation in vivo. Ex vivo analysis of FACS-sorted LSCs revealed loss of stemness and induction of differentiation following deletion of Plcg1. Likewise, Plcg1 appeared to be essential for maintenance of already established leukemia in vivo. In contrast, genetic inactivation of Plcg1 did not affect self-renewal or lineage-commitment in normal hematopoietic stem cells (HSCs).

Transcriptome profiling of LSCs by RNA-sequencing revealed transcriptional inactivation of the HoxA gene cluster following deletion of Plcg1. Gene-set enrichment analysis confirmed deregulation of Hox target genes in Plcg1-/- LSCs. Moreover, Plcg1 target genes were found to be negatively enriched following knockdown of RUNX1-ETO in Kasumi-1 cells.

Taken together, Plcg1 is specifically required for development and maintenance of RUNX1-ETO driven LSCs. As Plcg1 seems to be dispensable for normal HSCs, this selective vulnerability may offer novel therapeutic strategies to eradicate LSCs in this AML subtype.

Abstract No. T14/P069

C/EBPβ-LIP regulates the let-7/Lin28 circuit to control cellular metabolism

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Experiments with mouse models revealed that transgenic overexpression of the transcription factor C/EBPβ-LIP increases general tumor incidence, while in C/EBPβ-LIP deficient mice tumor incidence is reduced. The oncogenic mechanisms involved in these phenotypes have not been solved. Here we show that C/EBPβ-LIP enhances aerobic glycolysis and mitochondrial respiration (Seahorse XF analysis), resembling a shift to cancer/stem cell metabolism. By using an integrative analysis of genome wide transcriptome and whole cell proteome we show that although induction C/EBPβ-LIP does not significantly alter mRNA levels of glycolytic enzymes the protein levels of these enzymes are elevated. Further analysis of the C/EBPβ-LIP transcriptome revealed that C/EBPβ-LIP stimulates the expression of Lin28b, which is an oncofetal RNA-binding protein that enhances the translation of glycolytic and mitochondrial enzymes in order to increase the cellular metabolism and energy production. Moreover, Lin28b knockout by CRISPR/Cas9 genomic editing ablates C/EBPβ-LIP induced metabolic reprogramming in cells. We show that C/EBPβ-LIP controls Lin28b expression though transcriptionally repression of let-7. Let-7 and Lin28a/b have reciprocal functions in a regulatory circuitry where let-7 represses Lin28a/b-mRNAs, while Lin28a/b represses let-7 maturation. Finally, first analysis using a conditional C/EBPβ-LIP overexpressing mouse model show that let-7 levels are repressed and Lin28b levels are upregulated by C/EBPβ-LIP in vivo, which is associated with enhanced bioenergetics metabolism in bone marrow cells and results in hyperplasia in the examined tissues, skin and spleen.

Therefore, our data suggest a key role of C/EBPβ-LIP in controling the Lin28/let-7 regulatory circuit and thereby regulating cellular metabolism in context of stem cell function, tissue repair and tumor development.

Abstract No. T15

Stem cell based assays as the next generation phenotypic drug screens?

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Drug developers appreciate to optimize small molecules or therapeutic antibodies on a defined protein target. Targets are mandatory for antibody development and for a known target chemicals can be rationally optimized. Therefore industrial drug discovery campaigns often start by developing target-based assays to screen large libraries of diverse compounds. The primary hits from such cell-free high throughput screens (HTS) often have insufficient properties to show target-mediated activity in cells. They need chemical optimization before they can be safely applied to cellular assay formats. Although cellular HTS formats deliver more mature hits with respect to their physicochemical properties, there is still an ongoing debate in pharmaceutical industry on the usefulness of cellular/phenotypic screens since the molecular target mechanisms remain unknown ("black box" screens). Most recently, we have conducted > 10 black box HTS' at LDC. These cellular screens yield unbiased hits with respect to molecular mechanisms. When compared to primary hits from a biochemical HTS, these primary cellular hits are usually much more advanced (pharmacokinetics, physicochemistry, specificity). We managed to identify the molecular targets for cellular hits by means of chemoproteomic and pharmacophore modeling technologies, therefore wiping out the worries on the ignorance of the molecular mechanism of a future drug candidate. In addition, fewer cycles of medicinal chemistry are needed to optimize such cellular hits. Phenotypic, cellular screens much better represent certain disease pathologies. More recently, we have started to implement iPS-derived cells for HTS, which is even of higher translational relevance than phenotypic cell assays. IPS-derived cells best characterize a disease and hits from iPS-derived cellular HTS campaigns are most valuable. Having established the tools to identify the molecular targets for an iPSC-derived HTS hit, we believe that iPSC-based HTS is a more efficient and rewarding way to start a small molecule drug discovery project despite the high costs.

Abstract No. T16

Human iPSC-derived phagocytes as a cellular therapeutic to treat bacterial infections

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Hematopoietic differentiation of human induced pluripotent stem cells (hiPSC) opens new opportunities for innovative cell-based treatment strategies. However, clinical translation is currently hampered by the lack of therapeutically relevant quantities of functional effector cells. To overcome this hurdle, we here demonstrate the continuous production of iPSC-derived mature hematopoietic cells in fully-equipped stirred tank bioreactors and their subsequent therapeutic application as a novel cell-based treatment approach targeting acute respiratory infections.

First, we established a suspension-based hematopoietic differentiation protocol, which is able to continuously generate human iPSC-derived macrophages, granulocytes or erythrocytes from established "myeloid cell forming complex" aggregates, harboring $CD34^+/CD45^+$ hematopoietic progenitor cells.

Upscaling of this process into 120ml stirred tank bioreactors, resulted in the continuous harvest of 20-30 million iPSC-derived macrophages (iPSC-Mac) per week for five consecutive weeks. Produced iPSC-Mac displayed a highly pure $CD45^+CD11b^+CD14^+CD163^+TRA\text{-}1\text{-}60^-$ surface marker phenotype and a transcriptome profile, which was similar to peripheral blood derived phagocytes.

Upon pathogen challenge *in vitro*, iPSC-Mac efficiently phagocytosed *Pseudomonas aeruginosa* and secreted important pro-inflammatory cytokines. Moreover, iPSC-Mac were able to reshape their transcriptomic profile already one hour after pathogen contact and up-regulated defined gene sets associated with active innate immunity and pathogen clearance.

Of note, pulmonary iPSC-Mac transplantation (PiMT) rescued immunodeficient mice from established respiratory *Pseudomonas aeruginosa* infection already within 4-8hrs post transplantation as demonstrated by significantly reduced disease scores (1.8±0.2 vs. 8.1±0.2), normalized animal activity, as well as normal body temperature (35.4±0.5 vs. 29.4±1.7°C) and lung function values. Improved disease parameters were in line with a profound reduction in lung bacterial burden ($2x10^8±1x10^8$ vs $2x10^4±0.6x10^4$ CFU) and a normal lung histology in PiMT treated animals compared to solely infected mice. In summary, we demonstrate the continuous production of human iPSC-derived myeloid cells in industry-compatible bioreactors and introduce a cell-based and antibiotic independent treatment strategy targeting bacterial respiratory infections.

Abstract No. T17

HMGB1 accelerates regeneration of multiple tissues by transitioning stem cells to G_{Alert}

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Whilst stem cell therapy has been successful for some haematological disorders, considerable challenges remain for enhancing the repair of solid organs. Targeting endogenous cells overcomes many of the hurdles associated with exogenous stem cell therapy. Our objective was to identify the factor that transitions endogenous quiescent G_0 stem cells to an 'alert' phase thereby enhancing regeneration in multiple tissues, including bone, muscle and blood. Alarmins are endogenous molecules released upon tissue damage and are triggers of the immune response. We hypothesised they may have a role in tissue repair, and found the alarmin HMGB1 accelerated tissue regeneration in various systems by transitioning particular stem cell types to G_{Alert}. Alarmins were elevated post-fracture in both human and murine blood samples. In-vitro screening of candidate alarmins with human bone marrow-derived mesenchymal stromal cells showed that in response to osteogenic media, only pre-treatment with the fully reduced form of HMGB1 improved osteogenic differentiation. Next, using in-vivo microCT and biomechanical analysis, we found that exogenous addition of HMGB1 accelerated fracture healing via the CXCL12-CXCR4 axis. We confirmed our findings using conditional Hmgb1-/- mice and small molecule inhibitors. Analysis of cell cycle kinetics, cell size, ATP levels, mitochondrial DNA, and mTORC1 dependency revealed this was due to HMGB1 transitioning the murine skeletal stem cell to G_{Alert}. This effect also extended to murine muscle and haematopoietic stem cells, as well as human haematopoietic stem and progenitor cells and MSCs. HMGB1 also accelerated recovery in murine models when administered at the time, or 2 weeks before skeletal, muscle or haematological injury. In summary, HMGB1 accelerates the regeneration of multiple tissues whose cells it transitions to G_{Alert}, even if it is administered prior to injury, and only in response to injury. Therefore, the HMGB1-G_{Alert} pathway has broad potential therapeutic applications, such as in trauma, chemotherapy, and elective surgery.

Abstract No. T18

Metabolic targeting by mesenchymal stem cells in a mouse model of diet-induced NASH

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Background / Aim
Non-alcoholic steatohepatitis (NASH) is a severe liver disease associated with tissue inflammation, steatosis, fibrosis and eventually cirrhosis and HCC. End-stage NASH is increasingly an indication for liver transplantation. Since livers for organ transplantation are scarce, alternative therapy options are needed. MSC display immunomodulatory features. Hence, they may target macrophages, which play an important role in the pathogenesis of inflammation in NASH. This hypothesis should be tested and further potential targets of MSC be identified after their hepatic transplantation into mice suffering from NASH.

Methods
Immunodeficient mice received a methionine-choline-deficient diet (MCD) for 5 weeks to induce NASH. Following 1/3 partial hepatectomy, 1x106 human bone marrow-derived mesenchymal stem cells were transplanted into the livers via splenic application. MSC were prior differentiated into the hepatocytic lineage. One week later, organs were harvested. Global protein abundance in the liver was evaluated by proteome analysis and the type and number of macrophages were assessed by detection of specific markers.

Results
Mice receiving the MCD diet developed features of NASH like steatosis, fibrosis and inflammation. Both mitochondrial and peroxisomal fatty acid oxidation were attenuated driving lipid utilization into triglyceride storage. Apolipoprotein synthesis was dysregulated, thus augmenting fat accumulation. The number of macrophages expressing F4/80 increased, while the number of alternatively activated, “anti”-inflammatory CD163 expressing macrophages decreased. One week after MSC treatment, hepatic steatosis was reduced presumably via a significant increase of peroxisomal proteins Acaa1a and Acaa1b, thus stimulating peroxisomal lipid oxidation. In addition, apolipoprotein synthesis improved, which probably augmented lipid export. F4/80+ macrophages were even increased while CD163+ macrophages decreased further after MSC treatment.

Conclusions
MSC impacted on hepatic lipid metabolism by favoring lipid oxidation over storage thereby improving NASH. The increase in total macrophages concomitant with the decrease of “anti”-inflammatory macrophages probably supported resolution of fibrosis and tissue remodeling.

Abstract No. T19

Genetically encoded calcium indicators for drug screening in human engineered heart tissues

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Background: Investigating excitation contraction coupling in human iPS-derived cardiomyocytes (hiPSC-CM) can give important insight into the mechanisms of action of drugs. Our study aimed at establishing an in-vitro system for measurement of contraction force and calcium transient (CaT) in human engineered heart tissues (EHT) using genetically encoded calcium indicators (GECI).

Methods: Three-dimensional, force generating fibrin-based EHTs were prepared from hiPSC-CM and transduced with GECI (GCaMP5G or GCaMP6f). We measured contraction force (video-optical recording) and CaT (fluorescent light intensity) sequentially (10 sec delay) and analysed 6 compounds: EMD-57033 (myofilament Ca^{2+} sensitizer), Bay K-8644, nifedipine (L-type calcium channel agonist/antagonist), ryanodine (ryanodine receptor antagonist), beta-adrenergic agonist (isoprenaline) and SR Ca^{2+}-ATPase inhibitor (thapsigargin)

Results: GCaMP6f showed faster kinetics than GCaMP5G, better reflecting physiological CaT kinetics. EMD-57033 (10 µM) produced an increase in force (+170%) but no change of CaT. Bay K-8644 (300 nM) increased amplitude of force (+55%), relaxation time (+64%) and Calcium decay time (+55%). Nifedipine (100 nM) decreased force (-79%), contraction time (-30%), CaT (-76%) and calcium rise time (-26%). Ryanodine (10 µM) decreased force (-37%) and CaT (-49%). Isoprenaline (10 nM) increased force (+32%), decreased relaxation time (-17%), increased CaT (+13%) and decreased calcium decay time (-21%). Thapsigargin decreased force (-28%) and CaT (-33%). Force/calcium loops revealed compound-specific changes illustrating the mechanism of action.

Conclusion: The use of GCaMP6f in EHTs provides an efficient approach to optically analyse contractile parameters and calcium handling in stably beating engineered human heart muscles.

Abstract No. T20/P065

Establishment of murine 3D bronchoalveolar lung organoids from adult somatic stem cells for organ development and disease modeling

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Organoid models derived from murine and human stem cells have recently evolved as a powerful tool to study development and disease. We established a murine 3D bronchoalveolar lung organoid (BALO) model based on flow sorting of bronchoalveolar stem cells (BASC). Upon co-culture with lung mesenchymal cells (rMC), BASC underwent clonal expansion, gave rise to organoids, followed by branching and differentiation into lung organoids representing the bronchoalveolar compartment and allowing introduction of tissue-resident macrophages into the alveolar compartment. BALO were composed of club cells (CC10+), ciliated cells (β4-tubulin+, Foxj1+), goblet cells (mucin5ac+), alveolar epithelial cells type I (PDPN+, Hopx+) and II (SPC+), the latter showing surfactant production within the alveoli. Remarkably, rMC differentiated into myofibroblast (αSMA+PDGFRαhigh) and lipofibroblast-like (lipidTOX+PDGFRαlow) phenotypes and were indispensable for organoid generation. BALO were suitable for genetic manipulation and could be derived from several transgenic mice. Notably, BALO supported viral infection and replication as demonstrated by quantification of viral proteins and expression of interferon-β.

In summary, we defined an epithelial stem cell population giving rise to complete lung organoids recapitulating the 3D structure and cellular composition of the bronchoalveolar compartment which will allows us to explore new avenues for lung development and disease modeling.

Abstract No. T21

A xenotransplantation approach using human pluripotent stem cell-derived pancreatic organoids

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Introduction. Exocrine/ductal pancreatic differentiation from human pluripotent stem cells is a poorly understood process albeit various diseases arise from this compartment. We have previously designed a straightforward approach to direct human pluripotent stem cells (PSC) toward pancreatic organoids resembling exocrine and ductal progeny. Extensive phenotyping of the organoids not only shows the appropriate marker profile but also ultra-structural and functional hallmarks of human pancreas in the dish (Hohwieler *et al.*, GUT, 2017).

Aims & Methods. Xenotransplantation assays were applied to systematically transplant pancreatic organoids into immunodeficient mice aiming for the ultimate niche to host a human pancreas in mice.

Results. We systematically compared various PO transplantation niches (pancreas, kidney, anterior eye chamber). Tri-lineage differentiation potential of pancreatic progenitor cells, which gave rise to pancreatic organoids, is shown by immunohistochemistry for amylase, CK19 and insulin. Consequently, detailed immunophenotyping of the grafts was performed. In line with morphological appearance acinar-like cells stained positive for several acinar marker genes such amylase and chymotrypsin C, while ductal structures expressed CK19. To assess the maturity of the grafts, co-staining for acinar and ductal lineage markers with various progenitor cell markers such SOX9, PDX1 and NKX6.1 were performed. These data indicate a developmental stage of a human Carnegie Stage CS20 to CS23. Staining for α-smooth muscle actin (α-SMA) also revealed signs of neovascularisation in the grafts.

Conclusion. Xenotransplantation of human pancreatic organoids into the anterior eye chamber and/or the pancreas might become a future and humanized model system.

Company presentations

5th Annual Conference
German Stem Cell Network
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Company presentations: C1 – C9

C1 Tissue Engineering of primary human in vitro models – Influence of mechanical and biochemical stimulation on tissue functionality
Antje Appelt-Menzel

C2 Evolving media systems for modern stem cell applications
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C3 Current challenges in stem cell production for regenerative medicine and drug development
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C7 Avoid irrelevant, non-reproducible data - the neglected variable: physiologic oxygen
Paul Michaels

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Florent Bornes

C9 Highly efficient single-cell human pluripotent stem cell cloning and robust cardiomyocyte differentiation
Katharina Debowski

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Abstract No. C1

Tissue Engineering of primary human in vitro models – Influence of mechanical and biochemical stimulation on tissue functionality

Antje Appelt-Menzel[1,2,], Daniela Zdzieblo[2], Sebastian Schürlein[2], Thomas Schwarz[1,2], Jan Hansmann[1,2], Marco Metzger[1,2], and Heike Walles[1,2]*

[1]Translational Center Würzburg; Fraunhofer IGB
[2]Dep. Tissue Engineering and Regenerative Medicine (TERM); University Hospital Würzburg
*Presenting author

representing Peprotech GmbH

The main research focus of the Chair Tissue Engineering and Regenerative Medicine is the establishment of functional human tissue and organ models, that can be used as alternative test systems and that allow the establishment of innovative implant structures used for future therapeutic strategies. Our models are based on biological or synthetic carrier structures (e.g. BioVaSc® and electrospinning matrices) that are reseeded with cell lines, primary cells or iPSC-derived organ-specific cell types. Primary cells and iPS-derived cell progenies thereby allow the establishment of functional implants as well as test systems, that contribute to the activation of important biological mechanisms regulating in vivo-like tissue regeneration.
Specific bioreactors ensure cellular functionality in vitro by mimicking in vivo-like conditions of the microenvironment in the body. Furthermore, tissue relevant co-culture cells can be used to improve cell function as well as physiological tissue organization.
Besides the barrier systems skin, intestine, trachea and blood-brain barrier, we are also establishing 3D myocard models and tumor models of diverse organs.
Tissue maturation is routinely characterized by histological and immunohistological stainings, ultrastructural analyses and determination of characteristic gene expression patterns via qRT-PCR. The investigation of functionality, including electrophysiological analyses or validation of barrier integrity and transporter functionality via FDA approved reference drugs, are established.
Tissue maturation and cell differentiation can not only be supported by chemical and mechanical stimulation, as for example by a dynamic medium flow. Moreover, extracellular matrix (ECM) characteristics like organ-specific biochemical composition, structural information and biophysical cues are important regulatory factors for iPSC differentiation and consequent tissue characteristics. First in vitro experiments performed in our chair suggest ECM mediated impacts on pancreatic β-cell differentiation in terms of altered gene expression patterns. Together, this let us speculate that ECM-specific cues could be beneficial for the differentiation of hiPSCs towards functional and mature β-cells in vitro.

A. Appelt-Menzel, et al. (2017). "Establishment of a Human Blood-Brain Barrier Co-culture Model Mimicking the Neurovascular Unit Using Induced Pluri- and Multipotent Stem Cells." Stem Cell Reports 8(4): 894-906.
S. Schürlein, et al. (2017). "Generation of a Human Cardiac Patch Based on a Reendothelialized Biological Scaffold (BioVaSc)." Advanced Biosystems 1(3).

Abstract No. C2

Evolving media systems for modern stem cell applications

David Kuninger

R&D Cell Biology; Thermo Fisher Scientific, Frederick, USA

Culture systems for pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While a number of PSC feeder-free medium systems exist, there are many challenges encountered by stem cell scientists across the PSC workflow in today's applications. Here we sought to improve the robustness and versatility of traditional PSC culture medium systems identify and optimize critical medium components. Through assessment of over 65 different formulations, an optimum medium composition was identified which provides compatibility across the PSC workflow from somatic cell reprogramming, PSC expansion, downstream differentiation, as well as providing support in stressful applications such as gene editing. This system additionally provides versatility, allowing for every-other-day or weekend-free feed schedules and compatibility with a broad range of passaging reagents and matrices. Together this system provides a robust next-generation stem cell medium system for today's challenging PSC workflow needs.

Abstract No. C3

Current challenges in stem cell production for regenerative medicine and drug development

Christof Knocke

Vaudaux-Eppendorf AG, Schönenbuch, Switzerland

Stem cell-based technologies lay the basis for pioneering approaches in regenerative medicine, drug screening, and toxicology testing. The production of stem cells for regenerative medicine has to meet partly different requirements than their cultivation for basic research, because for industrial applications the cells have to be manufactured in much larger quantities in compliance with regulatory demands.

At the first Stem Cell Community Day, which took in place in Hamburg in spring this year, experts from industry and academia discussed recent achievements in stem cell bioprocessing for research and commercial manufacturing.

In our presentation we will summarize the expert's discussions concerning current challenges in the field. These include the identification of scalable culture systems, development of robust cell differentiation protocols, concepts for downstream processing, quality control, and the development of manufacturing platforms.

Abstract No. C4

Novel technologies for the cultivation and quality control of iPSCs and their derivatives

Sebastian Knöbel

Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany

Human pluripotent stem cell (hPSC)-derived cell products hold great promise for future clinical use. The regulatory requirements for such advanced-therapy medicinal products (ATMPs) imply the use of qualified raw materials, highly reproducible manufacturing processes and quality control assays.

Here we introduce a novel, quantifiable differentiation assay that is based on lineage-specific, complete media supporting directed differentiation into all three germ layers. The assay format allows quantitative flow cytometry analysis as well as immunocytochemistry assessment.

Furthermore, we present an automated workflow for the GMP-compliant expansion and differentiation of hPSCs on the versatile CliniMACS Prodigy® cell processing platform. Using this automated workflow we are able to generate mesencephalic dopaminergic (mesDA) progenitor cells up to clinically relevant scale. A dedicated marker panel allows for flow cytometry-based quality control of the resulting cell product.

Abstract No. C5

A novel maintenance medium extends the life-span and enables long term applications for both human primary hepatocytes and human pluripotent stem cell derived hepatocytes in conventional 2D cultures

Barbara Küppers-Munther

Takara Bio Europe AB, Göteborg, Sweden

Human primary hepatocytes are considered as the golden standard for *in vitro* model systems regarding drug development, toxicity assessment and metabolism studies. However, their rapid loss in cell viability in conventional 2D cultures limit the usage for these cells.

Human pluripotent stem (hPS) cell-derived hepatocytes have a great potential to become a future *in vitro* model for hepatocyte applications if they possess a relevant usage window and functionality, which has indeed been challenging to accomplish. However, our newly developed hepatocyte maintenance medium enables culturing of cryopreserved human primary hepatocytes as well as hPS cell-derived hepatocytes for 4 respectively 2 weeks with maintained viability and stable activities of several key cytochrome P450 enzymes (CYPs). Multiple analyses on cryopreserved hPS cell-derived hepatocytes, including RT-qPCR, immunostainings, functional assays such as albumin secretion and CYP activity assays demonstrate mature features and high functionality. Importantly, the hPS cell-derived hepatocytes show expression of essential genes of the drug metabolizing machinery, such as CYPs, phase II enzymes and transporters. An extended *in vitro* culture time for hepatocytes enables chronic toxicity testing and we show that our hPS cell-derived hepatocytes could be exposed to known hepatotoxins for up to 14 days. Cells respond expectedly to these toxic compounds demonstrating their utility for chronic toxicity studies. The hPS cell-derived hepatocytes also respond to insulin and have the ability to take up and store low-density lipoproteins as well as fatty acids. This novel maintenance medium presented here, maintains the viability of cryopreserved human primary hepatocytes for an outstanding time of culture and is in sharp contrast to existing hepatocyte maintenance media available on the market today. Also, this medium allows long term culture of cryopreserved hPS cell-derived hepatocytes, from multiple lines, with preserved functionality. This increased usage window of functional hepatocytes in 2D cultures will empower new areas of applications and research in the field of hepatocytes.

Abstract No. C6

Novel serum-free, xeno-free vitronectin-based culture system for hPSC

Suzanne Badoux

Biological Industries (BI), Kibbutz Beit Haemek, Israel

Human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC), collectively referred to as human pluripotent stem cells (hPSC), are able to differentiate into the three germ layers of the human embryo and are presumed to the capacity to self-renewal in vitro. Thus hold great potential for cell-based therapy.

The culture conditions including culture media have substantial effect on pluripotency. The most common freeder free matrices are Matrigel and recombinant proteins that support hPSC self-renewal such as laminin isoforms, and vitronectin. NutriStem® V9 XF is a defined, xeno-free, serum-free medium specially formulated to support the growth and expansion of hPSC using vitronectin and enzyme-free passage with EDTA. The medium contains low concentration of growth factors and only the essential components required for long term maintenance of hPSC.

NutriStem® V9 XF medium allows high proliferation rate over long-term culture, while maintaining stable karyotype, high pluripotency marker expression and tri-lineage differentiation potential of the hPSC. In addition, NutriStem® V9 XF medium supports the culture of hPSC with direct addition of Vitronectin ACF (animal component-free) to the medium without the need for cultureware pre-coating.

Abstract No. C7

Avoid irrelevant, non-reproducible data - the neglected variable: physiologic oxygen

Paul Michaels

BioSpherix, Ltd., Parish, USA

Irrelevant data in life sciences has led to a multitude of problems, including drugs being withdrawn from the market due to serious/fatal side effects, black box warnings, millions in lost revenue, billions awarded in law suits and the enormous time it takes to get a drug to market, just to name a few. Non-reproducible data has estimated losses in the billions with over 50% of research publications not having the ability to be replicate, contributing to significant loss of time to drug development. One factor that has been historically disregarded is the role of oxygen in cell based research methods. We'll explore the data and benefits of optimizing oxygen for reproducible and relevance in cell based research.

Abstract No. C8

Systems and solutions for culture and scale up of human pluripotent stem cells

Florent Bornes

Corning Life Sciences

There is an increasing need of culture solutions for pluripotent stem cells (PSCs) in research and cell therapy enablement. For the maintenance of PSCs, human ESC qualified Corning® Matrigel® matrix in combination with serum-free media has become the gold standard. New xeno-free solutions for scaling up PSCs take advantage of recombinant Laminin 521-coated culture vessels, which enables for single cell passaging without the need for a ROCK inhibitor. In addition, Corning provides a range of products for closed system solutions and scaling up of cell cultures.

Abstract No. C9

Highly efficient single-cell human pluripotent stem cell cloning and robust cardiomyocyte differentiation

Katharina Debowski

STEMCELL Technologies GmbH, Cologne, Germany

Recent advances in gene-editing techniques have led to more accessible and cost-effective methods to produce edited human pluripotent stem cell (hPSC) lines. However, low single-cell cloning efficiency (typically <1%) remains a major limitation of this technology. To address this challenge, we developed a novel hPSC cloning supplement, CloneR™, that yields single-cell cloning efficiencies of 20-40% in mTeSR™1 and TeSR™-E8™ across multiple matrices. Gene-edited hPSC clones can be differentiated to specific cell types for disease modelling, drug discovery, or toxicology screening purposes. The production and processing of hPSC-derived cardiomyocytes (hPSC-CMs) is both variable and cumbersome. To overcome this, we have developed an optimized workflow for hPSC-CM-based research, including cardiomyocyte differentiation, maintenance, dissociation, freezing, and support reagents. These STEMdiff™ Cardiomyocyte products facilitate reproducible and robust production and simple processing to yield high-quality hPSC-CMs (>80% cTNT+ and >1x10^6 hPSC-CMs/well of a 12-well plate). This talk will highlight hPSC gene-editing and cardiac differentiation workflows using the CloneR™ Supplement and the STEMdiff™ Cardiomyocyte product line.

Presentations strategic sessions

5th Annual Conference
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Presentations strategic sessions: S1 – S8

S1 Options and opportunities for scientists in science communication
Tobias Maier

S2 Tips and tools for efficient science communication in the public and private sector
Stefanie Seltmann

S3 Reconstructing human neurogenesis using single-cell transcriptomics
Barbara Treutlein

S4 Multimodal single cell genomics
Sascha Sauer

S5 tba
tbd

S6 Development of (stem) cell-based medicinal products: Regulatory aspects
Ralf Sanzenbacher

S7 Development of (stem) cell-based medicinal products: Turning research into GMP
Martin Hildebrandt

S8 Development of (stem) cell-based medicinal products: Clinical considerations for cell-based therapies
Mohamed Abou El-Enein

Abstract No. S1

Options and opportunities for scientists in science communication

Tobias Maier

Nationales Institut für Wissenschaftskommunikation (NaWik) gGmbH, Karlsruhe

Science communication is a growing field with diverse job opportunities for scientists. In his talk, Tobias Maier will outline how the field of science communication is changing and tell the story of his own journey from being a postdoc at a research institute to his current position as scientific head of the National Institute for Science Communication. He will further use his talk to introduce basic principles of good science communication and explain the benefits for scientists to actively communicate to a wider audience.

Bio Tobias Maier:

Tobias Maier is a science communication professional with a PhD in biochemistry and a ten-year track record in academic research. He is the scientific head of the National Institute for Science Communication (NaWik) in Karlsruhe, Germany. As a freelance trainer, Tobias offers career development workshops for scientists. In his free time, he writes a popular science blog in German called WeiterGen.

Abstract No. S2

Tips and tools for efficient science communication in the public and private sector

Stefanie Seltmann

External Communications; Pfizer Deutschland GmbH

Stefanie Seltmann, former head of press and public relations of the German Cancer Research Center (DKFZ), now the director of external communications at Pfizer Germany, reports on opportunities and pitfalls in science communication. How to translate the results of years of research into a one-page press release? How much prior knowledge can be expected when talking to the general public about science? Everyone can sell a Nobel Prize or a chancellor's visit, but how to inform lay audiences about long-term research projects without raising too much hope, particularly in the research on devastating diseases such as cancer? How do you distribute efficiently your valuable information? And how do you explain the value of a new drug when the law on medicinal products sets limits? Stefanie Seltmann reports on her own experiences and gives tips and valuable tools for getting started in science communication.

Abstract No. S3

Reconstructing human neurogenesis using single-cell transcriptomics

Barbara Treutlein

Max Planck Institute for Evolutionary Anthropology, Leipzig, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden and Technical University Munich, Department of Biosciences, Munich, Germany

Recent advances in the field of stem cell biology have made it possible to model human developmental processes in vitro. We combine stem cell based in vitro systems with single-cell transcriptomics analysis to reconstruct human organ development. In my talk, I will present our efforts on reconstructing human neurogenesis during health and disease.

We use cerebral organoids, three-dimensional cultures of human cerebral tissue derived from pluripotent stem cells, to model human brain development in vitro. We apply single-cell RNA-seq to dissect and compare cell composition and progenitor-to-neuron lineage relationships in human cerebral organoids and fetal neocortex in order to find out how well these in vitro systems recapitulate neural progenitor cell proliferation and neuronal differentiation programs observed in vivo. We identify cells in the cerebral organoids that derived from regions resembling the fetal neocortex and find that these cells use gene expression programs remarkably similar to those of the fetal tissue. We then apply our approach to cerebral organoids derived from patients with brain malformations to identify molecular mechanisms underlying these neurodevelopmental disorders. In summary, these data provide a high-resolution approach for understanding transcriptome states during neuronal lineage differentiation in the healthy and diseased human brain.

Abstract No. S4

Multimodal single cell genomics

Sascha Sauer

Scientific Genomics Platforms/Laboratory of Functional Genomics, Nutrigenomics and Systems Biology; Max Delbrück Center, Berlin

During the last decade, numerous deep-sequencing based genomics approaches have emerged, enabling the measurement of epigenetic marks, chromatin states and gene expression of thousands of genes simultaneously. For many of these procedures, progress in technology development has pushed sensitivity to the single cell level required to understand tissue complexity and identify disease causing cell populations. Now, one of the most urgent questions in the fields of epigenetics and transcriptional regulation is how single cell variations in chromatin structure, epigenetic states and transcriptional activity are causally related during tissue homeostasis and malignancy. Methodology platforms to (more) efficiently achieve this goal are key requirements for future systems studies including international initiatives such as the human cell atlas. After the advent of single cell genome/exome and transcriptome sequencing, multimodal single cell approaches shall allow for shedding light on so far hidden layers of cellular regulation of gene expression.

Abstract No. S5

tba

tbd

Abstract not available

Abstract No. S6

Development of (stem) cell-based medicinal products: Regulatory aspects

Ralf Sanzenbacher

Medical Biotechnology; Paul Ehrlich Institute, Federal Institute for Vaccines and Biomedicines, Langen/Germany

Realizing the strength of the research progress and increasing translational developments in regenerative medicine, cell and gene therapy, the European Union (EU) adopted the Regulation (EC) 1394/2007 on Advanced Therapy Medicinal Products (ATMP) which came into force in 2008. The ordinance is not specially addressed to stem-cell derived products, but includes those in the pharmaceutical categories of tissue engineered-products, gene therapies or somatic cell therapy, thereby providing a common framework for marketing of these products for all EU member states. This presentation aims to provide an overview on the current regulatory setting and summarizes important aspects in the quality development for production of (stem) cell-based medicinal products. Necessary time and efforts are frequently undervalued, especially at the transition phase from a lab scale prototype to an investigational product to be released for patient treatment within early clinical trials, but also beyond with a view to an economically viable commercial manufacturing setup. In first line, process steps may be critically addressed with a view on product safety, integrity, and functionality, starting from donor evaluation and procurement of cells or tissues, as well as the following manufacturing process including isolation, expansion, modification, formulation, storage, distribution, transport and reconstitution. Second, meaningful, reliable and robust characterization and control measures should to be established. Third, as legally requested, manufacturing has to be conducted in compliance with the requirements of good manufacturing practice (GMP). Additionally, the concept of offering patients non-authorized cell therapies directly at bedside gains momentum and will be tackled.

Abstract No. S7

Development of (stem) cell-based medicinal products: Turning research into GMP

Martin Hildebrandt

TUMCells Interdisciplinary Center for Cellular Therapies, TUM School of Medicine, Ismaninger Strasse 22, 81675 Munich, Germany

Translational research intends to turn projects into processes and products that comply with current regulations justifying their clinical use based on appropriate quality and preclinical data. When research is expected to reach the patient's bed, it is helpful to keep the rules pertinent to the manufacture of the future investigational product (GMP) and their use in clinical trials (GCP) in mind from the beginning. Tools are available to assist in this and to shape the developmental process in a consistent and goal-oriented manner. Issues to address include:

− the quality of documentation, record-keeping and source traceability that should be considered from the beginning;

− for the manufacture and quality control, the early choice of appropriate materials, equipment, vendors and in-process controls that can be crucial for the success of the entire project.

− Risk-based approaches lay the fundament to a proactive process development, a thorough validation concept and a robust manufacturing process. The risk analysis should include the various professions involved in the process, including regulatory knowledge, scientific advice and the clinical users.

− A straightforward definition of the intended product will allow for specifications to be developed, set and tested once the manufacturing process has been established. In many cases, this point defines the transition to GMP, with an established process ready to be validated, i.e. tested for its capacity to fulfill the pre-defined requirements.

In my presentation, I will touch upon existing guidance documents, useful tools and developmental algorithms that may be useful especially in cell-based medicines and regenerative medicine. I will try to dissect the manufacturing process of stem cell-based medicines, assess some of the various components, highlight critical points to be considered early on and provide examples of possible solutions.

Abstract No. S8

Development of (stem) cell-based medicinal products: Clinical considerations for cell-based therapies

Mohamed Abou El-Enein

Berlin-Brandenburg Center for Regenerative Therapies (BCRT)

The considerations when initiating a clinical trial for cell-based therapies are diverse and numerous. Alternative approaches to traditional clinical development based on sequential, seamless design are justified, including exploratory (phase I/IIa) trials examining both safety and efficacy while using an extensive biomarker portfolio, whenever applicable. Great emphasis should nevertheless be placed on patient safety, particularly in designing appropriate GMP-grade product and risk-benefit related stopping criteria. In this presentation some of these aspects will be discussed using a real-life example.

Poster presentations

5th Annual Conference
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Poster session I: P001 – P068

P001 – P028: Stem cells in regenerative therapies
P029 – P068: Stem cells in disease modeling and drug development

Even numbers: Monday, 11 September from 18:00 - 19:00
Odd numbers: 19:00 - 20:00

Posters can be mounted on 11 September 13h and should be removed until 12 September 13:30h.

Poster session II: P069 – P142

P069 – P076: Stem cells in diseases: cancer stem cells
P077 – P084: Computational stem cell biology and systems biology
P085 – P103: Somatic stem cells and development
P104 – P124: Pluripotency and reprograming
P125 – P142: Hematopoietic stem cells

Even numbers: Tuesday, 12 September from 17:00 - 18:00
Odd numbers: 18:00 - 19:00

Posters can be mounted on 12 September 14h and should be removed until 13 September 14:30h.

Poster session I: P001 – P068

P001 – P028: Stem cells in regenerative therapies

P001 The effects of amnion-derived mesenchymal stem cells on fibrosis mechanisms in nephrectomy rat model
Ezgi Akan

P002 Small sized population of human mesenchymal stem cell from umbilical cord blood improve stem cell properties and therapeutic benefit
Yun Kyung Bae

P003 Investigating the age-dependency of tubular regeneration in zebrafish kidney
Thomas Bates

P004 Generation of a NKX2.1/p63 knockin human induced pluripotent stem cell reporter line for monitoring the generation of respiratory cells
Ruth Olmer

P005 A pancreas-specific ECM scaffold – human iPS cell culture and pancreatic differentiation goes 3D
Constantin Berger

P006 Xeno-free and chemically-defined differentiation of human pluripotent stem cells into the definitive endoderm in static suspension culture
Ulf Diekmann

P007 Human iPSC- and hematopoietic stem cell-derived erythroid precursors differ in their DNA methylation profile
Isabel Dorn

P008 Mesenchymal stem/stromal cells release different small extracellular vesicle subtypes
Rita Ferrer-Tur

P009 Feasibility study for long-term expansion of human induced pluripotent stem cells (hiPSC) in 3D bioreactors
Nora Freyer

P010 Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) inhibits nitric oxide-induced apoptosis of chondrocytes via thrombospondin-1
Minju Lee

P011 iPSC-derived macrophages (iMACs) as a promising source to treat hereditary pulmonary alveolar proteinosis
Miriam Hetzel

P012 Modulation of YAP/TAZ-dependent transcription in adult muscle stem cell physiology during aging and pathophysiology of rhabdomyosarcoma
Soeren Huettner

P013 Role of merlin in muscle stem cells
Marie Juliane Jung

P014 Fully integrated closed system expansion and differentiation of pluripotent stem cells towards mesencephalic dopaminergic progenitor cells
Frank Jüngerkes

P015 Mechanotransductive induced expression of neuronal markers in human adipose tissue-derived mesenchymal stem cells
Kerstin Kraft

P016 From hair to repair: potentials of MSCORS in regenerative medicine
Hanluo Li

P017 Clinical-grade iPSC generated with a cGMP Sendai viral reprogramming kit
Tim Wessel

P018 Identification of a putative stem cell population in the adult mouse pancreas
Ekaterina Mameishvili

P019 Improved human pluripotent stem cell expansion in stirred bioreactors by process control
Felix Manstein

P020 GMP compliant gene editing of human induced pluripotent stem cells using CRISPR/Cas9 ribonucleoprotein
Lena Molitor

P021 AUTOSTEM: an integrative and closed approach for the robot-assisted manufacture of mesenchymal stem cells
Jelena Ochs

P022 Healing from hair follicle - stem cells from the outer root sheath to treat chronic wounds
Marie Schneider

P023 Comparison of growth conditions of adult stem cells in 2D and 3D systems
Henrieta Škovierová

P024 Intraoperative radiotherapy for breast cancer treatment efficiently prevents breast adipose tissue-derived mesenchymal stromal cells outgrowth
Karen Bieback

P025 Pathogen reduction through UV light irradiation retains the optimal efficacy of human platelet lysate for human mesenchymal stem cell expansion
Sabrina Viau

P026 A standardized and characterized clinical grade human platelet lysate for efficient expansion of human bone marrow mesenchymal stem cells
Sabrina Viau

P027 Inducible Caspase-9 safety switch in human iPS cells using TALEN technology
Madline Schubert

P028 Accelerating and synchronizing the differentiation of hPSC-drived neural stem cells into neurons by preventing cell proliferation
Katja Hufschmid

Abstract No. P001

The effects of amnion-derived mesenchymal stem cells on fibrosis mechanisms in nephrectomy rat model

Ezgi Akan[1,], Busra Cetinkaya[2], Sadi Koksoy[3], Inanc Mendilcioglu[4], Dijle Kipmen-Korgun[1], Gultekin Suleymanlar[5], and Emin Turkay Korgun[2]*

[1]Department of Medical Biochemistry; Akdeniz University
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*Presenting author

Chronic kidney failure is one of the major health problems all over the world. Since kidney has a complicated cellular composition, it is difficult to both understand and cure the kidney diseases. In last decade, stem cell therapies for kidney failure is promising. Many researchers have demonstrate that mesenchymal stem cells (MSC) are effective to recover the damage resulted from kidney failure. We aimed to investigate, the effects of amnion-derived mesenchymal stem cells on fibrosis mechanisms in nephrectomy rat model.

MSCs were isolated from human term placenta amniotic membrane, the characterization of cells were provided by flow cytometry. MSCs were used to assess their chondrogenic, osteogenic and adipogenic differentiation potential. Related with the fibrosis, in order to detect the elevated extracellular matrix (ECM) amount, Masson's Trichrome and to detect elevated collagen amount SiriusRed staining were performed. After the injection of the stem cells, expressions of BMP-7, collagen type I, and TGF-β1 proteins in kidneys were investigated by immunohistochemistry.

Masson's Trichrome and SiriusRed stainings showed that ECM amounts were increased in 5/6 Nephrectomy group and in the MSC injected group's ECM amount were decreased. TGF-β1 and collagen type I protein expressions were increased in nephroctomy group. MSC injected group had smaller expressions of these proteins. BMP-7 protein immunohistochemistry stainings were increased in MSC injected group in comparison to nephrectomy group.

Stem cell studies are an alternative treatment method that increases the population with time. It is the ideal organ for mesenchymal stem cell treatment because of the fact that the placenta is a post-natal organ and is easy to use ethically. Therefore, clarification of the relationship between chronic renal disease and MSCs may lead to better understanding of stem cell proliferation and cessation of progression of chronic renal failure.

Abstract No. P002

Small sized population of human mesenchymal stem cell from umbilical cord blood improve stem cell properties and therapeutic benefit

Yun Kyung Bae[1,], Mi Yeon Kim[1], Ji Hye Kwon[1], and Hye Jin Jin[1]*

[1]MEDIPOST Co., Ltd
*Presenting author

Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) presents a promising source to promote tissue regeneration. However, cellular heterogeneity of MSCs impedes their use for regenerative medicines. Further investigation of this phenotype is required to permit MSCs-based therapies with improved clinical efficacy. Although heterogeneity is caused by various factor, heterogeneity display a number of common characteristics that make them easily distinguishable from cell size. Here, we the first using filter with centrifugation in a close system as new strategy, prepared three population, mixed size (heterogeneous), to ≤ 8 um (small), to > 8 um (large) in diameter, and described characterization of small cell from hUCB-MSCs, their immune-phenotypes patterns, potentials for stem-ness, growth kinetic, multi-lineage differentiation, and senescence phenotypes. Moreover, the small size population revealed a higher cell growth and lower senescence, as well as exhibited grater stem cell properties including differentiation, stem-ness, and adhesion than others populations. By surface marker screening (252 antibody), we identified both EGFR and CD49f as a candidate marker in small size. Accordingly, the suppression of these marker altered the function of small size population, and resulted in depression of the effect of small cell. Interestingly, small population of hUCB-MSCs induced to a greater improvement in therapeutic effect by not only promoting the engraftment potential of infused stem cell, but also reducing lung damage in an emphysema mouse model. Therefore, small size populations of hUCB-MSCs could be a way to promote the efficiency of cell therapy in clinical use.

Abstract No. P003

Investigating the age-dependency of tubular regeneration in zebrafish kidney

Thomas Bates[1,], Uta Naumann[1], Beate Hoppe[1], and Christoph Englert[1]*

[1]Leibniz Institute on Aging (FLI)
*Presenting author

Purpose: Mammals have a limited ability to repair damaged kidney tubules, with tubular epithelial cells dedifferentiating and repopulating the affected tissue. This ability is reduced with age. In contrast, zebrafish exhibit remarkable regenerative capacity following acute kidney damage and can perform neonephrogensis, the formation of new nephrons, throughout life. We investigate the age-dependency of the regenerative capacity in the zebrafish kidney. Methods: Using zebrafish as a model organism, we employ intraperitoneal gentamicin injections to model acute kidney damage. Using novel functional and structural readouts of kidney regeneration, we measure the regenerative capacity of both young and old fish. These analyses are complemented with immunohistochemistry and expression analysis of FACS-isolated cell types from the kidney. Results: Following analysis of the kinetics of kidney regeneration, we have additionally looked on a molecular level with qRT-PCR. When performing functional age-related regeneration experiments, there was no clear difference in regenerative capacities with age. We are performing follow up experiments to further understand why this might be, hoping to uncover mechanisms or pathways that are involved. In addition, we are continuing the establishment of further novel assays for kidney function in the zebrafish. Conclusion: Our current results suggest that regeneration of the zebrafish kidney is not affected by age. We are currently trying to understand the underlying mechanisms in a model organism that does not exhibit an age-dependent decline in regeneration, which would act as a useful tool for future biomedical research.

Abstract No. P004

Generation of a NKX2.1/p63 knockin human induced pluripotent stem cell reporter line for monitoring the generation of respiratory cells

Sandra Baus[1], Saskia Ulrich[1], Sylvia Merkert[1], Ruth Olmer[1,], and Ulrich Martin[1]*

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One promising 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 underlying genetic mutation, in vitro differentiation into the needed airway epithelial cell type and replacement of the endogenous diseased cells. For long term restoration, most likely lung stem cells like basal cells will be required. A requirement of this strategy is the development of an efficient and robust protocol for the generation of basal cells from human iPSCs (hiPSCs). The transcription factor NK2 homeobox1 (NKX2.1) expressed by lung epithelial progenitor cells represents an appropriate marker for optimizing differentiation protocols towards lung epithelial cells. Combination with the tumor protein 63 (p63) should allow for monitoring of basal cell generation in sequential differentiation protocols. The aim of the present study was the generation of a hiPSC double transgenic reporter line targeting the NKX2.1 and p63 locus. Therefore we designed one targeting vector for a non-disruptive integration of an eGFP coding sequence into the NKX2.1 locus and one targeting vector for the disruptive integration of nuclear localized Venus coding sequence into the p63 locus. Furthermore, the p63 targeting vector introduces a Neomycin selection cassette under control of the endogenous p63 promoter by the use of a 2A-site located behind the Venus coding sequence. The correct integration of the NKX2.1 and p63 targeting vectors was verified using PCR and Southern blot analysis. The established hiPSC-NKX2.1/p63 reporter line represents an optimal tool for the improvement of protocols for the differentiation of hiPSCs into basal cells and enables their selection which is indispensable for further in vitro and in vivo analysis.

Abstract No. P005

A pancreas-specific ECM scaffold – human iPS cell culture and pancreatic differentiation goes 3D

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Human induced pluripotent stem cell- (hiPSC) derived ß-cells display a great hope in search of a causal treatment for diabetes mellitus. Current *in vitro* differentiation protocols enable the generation of immature ß-cells which require an additional *in vivo* maturation step to achieve full functionality. Structural 3-dimensional characteristics, biophysical cues and biological components of the organ-specific extracellular matrix (ECM) are known to play important roles during organogenesis *in vivo*. Therefore, we hypothesize that β-cell differentiation could be improved using ECM-based cell culture systems mimicking the in vivo microenvironment.

In this study, we report about a pancreas-specific ECM (PanMa) generated by perfusion-decellularization of porcine pancreata. The generated PanMa was used in pilot experiments as biological scaffold in 3D static bioreactor cultures for hiPS maintenance culture. ECM impact on hiPSC nature was assessed by analyzing cell type-specific gene and protein expression profiles under ECM-based conditions.

ECM characterization studies demonstrate that the decellularized PanMa scaffold maintained key extracellular matrix components found in native tissue, such as Elastin, Laminin, Collagen I and IV, as well as vascular structures. Further, hiPSCs cultured on PanMa scaffolds were viable for at least 6 days retaining pluripotency characteristics such as colony formation, proliferation and expression of pluripotency markers. In addition, elevated expression levels for *hSOX1*, *hNKX6.1* and *hInsulin* were detected after 6 days in culture indicating possible differentiation inductive signals inherent in the PanMa ECM.

In summary, we were able to establish a biological ECM from porcine pancreata retaining important ECM characteristics after decellularization suitable as an organ-specific, 3-dimensional scaffold. First *in vitro* experiments suggest that the ECM promotes differentiation towards a pancreatic lineage. Future experiments will assess the capacity of the pancreatic matrix to support directed differentiation.

Rezania et al. "Reversal of Diabetes with Insulin-Producing Cells Derived in Vitro from Human Pluripotent Stem Cells." Nat Biotech 32, no. 11 (November 2014): 1121–33. doi:10.1038/nbt.3033

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Abstract No. P006

Xeno-free and chemically-defined differentiation of human pluripotent stem cells into the definitive endoderm in static suspension culture

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Pluripotent stem cells (PSCs) hold great promises for a potential cell replacement therapy of type 1 diabetes. Most of the currently used differentiation protocols are either not xeno-free or not chemically-defined, complicating a clinical application of their differentiated progeny. Therefore, this study aimed to establish a xeno-free and chemically-defined differentiation condition to generate definitive endoderm (DE) and pancreatic progenitors (PP) cells.

Human PSCs were differentiated in adherent (2D) or static suspension (3D) culture towards the DE (CHIR-99021/ActivinA). To establish a xeno-free and chemically-defined condition various compositions were tested. Subsequent differentiation of the DE towards PDX1-positive PP-cells was performed with a modified version of our described protocol. The different steps were analyzed by flow cytometry, RT-qPCR and immunofluorescence.

To exclude the undefined Matrigel matrix from the differentiation our 2D-protocol was adapted to small-scale 3D-culture. This adaptation resulted in a comparable DE efficiency (~80% CXCR4+ DE cells) and similar expression levels of DE marker genes (SOX17 and FOXA2) as routinely obtained by 2D-culture. Next, different chemically-defined 2D-conditions were tested, in which RPMI performed better than MCDB as base media for DE induction. RPMI showed higher proliferation rates and the CHIR-99021 concentration could be decreased without negatively affecting differentiation. Using this condition for xeno-free and chemically-defined differentiation in 3D-culture yielded high numbers of DE cells (~80%) as well as strong expressions of DE marker genes comparable with the 2D-protocol. DE cells generated under this xeno-free and chemically-defined condition could be further differentiated into PP cells, as verified by PDX1 expression.

This study established a xeno-free and chemically-defined, small-scale 3D-culture to differentiate human PSCs into the DE and subsequently into PP cells. Establishing xeno-free and chemically-defined differentiation conditions represents one step towards a potential cell replacement therapy. Additionally, an adaptation of the small-scale 3D-protocol towards bioreactor systems offers an interesting up-scaling capability.

Abstract No. P007

Human iPSC- and hematopoietic stem cell-derived erythroid precursors differ in their DNA methylation profile

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To date, manufacturing of red blood cells (RBCs) from human iPSCs fails in terms of large scale expansion, terminal enucleation and switching from fetal (HbF) to adult hemoglobin (HbA). This is in contrast to highly efficient RBC generation from hematopoietic stem cells (HSCs) derived from cord blood (CB) or peripheral blood (PB). To gain more insight into regulatory differences between iPSC- and HSC-derived ex-vivo erythropoiesis, we performed global DNA methylation profiling of cultured RBCs.

Ex vivo erythropoiesis from PB-HSCs, CB-HSCs, ESCs H1 and iPSCs was performed using an already described culture system (Dorn et al., Haematologica 2015). Homogeneous cell fractions of mainly orthochromatic erythroblasts were collected and global DNA methylation analysis was performed using the Infinum MethylationEPIC Array.

PB-HSCs and CB-HSCs showed homogeneous differentiation into >98% GPA+ erythroid cells, of which ~80% underwent enucleation. PB-derived RBCs expressed predominantly HbA, CB-derived RBCs HbF. Despite comparable homogeneous maturation into more than 95% GPA+ cells, iPSC- and ESC-derived RBCs failed in enucleation (<15%) and expressed embryonic and fetal hemoglobin rather than HbA.

DNA methylation analysis allowed for the interrogation of 850,000 methylation sites (CpG Islands). Principle component analysis demonstrated tight clustering of PB-RBCs and CB-RBCs. In contrast, iPSC-RBCs clustered with ESC-RBCs.

Differential gene methylation analysis revealed a minority of ~17,000 differentially methylated genes (DMG) between PB-RBCs and CB-RBCs. This was in contrast to ~200,000 DMGs between iPSC-RBCs and CB-/PB-HSC derived RBCs.

Our preliminary data implicate great differences between cultured RBCs from HSCs and iPSCs/ESCs. These differences might indicate for a more primitive RBC phenotype from the latter sources or a failure of erythropoiesis from pluripotent cells due to the absence of the physiological microenvironment. Our ongoing work focusses on the identification of affected pathways during erythroid maturation in order to overcome existing hurdles in the ex vivo manufacturing of RBCs from hiPSCs.

Abstract No. P008

Mesenchymal stem/stromal cells release different small extracellular vesicle subtypes

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Due to their reported regenerative and immunomodulatory properties, mesenchymal stem/stromal cells (MSCs) represent a promising therapeutic agent for multiple diseases. Initially, the MSCs were thought to act in a cellular manner. However, recent investigations suggest that they act rather in a paracrine manner. Moreover, within the MSC conditioned medium (CM), the small extracellular vesicles (sEVs), such as exosomes and microvesicles, seem to be the key players for such therapeutic effects. On the other hand, the results from some MSC studies appear to be diverse and sometimes even controversial, suggesting that MSCs are actually a heterogeneous population, with only a sub-fraction providing therapeutic activity. Previous experiments performed by our group revealed great molecular and immunomodulatory differences between the sEV fractions isolated from 20 different MSC samples. Consequently, it is of our interest to identify sEV markers whose presence correlates with their functionality. However, none of the available methods allowed yet a detailed study of the molecular content of sEVs at a single vesicle level. Therefore, we established a new antibody-based approach for the analysis of single sEVs by imaging flow cytometry. This technique allowed, through the detection of CD81 and CD9 surface markers, the discrimination of MSC-sEVs and human platelet lysate (used as a culture supplement)-sEVs within the MSC-CM. Furthermore, with a three parameter antibody staining analysis, this method also allowed the identification of further MSC-sEV subpopulations. This technique rises as a powerful tool for the characterization of sEVs at a single vesicle level, including identification of novel sEV markers and subpopulations. Moreover, in the near future functional studies with the detected sEV subfractions will help to unravel their potency and to understand better the MSCs mode of action.

Abstract No. P009

Feasibility study for long-term expansion of human induced pluripotent stem cells (hiPSC) in 3D bioreactors

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For application of human induced pluripotent stem cells (hiPSC) in drug testing or in regenerative medicine, methods are demanded that allow for continuous cell production at a large scale. Here, we describe a method for long-term expansion of hiPSC in a multicompartment 3D-hollow-fiber bioreactor. The bioreactor technology is based on three interwoven capillary bundles for counter-current medium perfusion and gas supply to the cells cultured in the extra-capillary space (cell compartment).

Parameters, such as the applied culture medium (E8/mTeSR) and initial cell number (10^7 or $2x10^7$ initial cells), were optimized using a 3D-bioreactor with a cell compartment volume of 2 ml and the hiPSC line DF6-9-9T (WiCell). In a second step, EDTA treatment during expansion over 15 days was applied on day 5 and 10 to prevent cell differentiation or cell necrosis due to cell aggregates exceeding a critical size. The EDTA treated bioreactor culture was compared to an untreated control bioreactor in terms of energy metabolism and expression of pluripotency and differentiation markers. Cells were quantified indirectly via the glucose consumption during the experiment or directly via cell counting at the end of the experiment.

The results showed that the growth behavior of the applied hiPSC line was considerably better in mTeSR medium when compared to commercially available E8 medium, irrespective of the inoculated cell number. Expression of the pluripotency marker Oct-3/4 and the proliferation marker Ki-67 was preserved as indicated by mRNA and immunofluorescence analysis. Regular application of EDTA resulted in a prolonged increase in cell growth, as indicated by glucose consumption and lactate production, when compared to the untreated control bioreactor.

In conclusion, the feasibility of long-term expansion of hiPSC in 3D bioreactors was demonstrated. The methodology could be used for a further upscale of hiPSC expansion in a closed system according to GMP conditions.

Abstract No. P010

Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) inhibits nitric oxide-induced apoptosis of chondrocytes via thrombospondin-1

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To date, chondrocyte death has been focused as a main pathogenesis in development of OA. The main aim of this study was to investigate the anti-apoptotic effect of human umbilical cord blood-derived MSCs (hUCB-MSCs) on nitric oxide (NO)-induced chondrocyte apoptosis.

To investigate the anti-apoptotic effect of secreted factors from hUCB-MSCs, chondrocytes were co-cultured with hUCB-MSCs in separate system using transwell. The secretome of hUCB-MSCs was analyzed using a biotin label-based antibody array to identify paracrine factors underlying the anti-apoptotic effect of hUCB-MSCs. As a result, co-culture with hUCB-MSCs inhibited NO-induced rabbit chondrocyte apoptosis. By biotin label-based antibody array, we identified TSP-1 as an apoptosis inhibitory factor of hUCB-MSCs. In order to determine the anti-apoptotic role of thrombospondin-1 (TSP-1), knockdown studies using small interfering RNAs (siRNAs) targeting *TSP-1* were performed in hUCB-MSCs. Exogenous TSP-1 was added to a co-culture medium of chondrocyte and hUCB-MSCs. In an *in vitro* assay, the anti-apoptotic effect of hUCB-MSCs was analyzed using live/dead staining, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay, and western blotting. Knockdown of *TSP-1* expression in hUCB-MSCs abolished the anti-apoptotic effect of hUCB-MSCs on chondrocyte apoptosis. Exogenous addition of recombinant TSP-1 inhibited NO-induced chondrocyte apoptosis. To further validate the anti-apoptotic effect of hUCB-MSCs in an *in vivo* system, we made rat osteoarthritis animal model using anterior cruciate ligament transection (ACLT) and monosodium iodoacetate (MIA) injection. In parallel with in vitro results, cartilage regeneration by anti-apoptotic effect of hUCB-MSCs was also demonstrated in rat osteoarthritis animal model.

In conclusion, our findings suggested that hUCB-MSCs can inhibit NO-induced apoptosis of chondrocytes via TSP-1, which leads to inhibition of chondrocyte death in OA.

Abstract No. P011

iPSC-derived macrophages (iMACs) as a promising source to treat hereditary pulmonary alveolar proteinosis

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iPSCs have widely been employed for disease modeling, drug discovery and gene therapy studies in vitro. However, application of iPSC-derived, mature cells in vivo is scarcely investigated. Given the fact that tissue resident macrophages are of embryonic origin, are long-lived and robust differentiation protocols exist to generate high numbers of iMACs, they hold great promise for cell replacement therapies.

Previously, we have demonstrated that pulmonary macrophage transplantation (PMT) employing bone marrow-derived macrophages allows for long term correction of hereditary pulmonary alveolar proteinosis (herPAP) [Suzuki, Nature, 2014; Happle, Lachmann, STM, 2014], a rare lung disease characterized by the malfunction of alveolar macrophages (AM). We now present data that also iMACs can serve as a novel, safe and efficient cell source for PMT in herPAP.

In *Csf2rb*$^{-/-}$ mice, reflecting the herPAP disease phenotype, single PMT of 4x10^6 healthy/wild-type murine iMACs led to specific engraftment of donor-derived cells in the alveolar spaces for up to six month, while no teratoma formation or tissue toxicity was detected in transplanted mice. Additionally, iMACs adapted a typical AM morphology including Siglec-F expression upon PMT in vivo. Most importantly, a significant improvement of critical disease parameters such as protein, M-CSF, GM-CSF and surfactant protein-D (SP-D) concentration in the bronchoalveolar lavage fluid (BALF), PAS-positive material in lung sections, and lung density in CT scans was observed. Likewise, repetitive PMT of human iMACS led to significant engraftment in a humanized mouse model of PAP for up to two month and markedly diminished disease related parameters in the BALF and alveolar protein content in lung sections. Of note, in both models transplanted cells upon engraftment adopted a typical AM- phenotype and gene expression profile.

This data highlights the potential of iPSC derivatives in cell-based therapies and thus may have profound implications beyond the rare disease of PAP.

Abstract No. P012

Modulation of YAP/TAZ-dependent transcription in adult muscle stem cell physiology during aging and pathophysiology of rhabdomyosarcoma

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Satellite cells (SC) are skeletal muscle stem cells which are responsible for muscle development and a prerequisite for regeneration. Upon activation they are able to self-renew or give rise to myogenic progenitors. SC functionality is tightly controlled by cell intrinsic and extrinsic mechanisms.

During aging, SC functionality declines concomitant with sarcopenia, the age-dependent loss of muscle mass. There is a big need to improve our understanding of aging-associated changes within the stem cell compartment to develop targeted therapies aiming to improve physical conditions for the elderly or counteracting muscle wasting disorders.

The conserved Hippo pathway has been shown to play a central role in organ growth and size determination in vertebrates including mammals. Moreover, Hippo signaling is important for the maintenance and activation of satellite cells. The main pathway effectors are the transcriptional co-factors yes-associated protein 1 (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ). Importantly, we identified a novel negative regulator of YAP-dependent transcription. Furthermore we detected changes in the extracellular matrix composition of the aged stem cell niche, which could be a result of altered YAP/TAZ transcriptional output. Therefore this project aims to analyze the causal relationship between age-dependent modulation of YAP/TAZ activity and changes in the stem cell niche.

Moreover, we discovered differential expression of the novel YAP/TAZ negative regulator in a subset of human rhabdomyosarcoma cell lines. We are currently investigating, if this factor could be a potential therapeutic target to suppress cell hyperproliferation and enhance myogenic differentiation.

Abstract No. P013

Role of merlin in muscle stem cells

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Inactivation of Merlin results in Neurofibromatosis type 2 (NF2) characterized by vestibular bilateral schwannomas as well as other peripheral nerve tumors. Additionally NF2 patients often present a slow but progressive distal muscle atrophy and paresis in later stages of the disease.

We could show that merlin-deficient neurons after sciatic nerve crush injury showed a proper re-innervation concomitant with no difference in muscle weight and a regular fine architecture of muscle fibers. This indicated that merlin loss in neurons alone is not sufficient to cause muscle atrophy in NF2 patients. Therefore we started to investigate the intrinsic role of merlin in myogenesis. We could demonstrate that merlin is indeed expressed during in vitro myogenesis, we detected expression in myoblasts and myotubes. Our data also suggest that merlin expression and activity is increased during differentiation of myoblasts in vitro. Knockdown of Merlin expression in differentiating primary myoblasts resulted in an increased fusion index at early stages of differentiation. To further investigate the functional relevance of merlin expression in vivo we injured Pax7-creER;Nf2flox/flox mice. Loss of merlin expression in adult muscle stem cells lead to increased regeneration suggesting that loss of merlin in MSCs plays a crucial role in myogenesis and regeneration of skeletal muscle.

Abstract No. P014

Fully integrated closed system expansion and differentiation of pluripotent stem cells towards mesencephalic dopaminergic progenitor cells

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

Using our versatile and integrated GMP-compliant cell processing platform CliniMACS Prodigy® we previously developed a cultivation and expansion workflow for iPS cells. Now we have implemented the differentiation of PSCs into mesencephalic dopaminergic (mesDA) progenitor cells on the device. Adapting this differentiation protocol from embryonic body–based to fully adherent cultivation enabled straightforward upscaling of a lab protocol to a medium-scale production process within the closed system. Additionally, we have designed a concise marker panel for flow cytometry based quality control (QC) for characterization of the resulting mesDA progenitors.

One million PSCs were used as starting material for expansion in the Lam521-coated Centrifugation and Cultivation Unit (CCU). An in-process control revealed a pluripotent marker profile and a 30 to 60 fold expansion in the first cultivation phase. After 11 days of differentiation in a Lam111-coated CellStack (636cm2), we harvested around 800 million mesDA progenitor cells, before cells were passaged for final differentiation until d16. Extrapolating the cell numbers retrieved from the protoype process would correspond to 200-250 patient doses assuming 5 million cells per cryopreseved unit. However, the process holds further potential for upscaling.

Taken together, we have developed a method for adherent, closed-system cultivation of PSCs and differentiation to dopaminergic progenitor cells in combination with comprehensive QC assays.

Abstract No. P015

Mechanotransductive induced expression of neuronal markers in human adipose tissue-derived mesenchymal stem cells

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Nano-scale structural patterns are important hallmarks of the neuronal microenvironment in vivo. Cultivation of pluripotent stem cells or neuronal precursor cells on artificial nano-structures provides topographical cues directing cellular alignment and extension. Moreover, resulting physical load transfers tensile forces to the nucleus and influence signaling pathway, inducing neuronal lineage gene expression.

Mesenchymal stem cells (MSC) are of particular interest for regenerative medicine and exhibit a higher plasticity than previously anticipated. When cultured under appropriate conditions, MSC do not only differentiate along mesodermal lineages, but also show upregulation of endo- and ectodermal lineage markers including those usually expressed on neurons.

In this study Polydimethylsiloxane (PDMS) micro-structured surfaces were used to induce neuronal marker expression in human adipose tissue-derived mesenchymal stem cells (AD-MSC). AD-MSC cultivation on these surfaces induced extension and alignment of cellular processes, while increased expression of neurofilament and β-tubulin III were confirmed on gene and protein levels as compared to standard (flat surfaces) cultivation conditions.

Our results indicate that directed nano-structures might regulate AD-MSC differentiation in vitro. This is of relevance for regenerative medicine since such nano-structures could be used for pre-differentiation towards neuronal differentiation lineages, provide a novel tool for damaged neuronal tissue reconstitution, or for production of patient-specific neural cells.

Abstract No. P016

From hair to repair: potentials of MSCORS in regenerative medicine

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Human hair follicle Mesenchymal Stem Cells (MSCORS) from the Outer Root Sheath (ORS) compartment possess high therapeutical potential as an alternative somatic stem cell source compared with conventionally available stem cell pool such as bone marrow and adipose tissue. We have developed a non-invasive autologous stem cell technology platform using MSCORS from harmlessly obtained plucked hair follicles, to tackle tremendous unmet medical needs on efficient treatments for translational regenerative medicine, such as bone defects and cardiovascular disease.

By releasing the MSCORS with IP-protected technologies, we have isolated, cultivated, and characterized human MSCORS according to the ISCT criteria for MSC, including MSC surface markers and tri- differentiations towards mesoderm lineages. MSCORS exhibit mesenchymal stem cell-like properties expressing various pluripotent stem cells markers. MSCORS are capable of differentiating into chondrocytes, osteocytes and adipocytes, especially endothelial cells and smooth muscle cells. Using advanced biomaterial carriers and tissue engineered cultivation, we develop in vitro osteogenic hydrogel and artificial blood vessel as proof-of-concept models for future clinical translations. MSCORS differentiate towards osteoblast lineage and deposit calcium phosphate within the gelatin-based cross-linked hydrogels. This injectable, osteogenic, MSCORS capsulated hydrogel is promising in bone regeneration and therapies. MSCORS are also differentiated into fibroblasts, smooth muscle cells and endothelial cells, and reconstructed into artificial aorta with normal anatomic structure and partial functionality. This in vitro construct is providing non-invasive alternative for bypass surgery of cardiovascular disease.

Our future work will focus on of developing MSCORS as a non-invasive stem cell source for clinical translation, as well as optimizing the in vitro models of osteogenic injectable hydrogel and artificial aorta grafts via 3D cell culture and tissue engineering, which are the ultimate gateways towards the clinical applications in the treatments of bone defects and and cardiovascular disease.

Savkovic, V., Dieckmann, C., Milkova, L. and Simon, J. C. (2012), Improved method of differentiation, selection and amplification of human melanocytes from the hair follicle cell pool. Exp Dermatol, 21: 948–950. doi:10.1111/exd.12038

Savkovic, V., Li, H., Seon, J.K., Hacker, M., Franz, S. and Simon, J.C., 2014. Mesenchymal stem cells in cartilage regeneration. Current stem cell research & therapy, 9(6), pp.469-488.

Abstract No. P017

Clinical-grade iPSC generated with a cGMP Sendai viral reprogramming kit

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For the past decade, the induced pluripotent stem cell (iPSC) field has grown immensely, starting with basic research, and now moving more toward translational and clinical applications. As iPSC move towards the clinic, it is vital that the cells used are of high quality; free of reprogramming transgenes, free of adventitious agents, genetically stable, and functionally pluripotent. Traditional reprogramming workflows typically include one or more components of animal origin, such as fetal bovine serum (FBS) or bovine serum albumin (BSA), which can be sources of adventitious agents. This means that iPSC that are generated in a xeno-free, clinically relevant workflow will be much more readily applicable to clinical uses.

Here, we show the generation of high quality iPSC with a cGMP-manufactured Sendai reprogramming kit, which is free of animal origin components. The kit was used to reprogram both blood-derived cells and skin fibroblasts, in conjunction with xeno-free cell culture and reprogramming workflows, starting from initial cell isolation, all the way through to iPSC expansion and banking. The iPSC generated with this method were shown to have a normal karyotype, were free of Sendai viral vectors, expressed the standard self-renewal markers, and demonstrated functional pluripotency for all three germ layers. The ability to create high quality iPSC in clinically relevant, xeno-free workflows, will allow researchers to more smoothly transition cells to clinical applications.

Abstract No. P018

Identification of a putative stem cell population in the adult mouse pancreas

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The existence of stem cells in the adult pancreas has not been documented despite several lines of evidence suggesting the presence of such a population. Putative progenitor cells have been isolated from the centroacinar component based on their high aldehyde dehydrogenase (Aldh) enzymatic activity but the lack of a specific molecular marker has not allowed their characterization.

We have found that the mitochondrial *Aldh1b1* gene is expressed in all embryonic pancreas progenitors and its expression in the adult persists only in centroacinar-like cells. The number of Aldh1b1$^+$ cells expanded significantly following pharmacological beta or acinar cell ablation. We established an *Aldh1b1* lineage tracer line and found that the Aldh1b1$^+$ cell progeny expands over time and contributes to the endocrine lineage as alpha or beta cells as well as to the acinar and duct lineages under homeostatic conditions. Additionally, we found that Aldh$^+$ cells can form three-dimensional self-renewing spheroids *in vitro* and this activity is exclusively attributed to *Aldh1b1* expressing cells. Expanding spheroids can be passaged at least eight times; cells retain epithelial morphology and show high levels of *Pdx1* and *Aldh1b1* expression, comparable to that of embryonic pancreata.

These results suggest that we have identified an adult mouse pancreas stem cell population. To define the molecular signature and possible routes of differentiation of this population we have undertaken and are analyzing single cell transcriptomic profiling. Additionally, transplantation experiments are under way to determine the differentiation potential of these cells after expansion *in vitro*.

Abstract No. P019

Improved human pluripotent stem cell expansion in stirred bioreactors by process control

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Human pluripotent stem cells are a unique source for the production of tissue-specific human cell types, fueling the development of advanced in vitro disease models and novel regenerative therapies. Most applications will require the constant supply of billions of cells generated by robust, economically viable bioprocesses. The expansion of hPSCs as matrix-free cell-only aggregates in suspension culture (3D) is a potentially superior strategy for producing required cell numbers applying industry-compliant stirred-tank bioreactors (STBRs). STBRs ensure homogeneous distribution of cells, nutrients, and gases and enable continuous online monitoring and control of key process parameters such as pH and dissolved oxygen (DO)

We have recently established expansion and efficient cardiomyogenic differentiation of hPSCs in stirred tank bioreactors, demonstrating the universal utility of these systems for the mass production of hPSC-progenies. Moreover, daily medium replacement (termed repeated batch), which is typical for conventional tissue culture, was replaced by continuous medium exchange (termed perfusion). This advancement enabled significantly increased cell yields, and opened new possibility for process control.

Here we have combined perfusion with the control of specific process parameters such as pH or DO and the adaptation of medium components in the chemically-defined brew E8. The advanced process in 150 mL bioreactor scale resulted in more homogenous and more reproducible process conditions. Furthermore, it was able to determine important cell specific parameters in more detail. Detailed analysis of other process indicators will also be presented, including comprehensive assessment of hPSCs' pluripotency status and the energy metabolism.

Together, the study highlights the enormous potential for process development in hPS cell manufacturing, particular by using well monitored and controlled bioreactor systems, which also facilitates straightforward upscaling.

Abstract No. P020

GMP compliant gene editing of human induced pluripotent stem cells using CRISPR/Cas9 ribonucleoprotein

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Genetic engineering of iPSCs under defined manufacturing standards would open up new avenues for development of innovative therapies, such as the engraftment of autologous mutation corrected tissue derivatives of iPSCs. In order to adapt CRISPR/Cas9 gene modification to Good Manufacturing Practice (GMP) compliant processes we are evaluating a method that combines genome editing and reprogramming. Therefore, we use ribonucleoprotein (RNP) Cas9 and non-modified mRNAs (NM-RNAs) encoding the six factor reprogramming cocktail (OSKMNL). Combining these processes and using NM-RNAs to avoid viruses or plasmids, we foresee great benefit from the regulatory perspective by reducing the complexity of manufacturing processes in compliance with GMP and the regulatory threshold for clinical use. This will translate to reduced mutation burden by shortening the expansion campaign. Moreover it will result in lower costs compared to carrying these steps independently and analyzing the quality of iPSCs repeatedly (after reprogramming and after gene editing). We investigated time points for Cas9-mediated gene modification during the phase where fibroblasts are adapted to the reprogramming environment. We found that this enables genetic modification in fibroblasts with higher efficiency compared to Cas9 RNP mediated modification of iPSCs. Taken together, coupling of RNP-mediated gene modification and NM-RNA reprogramming of patient cells will help facilitate the development of safe autologous PSC-based therapies.

Abstract No. P021

AUTOSTEM: an integrative and closed approach for the robot-assisted manufacture of mesenchymal stem cells

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Mesenchymal stem cells (MSC) are of significant importance to the emerging field of regenerative therapy. In order to pave the way towards broad clinical application however, large quantities of MSCs are needed. Manufacturers are however confronted with many challenges such as donor-to-donor variability of the cell material. Additionally, the cell product is classified as an advanced therapy medicinal product (ATMP) and requires adherence to strict regulatory requirements.

Due to these reasons, a robotic platform is being developed within the pilot project AUTOSTEM, which will enable the automated production of MSC in multi-litre scale for therapeutic applications. The implementation of automated systems into biological processes opens up new possibilities with regard to higher reproducibility and increases the process robustness while minimizing the risk of human errors. In order to minimize the risk for contamination, the aseptic process is robot-assisted and requires no direct human interaction with the product. The platform covers the entire production chain from the inoculation to the two-step bioreactor expansion as well as the harvesting and formulation for cryopreservation. Therefore, the facility will consist of different areas with graduated clean room levels that allow the performance of both bioreactor-based processes as well as the open handling and manipulation of the cells. In order to achieve safe production and compliance with Good Manufacturing Practice (GMP), the components as well as the platform layout follow a hygienic design. A centralized control software programme allows control and monitoring of the process at all times.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 667932. The material presented and views expressed here are the responsibility of the author(s) only. The EU Commission takes no responsibility for any use made of the information set out.

Abstract No. P022

Healing from hair follicle - stem cells from the outer root sheath to treat chronic wounds

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Our research focuses on the generation of an autologous Advanced Therapy Medicinal Product (ATMP) designed for wound healing and repigmentation. We use hair follicles as starting biological material to isolate stem cells from the Outer Root Sheath (ORS) of human hair. The ORS presents an excellent non-invasively available source of material for regenerative therapies. It is obtained by harmless plucking of hair and harbours a collection of developmentally highly potent adult stem cells and precursors. Those cells are further differentiated into skin-relevant cells (keratinocytes, melanocytes, fibroblasts and mesenchymal stem cells) with the goal of generating a pre-clinically tested epidermal and dermoepidermal skin graft.

We have generated melanocytes, keratinocytes, fibroblasts and mesenchymal stem cells (MSCs) from hair follicle ORS by the means of an optimized explant method by Savkovic et al. All cultures were analyzed on the basis of their morphology, gene- and protein-marker expression. In order to create epidermal equivalents, melanocytes were co-cultivated with keratinocytes. Integration of fibroblasts generated the dermal compartment, producing a dermoepidermal equivalent.

Manufactured epidermal and dermoepidermal equivalents were anatomically and functionally comparable to human skin. Our autologous and non-invasively gained skin grafts offer an immense potential for personalized treatment of chronic wounds, accompanied by their use as toxicological tests in the industry and innovative cell-banking opportunities. Further use of the MSCs' exosomal content will improve the engraftment of the skin transplants.

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Schneider M, Dieckmann C, Rabe K, Simon JC, Savkovic V. Differentiating the stem cell pool of human hair follicle outer root sheath into functional melanocytes. Methods Mol Biol. 2014; 1210:203-27. Humana Press, Springer Link.

Abstract No. P023

Comparison of growth conditions of adult stem cells in 2D and 3D systems

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The three-dimensional (3D) cell culture systems have gained increased interest in drug discovery and tissue engineering due to their evident advantages in providing more physiologically relevant information and more predictive data for in vivo testing comparing to cells cultured in 2D. The 3D cultivated cells grow as spheroids on special non-adherent surface instead of cell monolayer of 2D system. Therefore, 3D cultures are more similar to in vivo behavior compared to 2D culture (Edmondson et al., 2014).

Dental pulp stem cells (DPSC) have a mesenchymal stem cells phenotype and are able to differentiate into neurons, cardiomyocytes, chondrocytes, osteoblasts, liver cells, or beta cells of pancreatic islets. Thus, DPSC have shown great potential for treatment of various human diseases in the field of regenerative medicine (Potdar and Jehlmalani, 2015).

In our project, we studied the biological properties of adult stem cells, human DPSC cell line (Lonza, USA), in 2D and 3D culture conditions on cellular level. We focused on the expression profile of specific markers during cultivation of DPSC in 2D versus 3D system. Moreover, we compare the presence of surface markers of adult stem cells to differentiated cells (human dermal fibroblasts) as a negative control to prove the stemness of DPSC in 2D and 3D growth conditions. The data from 3D cell culture system could be used as model system development in drug discovery to provide a simple, fast and cost-effective tools to avoid large-scale and cost-intensive animal testing as well as for future applications in regenerative medicine of dental tissues.

This work was supported by following grants: Research and Development Support Agency (APVV-15-0217), Slovak Scientific Grant Agency (VEGA 1/0178/17) and the project "Biomedical Center Martin", ITMS code 26220220187, co-financed from EU sources.

Abstract No. P024

Intraoperative radiotherapy for breast cancer treatment efficiently prevents breast adipose tissue-derived mesenchymal stromal cells outgrowth

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Mesenchymal stromal cells (MSC) in the bone marrow have been shown to be radioresistant related to pronounced DNA repair mechanisms. Intraoperative radiotherapy (IORT) during breast conserving surgery is an innovative technique applying low energy x-ray to the tumor bed immediately after removal of the tumour. Aim of this study was to investigate whether IORT affects the outgrowth potential of breast adipose tissue-derived MSC (bASC).

After surgical tumour resection, biopsies of the tumour bed before and after IORT with low energy x-rays were taken and processed applying well-established protocols for ASC isolation and characterisation.

100% of tumour bed samples pre-IORT yielded outgrowing bASC with typical ASC characteristics: fibroblastoid morphology, colony formation, proliferation, adipogenic and osteogenic differentiation and ASC surface marker expression. Interestingly, none of the post-IORT samples yielded outgrowth of bASC.

After breast conserving breast cancer surgery relapsing tumours emerge in 90% in close proximity to the initial tumor bed, potentially reflecting a significant contribution of the tumour bed to relapse Our data show that IORT, besides the proven effect on breast cancer cells, also modifies the tumour environment by having an impact on bASC of the tumour bed. This might help to reduce the risk of tumour relapse.

Abstract No. P025

Pathogen reduction through UV light irradiation retains the optimal efficacy of human platelet lysate for human mesenchymal stem cell expansion

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We recently developed and characterized a standardized and clinical grade human Platelet Lysate (hPL) that constitutes an advantageous substitute for fetal bovine serum (FBS) for human mesenchymal stem cell (hMSC) expansion required in cell therapy procedures, avoiding xenogenic risks (virological and immunological) and ethical issue. Because of the progressive use of pathogen reduced (PR) labile blood components, we evaluated the impact of the novel procedure THERAFLEX UV-Platelets for pathogen reduction on hPL quality (growth factors content) and efficacy (as a medium supplement for hMSC expansion). This technology is based on short-wave ultraviolet light (UV-C) and has the main advantage not to need the addition of any photosensitizing additive compounds (that might secondary interfere with hMSCs). We applied THERAFLEX UV-Platelets procedure on fresh platelet concentrates (PCs) suspended in platelet additive solution and prepared hPL from these treated PCs. We compared the quality and efficacy of PR-hPL with the corresponding non-PR ones. We showed no impact on the content in 5 cytokines tested (EGF, bFGF, PDGF-AB, VEGF and IGF-1) and a significant decrease in TGF-beta1 (-21%, n=16). We performed large scale culture of hMSCs during 3 passages and showed that hPL or PR-hPL at 8% triggered comparable hMSC proliferation than FBS at 10% plus bFGF (n=3). Moreover, after proliferation of hMSCs in hPL or PR-hPL containing medium, their profile of membrane marker expression, their clonogenic potential and immunosuppressive properties (inhibition of T-cell proliferation) were maintained, in comparison with hMSCs cultured in FBS conditions. We quantitatively compared the potential to differentiate in adipogenic and osteogenic lineages of hMSCs cultured in parallel in the 3 conditions and showed that they remained also identical. In conclusion, we demonstrated the feasibility to use UV-C treatment to subsequently obtain pathogen reduced hPL, while preserving its optimal quality and efficacy for hMSC expansion for cell therapy applications.

Abstract No. P026

A standardized and characterized clinical grade human platelet lysate for efficient expansion of human bone marrow mesenchymal stem cells

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Human platelet lysate (hPL) is rich in growth factors (GF) and nutritive elements and represents a powerful xeno-free alternative to fetal bovine serum (FBS) notably for mesenchymal stem cell (hMSC) proliferation. However, there is a large variability in hPL sources and production protocols, resulting in discrepancies in product quality, low management of product safety and poor batch-to-batch standardization. We describe here the development and the characterization of a standardized hPL prepared from transfusional grade screened normal human donor platelet concentrates (PCs), manufactured on an industrial scale (batch sizes of 10 L; 250 donors) and following a highly qualified process (clean room, trained operators, validated aseptic filtration). Platelets were lysed by a freeze / thaw cycle. Cell debris were removed by centrifugation and the supernatant (hPL) was recovered. Clinical grade 10L batches of aseptic filtered hPL were characterized. On the contrary to hPL prepared from a limited number of donors, we observed a robust standardization between 10L-industrial batches of hPL in terms of GF contents (bFGF, EGF, VEGF, PDGF-AB, TGF-beta1 and IGF-1), biochemical analyses (for instance total proteins, albumin, triglycerides, iron). We also compared the expansion and the functional characteristics of BM-hMSCs grown in clinical grade hPL versus MSC-screened FBS batch. We showed a reproducible and powerful increase in cell growth kinetics using hPL, a maintenance of BM-hMSC clonogenic potential and membrane marker expression (with however a strong overexpression of CD90). We observed a similar adipogenic and osteogenic differentiation potential and finally that immunosuppressive properties of BM-hMSCs (inhibition of T-cell proliferation) cultivated in parallel in both conditions remained also identical. Finally, we documented the stability over time of hPL stored at -80°C and -20°C. In conclusion, we demonstrated the feasibility to use a standardized, characterized, efficient and clinical grade hPL for research and cell therapy applications.

Abstract No. P027

Inducible Caspase-9 safety switch in human iPS cells using TALEN technology

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Human induced pluripotent stem cells (iPSCs) are considered as favorite cell source for regenerative therapies. However one major drawback of cell transplantations is the risk of tumor formation.

In order to improve the safety of iPSC-based therapies, an inducible Caspase9 suicide gene system was established for the eradication of iPSCs and their derivatives in case of potential side effects after in vivo application. So far, the inducible Caspase9 gene was applied by viral transduction of iPSCs. In our study, we used TALEN technology to stably integrate the iCaspase9 gene, under control of the ubiquitous CAG promoter, into the AAVS1 safe harbor locus of three different human iPSC lines. The established transgenic clones (iCaspase9-iPSCs) were characterized concerning their pluripotency status and the targeted integration of the transgene into the AAVS1 locus.

For the directed activation of the Caspase9, undifferentiated iCaspase9-iPSCs were exposed to specific chemical inducers of dimerization (CID) at different concentrations for 24 hours in vitro. Even the application of low concentrations of 1 nM CID, was sufficient to eliminate more than 99% of the cells immediately within 24 hours. Moreover, macrophages, cardiomyocytes and endothelial cells derived from iCaspase9-iPSCs were also exposed to the CID, and show the same results as their undifferentiated counterparts.

Our iCaspase9-iPSCs will be tested in mice as soon as possible. Therefore, arisen teratoma or transplanted macrophages will be eliminated via application of the CID in vivo, to prove the advantage of our established cell clones over former lentiviral approaches.

Abstract No. P028

Accelerating and synchronizing the differentiation of hPSC-drived neural stem cells into neurons by preventing cell proliferation

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Neural stem cells (NSCs) generated from human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), can be expanded in culture and further differentiated into neurons for disease modeling and chemical screening. Due to continuing proliferation of plated NSCs in culture in conventional neuronal differentiation medium, mixed cell populations including differentiated neurons and undifferentiated NSCs are often observed. Furthermore, cell aggregations are formed in culture and cell density increases along with the elongation of culture duration, which leads to the difficulty for interpreting end-point results. To solve these issues, we have developed a CultureOne™ supplement which can accelerate and synchronize the differentiation of hPSC derived NSCs into neurons by preventing the proliferation of undifferentiated NSCs in culture. With the treatment of CultureOne™ supplement, differentiated neurons are evenly distributed across the culture surface with extensive neurite networks and very little cell aggregation. Immonucytochemical staining showed that differentiated neurons expressed neuronal marker MAP2 or HuC&D with very few SOX1 positive undifferentiated NSCs at 2 weeks of differentiation. At 4-5 weeks of differentiation, the differentiated neurons expressed mature neuronal markers neurofilament and synaptophysin. To investigate the mechanism of CultureOne™ supplement actions, differentiating neurons were incubated with thymidine analog. By comparing with differentiating neurons without CultureOne™ supplement, the treatment with CultureOne™ supplement significantly decreased EdU positive cells without induction of cell death marker Caspase 9 expression, which suggests that the treatment with CultureOne™ supplement prevents cell proliferation. Upon depolarization with KCl, the signals of calcium influx of the differentiated neurons with the treatment of CultureOne™ supplement were much greater than untreated neurons, indicating the treatment with CultureOne™ supplement accelerates the maturation process of differentiating neurons. By using CultureOne™ supplement, the evenly distributed neurons with more maturity are more favorable to manual or automated imaging for quantification.

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Abstract No. P029

Stem cell-based modeling of DiGeorge syndrome

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Pluripotent stem cells (PSCs) can be differentiated into all cell types of the human body. PSCs can be derived from embryonic state (ESC) or from adult somatic cells by reprogramming them into pluripotent state (iPSC). Human iPSCs and their differentiated derivatives are new, promising models for studying disease-related phenotypes in vitro regarding such cell types which cannot be investigated directly or in long-term, or when appropriate animal models are not available. Our aim is to investigate in vitro cellular phenotypes in complex diseases such as DiGeorge syndrome affecting several organ systems (cardiovascular, nervous and immune systems). DiGeorge syndrome is caused by the deletion of the 22q11.2 chromosome region on one allele and the hemizygous presence of the affected genes is not sufficient for the healthy phenotype. We are especially interested in DGCR8 gene located in this region, which encodes a component of the microprocessor complex essential for microRNA biogenesis. For modeling this disease we generated iPSCs from peripheral blood mononuclear cells of a mother and her daughter (both suffering from disease) as well as from spouse (not affected by DiGeorge syndrome) by the expression of four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc) via Sendai virus vector (SeV) transduction. The generated iPSCs were characterized by RT-PCR, immunocytochemistry and FACS. iPSCs are being differentiated into cell types relevant to the diseases such as endothelial, cardiac and neural cell types and characterized based on morphology, viability, transcriptomics, proteomics and functionality. Our study can provide insights to the development of the disease and discovery of biomarkers.

This study has been funded by the National Brain Research Program (NAP) of Hungary (KTIA_NAP_13-1-2013-0001 to LH) and National Heart Program (NVKP_16-1-2016-0017).

Abstract No. P030

Custom-tailored cardiomyocytes: A directed differentiation of human pluripotent stem cells into defined atrial and ventricular cardiomyocyte subtypes

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Generation of homogenous populations of subtype-specific cardiomyocytes (CMs) from human iPSCs and their comprehensive phenotypic comparison is crucial for a better understanding of the predominantly cardiac subtype-restricted disease mechanisms as well as for regenerative and pharmacological applications. Current differentiation protocols produce a heterogeneous population of ventricular, atrial and pacemaker-like CMs. **Goal of our study** is to develop an efficient and cost-effective method for directed in vitro differentiation of iPSCs into defined functional CM subtypes in feeder-free culture conditions. Furthermore, we aim to obtain comprehensive understanding of the molecular and functional characteristics of iPSC-derived CM subtypes which is key to future iPSC-CM-based approaches.

On the basis of temporal modulation of canonical WNT and retinoid acid signaling throughout differentiation of iPSCs, we were able to guide the cardiac progenitor cells towards distinct cell fates resulting in homogeneous populations of either ventricular or atrial CMs. The atrial and ventricular iPSC-CMs were deeply investigated for their cardiac subtype identities based on their characteristic cell biological and functional properties. The functional and pharmacological characterization of the iPSC-derived CM populations via optical action potential and calcium imaging as well as engineered heart muscle analysis revealed that ventricular iPSC-CMs display properties of CMs from the human ventricle, whereas atrial iPSC-CMs highly correspond to the atrial heart muscle. RNA sequencing and SILAC-based proteomics of atrial and ventricular iPSC-CMs not only highly correlated with expression profiles of human ventricle and atria, respectively, but also identified novel candidates with potential crucial roles in cardiac subtype specification or cellular function.

This study provides an efficient tool of a **directed differentiation of iPSCs into custom-tailored functional CM subtypes** and provides new insights in the comprehensive **understanding of the molecular and functional characteristics** of atrial and ventricular iPSC-CMs for a more precise disease modeling, drug screening as well as for cell-based therapeutic approaches.

Abstract No. P031

A novel reporter system for CRISPR/Cas9-stimulated gene editing

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Recently, we have described an improved *in cella* platform for the evaluation of homology directed repair (HDR) stimulation at genomic target loci by Cas9 nickase. However, global and unselected HDR efficiencies were in the only in the range of 0.5-1%. Thus, potential *in vivo* applications of CRISPR stimulated gene editing demand for higher efficiencies in order to correct a therapeutically relevant number of cells. Here, we report a novel fluorescence-based reporter system for analysis of substances, which could influence HDR and NHEJ. Importantly, the system is based on a single point-mutation which completely ablates fluorescence of the HDR-reporter fluorophore and which can efficiently be corrected by a proximal sgRNA. Following lentiviral vector transduction of this reporter into cell lines of choice we were able to optimize length and concentration of ssODN donors for better HDR-stimulation efficiency using Cas9_D10A nickase. Moreover, we also evaluated some previously reported chemical and biological stimulants of HDR. Intriguingly, the small molecule-compound L755507 suppressed HDR in our reporter system when using Cas9 nickase, which stands in contrast to previous reports. Also, we found no significant effect for SCR-7 compound and for Ad4 proteins. Currently, we are adapting the system for use in human iPSC and other cell types and for additional investigation of NHEJ events. Together, our point-mutation-based HDR reporter-system allows for fast and accurate evaluation of HDR efficiencies.

Abstract No. P032

IPS-derived microglia in vitro model for the analysis of AD

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Alzheimer´s disease (AD) is the most common form of dementia. Environmental and genetic factors contribute to the risk for AD, but the underlying disease mechanisms are poorly understood. Glia cells are affected in AD patients contributing to the tau pathology and the accumulation of neurotoxic amyloid as well. Recently, genome-wide association studies allowed the identification of DNA variations associated with an elevated risk for AD. Some of the identified susceptibility genes including CD33, ABCA7, and TREM2 highly recommend that the alterations of the immune system play a major role in AD.

AD patient-specific blood carrying risk variants in AD susceptibility genes were applied for the generation of induced pluripotent stem (iPS) cells. SNP variants in those genes were genotyped based on their potential function according genomic localization and tested for association to AD. Alkaline phosphatase staining, the expression of pluripotency markers (OCT4, SOX2, NANOG, KLF4, MYC, and LIN28), and the differentiation into the three germ layers verified pluripotency of iPS cells. AD iPS cells were differentiated into astrocytes and microglia and the differentiation was characterized by the expression of glia cell markers. We established a 4-step protocol for the generation of AD-specific microglia enabling the focused analysis of AD-associated risk variants, which are a powerful tool for the analysis of molecular and cellular disease mechanisms.

The protein expression of pluripotency markers was successfully induced shown by IF and WB analysis. Cells were also screened for the most efficient induction of the three germ layers and the induction of neural cell fates including glia cell fates. We aim at the molecular characterization of SNPs and functionally mutations in CD33 and TREM2 which are surface markers that are necessary for the clearance function of microglia. Functional variants most probably influence the interplay of CD33 and TREM2 for the regulation of phagocytosis.

Abstract No. P033

A novel in vitro cardiomyocyte model using human induced pluripotent stem cells for the investigation of cardiac metabolism in diabetes mellitus

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The availability of human-induced pluripotent stem cells (h-iPSCs) to generate and isolate human cardiac cells gives the unique opportunity to elucidate the complex mechanisms in the development of diabetic cardiomyopathy. In order to mimic the phenotype of diabetic cardiomyopathy in vitro, we exposed cardiomyocytes to diabetic medium containing insulin, fatty acids, glucose, endothelin 1 and cortisol for two days.

The h-iPSC-derived cardiomyocytes exposed to diabetic medium significantly increased accumulation of lipid droplets and cellular area of cardiomyocytes. Further, these cells showed a loss of the regular striped pattern of α-actinin staining indicating that diabetic medium may cause a hypertrophic phenotype. These changes in cellular structure were accompanied by decreases in beaten frequency of cardiomyocytes. In addition, we identified a change in gene expression related to cellular metabolism (i.e. glycolytic pathways versus fatty acid metabolism) following 2 days of exposure of cardiomyocytes to diabetic medium.

Gene expression related to glucose metabolism such as the enolase (ENO1) and solute carrier family 4 (GLUT4) were significantly down-regulated. In contrast, genes involved in regulation of FA metabolism such as long-chain fatty acid transporter CD36 increased under diabetic conditions. h-iPSC-derived cardiomyocytes exposed to diabetic medium showed reduction of sarcoplasmic reticulum calcium contents, such as SERCA2A. To further investigate the effect of diabetic medium on gene expression of ventricular cardiac muscle structure and function, myosin light chain (MYL3), sodium voltage-gated channel alpha subunit 5 (SCN5A) and sodium voltage-gated channel beta subunit 2 (SCN2B) were measured and a clear reduction in gene expression was found.

Interestingly, gene expression as well as phosphorylated-protein levels of Akt was down-regulated when h-iPSCs-derived cardiomyocytes were cultured under diabetic conditions as described above. Additionally, protein expression of the ceramide synthases (CerS), known regulators of cardiac lipotoxicity, showed a significant increase in h-iPSC-derived cardiomyocytes under diabetic conditions.

Abstract No. P034

Innate immune signaling pathways in iPSC-derived hepatocytes

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Stem cell-derived hepatocytes are a powerful tool for generating patient-specific models to study host-pathogen interactions. A key requirement for such studies is demonstration of functional microbe detection molecules and innate immune signaling pathways in the derived hepatocytes.

Here we investigated TNF-alpha and TLR7 signaling in iPSC-derived hepatocytes.

On d21 of the differentiation protocol stem cell-derived hepatocytes were characterised by CYP-assays and immunostaining for hepatocyte markers.

TNF-alpha stimulation of iPSC-derived hepatocytes induced NFkB-signaling and expression of inflammatory genes such as IL8 and CXCL10. While TNF-alpha stimulation of Huh7 cells induced expression of the same set of genes, protein-levels differed considerably. The TLR7 agonist R848 alone could not induce chemokine expression in stem cell-derived hepatocytes or Huh7 cells. While upregulation of TLR7 expression by TNF-alpha could be potently achieved in Huh7 cells, stem cell-derived hepatocytes responded to TNF-alpha with an only weak upregulation of TLR7. Likewise, incubation of stem cell-derived hepatocytes with TNF-alpha prior to TLR7 stimulation was not sufficient to render the cells responsive to the TLR7-agonist.

Presence of pattern recognition receptors and innate immune pathways in stem cell-derived hepatocytes is a prerequisite to study host-pathogen interactions and mechanisms that underlie differences in susceptibility to infection across complex populations of individuals. While IFN-expression via the RIG-I pathway and ISG-expression via JAK/STAT can be potently induced in stem cell-derived hepatocytes, TLR-expression and signaling is impaired, requiring improvements in differentiation protocols to obtain fully mature hepatocytes with inducible TLR-responses.

Abstract No. P035

Neural stem cell maintenance by the primary cilium in iPSC-derived human brain organoids

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A cohort of cell cycle control mechanisms within the complex neural epithelial tissue controls the fine regulation of a stem cell's ability to divide asymmetrically to generate both self-renewing and differentiating daughter cells. Disruption of these mechanisms can elicit neurodevelopmental disorders. The primary cilium is a microtubule-based organelle that is dynamically regulated, with assembly occurring during cell cycle exit, and disassembly coinciding with cell cycle re-entry. We recently identified an unexpected role for the cilium functioning as a molecular switch whose timely disassembly via a CPAP-mediated Cilium-Disassembly-Complex (CDC) is required to maintain self-renewing neural progenitor pool in induced pluripotent stem cells (iPSC)-derived human brain organoids. In my talk, I will show you how a timely cilia disassembly critically regulates neural stem cell homeostasis in developing human brain organoids.

Abstract No. P036

Generation of disease-specific blood-brain barrier cells using patient-derived induced pluripotent stem cells for the analysis of Alzheimer's disease

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The most common form of dementia is Alzheimer's disease (AD), which shows an increasing incidence with age. One hallmark of AD is the accumulation of amyloid-β (Aβ) peptides due to a dysregulated blood-brain barrier (BBB). Genome-wide association studies carried out by our and other research teams identified several risk variants for the sporadic, late-onset AD (LOAD) in ABCA7, which is an ABC transporter responsible for Aβ efflux at the BBB. We generated patient-specific induced pluripotent stem (iPS) cells from AD patients and controls and differentiated them into BBB cells to study disease mechanisms related to ABCA7 and AD.

First, episomal vectors were used for the generation of iPS cells from B-lymphoblastoid cell lines (B-LCLs), which were obtained from AD patients and healthy donors. Pluripotency was verified by alkaline phosphatase activity, transcript, protein, and immunofluorescence analysis, and the induction of three germ layers. Immunofluorescence staining showed nuclear localization of pluripotency-related transcription factors like SOX2 and NANOG. Transcript and protein analysis confirmed permanent induction of pluripotency marker genes including OCT4. Successfully established iPS cells were subsequently differentiated into BBB cells. The efficient differentiation into endothelial cells and astrocytes was characterized by flow cytometry, transcript, immunofluorescence, and protein analysis. The expression of cell specific markers like von Willebrand factor and GFAP was observed.

Overall, we successfully generated AD iPS cells and established the differentiation of the obtained AD iPS cells into BBB-related endothelial cells and astrocytes. The generated iPS cells, endothelial cells, and astrocytes mimic a patient- and disease-specific background and are suitable to study AD-associated and patient-specific genetic risk variants and the resulting pathogenic phenotypes *in vitro*. BBB models provide a powerful tool for the understanding of AD disease mechanisms and they will help deepen our knowledge about the AD-related dysregulation of the BBB.

Abstract No. P037

A 3D lt-NES cell model of human Alzheimer's disease recapitulates amyloid deposition and phospho-tau accumulation

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder. Most cases occur sporadically, but specific mutations account for early-onset disease variants (familial AD; fAD). AD patients typically develop brain deposits of amyloid β (Aβ) and neurofibrillary tangles composed of accumulated hyperphosphorylated tau (p-tau). Here, we set out to model AD in a 3D hydrogel matrix to protect early Aβ aggregates during cell culture maintenance and to enable progression of amyloid pathology. Since amyloid deposition critically depends on high Aβ concentrations, we conditionally overexpressed amyloid precursor protein (APPSwe/Lon) and Presenilin-1 (PS-1ΔE9) mutations via targeted insertion into the AAVS1 safe-harbor locus of human induced pluripotent stem cells (iPSCs). Homozygous mutant iPSCs were differentiated into long-term self-renewing neuroepithelial stem cells (lt-NES cells; Koch et al., PNAS 2009), which were subsequently embedded in matrigel for long-term differentiation as described (Choi et al., Nature 2014). Compared to doxycycline-induced conventional 2D cultures, which efficiently released Aβ into the cell-culture medium, supernatants of induced 3D cultures contained only small amounts of Aβ. However, 2 months after differentiation, the 3D cultures exhibited prominent ThT-stained plaque-like deposits with filamentous structure and characteristic DAPI autofluorescence. We could distinguish Aβ-positive compact and diffuse deposits as well as large numbers of Aβ-positive punctae. After 16 weeks of differentiation, induced 3D cultures had accumulated TBS-insoluble Aβ and exhibited robustly increased p-tau in the majority of the cells. No overt differences in cell viability were detected in induced vs. non-induced cultures. These data indicate that 3D matrix cultures of lt-NES-derived neurons may serve as a suitable model for studying intra- and extracellular AD pathology in an authentic human neuronal system. Due to the standardized and controllable transgene expression and its ease of handling, this system may prove particularly useful in the context of industrial applications such as automated drug screening.

Koch, P. et al. A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. Proc Natl Acad Sci U S A 106, 3225–30 (2009).

Choi, S. H. et al. A three-dimensional human neural cell culture model of Alzheimer's disease. Nature 515, 274–278 (2014).

Abstract No. P038

Does the contractile function of the sarcomeres differ between human ESC-derived cardiomyocytes and adult human ventricular myofibrils?

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Characterization of the contractile function of human stem cell-derived cardiomyocytes helps in using them as cellular disease models, to advance stem-cell based therapies addressed to cardiovascular diseases or to assess novel pharmacological or genomic interventions targeting heart diseases.

Question: The aim of the present study was to understand whether β-myosin heavy chain isoform-expressing myofibrils within human embryonic stem cell-derived cardiomyocytes (hESC-CMs) do recapitulate the contractile function of the adult human ventricular myofibrils (hvMFs) isolated from donor hearts.

Methods: We have identified principal sarcomeric protein isoforms involved in the modulation of the force development and analyzed the steady-state and kinetic parameters of the forces generated by the myofibrils within of single demembranated hESC-CMs and by small hvMFs bundles using the same micromechanical method.

Results: Our results indicated that at saturating Ca(2+) concentration, both hvMFs and myofibrils within hESC-CMs developed force with similar kinetics, but maximum isometric force was smaller for myofibrils of hESC-CMs than for hvMFs. At submaximal Ca(2+) activation force levels, where intact cardiomyocytes normally operate, contractile kinetic parameters of demembranated hESC-CMs and hvMFs exhibited differences, which can be attributed to a sarcomeric protein isoforms pattern of hESC-CMs specific to an early developing stage of human ventricular cardiomyocytes.

Conclusions: Myofibrils within hESC-CMs only partially recapitulate the contractile features of adult hvMFs. We highlight the importance of correlating sarcomeric protein isoforms content with the contractile function of myofibrils within human stem cell-derived cardiomyocytes differentiated in vitro, because their morphological maturation may not necessarily determine and parallel the adult ventricular-like contractile function at myofibrillar level. Human stem cell-derived cardiomyocytes at an early developing stage could provide the basis for extended studies with patient-specific cardiomyocytes carrying for instance, familiar hypertrophic cardiomyopathy-related missense mutations in sarcomeric proteins, addressing questions related to the onset of the disease during early developmental stages.

Abstract No. P039

Stem cell-derived neural in vitro models for the patient-specific analysis of psychiatric genetics in schizophrenia

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Aim:
Schizophrenia is a neurodevelopmental disease caused by environmental and genetic factors. Characteristics of schizophrenia include a deregulation of the glutamatergic and the GABAergic neurotransmission. Recently, 108 loci referring to single nucleotide polymorphisms (SNPs) and 8 loci containing copy number variations (CNVs) have been associated with schizophrenia. Together with other research teams, we identified CNVs in NRXN1 suggesting the detailed analysis of NRXN1-related disease mechanisms. We established patient-specific induced pluripotent stem (iPS) cells from schizophrenia patients for mimicking cellular and molecular phenotypes related to schizophrenia.

Methods:
We generated iPS cells from B-lymphoblastoid cell lines (B-LCLs) obtained from patients carrying CNVs in NRXN1. Alkaline phosphatase staining, pluripotency marker expression, and the induction of three germ layers was used to characterize generated iPS cells. A neuronal screening protocol was applied to select appropriate iPS cell clones. Established lines were differentiated into neural stem cells (NSCs) and mature neurons. Morphology, transcript analysis, western blot analysis, immunofluorescence analysis, ELISA, electrophysiology, and mitochondrial respiration were used to characterize terminal differentiated schizophrenia-specific neurons.

Results and Conclusion:
Alkaline phosphatase staining, immunofluorescence analysis, transcript analysis, western blot analysis, and the induction of three germ layers verified the pluripotency of stem cells including OCT4, NANOG, and SOX2. SOX17, PRRX1, and PAX6 demonstrated the spontaneous differentiation into derivatives of the three germ layers. Screening of iPS clones revealed differences for the induction of NSC markers such as ASCL1. Neurodevelopmental genes including NESTIN, GFAP, and TUBB3 were used to monitor neural differentiation. Mitochondrial respiration changed during differentiation. Mature neurons expressed GABA and glutamate receptors and were excitable for GABA and glutamate. Schizophrenia-specific neurons showed altered regulation of cellular neurexin interaction partners and altered release of neurotransmitters.

Together, we established a functional in vitro model for studying psychiatric genetics in schizophrenia enabling the identification of potential therapeutic targets.

Abstract No. P040

iPSC-based disease modeling of inborn errors of neurotransmitter biosynthesis

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Inborn errors of neurotransmitter biosynthesis present early after birth or in early infancy with a broad spectrum of predominantly neurological symptoms. Treatment is challenging and aims at reversing the disturbances of monoamine synthesis and/or metabolism. Here, we focus on tyrosine hydroxylase (TH) and GTP cyclohydrolase 1 (GCH1) deficiency. TH is the rate-limiting enzyme for the synthesis of dopamine and catalyses the hydroxylation of L-tyrosine to L-dopa. GCHI is the first and rate-limiting enzyme of tetrahydrobiopterin (BH_4) biosynthesis. BH_4 is the essential cofactor of TH for the generation of L-dopa. Therefore, inactivity of TH or GCH1 results in deficiency of dopamine and consecutively to deficiencies of epinephrine and norepinephrine.

As induced pluripotent stem cells (iPSCs) can be used as model to study the pathophysiology of inborn errors of neurotransmitter biosynthesis we have generated iPSCs from fibroblasts of patients with TH, GCH1 deficiency and healthy controls, which were differentiated into neural progenitor cells thereafter. To identify patterns specific for the respective disease, transcriptome sequencing was performed and a broad spectrum of metabolite data (amino acids, organic acids, biogenic amines, pterins) in the patient´s and control fibroblasts, iPSCs and NPCs were sampled. Integrative supervised and unsupervised bioinformatics analysis of transcriptome sequencing and metabolic data is in progress. Furthermore, reference values for a panel of metabolites in the cytoplasm were collected using multiple healthy control cells.

Abstract No. P041

Angiogenic properties of human trophoblast progenitor cells

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Placenta is originated from polar trophoectoderm and at term it includes progenitor cells with ability to transform into all trophoblast subtypes. Placental vascular network development is a tightly controlled vasculogenic and angiogenic process. During the course of angiogenesis, angiogenic factors produced by placental cells (trophoblasts, Hofbauer cells, pericytes, and endothelial cells) play an important role. The aim of this study is to observe the angiogenic properties of human trophoblast progenitor cells (hTPCs) isolated from term placenta.

hTPCs were isolated from human term placenta, the characterization of cells were provided by flow cytometry with CDX2 and EOMES antibodies. VEGF, VEGFR1 and VEGFR2 proteins and mRNAs in hTPCs were identified by western blot, and quantitative real-time PCR.

hTPCs were positive for trophoblast stem cell markers CDX2 and EOMES in 92.5% and 92.7%, respectively. VEGF, VEGFR1 and VEGFR2 protein expression and mRNA levels were detected to be higher with respect to control HUVEC cells.

In this study, we demonstrate the trophoblast progenitor cells express and release angiogenic factors for the first time. With this information, trophoblast progenitor cells seem to promising for recruitment therapies with enhancing angiogenesis.

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) the project number 114S547.

Abstract No. P042

Studies on neurodegeneration due to oxidative stress in triple A syndrome

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The triple A syndrome is a rare autosomal recessive disorder characterized by the three main symptoms adrenal insufficiency, alacrima and achalasia. In 60% of all cases the disorder is associated with progressive dysfunction of the central, peripheral and autonomic nervous systems. Classical triple A syndrome is caused by mutations in *AAAS* gene encoding the nucleoporin ALADIN. Previous work has shown that there is an involvement of oxidative stress in the pathogenesis of the disease. However, the exact cellular mechanisms leading from ALADIN mutations to degeneration of neurons are not well understood. To address this question we generated iPSCs from patient and wild-type (WT) fibroblasts, differentiated these cells into neuronal precursor cells (NPCs) and performed different tests with and without an oxidative stress (paraquat) treatment. Viability of patient NPCs was lower compared to WT cells; however, due to a large variance these changes were not significant. Although the H_2O_2 content after paraquat treatment increased equally in patient and WT cells, the amount of reduced form of glutathione (GSH) was lower in untreated patient cells than in WT cells. The ratio of reduced to oxidized glutathione (GSH/GSSG) differed considerably between individual cell clones. However, individual patient clones could be identified which, in contrast to WT cells, showed a massive reduction of the GSH/GSSG ratio in case of oxidative stress. In summary, we demonstrated that NPCs derived from patient-specific iPSCs already have an increased basal oxidative stress level and that their anti-oxidative potential is reduced. These results are in agreement with our published findings in the adrenal cell model of triple A syndrome. Further studies on mature motor neurons will prove our hypothesis that oxidative stress in patient cells leads to stress induced premature senescence (SIPS) and ultimately to neurodegeneration in triple A syndrome.

Abstract No. P043

Functional hiPSC-hepatocytes generated for disease modeling and drug development using a universal differentiation protocol

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Hepatocytes derived from human pluripotent stem cells (hPSC) have the potential to serve as a predictive human in vitro model systems, provided that they possess relevant hepatocyte functions. The liver performs over 500 functions and many in vitro models lack the ability to recapitulate these functions. In addition, existing differentiation protocols have not been robust enough for use with multiple hPSC lines, further hampering the use of hPSC-derived hepatocytes.

Therefore, we have developed a robust differentiation protocol which recapitulates in vivo liver development and allows derivation of hepatocytes from multiple hPSC lines. Of 25 different lines tested, all were efficiently differentiated into highly homogenous hepatocyte cultures that exhibit important adult hepatocyte features, such as substantial CYP activities, low expression of fetal genes, and high expression of adult genes. More importantly, hepatocytes derived from multiple hPSC lines show diverse CYP activity profiles, thus reflecting the inter-individual variation present in the population. To allow generation of panels of cryopreserved hepatocytes from multiple lines, we have also developed a cryopreservation method for hPSC-derived hepatocytes. Like their fresh counterparts, the cryopreserved hPSC-derived hepatocytes have substantial CYP activities in the same range as in human primary hepatocytes. Importantly, a novel maintenance medium significantly improves hepatocyte functions, such as albumin and urea secretion, gluconeogenesis, glycogen storage, LDL-uptake, and lipid storage. These functions are maintained for up to two weeks, much longer than in primary hepatocytes which rapidly lose their functionality within days in conventional 2D cultures. We also show the utility of the cryopreserved hPSC-derived hepatocytes for chronic toxicity studies.

Taken together, our robust differentiation protocol together with the improved maintenance medium allow the reliable generation of mature hepatocytes from multiple hPSC lines. This can provide an inexhaustible source of human hepatocytes for use in in vitro disease modeling, drug discovery, metabolism, and chronic toxicity studies.

Abstract No. P044

A novel mechanism for the pathogenesis of branchio-oculo facial syndrome (BOFS) and other human neurocristopathies

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BOFS is a rare autosomal dominant congenital disease, characterised by craniofacial abnormalities reflecting defects in neural crest cell (NCC) development (neurocristopathies). All reported patients carry heterozygous mutations or deletions within the *TFAP2A* gene, a master regulator of NCCs and craniofacial development. However, here we present a unique BOFS patient harbouring a de novo heterozygous 80Mb inversion of the *TFAP2A* allele. Combining public Topologically Associating Domain (TAD) maps with our epigenomic data we found that *TFAP2A* is part of a TAD containing many NCC-active enhancers.

As one of the inversion breakpoints is located between *TFAP2A* and its putative NCC enhancers, we hypothesize that the inversion alters the enhancer-gene interactions, resulting in abnormal expression of this gene during embryogenesis and ultimately in BOFS. This hypothesis is further supported by Circular Chromosome Conformation Capture sequencing (4C-seq) data of NCCs derived from wild type (WT) human induced pluripotent stem cells (hiPSC), in which we show strong physical interaction between the *TFAP2A* promoter and NCC-active enhancers. Moreover, the in vivo relevance of several of those enhancers was confirmed by reporter assays in chicken, demonstrating the enhancer activity in migrating NCCs.

Next, we derived patient-specific hiPSC and differentiated them into NCCs. Allele-specific PCR revealed monoallelic *TFAP2A* expression in patient while biallelic in WT. Furthermore, immunofluorescence showed reduced TFAP2A expression in the patient, hence we will test by allele-specific 4C-seq if the inverted allele is silenced. To further study the regulatory landscape of TFAP2A we have generated heterozygous and homozygous deletions of *TFAP2A* and its NCC-active enhancers in WT hiPSC using CRISPRs/Cas9. In vitro differentiation studies of NCCs, NCC-derived mesenchyme and cartilage, combined with genomic approaches (4C-seq, RNA-seq, TFAP2A ChIP-seq) should reveal the major regulatory networks controlled by *TFAP2A* and provide mechanistic insights into how defects in *TFAP2A* regulatory landscape contribute to BOFS and other neurocristopathies.

Abstract No. P045

Model embryonic development with hiPSC

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Human induced pluripotent stem cells (hiPSC) hold great promise in producing cells, tissues and organs for basic and applied research as well as regenerative medicine. Notably, the derived cells recapitulate mostly embryonic cell types rather than adult tissues. We exploit this characteristic feature of hiPSC to model the development of early embryonic cells and tissues.

In vitro models of early embryonic cells and tissues are needed in toxicology to assess the safety of chemicals to developing embryos and foetuses. These can be exposed during pregnancy to chemicals and drugs, and are particularly vulnerable to agents that disturb development within a narrow programming window during foetal life, with potentially harmful effects on the developing human being. However, there are currently only limited options to test for toxicity to developing embryos and foetuses, and most of them rely on animal experiments.

Therefore, we are developing an assay that models early embryonic development in order to test for developmental toxicity. We use hiPSC differentiation into early developing cell types in a three-dimensional environment, in order to mimic the in vivo situation as closely as possible. We also aim to explore how the extracellular matrix and cell-cell contacts contribute to the differentiation. Our approach will add to the increasing understanding of how relevant in vitro models are to mimic human organs and tissues. Lastly, the developed assay will have great value in testing drugs and chemicals for human safety and to replace animal experiments.

Abstract No. P046

Sodium channels in Huntington's disease

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Huntington's Disease (HD) is a fatal neurodegenerative genetic disorder characterized by motor impairments, cognitive decline and psychiatric symptoms. Mutation of huntingtin gene with a CAA-CAG glutamine triplet expansion higher than 36 repeats leads to a pathological phenotype. Within the brain, the striatal Medium Spiny Neurons (MSNs) progressively degenerates, possibly due to increased intracellular levels of calcium.

It was already shown that the sodium channel beta-4 subunit, encoded by the SCN4B gene and involved in both voltage-gated sodium channel activity and dendritic growth modulation, is down-regulated in both human post-mortem tissue and in a mouse model of HD. We confirm the beta-4 down regulation by RT-qPCR in the BACHD mouse model. This finding raises the hypothesis that beta-4 plays an important role in the pathophysiology of HD and eventually in MSN degeneration. The beta-4 subunit is involved in the generation of so-called resurgent currents, a sodium channel gating mode which supports neuronal excitability. Since elevated intracellular calcium levels are typical for HD, we tested the effect of calcium on resurgent currents in heterologous expression systems of different sodium channel subtypes.

To investigate HD-related changes in a more physiological system, we differentiated induced pluripotent stem cells (iPSCs) into MSN-like cells to further study the impact of this subunit on sodium channel activity in MSNs. iPSC-derived MSNs show action potential firing and voltage-gated sodium and potassium currents. We performed immunostaining with the neuronal marker NeuN and DARPP32 to test for MSN identity. On the meeting, a thorough characterization of the MSNs, their sodium channel's gating and marker expression will be presented.

Abstract No. P047

The involvement of microRNAs in equine cord blood-derived multipotent mesenchymal stromal cell's mononuclear cell suppressive potency

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BACKGROUND: Inflammation-associated disorders are significant causes of morbidity in horses. Equine mesenchymal stromal cells (MSC) holds promise as cell-therapy candidates due to their secretory non-progenitor functions. MSC derived from equine umbilical cord blood (eUCB) are of clinical interest due to ease of procurement, multipotency, and immunomodulatory ability [1]. Using lymphocyte suppression assay (LSA), we have shown that eUCB-MSCs are mononuclear cell (MNC) suppressive *in vitro*. MicroRNAs (miRNAs) play a key role in regulating cellular function [2]. This study focuses on miRNA's involvement in the immunomodulatory functions of eUCB-MSC.

HYPOTHESIS: We hypothesize that miRNA expressions within eUCB-MSCs are associated with their MNC suppressive potency.

APPROACH: Individual eUCB-MSC cultures are categorized as having high or low MNC suppressive potencies based on lymphocyte suppression assays using ConA to stimulate responder lymphocytes in order to determine the suppressive effect of MSCs. Once MSC cultures are categorized as high or low, candidate miRNAs are examined using qRT-PCR and novel transcripts are investigated using next generation sequencing.

CONCLUSION: This work aim to identify miRNAs with predictive value of MSCs' mononuclear suppressive properties. This knowledge could be used in production monitoring of cellular potency and as release criteria prior to clinical use.

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Abstract No. P048

Calcium-based high-throughput compound screenings on patient neuronal cells carrying mtDNA mutations

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Mutations in mitochondrial DNA (mtDNA) are strongly linked to diseases affecting the nervous system and for which no effective treatment exists. Among these diseases, the most sever is maternally inherited Leigh syndrome (MILS), a pediatric neurodegenerative disorder that is caused by mutations in mtDNA genes, such as MT-ATP6 in complex V of the electron transport chain (ETC). The lack of therapy for MILS stems from the paucity of model systems. No animal models exist due to the difficulty of engineering mtDNA, and the existing cellular models lack the metabolic features of neural cells and do not provide the patient-specific match between mitochondrial and nuclear genomes.

We recently showed that neural progenitor cells (NPCs) differentiated from iPSCs are an effective modeling tool for neurological disease associated with mtDNA mutations, as they rely on mitochondrial respiration and maintain the original parental mtDNA profile (Lorenz et al, Cell Stem Cell, 2017). In particular, by using NPCs derived from MILS patients carrying a mutation in the mitochondrial gene MT-ATP6 (m.9185T>C) we unveiled mutation-associated neural phenotypes, including mitochondrial calcium handling defects.

We have now generated additional NPC lines from other MILS patients carrying other MT-ATP6 mutations (m.8993T>C and m.8993T>G). We confirmed the presence of the same mitochondrial calcium phenotypes in all MILS NPCs. In silico modeling confirmed that Complex V mutations can cause mitochondrial calcium defects. Finally, we confirmed our results using the high-throughput (HT) cellular screening system FLIPR (Fluorescence Imaging Plate Reader). With this system we are now ready to perform a large-scale HT screening using libraries of FDA-approved compounds, natural compounds, and drug-like compounds.

With this approach, we have the chance to identify for the first time potential drugs effective against the neural impairment of MILS, an incurable and severe disease that suffers from a lack of effective model systems.

Human iPSC-Derived Neural Progenitors Are an Effective Drug Discovery Model for Neurological mtDNA Disorders. Lorenz C, ..., Prigione A. Cell Stem Cell. 2017 May 4;20(5):659-674.e9.

Concise Review: Induced Pluripotent Stem Cell-Based Drug Discovery for Mitochondrial Disease. Inak G, Lorenz C, Lisowski P, Zink A, Mlody B, Prigione A. Stem Cells. 2017 Jul;35(7):1655-1662.

Abstract No. P049

Important aspects of the Foxg1 knockout phenotype can be recapitulated with neuronal differentiation of murine pluripotent stem cells

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Foxg1 is a transcription factor critical for the development of the mammalian telencephalon. It controls proliferation of the dorsal telencephalon progenitor cells and specification of the ventral telencephalon. A homozygous knockout of Foxg1 in mice leads to severe microcephaly, attributed to nearly complete loss of telencephalic structures, such as the cerebral cortex, due to premature differentiation of cortical progenitors. Other organ systems are developed normally, but animals do not initiate breathing and die shortly after birth.

Here, we analyze whether the Foxg1 knockout phenotype can be recapitulated by an in vitro model of neuronal development. We used a serum free and morphogen reduced embryoid body like culture to study the effects of the Foxg1 knockout in different mPSCs. This protocol was described to recapitulate important steps of telencephalon development in vitro.

We validated our protocol with a Foxg1 reporter line and were able to derive up to 90 % Foxg1 positive cells in the established culture system. Differentiation efficiencies varied in between different mPSC lines, with miPSCs showing slower differentiation than mESCs. Several mPSC lines with homozygous mutations in the Foxg1 coding exon were produced using the CRISPR/Cas9 system leading to a truncated protein with loss of functional domains (Foxg1 knockout). Quantitative reverse transcription (q) PCR revealed that different Foxg1 knockout mPSCs commonly express significantly lower levels of Foxg1, Emx1, and VGlut1 compared to wildtype controls, indicating reduced differentiation towards dorsal telencephalic progenitors.

These results show that the loss of dorsal telencephalic progenitors can be recapitulated with a rather simple and rapid differentiation protocol.

Abstract No. P050

Bohring Opitz syndrome-causing mutations in additional sex combs-like 1 impair human neural crest differentiation

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The severe developmental disorder Bohring Opitz syndrome (BOS) has recently been linked to heterozygous mutations in additional sex combs-like 1 (ASXL1). This epigenetic recruitment factor is involved in different mechanisms of Polycomb-mediated gene repression, however, its role in regulation of developmental transcription programs is thus far unknown.

We found that ASXL1 mutations in BOS-patient derived induced pluripotent stem cells (BOS-iPSC) led to expression of dominantly acting, truncated ASXL1 proteins. Introduction of similar ASXL1 mutations in human embryonic stem cells strongly impaired differentiation to migrating neural crest cells in vitro and also in vivo in developing chicken embryos. We could link this phenotype, which explains craniofacial and further symptoms found in BOS-patients, to downregulation of a cohort of neural crest specifiers, most importantly the master regulator ZIC1. Several repressed genes showed increased levels of repressive H3K27me3 modifications, indicating that a dominantly acting, truncated ASXL1 that modulates Polycomb regulation of specific loci is underlying disturbed neural crest development and associated symptoms in BOS.

Abstract No. P051

Rethinking the role of sodium channels in neuronal excitability and pain: new insights from iPSC-derived nociceptors of erythromelalgia patients

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In recent years, the chronic inherited pain syndrome erythromelalgia (IEM) was linked to mutations in the voltage-gated sodium channel (Nav) subtype Nav1.7. We established a patient-specific, stem cell-derived human model system to investigate the effect of the IEM mutation Nav1.7/I848T: differentiation of patient-derived iPSCs into human nociceptors. To this end, we reprogrammed fibroblasts of a skin biopsy of two patients (mother and daughter) carrying the I848T mutation. Applying a small molecule differentiation protocol allowed for the generation of peripheral sensory neurons, which well characterize as nociceptor-like cells. Using whole-cell patch-clamp electrophysiology, we show that nociceptors from IEM patients display a decreased action potential threshold as well as higher action potential amplitude and stronger afterhyperpolarization. These findings could well explain the increased pain experienced by the patients.

In order to characterize the voltage-dependence of Nav activation, we used an adapted voltage-clamp pre-pulse protocol to avoid specific technical challenges that arise from the fast gating of Navs and the long neurites of the cells. We were able to faithfully measure even small changes in voltage-dependence of activation of TTX-sensitive Navs. iPSC-derived human nociceptors of the IEM patients displayed a significantly hyperpolarized Nav activation compared to wildtype controls, thus confirming that the IEM mutation induces this shift in a human, patient-specific system. Hence, we are able to faithfully mimic and pharmacologically influence the mutation-induced biophysical changes of Nav1.7 in human stem cell-derived nociceptors, thereby providing a promising platform for innovative, potentially patient-specific drug design. Furthermore, specifically blocking Nav1.7 allowed us to measure the voltage-dependence of other TTX-sensitive Nav isoforms. The results of these experiments change our current understanding of the role of the different Navs in the generation of action potentials and suggest that in human nociceptors Nav1.7 plays a particular role, which we may need to redefine.

Abstract No. P052

Generation of disease-specific iPSCs and development of transgenic reporter cell lines for pulmonary hypertension disease modeling and drug screening

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Background: Pulmonary hypertension (PH) is a progressive disease characterized by abnormal remodeling of small vessels in the lung caused by dysfunctional migration and proliferation of vascular smooth muscle (vSMCs) and endothelial cells (ECs). Studies have linked mutations in the bone morphogenetic protein receptor type 2 (BMPR2) to heritable PH. Despite molecular insights mainly from studies with primary cells or immortalized cell lines knowledge about the underlying mechanisms of PH is limited. Induced pluripotent stem cells (iPSCs) can proliferate and differentiate into cell types of all three germ layers in vitro. Therefore, iPSCs offer an attractive cell source for the generation of functional human ECs from PH patients and healthy individuals. This project aims at generating ECs from PH patient-specific iPSCs harbouring BMPR2 mutations as an in vitro disease model to elucidate the role of EC malfunction in PH and for high throughput drug screening.

Methods: PH patient-specific iPSCs were generated from CD34pos cells using sendai virus reprogramming vectors. By timed addition of growth factors (BMP4/VEGFA) and activation of the WNT pathway, BMPR2mutated iPSCs could be differentiated towards ECs. Applying a reporter assay for Smad4-dependent transcription with Luciferase readout allows for studying impaired BMP-target gene transcription in ECs derived from BMPR2mutated compared to BMPR2wildtype iPSCs.

Results: PH-patient specific iPSCs showed expression of pluripotency associated markers on both mRNA and protein levels. Generated BMPR2mutated ECs showed expression of typical EC markers (VEcadherin, CD31), uptake of DiI-LDL as well as network formation in matrigel assays. Also, preliminary studies show a dose-dependent response in luciferase activity in cells stimulated with BMP4 after transient transfection with vector plasmid constructs encoding BMP-response elements (BRE) and Smad4-binding elements (SBE4), and luciferase.

Conclusions: Utilizing BMPR2mutated ECs derived from PH patient-specific iPSCs might represent a valuable tool for disease modeling and drug discovery for treatment of heritable PH.

Abstract No. P053

Differentiation of iPSC-MRAS KO cell line into vascular calcifiying cells - work in progress

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Genome-wide association studies (GWAS) on five international cohorts identified genetic variants within the MRAS gene to be associated with coronary artery diseases (CAD). One of the identified significant single nucleotide polymorphisms (SNPs) is located on 3q22.3 near the MRAS gene (PMID: 22144573). MRAS was also found to be highly associated with coronary artery calcification (CAC) and myocardial infarction (MI). MRAS encodes the Ras-related protein M-Ras which is a GTP binding protein and belongs to the RAS superfamily. It functions as a signal transducer for novel upstream stimuli in controlling cell proliferation (PMID:16630891).

Here we generated an iPSCs-MRAS knockout cell line using CRISPR/Cas. We aimed to use a well-established protocol in our research group to differentiate these cells into vascular smooth muscle cells (VSMCs). Subsequently, calcification will be induced in these iPSC-derived VSMCs using calcifying media and the effect of MRAS deficiency on calcification will be studied and compared to wildtype cells.

During the differentiation step from iPSCs into VSMCs, we acknowledge that the previously established protocol cannot straightforwardly be adapted to the MRAS KO cell line. The protocol needs to be newly optimized for every cell line coming from different donors. Hence, the current protocol is going to be optimized to work for the MRAS KO cell line as well. Interestingly, the protocol works well for the wildtype cell lines. We will take advantage of these different wildtype cell lines to determine the basal deviation and difference on calcification between the different wildtype cells lines in order to estimate the real biological relevant difference expected from the KO. Immunofluorescence staining on VSMC markers like CNN1 or SM22α will be performed. In addition, analyses of the calcification by Calcein and Alizarin Red staining will be carried out, as well as at molecular level using known osteogenic markers like OPN or RUNX2.

O'Donnell CJ et al., 2011, Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction.

Abstract No. P054

Investigation of the role of DGCR8 in DiGeorge syndrome using DGCR8 deficient pluripotent stem cell based disease models

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Human pluripotent stem cells give us the opportunity to study early stages of embryonic development and can be used for drug screening, toxicity testing, and disease modeling. The 22Q11 deletion (or DiGeorge - DG) syndrome is the most common micro-deletion syndrome associated with a broad range of developmental features affecting the cardiovascular, nervous and immune systems (1). These abnormalities are caused by genetic deletions affecting about 40-50 protein coding genes. Primary candidate gene in the deleted region is the DG syndrome critical region gene 8 (DGCR8), which encodes a component of the microprocessor complex essential for biogenesis of microRNAs, which represent an abundant class of key regulators controlling diverse cellular functions in eukaryotes such as differentiation, development and antiviral defense. Our aim is to investigate in vitro cellular phenotypes in complex multi-organ diseases such as DG syndrome. For this purpose we plan to establish a DGCR8 knock-out human embryonic stem cell line (hES), by the insertion of a CAG-EGFP-CAG-Puromycin resistance gene cassette into exon 3. This genetic modification will be implemented by CrispR/Cas9, which enables precise genome editing (2). We transfected hES cells with the CrispR/Cas9 and donor plasmid constructs, selected them based on puromycin resistance and EGFP expression and cloned them. Next, we plan to characterize our DGCR8-KO clones and differentiate them into neurons, cardiomyocytes and endothelial cells. This genetic modification may cause several changes in cell growth, survival, differentiation, or in phenotype and function of the derived mature cell types. Examination of these „in vitro anomalies" by immunocytochemistry, Ca-imaging, and patch-clamp technique may bring us closer to understand the molecular mechanisms underlying the complex symptoms of the DG syndrome. This in vitro model system will also be suitable for drug screening and pharmacological testing.

This study has been funded by the National Brain Research Program of Hungary (KTIA_NAP_13-1-2013-0001,KTIA_NAP_13-2014-0011).

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Abstract No. P055

Role of the transcription factor NRF2 in hippocampal neurogenesis and in a mouse model of Alzheimer´s Disease

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Neural stem/progenitor cells (NSPCs) located at the subgranular zone (SGZ) of the hippocampus, differentiate into granular neurons, which in turn integrate in circuits that participate in learning and memory functions. Considering that oxidative stress, neuroinflammation and proteinopathy alter the activity of the SGZ neurogenic niche, in this study we hypothesized that Nuclear Factor-Erythroid 2-Related Factor 2 (NRF2), a master regulator of homeostatic responses, might modulate the fate of NSPCs at the SGZ. We have produced genetically modified mice that express human mutated forms of TAU(P301L) and the amyloid protein precursor APP(V717I) in the presence or absence of NRF2. We report that parental *Nrf2*$^{-/-}$ and APP/TAU/NRF2-deficient mice have altered synaptic plasticity and cognition as they present a decrease in hippocampal long term potentiation and poor performance in the Morris water maze test. At the neuropathological level, we found an accelerated loss of NSPCs pool and neuronal differentiation during mouse ageing from 3 to 12 months. In SGZ-derived neurospheres the clonogenic and proliferative capacity of NSPCs was severely reduced in *Nrf2*$^{-/-}$ mice compared to *Nrf2*$^{+/+}$, and the neuron/astroglia ratio was reduced. This effect was reversed by transducing *Nrf2*$^{-/-}$ NSPCs with a lentiviral vector expressing NRF2. Our findings demonstrate the importance of NRF2 in the maintenance of proper proliferation and differentiation rates of hippocampal NSPCs. Pharmacological interventions aimed at up-regulating NRF2 may preserve the neurogenic functionality of the hippocampus and improve cognitive functions in AD.

Abstract No. P056

Schizophrenia genetics: neural stem and progenitor cells for the analysis of human neurogenesis

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Neurodevelopmental diseases such as schizophrenia are often associated with genetic variations. Single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) presumably affect the neural development, resulting in extensive cognitive impairments that may have a great impact on cell functions. Using the example of schizophrenia, several risk genes are associated with the disease, including some that are involved in neural development, neurotransmission and/or the immune system. Reprogramming of human somatic cells enables the generation of induced pluripotent stem cells (iPSCs) bearing the patient-specific genetic information. The neural differentiation of iPSCs might elucidate the impact of such variations and moreover enables the improvement of antipsychotic drug treatment by sub-classifying patients at a molecular level.

Therefore, patient-specific B cell-derived iPSCs were differentiated into neuroepithelial rosette-like structures containing self-renewing cells and further cultured as free-floating neurospheres to improve cell proliferation. Neurosphere single cells were maintained as stable populations of neural stem cells (NSCs), which were differentiated into functional progenitor and mature neural cells. Transcript and protein analysis as well as double immunostaining of specific markers confirmed the successful generation of NSCs (SOX2, MSI-1, NEUROG3, and NES) as well as glial and neuronal cells (GFAP, O4, TUBB3, STX, and NEUN). Different neuron subtypes such as inhibitory GABAergic and excitatory glutamatergic cortical neurons were detectable (GABBR1, GRIA2), further verified through patch-clamp recordings of mature neurons. Regional identity of these subtypes was confirmed through the expression of telencephalic and hippocampal markers (FOXG1, OTX1, PROX1, and AUTS2). Differences in regard to the expression levels of neurotransmitter receptors GABBR1 and GRIA2 were demonstrated between healthy and diseases neural cells. Together, these results confirmed the efficient neural differentiation of human iPSCs, enabling functional studies of healthy and diseased human cortical development concerning the impact of specific genetic variations.

Abstract No. P057

CRISPR/Cas9 knock-out of Nexilin in hiPSC and cardiomyocyte differentiation

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The protein Nexilin was identified to be a structural protein in the Z-disk in the sarcomere of cardiomyocytes. It is known to play an important role in sarcomere integrity as it was shown that loss of Nexilin leads to blurry Z-disks in zebrafish. Mutations in Nexilin were found to lead to dilated cardiomyopathy (DCM) in humans, zebrafish and mice. The underlying pathomechanisms are still unknown. The main aim of this study is to generate hiPSCs lacking Nexilin and functionally characterize derived cardiomyocytes to get a better understanding of the molecular mechanisms leading to DCM in humans.

In a first step of the project we aimed to establish the protocol of differentiation and characterize cardiomyocytes derived from human iPSCs. Therefore, modulation of canonical Wnt signaling is performed, leading to efficient differentiation of hiPSC to cardiomyocytes. Characterization of cells was performed before differentiation at RNA level using the markers Oct4, Klf4 and Nanog to show pluripotency of hiPSCs. After differentiation, characterization is performed using heart specific expression markers such as MYL2, Troponin T, Desmin, GATA4 and HCN4 at RNA level.

The next step was the generation of Nexilin knockout iPSCs using the CRISPR/Cas9 system. The resulted cell lines will then be differentiated into cardiomyocytes and the same afore mentioned markers will be used for detailed molecular characterization of Nexn-KO iPSC-derived cardiomyocytes and compared to controls. Additionally, phenotypic characterization, sarcomere integrity and expression levels of cardiac transcription factors such as Smad proteins and Tbx5 will be analyzed to understand the function of Nexilin better.

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Abstract No. P058

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

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The genetic disorder Cystic Fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene coding for a cAMP-activated chloride-channel. So far, immortalized cell lines overexpressing mutant CFTR-variants have been used to screen compound libraries. In fact, CFTR-modulators have been identified, but show modest effects at best. Obviously, the complexity of the mutant CFTR-maturation and turnover kinetics including the influence of genetic modifiers require the use of advanced personalized cellular models. To address these unmet needs we focus on the generation of induced pluripotent stem cell (iPSC) lines from CF-patients homozygous for F508del mutation. CF-iPSCs were generated via reprogramming of $CD34^{pos}$ cells isolated from small volumes of non-mobilized peripheral blood. The resulting CF-iPSCs were analysed regarding their karyotype, pluripotency status and potential to differentiate. TALEN-based genome engineering was applied for targeted introduction of reporter transgenes. Several embryonic stem cell (ESC) and iPSC lines including an iPSC line with homozygous F508del mutation were generated expressing a tomato-fluorescence-reporter under control of one allele of the CFTR-locus. Moreover, a halide sensitive yellow fluorescent protein (YFP) was introduced into the AAVS1-locus to monitor CFTR-function. Finally, the F508del mutation in the CF-iPSC reporter line was genetically corrected using an oligonucleotide-based 'footprintless' approach. The stable expression and the functionality of the YFP-reporter could be demonstrated. Directed differentiation of reporter iPSCs towards intestinal/biliary epithelium revealed YFP^{pos}/$tomato^{pos}$ cells, displaying CFTR-channel specific response after Forskolin application, which was inhibited after CFTR(inh)-172 treatment. Furthermore, heterozygous correction of F508del mutation resulted in recovery of CFTR function comparable to wild type CFTR. These results represent a proof of concept for the applicability of genetically engineered patient-specific iPSC lines in disease modeling with regard to the individual genetic context. Furthermore, we are preparing for first iPSC-based high-throughput screens aiming at the identification of novel correctors and potentiators of CFTR-trafficking-mutations.

Abstract No. P059

Assessment of intra-patient clonal variability in human iPS cells at an undifferentiated and differentiated level

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In the field of cardiovascular research, human induced pluripotent stem cell (hiPSC) derived cardiomyocytes show great potential for individualized, patient-specific disease modeling in combination with high-throughput drug discovery. One drawback, however, is that technical variations (i.e. in the process of reprogramming) and the status of pluripotency influence not only cardiogenic potential but also intra-patient clonal variability and therefore might cloud inter-patient functional differences.

This study was undertaken to analyze intra-patient clonal variability at both an undifferentiated and differentiated level. Therefore various iPS cell clones of donors are evaluated for pluripotency characteristics by transcriptional profiling (PluriTest), high content imaging and flow cytometry based quantification. Furthermore, iPS cell clones are differentiated into cardiomyocytes and analyzed for their variability in EHTs at a functional level. Characteristics like contractile force development, calcium sensitivity, force-frequency relationship, and response to the ß-adrenergic stimulator isoprenaline of the various clones are compared. First results of two iPS cell clones of one donor hypothesize only a small intra-patient variability at the differentiated level.

All in all, understanding better intra-patient clonal variability is an important step to enable the interpretation of human iPS cell research. The comparable characteristics at the differentiated level of two clones of one donor in the EHT system hypothesize that cardiovascular disease phenotypes and inter-patient functional differences, i.e. in drug responsiveness, might potentially be unmasked with our method.

Abstract No. P060

Exploration of Nrf2 pathway activation during neuronal differentiation of human neural stem cells

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Organisms are exposed to reactive oxygen species (ROS) from internal metabolism and environmental toxicants. Exposure to ROS is counterbalanced by antioxidant defence systems such as the Nrf2/antioxidant response element pathway (ARE), a prime regulator of endogenous antioxidant responses. ROS are also important regulators of intracellular signaling pathways involved in early neuronal development. The regulation of neuronal redox homeostasis via the Nrf2/ARE pathway in hPSC-derived neurons is not well understood and has major implications for the use of stem cell-based approaches in basic and translational research.

We performed comprehensive time-course microarray studies in hPSC-derived long-term neuroepithelial stem cells (lt-NES) differentiated for up to 12 weeks. Concurrent with neuronal maturation we observed expression changes of components of the Nrf2 pathway. Dose-response analysis revealed an increased resistance of more mature cultures to the OS inducers Rotenone and tert-butyl hydrogen peroxide as determined by AlamarBlue assay. To assess if this phenotype is associated with altered Nrf2-signaling, we performed qRT-PCR analyses of downstream targets after treatment with the Nrf2 inducer tert-butylhydroquinone (tBHQ). Interestingly, we observed that induction of the Nrf2 target genes NQO1 and HMOX1 upon 8h of tBHQ treatment decreases with progressing neuronal maturation. Since the Nrf2 pathway is known to intersect with autophagy through the direct interaction between the autophagy adaptor protein p62 and the Nrf2 repressor Keap1, we wanted to assess the state of these regulators during lt-NES differentiation. We observed an increase in p62 levels with a concomitant decrease of Keap1 along neuronal maturation. At the same time LC3-II levels showed a decrease with differentiation indicative of a reduction in the autophagic flux. Further elucidation of the maturation-dependent dynamics of the Nrf2 pathway and OS-regulation is expected to provide an important basis for interpreting and counteracting OS-mediated effects in disease modeling and cell-based regeneration.

Abstract No. P061

Human autologous Parkinson´s disease co-culture model reveals Th17 cell-induced neuronal death

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Parkinson´s disease (PD) is the most common neurodegenerative movement disorder, characterized by severe motor symptoms. Neuroinflammation developed to an important contributor in PD pathology. While the contribution of innate immune cells is well established, the role of adaptive immune cells for PD remains elusive. We hypothesize that T lymphocytes have a detrimental effect on neurons and aim to decipher their specific mechanism of action in sporadic PD.

To study the effect of T lymphocytes in PD pathology, a human autologous co-culture model was established by the co-culture of human induced pluripotent stem cell (hiPSC)-derived midbrain neurons from PD patients with autologous T lymphocytes.

Our findings reveal that T lymphocytes induce neuronal cell death and neurite degeneration of midbrain neurons in PD. Interestingly, when culturing PD patient-derived T lymphocytes with control neurons or vice versa, no neuronal cell death was detected, indicating that T cell-induced neurotoxic effect is PD-specific. When investigating isolated T lymphocytes derived from the peripheral blood of PD patients and controls, increased frequencies of IL-17-producing CD4+ T cells were found in PD patients. Accordingly, we show that in the co-cultures of PD patient-derived T lymphocytes increased neuronal cell death is induced by the cytokine IL-17, which binds to IL-17 receptor (IL-17R) expressed on PD neurons. Downstream, IL-17 – IL-17R signaling leads to an activation of NFkB. Finally, blockage of IL-17 or IL-17R rescued the T cell-induced neurotoxicity in PD neurons.

IL-17 producing T lymphocytes (Th17 cells) are known as essential mediators of autoimmune diseases like multiple sclerosis, and are here, for the first time, delineated as a crucial factor for activated adaptive immunity in sporadic PD.

Abstract No. P062

In vitro models of schizophrenia

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Induced pluripotent stem cells (IPSCs) can be differentiated into any cell types of human body, such as neuronal progenitor cells, and then to mature neurons. Using this property we can investigate the effects of specific mutations on neuronal differentiation. For disease modeling purposes we can generate IPSCs from patients bearing de novo mutations (DNMs) in the gene of interest, or use genome editing to create cell lines with desired mutations.

Schizophrenia (SCZ) is a psychiatric disorder with a complex genetic background. While several mutations have been related to SCZ (1), in most cases their biological significance remains unclear. Using somatic cell reprogramming to generate IPSC lines from a case-control trio, we aim to investigate the effects of DNMs. We describe a patient, carrying 3 non-synonymous DNMs, one of them in the KH-type splicing regulatory protein (KHSRP) gene which has deleterious effects. KHSRP is implicated in alternative RNA splicing which has critical importance in mRNA localization that is essential in neural development and in neuroplasticity.

With another approach we aim to characterize the possible neuron-specific role of the disrupted in schizophrenia 1 (DISC1) gene. DISC1 is involved in neurite outgrowth and cortical development through its interaction with other proteins. In this experimental setup we use an isogenic cell line pair of DISC1 exon-2 biallelic knockout (XCL1 DISC1 exon-2 KO/KO) and wild type.

Given that impaired hippocampal neurogenesis is implicated in the pathogenesis of SCZ we have adopted an established protocol to differentiate hippocampal dentate gyrus granule cells (2). Currently we are investigating the effects of the DNMs or genome editing on the target proteins and studying the phenotypic alterations including neuronal morphology, neurite outgrowth, synaptic connectivity and electrophysiological activity on differentiated neuronal cells.

Grants: National Brain Research Program of Hungary (Grant NAP-B KTIA_NAP_13-2014-0011 and Grant NAP-A KTIA_13_NAP-A-I/6).

Singh S., Kumar A., Agarwal S., Phadke SR., Jaiswal Y.: Genetic insight of schizophrenia: past and future perspectives. Gene. 2014 Feb 10;535(2):97-100.

D.X. Yu, F.P. Di Giorgio, J. Yao, et al.: Modeling hippocampal neurogenesis using human pluripotent stem cells, Stem Cell Reports. 2 (2014) 295-310.

Abstract No. P063

Functional role of the CAD risk locus 9p21 in calcifying iPSC-derived SMCs

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Using genome wide association studies (GWAS) our group contributed to the identification of 46 coronary artery disease (CAD) loci (PMID: 17634449, PMID: 21378990, PMID:23202125). The 9p21 locus was among these loci with a strong association to CAD and spans a region of 60 Kb. It is known that cardiovascular risk factors that contribute to the manifestation of CAD increase the risk of coronary or aortic calcification. The CAD risk locus 9p21 was reported very recently to be associated with coronary artery calcification (CAC) (PMID: 23561647).

In this work we aim to functionally analyze the effect of 9p21 locus on calcification using iPS-derived vascular smooth muscle cells (VSMC). Induced-pluripotent stem cells (iPSCs) with a deletion of 60 Kb region within the 9p21 locus were successfully generated and validated. A protocol for differentiating iPSCs into calcifying VSMCs was established in our laboratory and studies on calcification in-vitro using iPSC-derived VSMCs from WT, KO and Heterozygous iPSC lines for 9p21 are ongoing. The iPS-derived VSMCs express, as expected, SMC markers both at RNA and protein level. For the calcification assay calcifying media containing Pi or ß-glycerophosphate/L-ascorbic acid phosphate were used to induce calcification in iPS-derived VSMCs. Calcification deposits were confirmed using calcification specific staining such as Alizarin-Red-S and Calcein, and quantified using a calcium assay kit. Results from the three 9p21 genotypes WT, Het and KO will be compared. The assay may be used in future as read out to screen for calcification inhibitors.

1 *Schunkert, H. et al., 2011. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nature Genetics 43(3): 333-8*

2 *Cheung, C. et al., 2014. Directed differentiation of embryonic origin-specific vascular smooth muscle subtypes from human pluripotent stem cells. Nature Protocols 9(4): 929-938*

Abstract No. P064

Contractile work drives maturation of energy metabolism in hiPSC-derived cardiomyocytes mimicking developmental hypertrophy

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Energy metabolism is a key aspect of cardiomyocyte biology. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) are a promising tool for biomedical application, but are immature and have not undergone changes related to postnatal developmental hypertrophy.This study compared the mitochondrial and metabolic state of hiPSC-CM in standard 2D culture and cultured in 3D engineered heart tissue (EHT) format and determined the influence of contractile activity.

Methods and Results: HiPSC-CM in EHTs showed higher numbers of mitochondria (electron microscopy), mitochondrial mass (mitotracker), DNA (Mt-ND1, Mt-ND2), and protein abundance (proteome) than in 2D culture. Ingenuity pathway analysis revealed the PGC-1α transcriptional regulatory cascade as an important signaling pathway. While hiPSC-CM exhibited the principal ability to use glucose, lactate and fatty acids as energy substrates irrespective of culture format, hiPSC-CM in 3D performed more oxidation of glucose, lactate and fatty acid, and less anaerobic glycolysis. The increase in mitochondrial mass and DNA in 3D was diminished by reduction of contractile force with blebbistatin, pointing to the importance of contractile work for mitochondrial development.

In summary, the study provides several lines of evidence that a 3D culture format induces in hiPSC-CM maturation of energy metabolism towards a more adult like state mediated by contractile work. The establishment of a hiPSC-CM with advanced metabolic maturation model should be an important asset to the hiPSC-CM toolbox since it allows one to study metabolic changes mediated by drugs or cardiac diseases.

Abstract No. P065/T20

Establishment of murine 3D bronchoalveolar lung organoids from adult somatic stem cells for organ development and disease modeling

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Organoid models derived from murine and human stem cells have recently evolved as a powerful tool to study development and disease. We established a murine 3D bronchoalveolar lung organoid (BALO) model based on flow sorting of bronchoalveolar stem cells (BASC). Upon co-culture with lung mesenchymal cells (rMC), BASC underwent clonal expansion, gave rise to organoids, followed by branching and differentiation into lung organoids representing the bronchoalveolar compartment and allowing introduction of tissue-resident macrophages into the alveolar compartment. BALO were composed of club cells (CC10+), ciliated cells (β4-tubulin+, Foxj1+), goblet cells (mucin5ac+), alveolar epithelial cells type I (PDPN+, Hopx+) and II (SPC+), the latter showing surfactant production within the alveoli. Remarkably, rMC differentiated into myofibroblast (αSMA+PDGFRαhigh) and lipofibroblast-like (lipidTOX+PDGFRαlow) phenotypes and were indispensable for organoid generation. BALO were suitable for genetic manipulation and could be derived from several transgenic mice. Notably, BALO supported viral infection and replication as demonstrated by quantification of viral proteins and expression of interferon-β.

In summary, we defined an epithelial stem cell population giving rise to complete lung organoids recapitulating the 3D structure and cellular composition of the bronchoalveolar compartment which will allows us to explore new avenues for lung development and disease modeling.

Abstract No. P066

Single cell mapping allows correlation between twitch contractions, mRNA and protein expression of cardiac myosin heavy chain of the same hES-CM

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Here we wanted (i) to gain insight into the variability of α- and β-myosin heavy chain (α-/β-MyHC) protein and the corresponding mRNA expression among individual human stem cell-derived cardiomyocytes (hES-CMs) and (ii) to investigate how MyHC composition of the individual CMs affects their twitch kinetics. For this, we developed a novel, setup-independent single-cell mapping technique to detect different parameters of the same individual hES-CMs.

After cultivation of hES-CMs for ~35 days either in cardiac bodies (CBs) or plated on glass coverslips, twitch contractions were assessed. Then X-Y-coordinates of the CMs were recorded for remapping of the same CMs on different microscopes after fluorescence in situ hybridization using specific probe sets against MYH6- and MYH7-mRNAs and again after fluorescent immunostaining of MyHC proteins.

Single long-plated CMs showed 83±22% MYH7 and 17±22% MYH6-mRNA expression (n=84). We detected on average 489±500 MYH7- and 108±217 MYH6-mRNA molecules per cell. Correlation of mRNA to protein expression showed only 5±11% MYH6-mRNA of total myosin-mRNA for pure β-MyHC expressing CMs (n=31). hES-CMs cultivated in CBs showed 61±29% MYH7 and 39±29% MYH6-mRNA expression, with 27±31% MYH6-mRNA of total myosin-mRNA in pure β-MyHC CMs (n=49). Pure α-MyHC CMs from CBs had 75±17% MYH6-mRNA (n=7). In CB-derived CMs we detected on average 615±582 MYH7- and 356±403 MYH6-mRNA molecules per cell. Our results of all analyzed CMs together showed highly variable fractions for both MYH6- and MYH7-mRNAs from cell to cell, ranging from 0% to 100% (n=49 and 84). So far, no correlation between twitch contraction kinetics and the expressed MyHC-isoform in the individual cardiomyocytes was observed.

In conclusion, the large variability of copy numbers per individual hES-CM of both mRNAs suggests that transcription of the two genes occurs in a burst-like manner. Surprisingly, twitch contraction kinetics seem to be dominated by other parameters than the MyHC-composition.

Weber, N., Schwanke, K., Greten, S., Wendland, M., Iorga, B., Fischer, M., et al. (2016). Stiff matrix induces switch to pure beta-cardiac myosin heavy chain expression in human ESC-derived cardiomyocytes. Basic Res Cardiol 111(6), 68.

Abstract No. P067

Engineered heart tissue from fluorescent labelled human iPSC-derived cardiomyocytes, endothelial cells, smooth muscle cells and fibroblasts

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Background

Engineered heart tissue (EHT) from hiPSC-derived cells can be used for drug screening and disease modeling. The aim of the current study was to generate hEHTs comprising the main four cardiac cell types marked with different colours to investigate cellular composition dynamics and tissue organization with the goal to establish a tissue model close to the native human heart. Therefore, four distinct differentiation protocols were carried out to obtain cardiomyocytes, endothelial cells, smooth muscle cells and fibroblasts, all derived from one hiPSC line.

Methods and results

HiPSCs were transduced with lentiviral vectors encoding four different fluorescent proteins. Following the common mesodermal induction, blue hiPSCs were differentiated to cardiomyocytes (CMs) via inhibition of wnt-signaling, while green mesodermal progenitors were treated with VEGF to generate endothelial cells (ECs). For the differentiation of orange smooth muscle cells (SMCs) and red fibroblasts, wnt-signaling was further activated, followed by treatment with either a combination of TGFβ and bFGF or bFGF only. All four were differentiated successfully and characterized using marker genes on mRNA and protein level as well as cell type-specific properties such as the ability for contraction, tube formation or collagen production. Fibrin-based multicellular hEHTs were generated from 50% ECs, 30% CMs, 10% SMCs and 10% fibroblasts. The stable transduction of hiPSCs allowed for the analysis of cell organization in living tissue via confocal microscopy.

Conclusion

Colour-coded hiPSC are feasible for the generation of multicellular hEHTs and live-analysis of the fate of different hiPSC-derived cell types. Future experiments will systematically investigate the impact of different cell composition on contractile function, cell-cell interactions and tissue organization.

Abstract No. P068

Gene editing and clonal isolation of human induced pluripotent stem cells using CRISPR/Cas9

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Human induced pluripotent stem cells (hiPSCs) represent an ideal in vitro platform to study human genetics and biology. The recent advent of programmable nucleases makes also the human genome amenable to experimental genetics through either the correction of mutations in patient-derived iPSC lines or the de novo introduction of mutations into otherwise healthy iPSCs. The production of specific and sometimes complex genotypes in multiple cell lines requires efficient and streamlined gene editing technologies. We presently achieve high rates of gene editing at up to three loci using a modified iCRISPR system. This system includes a doxycycline inducible Cas9 and sgRNA/reporter plasmids for the enrichment of transfected cells by fluorescence-activated cell sorting (FACS).

Poster session II: P069 – P142

P069 – P076: Stem cells in diseases: Cancer stem cells

P069/T14 C/EBPβ-LIP regulates the let-7/Lin28 circuit to control cellular metabolism
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P070 In vitro system to dissect the molecular basis of Vanilloid/TRPV1 mediated anti-tumoral activity of human neural progenitor cells in GBM.
Luis Azmitia

P071 The self-renewal-controlling miRNA-193b is a potent tumor-suppressor and a biomarker for poor prognosis in acute myeloid leukemia
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P073 High grade serous ovarian cancer development involves p53 and PTEN dependent switch in stemness regulation in the fallopian tube epithelium
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P074 Novel role of NRF2 transcription factor in modulation of cancer stem cells through TAZ regulation
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P075 Differential analysis of physiologic and oncogenic Wnt signaling in human colon organoids by transcriptome and proteome profiling
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P076 p18 loss improves stem cell self renewal, tissue maintenance, and lifespan in the context of telomere dysfunction induced aging
Omid Omrani

Abstract No. P069/T14

C/EBPβ-LIP regulates the let-7/Lin28 circuit to control cellular metabolism

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Experiments with mouse models revealed that transgenic overexpression of the transcription factor C/EBPβ-LIP increases general tumor incidence, while in C/EBPβ-LIP deficient mice tumor incidence is reduced. The oncogenic mechanisms involved in these phenotypes have not been solved. Here we show that C/EBPβ-LIP enhances aerobic glycolysis and mitochondrial respiration (Seahorse XF analysis), resembling a shift to cancer/stem cell metabolism. By using an integrative analysis of genome wide transcriptome and whole cell proteome we show that although induction C/EBPβ-LIP does not significantly alter mRNA levels of glycolytic enzymes the protein levels of these enzymes are elevated. Further analysis of the C/EBPβ-LIP transcriptome revealed that C/EBPβ-LIP stimulates the expression of Lin28b, which is an oncofetal RNA-binding protein that enhances the translation of glycolytic and mitochondrial enzymes in order to increase the cellular metabolism and energy production. Moreover, Lin28b knockout by CRISPR/Cas9 genomic editing ablates C/EBPβ-LIP induced metabolic reprogramming in cells. We show that C/EBPβ-LIP controls Lin28b expression though transcriptionally repression of let-7. Let-7 and Lin28a/b have reciprocal functions in a regulatory circuitry where let-7 represses Lin28a/b-mRNAs, while Lin28a/b represses let-7 maturation. Finally, first analysis using a conditional C/EBPβ-LIP overexpressing mouse model show that let-7 levels are repressed and Lin28b levels are upregulated by C/EBPβ-LIP in vivo, which is associated with enhanced bioenergetics metabolism in bone marrow cells and results in hyperplasia in the examined tissues, skin and spleen.

Therefore, our data suggest a key role of C/EBPβ-LIP in controling the Lin28/let-7 regulatory circuit and thereby regulating cellular metabolism in context of stem cell function, tissue repair and tumor development.

Abstract No. P070

In vitro system to dissect the molecular basis of Vanilloid/TRPV1 mediated anti-tumoral activity of human neural progenitor cells in GBM.

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Objective: Malignant gliomas **(GBM)** are the most frequent primary brain tumor in adults, with no curative therapy and an average survival rate of 15 months (Curado *et al.* 2007). It has been shown that endogenous vanilloids (fatty acid amides) secreted by neural progenitor cells **(NPC)** induce cytotoxicity in GBM cells. This antitumoral effect appears to be mediated by the direct stimulation of the Transient Receptor Potential Vanilloid 1 **(TRPV1)** specifically present on GBM cells (Stock *et al.* 2012). As NPC represent only a small and difficult to access cell population in the adult human brain we turned to NPC differentiated from human induced pluripotent stem cells **(iPS cells)** as well as directly induced from fibroblasts as in vitro models for dissecting Vanilloid-mediated antitumoral activity in humans.

Methods: iPS cells were differentiated from patient derived dermatomal fibroblasts into long-term expandable neural stem cells **(lt-NSC)** following published protocols (Koch *et al.* 2009) and directly reprogrammed into induced NPC **(iNSC)** with a viral free insertion method (Capetian & Azmitia *et al.* 2017). Both lt-NSC and iNSC were expandable and expressed NPC markers.

Results: Human primary glioma initiating cells **(GIC)** were exposed to conditioned media from both lt-NSC and iNSC. We observed an increased cell death in GIC suggesting an involvement of endogenous vanilloids secreted by lt-NSC and iNSC.

Conclusion: In summary, we have established a human in vitro model with two different source for human NPC in order to molecularly dissect vanilloid –mediated GBM cytotoxicity. This system may be either exploited via transplanted, isogenic neural progenitors in GBM patients or, more ideally, used to identify metablic pathways leading to the synthesis of individual, highly bioactive endogenous vanilloids as potential chemotherapeutic agents for the treatment of GBM.

Abstract No. P071

The self-renewal-controlling miRNA-193b is a potent tumor-suppressor and a biomarker for poor prognosis in acute myeloid leukemia

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Dysregulated microRNAs (miRNAs) are implicated in the pathogenesis of acute myeloid leukemia (AML). We previously showed that miR-193b controls hematopoietic stem and progenitor cell (HSPC) expansion by modulating cytokine receptor signaling. Therefore we hypothesized that miR-193b is a potent tumor suppressor in AML. To investigate miR-193b in AML, we profiled its expression in cytogenetically and clinically characterized de novo pediatric (n=161) and adult AML patients (n=40) via quantitative real-time PCR (qRT-PCR) and validated our findings in an independent cohort of n=187 patients. We investigated the tumor suppressive function of miR-193b in human AML blasts in vitro and miR-193b knockout mice in vivo.

Here we demonstrate that miR-193b exerts important tumor-suppressive functions in the hematopoietic system. miR-193b is downregulated in several cytogenetically-defined subgroups of pediatric and adult AML, and low expression was an independent indicator for poor prognosis and survival. In pediatric AML miR-193b expression could identify patients with a very high risk of relapse or death within the ELN high risk patients or patients with a high 17-gene leukemic stem cell score. In knockout mice, loss of miR-193b cooperated with Hoxa9/Meis1 during leukemogenesis, whereas restoring miR-193b expression prolonged the survival of Meis1-induced leukemia mice and of patient-derived AML xenografts. Mechanistically, we show that miR-193b induces apoptosis and a G1/S-phase block in various human AML subgroups by targeting multiple factors of the KIT-RAS-RAF-MEK-ERK signaling cascade and the downstream cell cycle regulator CCND1.

As the tumor-suppressive function is independent of patient age or cytogenetics, restoring miR-193b would assure high anti-leukemic efficacy while preventing the emergence of resistance mechanisms.

Abstract No. P072

Merlin cooperates with neurofibromin to suppress the Ras-MAPK pathway

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The Ras-MAPK pathway is frequently activated in human tumors. Neurofibromatosis (NF) patients develop multiple tumors of Schwann cell origin. The NF1 tumor suppressor neurofibromin is the major Ras-GAP promoting Ras inactivation, while the NF2 tumor suppressor merlin is a scaffold protein coordinating multiple signaling pathways. Although merlin has long been implicated in antagonizing Ras-mediated transformation, the underlying molecular mechanism remains elusive. Here we show merlin can directly interact with Ras, neurofibromin, and Raf1. Merlin does not increase the Ras-GAP activity of neurofibromin but instead cooperates with it to suppress Raf1 activation by blocking the binding of Ras to Raf1. Suppression of the Ras-MAPK pathway likely contributes to merlin's tumor suppressor function, and this pathway may serve as a drug target for NF2.

Abstract No. P073

High grade serous ovarian cancer development involves p53 and PTEN dependent switch in stemness regulation in the fallopian tube epithelium

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A large body of evidence points to the fallopian tube epithelium as a site of origin of high-grade serous ovarian cancer (HGSOC). Yet, understanding of the molecular mechanisms of initial cellular and successive malignant transformation remains obscure.

Having established a long-term organoid model of human primary Fallopian tube epithelial cells (FTECs) (Kessler et al., 2015), we are able to genetically engineer these cells and thus model transformation processes from a completely healthy towards a cancerous tubal epithelium. By perturbing the pathways of tumor suppressor genes known to be frequently mutated or deregulated in HGSOC, we intend to investigate the contribution of such genetic changes on the carcinogenic potential of FTECs.

After genetic manipulation of healthy FT organoids by stable shRNA-mediated depletion of p53, PTEN and pRb, major components of HGSOC driver pathways, we identify a shift in growth requirements and regulatory mechanisms, which control growth of the organoids.

Complementary to our findings from stepwise analysis of tumorigenesis process we have discovered that solid tumour deposits of ovarian cancer have altered mechanisms of stemness regulation due to a change in the activity and crosstalk of specific paracrine signaling pathways. Based on this finding we were able to generate first set of 15 different stable patient derived organoid cultures.

The phenotypes of the genetically modified, healthy FT and cancer organoids cultured in different growth media are analysed and compared with regard to cell organisation and composition, gene expression as well as metabolic changes using immunofluorescence, microarray, RT-PCR, Western Blot and seahorse metabolic assays, respectively.

These important findings provide first functional evidence of how reprogramming could occur in vivo connecting individual tumor suppressors with a paracrine pathways which regulate stemness.

Abstract No. P074

Novel role of NRF2 transcription factor in modulation of cancer stem cells through TAZ regulation

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Most tumors are initiated and maintained by a subpopulation of migratory and invasive mesenchymal cancer stem cells (CSCs). CSCs are responsible for the acquisition of aggressive phenotypes, which are resistant to anti-tumoral therapies, promoting tumor recurrence. NRF2 (nuclear factor (erythroid-derived 2)-like 2) and TAZ (transcriptional coactivator with PDZ-binding motif) transcription factors are involved in tumor development but their role in regulation of CSCs and their possible crosstalk have not been explored. The cytoprotective actions of NRF2 allow cancer cell survival and resistance to drugs, while TAZ is essential for cancer initiation and growth of most solid tumors. Our studies demonstrate a correlation between NRF2 and TAZ expression and the prognosis of patients with glioma. In addition, we identified functional NRF2-binding site (Antioxidant Response Element, ARE) in the promoter region of the TAZ encoding gene (*WWTR1*). Our findings suggest that at least part of the NRF2-tumorogenic capacity is channeled through induction of TAZ expression. Thus, we propose the combination of NRF2 and TAZ expression as a novel prognostic marker and therapeutic target for gliomas.

Abstract No. P075

Differential analysis of physiologic and oncogenic Wnt signaling in human colon organoids by transcriptome and proteome profiling

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Constitutively active Wnt signaling due to loss-of function mutations in the APC gene have been recognized as the main driver of colorectal tumorigenesis. Targeting oncogenic Wnt has proven difficult because the pathway is also critical for stem cell homeostasis in many tissues including the normal gastrointestinal epithelium. In order to discriminate oncogenic from physiologic Wnt signaling, we have performed comprehensive transcriptome and proteome profiling in primary human colon organoids.

For this purpose we have engineered APC mutations in 3 normal human donor organoid lines to account for variability of the human genetic background. These isogenic lines were subjected to paired RNA sequencing and mass spectrometry analyses. Culture in the presence and absence of exogenous Wnt allowed us to discriminate receptor-mediated signaling from oncogene-induced responses. Overall we found moderate overlap between RNA and protein changes. However, this differential approach revealed that normal stem cells and adenoma cells are characterized by distinct proteomic signatures: While Wnt-receptor stimulation activated Hippo and small GTPase signaling, APC mutations affected nuclear receptor signaling and lipid metabolism.

By qRT-PCR and immunohistochemistry on patient material we could validate new markers for adenoma and normal colonic stem cells, as well as cell surface molecules that allow their selective purification. Together, our data represents a valuable resource for biomarkers of human colonic stem cells and adenoma cells that could allow more precise targeting of pathway deregulations in colorectal cancer.

Abstract No. P076

p18 loss improves stem cell self renewal, tissue maintenance, and lifespan in the context of telomere dysfunction induced aging

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Cdkn2c ($p18^{INK4c}$) controls G1 cell cycle transition and self renewal of stem and progenitor cells but its role in aging is unidentified. Here we show that the removal of $p18^{INK4c}$ leads to improvements in organ homeostasis and lifespan of aging telomerase knockout mice with dysfunctional telomeres. This rescue occurs independent of the continuous accumulation of DNA damage and the activation of DNA damage checkpoints. The study shows that $p18^{INK4c}$ is not induced in response to DNA damage and $p18^{INK4c}$ itself does not contribute in the activation of canonical DNA damage signals. Instead, deletion of $p18^{INK4c}$ leads to an improved maintenance of intestinal stem cells despite continuous activation of DNA damage signals. Proteomics and DNA methylation analysis using intestinal stem cells indicates induction of ALT (alternative lengthening of telomere) in $p18^{INK4c}$ knockout mice in compared to age-match wild-type mice. These findings support the concept that enhancements in stem cell self-renewal can prevail the induction of DNA damage checkpoint thereby slowing the depletion of damaged stem cells, which leads to prolonged organ homeostasis and lifespan elongation in the context of telomere dysfunction induced aging.

P077 – P084: Computational stem cell biology and systems biology

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Abstract No. P077

Workflow for integrative analysis of multi-omics data from skin fibroblasts, iPSCs and neuronal progenitor cells

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The comprehensive assessment of cellular states requires the integrative analysis of multiple omics data layers. Transcriptomics reveals cell-specific gene expression profiles which are at least partially a proxy for protein expression, whereas metabolomics allows to study the products of cellular processes and thus provides a measurement of enzymatic activity. Combination of these complementary layers of information can reveal new insights into cellular processes and their regulation. We built a generic workflow for unsupervised and supervised integrated analysis of transcriptome and metabolome data. The workflow contains (i) pre-processing and normalization, (ii) unsupervised analysis with non-negative matrix factorization (NMF) using the R package Bratwurst (manuscript in preparation), (iv) association of signatures to subgroups, (v) feature selection and (vi) multi-omics integration. For the latter, the link between transcriptomic and metabolomic data was established by utilizing the web application programming interface (API) of the database STITCH to find cross-links between both data types. Alternatively, a supervised analysis for the identification of differentially expressed genes or differentially abundant metabolites can be run. Furthermore, we present a data structure implemented in R derived from MultiAssayExperiment to store and handle complex datasets and corresponding metadata. In the present work, induced pluripotent stem cells (iPSCs) were generated from skin fibroblasts and differentiated into neuronal progenitor cells. Transcriptome and metabolome data was acquired for all differentiation stages as well as activated fibroblasts. Application of the above workflow will reveal cellular processes specific to the differentiation stages.

Abstract No. P078

Reconstruction of hierarchical differentiation processes based on the temporal clonal readout in distinct hematopoietic lineages

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The differentiation process of hematopoietic stem cells towards mature blood cells is generally depicted as a hierarchical decision process. Although the principle structure of such a branching tree is widely accepted, increasing evidence about complementary and alternative differentiation pathways question the concept of a fixed hierarchy. Clonal tracing studies allow quantifying to which extend and in which lineages marked hematopoietic stem cells contribute to the production of mature blood cells. However, the reconstruction of a hierarchical decision tree based on the temporal clonal readout in distinct blood lineages is computationally challenging.

We use a computational approach to investigate how and under which conditions the original branching tree can be faithfully reconstructed. To this end we use a mathematically model to generate prototypic differentiation processes through a hierarchical decision tree and produce clonal readouts in different lineages over time that closely resample the data available from corresponding experimental and clinical studies. Applying a numerical optimization procedure, we estimate the extend of necessary data (e.g. with respect to measured compartments and timing of subsequent measures) that is needed for the reconstruction process in order to obtain identifiable results. We complement the model-based investigations with available data from clonal tracing studies and test our suggested approaches for reconstruction of hierarchical decision trees in real-world settings.

Our analysis is a necessary and complementary prerequisite to support experimental approaches for the reconstruction of hierarchical, hematopoietic decision trees. In particular, the modeling approach allows, based on the available data, to estimate whether certain decision processes can be uniquely reconstructed or not.

Abstract No. P079

Quantitative assessment of batch effects in single-cell RNA-sequencing

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RNA-seq is used to quantify the transcriptome of single cells and for exploring heterogeneous cell populations. However, this method suffers from technical noise and batch effects. The success of batch effect correction is usually evaluated by visual inspection of dimension-reduced representations, for example, principal component analysis. This qualitative approach is necessarily imprecise as the first principal components explain only a low percentage of variance in the data.

Here, we present *kBET*, a method to quantify batch effects that outperforms existing approaches. *kBET* uses the batch information of all samples to compare the global batch label frequency with the local frequency in a set of neighbouring samples. Shifts in the local frequencies indicate a bias caused by a batch effect. We show that *kBET* is highly sensitive to bias in gene expression, performs more robust on high dimensional gene expression data than previous approaches, and is easy to interpret. We assess commonly used batch correction and normalization approaches by their performance to remove batch effects while preserving biological variations.

In conclusion, we provide a series of improvements to single-cell RNA-seq data processing that can be easily extended to related data types such as bulk RNA-seq, ChIP-seq or single-cell ATAC-seq.

Abstract No. P080

How spatial statistics can help to identify haematopoetic stem cell niches

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A small population of haematopoetic stem cells (HSCs) sustain blood production throughout life. Although it is known that HSCs reside in specialized niches in the bone marrow (BM) that regulate their behavior, it remains unclear what BM cells make up these niches.

Proposals for possible niche cells such as the endosteum, vascular and mesenchymal cells are typically based on their observed proximity to HSCs. However, so far, the complex reticular 3D structures of the bone marrow have made it hard to accurately observe and measure cellular components and understand their spatial relationships. With recent advances in microscopy and quantitative image analysis, localization of HSCs and potential niche cells is now becoming feasible. Nevertheless, drawing strong conclusions based on these data requires specialized statistical testing of spatial relationships.

Here, we present a statistical framework for the identification of potential HSCs niche cells based on analysis of point processes. These methods allow one to test e.g. whether observed spatial patterns deviate from the expectation under complete spatial randomness (CSR) and, if so, whether they are more clustered or dispersed than expected, within the complex 3D spatial domain. Moreover, point process analysis can reveal interactions between cells (repulsion, attraction) and can be used to detect the influence of co-variates such as proximity to the vasculature or concentrations of growth factors. Importantly, this statistical framework provides a guide to biologists to help design experiments and set up quantitative analysis pipelines.

Abstract No. P081

Mathematics of CML: a mechanistic interpretation of the bi-exponential decline can help to optimize dose reduction and adaptive treatments

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The availability of tyrosine kinase inhibitors (TKIs) since the early 2000s has revolutionized Chronic Myeloid Leukemia (CML) therapy. However, permanent control of the disease requires continuing and potentially life-long TKI therapy. In most CML patients treated with TKIs, the most common observed response is a bi-phasic decline in the BCR-ABL ratio, which can be parameterized for each patient. Here, we study a simple mathematical model that describes the treatment response in CML patients as a mechanistic phenomena resulting from the interaction of activated CML stem cells with the TKI. The simplicity of the model allows us to obtain explicit formulas that attribute specific functional aspects of the treatment with the bi-phasic decline characteristics. Our analysis reveals that the long term decline in CML tumor load is limited by the activation of quiescent stem cells. Based on this finding we suggest dose de-escalation schedules in which the treatment intensity can be substantially reduced after the first decline while the leukemic stem cell response is not altered. Our mathematical model confirms results from the DESTINY trial that showed the potential for the safety of dosage halving in CML patients in sustained remission. Our model suggests that the dose de-escalation can even be larger in most patients. We propose a simple method to calculate this optimal de-escalation ratio for each individual patient, based on its observed and continuously monitored treatment response. Our results illustrate how mathematical models can be used to substantiate and optimize clinical decision making.

Clark, Richard E. et al "Chronic Myeloid Leukaemia Patients with Stable Molecular Responses (at least MR3) May Safely Decrease the Dose of Their Tyrosine Kinase Inhibitor: Data from the British Destiny Study." Blood 128.22 (2016): 938.

Komarova, Natalia L., and Dominik Wodarz. "Effect of cellular quiescence on the success of targeted CML therapy." PLoS One 2.10 (2007): e990.

Abstract No. P082

Age and diet affect the intestinal stem cell proteome

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The small intestine is responsible for nutrient sensing and absorption, and it is one of the most important interfaces between the environment and our body. During aging, there is a progressive loss of function of molecules, cells and organs that leads to increased morbidity. Intestinal aging is characterized by proliferative imbalances, leading to persistent inflammation and tumorigenesis. It remains unclear which molecular alterations lead to loss of intestinal function. Therefore, it is crucial to study age-related changes on a cell-type specific level, particularly stem cells due to their role in maintaining organ homeostasis.

In this study, we isolated crypts and stem cells from the small intestine of mice and *D. melanogaster* midgut. We compared proteomic profiles of tissues and cells from different age groups using state-of-the-art mass spectrometry. We found proteome signatures in the intestinal epithelium, which indicate that aging affects metabolic networks, stem cell proliferation, and epithelial immune responses. Of note, some of these aging-associated alterations (e.g., immune modulatory responses) are reverted by dietary restriction, a health span extending intervention conserved across species. The identified changes are currently investigated regarding their relevance for intestinal stem cell function using *in vitro* and *in vivo* assays.

In the long term, our work could lead to the identification of novel interventions (e.g. nutrition) that are able to revert or slow down age-associated molecular alterations, and ultimately lead to extended healthy lifespan in mammals.

Abstract No. P083

The extracellular matrix protein Smoc2 affects muscle stem cell functionality during aging

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Skeletal muscles decrease in size and function during aging, leading to frailty in the elderly. Satellite cells (SCs, stem cells of skeletal muscles) are affected by aging resulting in decreased regenerative capacity of the muscle after injury. The molecular mechanisms responsible for the decline of SCs and muscle function remain poorly understood.

Here, we used mass spectrometry to analyze the aging proteome of skeletal muscles and SCs. We investigated the impact of aging on four different muscle types from mice having three different ages: young (3 months), old (18 months) and geriatric (26 months). Using multiplexing by tandem mass tags, we achieved high proteome coverage in our analysis (typically > 4,000 proteins). We found that different muscles undergo specific changes during aging (e.g., energy metabolism), while other changes e.g. in the extracellular matrix were conserved across muscle types. In particular, we found the abundance of the extracellular matrix protein Smoc2 to steadily increase during aging in all the muscle types tested. We showed that exogenous supplementation of Smoc2 can activate MAPK signaling in myoblasts, and influence the active state of the satellite cells and their differentiation in fiber culture assays.

The combination of unbiased proteomic approach with functional studies allowed me to identify a novel regulator of skeletal muscle homeostasis. In the long term, my findings could help the design of therapies aimed at reversing or decelerating age-related muscle wasting to improve quality of life of the elderly.

Abstract No. P084

Recovering clonality by evaluation of multiple integration sites in tracing studies

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Viral integration sites represent a suitable genomic marker to identify the clonal progeny of initially marked cells. Such marking strategies in various experimental settings reveal much information about the clonality of the hematopoietic stem cell (HSC) compartment, but also represent a sensitive method to continuously monitor patients after receiving gene therapy. However, especially in clinical situations a high multiplicity of infection (MOI) leads to parallel integration of more than one virus within the target cells. Tracing of individual integration sites thereby overestimates the number of present clones, which often carry multiple integrations.

We present a method to numerically reconstruct common integrations within one clone, thereby estimating the clonal structure in situations with high transduction rates. In order to test and validate our approach, we use a simplified model of clonal persistence that includes different sources of variability that are frequently observed in comparable experimental settings (such as varying clonal growth rate, amplification bias of different integration sites, or sampling error). Using this simulation data we identify minimal constraints that are required to estimate the clonal affiliations. In particular, we demonstrate that multiple temporal or spatial measurements with different clonal abundances are necessary to faithfully reconstruct the underlying relation between integration sites and clones. We illustrate the application of our approach for different representative model scenarios.

Our analysis shows that numerical models are a valuable tool to develop sensitive and biologically meaningful measures in a controlled environment. In the particular application, we illustrate the potential and challenges in the reconstruction of clonal identity with multiple viral integrations. Our algorithm is a prerequisite for the unbiased clonal analysis especially for continuously monitored patients after gene therapy.

P085 – P103: Somatic stem cells and development

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Abstract No. P085

Transdifferentiation of dermal cat fibroblasts to chondrogenic lineage under the effect of valproic acid and platelet rich plasma-PRP

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Cell reprogramming has allowed for morphological and functional changes in cells. These changes influence the level of cellular potency and can give rise to cells with greater capacity of differentiation towards other lineages, what has profound implications for cellular therapy. The effect of valproic acid and PRP has not been described in dermal cat fibroblast. We hypothesized that the use of these two molecules could favor the differentiation capacity of the fibroblasts towards chondrogenic lineage. Fibroblasts were isolated from abdominal region of a 3-years old female cat and cultured in DMEM/F12 medium + 20% FBS until confluence. Then, cells were cultured in DMEM/F12 medium with 1mM valproic acid for 5 days, and changed to 1%PRP for additional 7 days. After this, cells were cultured in differentiation medium (adipogenic for 7 days, chondrogenic for 30 days and osteogenic for 21 days) and stained to evaluate differentiation with oil red, alcian blue and alizarin red respectively. Cells treated with valproic acid and PRP showed similar growth rate than control group. Adipogenic differentiation and morphological changes were not detected after staining. However, weak staining was observed in osteogenic differentiation and cells lost there their fibroblastoid morphology. Chondrogenic differentiation was evident at 10 days of induction by pellet formation and at the day 30 had a strong staining. Some components of platelet-rich plasma such as TGF-beta, PDGF and IGF may favor chondrogenic differentiation, which is reported for mesenchymal stem cells but has never been reported neither for feline fibroblasts, nor using this combination of molecules.

Abstract No. P086

The regenerative capacity of human embryonic stem cell-derived neural stem cells in response to ionizing radiation

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Ionizing radiation is used to treat cerebral cancers, i.e. to kill tumor cells. Inevitably, also normal brain tissue surrounding the tumor will be exposed and may result in a long-term decline in neurocognitive function. Damage of neural stem cells (NSCs) in the brain, specifically their inability to replenish lost neurons and glia cells is discussed as the underlying mechanism of the cognitive dysfunction observed in survivors of childhood cancer.

To enhance the knowledge in this field, NSCs derived from human embryonic stem cells (hESC, H9) were used in this study. Two subculture methods were compared: the commonly applied enzymatic passaging that may render the cells prone to karyotype instability and mechanical scrapping that has shown to favour genetic stability. Yet, on chromosomal level no difference was observed between both methods and in both cases NSCs expressed the characteristic markers nestin, SOX2 and PAX6.

After exposure to therapeutically relevant X-ray doses (1 and 2 Gy), a dose-dependent increase in the aberration yield was observed. The removal of damaged cells by apoptosis started 12 hours after exposure as judged by the number of TUNEL-positive cells, lasting up to 3 days. Noteworthy, the aberration yields were slightly higher in the progeny of irradiated NSCs compared to the control, which may impact on their stem cell character, for example on the ability to proliferate and differentiate within neurospheres. In fact, the number and size of neurospheres formed after X-ray exposure diminished in a dose-dependent manner.

In summary, our data indicate that the majority of injured NSCs are removed from the proliferating pool, but the stemness properties, and hence the regeneration capacity of the surviving cells is hindered. Ongoing studies on NS formation as well as gene expression analysis will help elucidate the mechanisms affecting neural regeneration after an insult.

Work supported by BMBF, Germany(02NUK034C).

Abstract No. P087

Biochemical niche factors trigger epigenetic alterations of aged muscle stem cells

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Muscle stem cells (MuSCs) are responsible for the formation and regeneration of skeletal muscle in response to exercise and injury. Their number as wells as functionality is reduced in age, thus contributing to impaired muscle regeneration.

Epigenetics describe mechanisms that orchestrate dynamic changes of gene activity by altering structural properties of the chromatin during development, differentiation and disease. DNA methylation and the posttranslational modification of histones are likely to be the main epigenetic features and mounting evidence supports the idea of an epigenetic landscape that arises from their crosstalk.

In our studies we found that in response to activation, aged MuSCs display epigenetic alterations which lead to the aberrant induction of developmental pathways.

Consequently, we aim to investigate the upstream mechanisms that are involved in the alterations of the epigenetic landscape with age. Our data indicates that biochemical properties of the myogenic niche trigger epigenetic changes in aged MuSCs upon activation.

Abstract No. P088

Effect of 5-azacytidine and platelet-rich plasma on the expression of cell surface markers and mesodermal differentiation of feline fibroblasts

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Mouse embryonic fibroblast as well as terminally differentiated cells can be artificially reprogrammed towards higher potency levels and then differentiated into mesodermic lineages with the use of 5-azacytidine an epigenetic reprogramming agent. Cell reprogramming paves way for other cell therapy options. The goal here was to evaluate the effect of inhibitor of DNA methyltransferase in conjunction with PRP on the expression of feline fibroblast surface markers and subsequent differentiation ability to mesodermic lineages. Fibroblasts were isolated from abdominal region of a female cat, then cultured and expanded in DMEM/F12 + 20% FBS. For reprogramming, cells were cultured in DMEM/F12 with 10 µM 5-azacytidine for five days and changed to 1% PRP for additional 7 days. Cells were evaluated by flow cytometry at days 0, 5 and 12 of reprogramming. Markers assayed were: CD44, CD45, CD90 and MHCII. After reprogramming the cells were cultured in differentiation medium (adipogenic for 7 days, chondrogenic for 30 days and osteogenic for 21 days) and stained to evaluated differentiation with oil red, alcian blue and alizarin red respectively. The percentage of expression of the markers at day 0 was CD90 10%, CD44 5.6%, CD45 6.8% and MHCII 4.4%; day 7: CD90 21.6%, CD44 0.7%, CD45 2% and MHCII 0.7%; and day 12: CD90 17.2%, CD44 0.1%, CD45 1.2% and MHCII 4%. Adipogenic and osteogenic differentiation were not evident with stain or by morphological changes. After chondrogenic differentiation a small pellet was visualized which stained blue with Alcian staining. The control group showed no morphological changes or staining. In conclusion, there were not significant changes in expression of surface markers upon reprogramming and differentiation of cat fibroblasts, nevertheless, chondrogenic differentiation was achieved. Since no titration of 5-azacytidine concentration was conducted, probably this variable should be considered in future experiments.

Abstract No. P089

Dental pulp stem cells: characterization and neuronal differentiation

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Mesenchymal stem cells (MSC) represent a population of multipotent cells that have a wide distribution in adult tissues. Dental pulp mesenchymal stem cells (DPSC) have been the target of numerous researches because they are easily isolated and expanded and by the elevated potential for therapy. Furthermore, due to its common ectodermal origin with neurons, it could be used as treatment of neurodegenerative diseases. This study aimed to characterize DPSC phenotypically, functionally and analyzing the expression of the neuronal marker βtubulinIII and nestin after the induction of neuronal differentiation. This study had the approval of the local Ethics Committee (CAAE:42751615.8.0000.0020). Dental pulps were extracted, sectioned into small pieces and dissociated with collagenase type I. Tests with the cells were carried out between P3 and P5. For the immunophenotypic characterization, a panel of antibodies suggested by the International Society for Cellular Therapy for the definition of MSC was used. The DPSC were differentiated into four lineages (adipogenic, osteogenic, chondrogenic and neuronal) for 21 days. For neuronal differentiation, the cells were analyzed by immunofluorescence technique with anti-βtubulinIII antibody and qRT-PCR with nestin gene. The DPSC had plastic adherence, fibroblast-like morphology, immunophenotypic profile positive for CD29, CD73, CD90, CD105 and reduced expression for CD14, CD19, CD34, CD45, HLADR. In the DPSC, it was possible to observe the osteogenic and chondrogenic differentiation but not the adipogenic. After neuronal differentiation, it was possible to observe the expression of βtubulinIII and nestin in differentiated DPSC. This study demonstrates that DPSC are easy to obtain and expand in culture. In addition, they have characteristics in common to MSC obtained from other tissues and can be used as an alternative source of stem cells. Due to their great potential for neuronal differentiation, DPSC may be an excellent option for studies of neurological diseases or injuries.

Abstract No. P090

Role of microRNA-302 family in mesenchymal stem cell differentiation into osteogenic and adipogenic lineages

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Human bone marrow mesenchymal stem cells (hBMSCs) are adult stem cells, characterised by self-renewal and differentiation capacity into three mesenchymal lineages: osteoblast, chondrocyte and adipocyte. Micro RNAs (miRNAs) are a class of non-coding short RNAs (20-22 nucleotides) which target messenger RNAs to suppress protein synthesis and regulate cell function. Recently, MSCs are becoming an appealing tool for clinical application. However, controlling their behaviour whilst delivered into the body is a challenge. Therefor our aim is to investigate miRNA potential role in regulation of MSCs fate decisions.

First clonally derived MSCs were tested using miRNA array. We identified 5 miRNAs which had not previously been reported to be involved in MSC differentiation. From these we selected 2 family members of miRNA-302 (a and b) for further investigation. Then we validated their expression by qRT-PCR and found that miRNA-302a and b were down regulated during differentiation.

To determine the role of the miR-302 family during MSC differentiation, their functional activity was tested by knockdown and over expression, via transfection of miRNA-302 family inhibitor or mimic into MSCs and the cells were treated with appropriate media for 14 days. Then lineage specific gene expression was measured by qRT-PCR. Knock-down of miRNA-302 family in MSCs caused a significant upregulation of between 2.5-3.5 times of RUNX2 expression and between 3-8.5 times of ALP expression during osteogenesis of MSCs. However, there were no notable effects of miR-302 family knockdown during adipogenesis of MSCs. On the other hand, overexpression of miR-302b did not make any significant changes in osteogenic and adipogenic specific gene expression during differentiation.

The results suggest that the miR-302 family may act to maintain MSC naivety and regulate osteogenic gene expression. However, miR-302b may not directly affect MSC differentiation and suggests intra-familial miRNA differences may be present that have distinct effects upon cell fate.

Abstract No. P091

Mechanisms of planarian stem cell regulation during starvation

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Dietary restriction is the most powerful anti-aging strategy, which is conserved throughout evolution in the animal kingdom. It is known that dietary restriction extends the life span of vertebrate and invertebrate animals and protects against some age-related diseases. Dietary restriction beneficial effects are due at least in part to an increase in stem cell function. However, little is known about the cellular and molecular mechanisms that dietary restriction uses to regulate stem cell function. To address this, we use the freshwater planarian Schmidtea mediterranea. Planarian represents an attractive model to study stem cell biology since 25 % of the planarian parenchyma consists of adult stem cells. This characteristic confers planarians great plasticity: they can regenerate a new full planarian from very tiny fragments of their bodies and they can stand long periods of starvation. Remarkably, during these periods of starvation they do not show any physiological impairment and maintain a stable population of proliferating stem cells.

Through a RNA-seq screen we aimed to find novel genes involved in the enhancement of stem cell function during starvation. We are comparing the transcriptome of stem cells and differentiated cells from starved planarians to the transcriptome of stem cells and differentiated cells from well-fed planarians. So far, we have identified the main signaling pathways involved in stem cell regulation during starvation. Currently, we are characterizing the function of the top candidate genes through RNAi experiments.

In conclusion, this study will clarify how dietary restriction regulates stem cell function and will allow a better understanding of its influence in processes such as regeneration and aging.

Abstract No. P092

Towards identifying GCNF target genes and function in human pluripotent and neural stem cells

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We aim at deciphering the function of the orphan nuclear factor GCNF/NR6A1 during neural induction and neuronal differentiation using human pluripotent stem cells (hPSCs) and hPSC-derived neural stem cells (NSCs). GCNF represents a promising candidate involved in neuronal lineage development. It displays a distinct expression pattern in the developing brain, and GCNF knockout approaches have been shown to disrupt neurogenesis in several species. GCNF is known for its function as transcriptional repressor of Oct4, thereby silencing pluripotency during early development. However, GCNF expression persists after gastrulation in the neuroepithelium, which points to an important role of GCNF during brain development and raises the questions as to which other target genes are regulated by GCNF. Thus, we aim at annotating the GCNF target repertoire in a genome wide manner by using chromatin immunoprecipitation (ChIP) followed by DNA sequencing. Since there are no ChIP-grade antibodies for GCNF available, we took advantage of the CRISPR/Cas9 genome editing technology to introduce an epitope-tag into the GCNF locus to facilitate high quality ChIP. As the editing frequency in hPSCs is low, we have established a selectable CRISPR/Cas9 system to enrich for edited cells by delivery of a puromycin resistance gene. After puromycin selection, the loxP flanked resistance gene was excised using Cre recombinase. In addition, we have generated GCNF knockout hPSCs using CRISPR/Cas9 as well as several lentiviral constructs for conditional modulation of GCNF expression in hPSCs and hPSC-derived NSCs. Using these cells, we will analyze how GCNF affects early neural induction, neuronal lineage specification and differentiation. We will in particular analyze whether GCNF ablation impairs the propensity of hPSCs to undergo neural differentiation. We expect that these studies will elucidate the role of GCNF in neuronal lineage specification, thereby further contributing to our understanding of human brain development.

Abstract No. P093

An automated walkaway system to perform differentiation of 3D mesenchymal stem cell spheroids

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Human mesenchymal stem cells (hMSCs) are multipotent and found in multiple areas of the body. Including bone marrow, skeletal muscle, dermis, and blood. The cells are known for their ease of isolation and ability to differentiate and mature into multiple lineages including adipocytes, chondrocytes, osteocytes. hMSCs play a critical role in adult tissue repair, therefore are of great interest in tissue engineering applications. For example, as adult cartilage cannot repair itself, chondrocyte-derived hMSCs may be used for cartilage repair applications. Transplantation of spheroidal chondrocytes is already being studied as a treatment for hip joint cartilage defects. Initial hMSC experimentation involved two-dimensional (2D) cell culture in a monolayer. Culturing the cells in this manner results in a loss of replicative ability, and differentiation capability over time. Numbers of techniques to culture hMSCs in a three-dimensional (3D) format were then incorporated, such as pellet and micro mass culture. These methods increased the differentiation process, but required large numbers of cells, extensive manual processing steps, and a high overall cost per method. Recently developed 3D cell culture technologies, which have the ability to create spheroids of smaller cell numbers in high density microplates, can overcome the limitations of earlier methods while providing the necessary environment for proper stem cell differentiation.

Complete differentiation from multipotent hMSCs to chondrocytes, typically takes 14-28d. With media exchanges required every 2-3d, manual processes are not only tedious, but when working with nonattached cells, increases the risk of accidental spheroid removal. Automating the processing steps and incorporating a 3D magnetic bioprinting method, (384-Well-BiO-Assay-Kit) and NanoShuttle-PL-particles (nano3D Biosciences), frees researchers to perform other tasks and reduces the risk of spheroid loss.

Here we demonstrate the validation of a combined solution to perform automated chondrocyte differentiation from 3D hMSC spheroids, where all instrumentation was contained within a laminar flow hood. A washer/dispenser was used for media exchanges, an automated incubator maintained proper environmental conditions between exchanges. Label-free cellular imaging and immunofluorescence imaging was performed.

Abstract No. P094

The role of HAT in the regulation of adult neurogenesis

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Histone acetyltransferases (HAT) and histone deacetylases (HDACs) are important mechanisms to regulate development and cell differentiation. Hypo-acetylation has been linked with a decline of the adult neurogenesis and neurodegenerative diseases. HDAC inhibitors can increase the production of adult neurons and improve memory formation. Moreover, HDACs have been used as therapeutic tools for various neuropathies, including Alzheimer disease, Parkinson's disease or schizophrenia, among others.

TRRAP is a common cofactor of the GNAT and MYST families of HATs. To investigate the mechanisms by which HAT regulates adult neurogenesis, we specifically deleted Trrap in the adult neural stem cells (aNSCs) in the subventricular zone and the subgranular zone within the dentate gyrus. We found that Trrap deletion leads to a gradual reduction and eventually halt of the newborn neuron production at 7 months of age, in contrast to 18 months of wild-type controls, showing an accelerating aging process. Moreover, Trrap-null aNSCs formed fewer and smaller neurospheres in cultures compared to wild type aNSCs, indicating a compromised self-renewal of aNSCs. Although Trrap-deleted neurospheres are viable, they underwent cell death when disassociated in culture, suggesting that the cell-cell interaction is essential for Trrap-null aNSCs. Intriguingly, the proliferation defects and lethality of Trrap mutant aNSCs could be rescued by an aggregation with its wild-type counterpart in vitro, suggesting a non-cell autonomous effect of the Trrap deletion in aNSCs. Currently we are investigating the molecular pathways by which TRRAP-HAT controls stemness and fitness of aNSCs in aging processes.

Abstract No. P095

Comprehensive cell surface antigen screening of the human neural lineage reveals dynamic expression patterns of transferrin receptor 1 (CD71)

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Cell surface antigens are prominently involved in cellular cross-talk and adhesion and can act as receptors for the cytoplasmic transmission of extracellular signals. Many of these antigens have been classified under the CD (cluster of differentiation) nomenclature and are frequently used for immunophenotyping. By exploiting the capabilities of this characterization technique, we intend to resolve the heterogeneity of the human neural lineage and highlight the potential functional differences attributed to varying surface antigen expression. To this end, a comprehensive analysis of >200 CD antigens was conducted on neural stem cells (NSC) and neurons (NEU) derived from human pluripotent stem cells, resulting in differential surface antigen profiles of neural stemness vs. neuronal differentiation. Gene ontology analysis of the identified neural cell surface antigens revealed a broad range of surface antigen-mediated biological processes. We further validated the expression of 45 antigens by low-throughput cytometric analysis and identified profound down-regulation of transferrin receptor 1 (TFR1; CD71) and integrin alpha 2 (ITGA2; CD49b) upon differentiation to neurons. Expression of both these proteins was maintained in neural crest cells, NSC and pluripotent stem cells. Capitalizing on the distinctive expression of CD71, a marker otherwise widely expressed due to its role in iron homeostasis, we were able to enrich neuronal subpopulations of interest and exploit CD71 as a marker to assess selective vulnerability to neurotoxic agents in heterogeneous neural cultures. Moreover, we could even demonstrate that MYCN was critical for the maintenance of CD71 expression in proliferating neural cells. In summary, our data provides candidates for further study of cell-cell communication in neural development as well as novel surface antigens 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. P096

Meaningful modeling of cortical development and microcephaly in rosettes and organoids derived by combined pathway inhibition

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Methods for deriving early cortical progenitors from PSCs are diverse, lack robust readouts and largely yield heterogeneous populations. We show that efficient transition of PSCs towards homogeneous dorsal cortical progenitors is reflected by dramatic enhancement of Notch activation and radial organization capacity in a process that requires WNT inhibition on top of dual SMAD inhibition. This paradigm works well for deriving both cortical neural rosette monolayers (2D) as well as cerebral organoids (3D), both of which upon triple inhibition exhibit enhanced Notch activation and radial organization accompanied by homogeneous cortical marker expression. Particularly evident is the expression of the definitive upper cortical layer marker CUX2. Importantly, dual SMAD inhibition alone enhances Notch activation but not radial organization, and in concordance enriches for caudal fates. On the other hand, WNT inhibition alone does enrich for anterior fates but lacks the robust CNS cell induction and expansion induced by dual SMAD inhibition. Microcephaly organoids harboring centrosomal defects show massive cell death, significant size reduction, cell cycle arrest and expansion of more caudal regions of the cortex – all primarily under triple pathway inhibition. In contrast, more posterior regions that can be readily induced by dual SMAD inhibition alone remain intact in microcephaly organoids, demonstrating cortex specific cell death. Transcriptional profiling of normal and microcephaly organoids reveals an intriguing set of dysregulated genes that suggest mechanisms underlying the specific death of cortical progeny. Thus, combined inhibition is indispensable for standardized modeling of corticogenesis and for demonstrating cortex-specific defects associated with microcephaly.

Abstract No. P097

The surface proteome of adult neural stem cells in zebrafish reveals long range cell-cell connections and age-related changes in responsiveness to IGF

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The generation of new functional cells in adult tissues happens in a restricted fashion, firstly due to the limited presence of stem cells and secondly due to their parsimonious recruitment into cell cycle. Furthermore, the recruitment of stem cells becomes even more restricted as aging progresses. The precise age-related changes associated with the declining frequency of cell cycle entries are incompletely understood. It is thus important to elucidate how the recruitment from quiescence into cell cycle is regulated in adult populations of stem cells. This recruitment might involve fluctuations in growth factor signaling pathways, or also direct cell-to-cell communication that coordinates and limits the numbers of recruited cells. To identify those mechanisms, we developed a method to compare the surface proteome of young and aged adult neural stem cells, making use of the advantageous architecture of the adult zebrafish telencephalon, where the radial glial cells are located directly on the surface of the brain and function as stem cells. We validated our method by comparing the dataset with fluorescence-activated cell sorting (FACS) using the gfap:GFP transgenic line labelling the radial glial cells. We firstly identified proteins involved in filopodial extensions and validated this finding by the morphological analysis of single cells lipofected in vivo. Secondly, we observed an age-related decrease in the expression of IGF-receptors, which we examined functionally. Overexpressing the ligand IGF2b increased complete cell cycle activity in young brains, while incomplete cell cycles in old brains resulted in multinucleated cells, underscoring the important contribution of cell-intrinsic changes during aging.

Abstract No. P098

Successions between En1 +/- fibroblastic lineages drives dermal development, and its phenotypic transition from regeneration to scarring

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All mammals and humans undergo metamorphosis in response to injury, from regeneration to scarring (RTS). Here we follow two functionally diverged fibroblastic lineages (ENFs & EPFs) and document their lineage successions during backskin development that coincides with RTS. We show that ENFs are dermal sculptures that develop and regenerate native architectures during fetal life, and that their lineage decline over time imposes a dermal tissue absent of such events. We show that EPFs are scar producers even at fetal stages, wherein their numbers are bellow a threshold needed to generate macroscopic scars, but that their dynamics predicts scar emergence. We show that clonal advantages to EPFs rather then programed cell-lineage death, most likely are primary succession mechanisms, and that RTS can be partly circumvented by transplantations of fetal ENFs or decellularised fetal dermis. Our findings provide a mechanism for regenerative decline in mammals, carry clinical implications by suggesting that human dermal regeneration could be reached by coxing or transplanting ENFs alone, and provide a model for comparative regeneration studies between taxon groups.

Abstract No. P099

Neural stem cells induce the formation of a life-long stem cell niche

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Adult stem cells are invariably located in specialized micro-environments termed niches. Physical contact with the niche has been shown to be essential for maintaining stemness, indeed stem cells displaced from the niche start undergoing differentiation programs. During the past two decades, huge progress has been made in identifying signaling molecules that emanate from niche cells and act on the stem cells they harbor to maintain their self-renewal capabilities. Intriguingly, results from different models have also revealed that most niches have a different lineage than their respective stem cells. This realization raises a series of unresolved fundamental developmental questions. Namely, do stem cells and their niches form independently during development? And if so how do they locate to their final position? It is also presently unclear whether stem cells or their niches form first and the nature of their initial interaction during development remains largely unknown. Here we make use of the morphogenesis of neuromast organs during the embryonic development and post-embryonic growth of Medaka fish to shed light on these difficult questions. Using a combination of novel tissue specific transgenic lines, lineage tracing, long-term live-imaging and 2-photon laser ablations we reveal the location and action of neuromast stem cells under homeostatic and regenerative conditions. In addition, we report that these neural stem cells are able to induce a stable fate change in the surrounding epithelium during development, demonstrating a hierarchical organization guiding the in vivo formation of a life-long stem cell niche in a vertebrate model. We speculate that transformation of surrounding tissues by stem cells plays a major role during the establishment of vertebrate stem cell niches.

Abstract No. P100

Cytokines select for clones with common MSC progenitors, change the cytokine production profile and homing of the cells in tumor bearing mice

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Mesenchymal stromal cells (MSC) are used in hundreds of clinical studies, but reproducibility and efficiency remain critical issues. Results are influenced by isolation of MSC from many different vascularized tissues and a variety of culture conditions. The umbilical cord is an easily available MSC source and contains a proposed perivascular stem cell niche, which allows continuous outgrowth of MSC explant monolayers (MSC-EM) over a period of several months. This opportunity for long-term ex vivo cultivation enables the analysis of different culture conditions already at the MSC progenitor level. We marked potential MSC progenitors with genetic barcode vectors and analyzed the influence of frequently used cytokines (EGF, bFGF or TGF-b1) on MSC-EM. The addition of bFGF or EGF to our standard medium (MSC10 = aMEM, 10% hAB-Serum) increased, whereas TGF-b1 decreased the growth compared to MSC10 medium. Barcode analysis by high-throughput sequencing revealed a progenitor specific selection of MSC-EM. Bio-PlexTM assays were accomplished to quantify levels of 27 cytokines secreted by MSC and demonstrated that cytokine-selected cells often exhibited greatly altered secretion profiles. A murine xenotransplant model of human breast cancer was used to investigate potential differences between the capacity of TGF-b1-selected cells (TSC) and MSC10-selected cells to home to tumors. Biodistribution of TSC was significantly lower in the peripheral blood, bone marrow, liver, spleen and lung. In the tumors, we found either lower or equal numbers of TSC. Our results show a progenitor-specific selection of MSC-EM by different cytokines. Additionally, the selected cells exhibit distinct properties like cytokine production and homing to various tissues. These important insights into MSC development might aid creation of more robust MSC therapies and provide an impetus to generate MSC with tailored functions for precision medicine.

Abstract No. P101

Clonal analysis of the Medaka gill reveals fate restriction during post embryonic growth

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Post embryonic organogenesis occurs in all organisms by either increasing the size of an organ or by adding new functional units to it. Unlike any other vertebrates, fish grow during their whole life. But how do organs adapt to a constant growth and what are the mechanisms behind this homeostatic growth? Long term cell tracking and lineage tracing *in vivo* is a powerful tool to reveal stem cell fate and understand their contribution to postembryonic growth.

The Medaka gill, a new model for post embryonic growth, offers several advantages such as structural simplicity, compartmentalization and easy access for imaging and experimental manipulation. In this study we determine post embryonic growth in the medaka gill and identify stem cells and their niches which constitute to the formation of new functional units within the organ. Furthermore, we characterize fate restriction of stem cells during homeostatic growth.

The experimental strategy consisted of IdU incorporation experiments, inducible labeling and subsequent lineage tracing. By combining these approaches with confocal microscopy, we demonstrate that the organ increases in size by addition of new filament stacks at the extremes, and in turn, those filaments increase in a proximal-distal manner by adding new lamellae. We also prove that stem cells generating new filament tissue are located at the tip of each filament.

Furthermore, we characterize four fate restricted stem cell populations residing in the gill, each giving rise to distinct patterns of cell types. These patterns occur at different rates, indicating differences in stem cell numbers.

Ultimately, these findings will contribute to understanding post embryonic growth, stem cell behaviour and tissue homeostasis *in vivo*.

Abstract No. P102

Reliable and robust animal- and human-component-free hMSC-BM expansion on a new synthetic ready-to-use FN1 motifs surface

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Human mesenchymal stem cells (hMSCs) consist in a heterogeneous population of multipotent cells, easily isolated from various tissues as adult bone marrow, adult adipose tissue, dental pulp, fetal or neonatal tissues. Combining multi-lineage differentiation potential, low immunogenicity, great immunomodulatory potential and tissue repair promotion capabilities, hMSCs represent a promising stem cell population in term of regenerative medicine, cell therapy and tissue engineering applications. Relatively in low abundance in their natural niches, hMSCs require a robust *in vitro* cell culture expansion process to obtain sufficient high-quality cell numbers for research and clinical applications. In order to reach efficient cell expansion with a high level of consistency and reproducibility, well-defined, serum-free, xenogenic-free (XF) or animal-component-free (ACF) hMSC culture systems are recommended. Therefore, a completely new human- and animal-component-free, ready-to-use surface had been developed. This proprietary coating technology is made up of synthetic fibronectin-derived motifs, specifically designed to mimic the cell attachment site of native extracellular matrix (ECM) proteins. This constitutes a completely defined xeno-free substrate to culture ECM protein-dependent cell types, especially in restrictive culture settings as serum-free conditions. The present experimental work demonstrates that this fully synthetic surface, used in combination with well-defined culture medium and dissociation solution, represents a highly effective and reliable alternative to biological coating-dependent hMSC culture systems.

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Abstract No. P103/T07

Regulation of epidermal stem cells by flightless I protein during wound repair

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Epidermal stem cells (EpSCs) reside in niches of interfollicular and follicular epidermis. These cells proliferate and migrate out of their niches during re-epithelialization of wound healing which requires the remodeling of the actin cytoskeleton. Initially identified as an essential actin-remodeling protein and a negative regulator of wound healing, Flightless I (Flii) protein has also been found to be an important regulator during hair follicle homeostasis and a positive regulator of digital tissue regeneration. Here, we investigated the mechanisms behind Flii involvement in EpSCs during wound repair. Using an incisional wound healing model in genetic Flii mice (Flii knockdown: Flii+/-, wild type: WT, Flii transgenic: FliiTg/Tg) our current study assessed the effect of differential Flii levels on the quiescent and proliferative property of EpSCs during different stages of wound repair. Flii was found to be highly expressed by purified CD34, K15 and Integrin a6 positive EpSCs as well as native follicular EpSCs in the skin. Interestingly, differential Flii levels did not change the number of epithelial stem cells in unwounded mice epidermis. Noticeably, reduced Flii resulted in decreased expression of Lrig1, a positive regulator of stem cell quiescence, in the EpSCs adjacent to the wound. Consistently, K14 expression which marks the population of undifferentiated cells in the epidermis was also reduced in Flii+/- EpSCs regions. Additional proliferative PCNA and EGFR1 positive were identified in EpSCs zones adjacent to the wound in Flii+/- mice compared to WT and FliiTg/Tg counterparts. The nuclearization of β-catenin and expression of proteins involved in Wnt/b-catenin signalling including Axin2, Lgr6 & Flap2 in the EpSCs were found to be shifted towards Wnt activation in response to reduced Flii. Together, our data suggests that Flii may participate in epidermal stem cell activation during wound repair which hold promise for design of novel therapies for improved wound healing outcomes.

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Abstract No. P104

Global gene expression and in vitro chondrogenesis of immature non-MSC-like iPSC-derived mesenchymal progenitors

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Current in vitro chondrogenesis of induced pluripotent stem cells (iPSCs) lacks robustness and reproducibility. Standardly, iPSCs are first induced into mesenchymal progenitor cells (iMPCs), which are expanded before differentiation into chondrocytes. During expansion, iMPCs gain a mesenchymal surface marker profile and express CD105 and CD73 in passage 3, but not in p0. We here asked what global gene expression changes are connected with iMPC maturation during expansion and whether maturity is a necessary prerequisite for subsequent chondrogenesis.

Mesenchymal surface markers were detected in human iMPCs at p0 and p3 by flow cytometry. Global gene expression was profiled after p0, p1, and p3 with the Illumina Human Sentrix 12 Expression Beadarray. Chondrogenic differentiation capacity was tested in standard 3D micromass culture with TGF-beta with or without BMP-4 in chondrogenic medium after each passage.

Gene expression and gene set enrichment analyses revealed muscle-associated and mesenchymal migration markers (ACTA1, ACTA2, ACTC1, MYL7, and others) among the top down-regulated genes during iMPC maturation. Conversely, TGF-beta and BMP signaling-associated genes (ALK1, TGFBI, ENG, TGFBR2, BMPR2, and others) were enriched within the top up-regulated genes. iMPCs from all passages (p0-p3) successfully formed cartilage tissue and deposited collagen II and proteoglycans when treated with TGF-beta in chondrogenic medium. Interestingly, addition of BMP-4 strongly inhibited chondrogenesis of p0 iMPCs, but tended to improve cartilage formation of cells after p1, p2, and p3.

These data suggested that during expansion iMPCs turned away from an initial muscular cell fate and matured into mesenchymal cells with broad expression of TGF-beta/BMP signaling components. Surprisingly, mesenchymal maturity seemed not a necessary prerequisite for in vitro chondrogenesis and p0 cells were also inducible to form cartilage. However, for such immature cells, chondrogenic media should not contain BMP-4. Further studies are now needed to test efficiency and quality of cartilage formation by immature iMPCs.

Abstract No. P105

Differentiation-defective human iPS cells reveal strengths and weaknesses of the teratoma assay and alternative in vitro pluripotency assays

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For optimal use of human induced pluripotent stem cells (hiPSCs) it is essential to identify lines that are fully reprogrammed and of high quality with proven pluripotency in terms of differentiation. The ability to form teratomas in vivo is regarded as functional evidence of pluripotency for human pluripotent stem cells (hPSCs). Since the Teratoma assay is animal-dependent, laborious and only qualitative, there is an ongoing debate whether it is an acceptable tool. This has led to the development of an assay analyzing teratomas quantitatively (TeratoScore) as well as in vitro alternatives such as the PluriTest and the hPSC Scorecard. Here we compared normal hPSCs, hiPSCs with reactivated reprogramming transgenes and nullipotent human embryonal carcinoma (hEC) cells in these assays. Cells were cultured on Vitronectin in TESR-E8 media (hPSCs) or in DMEM/10% FCS (hEC cells). The quality of undifferentiated cells was analyzed by FACS for OCT3/4 as well as karyotyping. As assessed by immunohistochemistry and immunfluorescent staining the normal hPSCs gave rise to typical teratomas whereas the xenografts of the hEC cells and the hiPSCs with reactivated reprogramming transgenes were largely undifferentiated and malignant. TeratoScore confirmed typical teratomas and tumors lacking differentiation but was unable to identify partially differentiated tumors. The hPSC Scorecard assay confirmed the line-specific differentiation propensities in vitro. However, when undifferentiated cells were analysed with PluriTest only hEC cells were identified as abnormal whereas all other cell lines were undistinguishable and resembled normal hPSCs. Based on our results we propose PluriTest in combination with the hPSC Scorecard assay for characterization of pluripotency status and function of hPSCs used for in vitro disease modeling and drug testing. By contrast, the Teratoma assay is the only method which is able to determine whether hPSCs could potentially develop a malignant phenotype, one of the critical criteria for any clinical application.

Abstract No. P106

Genome-wide tracking of dCas-Dnmt3a footprints in mESCs

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Targeted editing of DNA methylation is of major interest and has recently become possible through combining genome-engineering tools, such as nuclease inactive dCas9, and the catalytic domain of the de novo DNA methyltransferases. In contrast to genetic editing with nuclease competent Cas9, tracing the activity of epigenome editing tools is challenging within a highly modified mammalian genome. Here, we took advantage of an engineered cell line with little background DNA methylation and find a surprisingly ubiquitous nuclear activity of dCas9 fused to the catalytic domain of Dnmt3a.

Abstract No. P107

Enhanced neurogenesis in degenerated hippocampi following pretreatment with miR-302/367 expressing lentiviral vector in mice

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Astrogliosis is the main landmark of neurodegenerative diseases. In vivo reprogramming of reactive astrocytes to functional neurons opened new horizons in regenerative medicine. However there is little evidence that show possible application of in vivo reprogramming approaches for enhancement of neurogenesis. Cluster miR-302/367 as embryonic stem cells specific microRNAs showed high capability in cell reprogramming. Here we show that application of lentiviral particles expressing cluster miR-302/367 along with systemic valproate enhanced the capability of mice brains for neurogenesis in CA3 area following kainic acid induced hippocampal neurodegeneration. Following pretreatment with miR-302/367 expressing viral particles and valproate, transduced cells showed neuroblast and mature neuron markers when neuronal loss was induced by kainic acid. Comparing the neuron counts in CA3 region also showed that neurogenesis was increased in CA3 region in animals which were pretreated with miR-302/367 vector and valproate, only in injected side of the brain. Our data suggest that targeted application of miR-302/367 expressing vector may enhance the capacity of hippocampus and other brain structures for regeneration following neuronal loss.

Abstract No. P108

Soft hydrogels support differentiation of induced pluripotent stem cells toward mesenchymal stromal cells

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Induced pluripotent stem cells (iPSCs) can be differentiated toward mesenchymal stromal cells (MSCs), but on epigenetic level this transition remains incomplete. Hydrogels provide a more physiological three-dimensional environment for *in vitro* cell culture than conventional tissue culture plastic (TCP). In this study, we followed the hypothesis that growth and differentiation of primary MSCs and of iPSC-derived MSCs (iMSCs) can be enhanced on hydrogels. To this end, we used a fibrin-based gel of human platelet lysate (hPL), consisting of the same components as the over-layered culture medium. hPL-gel supported growth of primary MSCs and facilitated more pronounced deposition of extracellular matrix (ECM) components than TCP. Furthermore, iPSCs were effectively differentiated toward MSC-like cells if cultured on hPL-gel, whereas they did not grow if seeded into hPL-gel. Unexpectedly, the differentiation process of iPSCs toward MSCs was hardly affected by the substrate: iMSCs that were either generated on TCP or hPL-gel revealed similar morphology, immunophenotype, differentiation potential, and gene expression profiles. Moreover, DNA methylation patterns were essentially identical in iMSCs generated on TCP or hPL-gel. Taken together, hPL-gel provides a powerful matrix to support growth, ECM deposition, and differentiation of MSCs and iMSCs. On the other hand, the soft hydrogels did not impact on cell fate decision during differentiation of iPSCs toward MSCs.

Abstract No. P109

Role of Smg6-mediated mRNA decay in neural stem cell differentiation

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Mammalian embryo development is a complex process from a single cell type to a multicomplex organism. Organogenesis follows cell proliferation and differentiation pathways. The pluripotent embryonic stem cells (ESCs) produce committed progenitors in three different germ layers resulting in all tissues and organs. Transcription factors, growth factors, epigenetic modulations and apoptotic pathways have been shown to regulate embryogenesis.

Smg6 encodes an RNA endonuclease, a component of nonsense-mediated mRNA decay (NMD), which functions in conducting post-transcriptional degradation of aberrant transcripts and thereby regulating cellular RNA repertoire. We showed previously that Smg6 complete deletion causes embryonic lethality by blocking ESCs differentiation program. Here we aim to study how Smg6-mediated NMD regulates neurogenesis from committed neuroprogenitors. We generated and characterized a mouse model in which Smg6 is deleted in the progenitors of the central nervous system (CNS) (Smg6-CNSΔ).

Smg6-CNSΔ mice showed perinatal lethality and a slight, however, significant decrease of the cortex size at E18.5. Analysis of the cortex revealed a decrease of the $Tbr2^+$ progenitor populations in subventricular zone starting already at E14.5. Although not significant, we detected a consistently marginal decrease of $Cux1^+$ late born neurons in the upper cortical layers II/III and IV. In contrast to wild type controls, the numbers of $Tbr1^+$ early born neurons in the layer VI of E18.5 cortex were higher suggesting that Smg6 ablation doesn't block neuronal differentiation but seems to affect the cell fate decision of neural stem cells (NSCs) at different embryonic stages.

In vitro studies revealed that Smg6 deletion in NSCs reduced neuronal differentiation associated with a defective neuronal maturation. Consistently, $Smg6^{-/-}$ primary neurons exhibited more apoptosis, also suggesting a role for Smg6-NMD in neuronal maturation.

Altogether, our results show that unlike ESCs, Smg6 is not essential for the general differentiation, but involved in fine-tuning of differentiation program of committed lineage progenitors.

Li et al, EMBO J. 2015; 34(12):1630-47.

Abstract No. P110

Development of an improved feeder-free culture system for mouse pluripotent stem cells

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We developed simplified new feeder free mouse ESC culture medium. In assessing and optimizing mESC culture, we focused on 3 major attributes: (1) cell growth & colony morphology, (2) maintenance of pluripotency, and (3) ability to support downstream differentiation. Incorporation of multi-parametric Design of Experiment (DOE) approaches with robust cellular assays and automated imaging & analysis enables us to test multiple components in parallel and helped identify optimal conditions through iterative experimental rounds. Taken together this work highlights both (a) our design philosophy for culture media development- identify key functional endpoints, develop or incorporate robust, scalable assays, and test a wide array of components and workflow parameters; and (b) our results to date with this new system.

Abstract No. P111

Quantitative assessment of human pluripotent stem cell differentiation potential

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Human pluripotent stem cells (hPSC) play an important role in disease modeling, drug discovery and cell therapy applications. Today, generation of hiPSC has become a standard procedure in stem cell laboratories. Resulting cell lines need to be characterized after reprogramming and maintenance culture and must fulfill certain criteria, such as characteristic stem cell morphology, long-term self-renewal, karyotypic stability, expression of a specific marker profile, and differentiation capacity into all three germ layers.

Differentiation capacity can be assessed in vivo using the Teratoma assay or in vitro using spontaneous or directed differentiation assays. Teratoma assays are hard to standardize and quantify, extremely costly and ethically controversial. Spontaneous differentiation assays in vitro are highly variable and also hard to read out. Evaluation of differentiation capacity is mostly done using qualitative immunocytochemistry since quantification requires sophisticated microscopy tools. Commonly used real-time PCR panels have the disadvantage of detecting only average gene expression values rather than revealing defined cell fates.

To overcome these constraints, we aimed at developing a standardized, quantifiable differentiation assay based on lineage specific, complete media which support directed 2D differentiation in all three germ layers within 7 days. The assay format allows quantitative flow cytometry analysis as well as immunocytochemistry assessment.

As proof of principle four hiPSC lines were differentiated repeatedly and analyzed by flow cytometry and immunocytochemistry. The quantitative, flow-based analysis confirmed reproducible differentiation properties of all four hiPSC lines into Ectoderm, Mesoderm and Endoderm. Importantly, the assay revealed subtle differences in their intrinsic propensity to give rise to cells of the three germ layers, illustrating a convenient way to assess the differentiation potential of freshly reprogrammed hiPSC as well as established hPSC lines.

Abstract No. P112

Development of novel E-cadherin binding peptides for the culture of pluripotent stem cells

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Introduction:
Embryonic stem cells (ESCs) are a useful model to study processes associated with pluripotency and differentiation. The Ward lab has previously shown that the cell surface protein E-cadherin regulates naïve pluripotency pathways in mouse (m)ESCs. Manipulation of E-cadherin protein in ESCs using exogenous peptides represents a useful method for studying pluripotency and differentiation pathway changes. Our lab has recently described several novel E-cadherin antagonising peptides: Peptide A (SWELYYPLRANL), Peptide B (S**A**ELYYPLRANL) and a putative agonising peptide: Peptide Z (SWELY**A**PLRANL) that exhibit specific alterations in naïve pluripotency pathways and gene transcript expression in mESCs.

Methods:
Peptide solubility was assessed in water and dimethyl sulfoxide (DMSO) in the stock solution and at the final working concentration within culture medium by centrifugation. Peptides were applied to mESCs at 0.25mM and 0.50mM concentrations. D3 mESCs were seeded into 6-well plates and peptide added daily at the relevant concentration for 5 days. Transcript expression assessed using quantitative polymerase chain reaction (qPCR).

Results:
Peptide Z was relatively insoluble in water and was found to be soluble in DMSO. Treatment of wtD3 cells with 0.5% v/v DMSO lead to significant cell death whereas 0.25% v/v DMSO exhibited less toxicity. Additionally, 0.25mM of Peptide A inhibited cell-cell contact in mESCs whereas Peptide B had no observable effect. By contrast, Peptide Z induced loss of cell-cell contact and led to reduced cell numbers compared to both Peptide A and B treatment. Quantitative PCR analysis revealed significant transcript expression alterations of the pluripotency markers.

Conclusion:
Alterations in transcript expression might not reflect the actual function of the peptides due to DMSO toxicity to the cells. As a result, DMSO is not suitable as a peptide solvent for use with mESCs. Therefore, water solubility of Peptide Z remains an issue and alternative methods for increasing solubility need to be investigated.

Hawkins, K., Mohamet, L., Ritson, S., Merry, C. L. & Ward, C. M. (2012). E-cadherin and, in its absence, N-cadherin promotes Nanog expression in mouse embryonic stem cells via STAT3 phosphorylation. Stem Cells, 30(9), 1842-51

Segal, J. M. & Ward, C. M. (2017). Novel peptides for deciphering structural and signaling functions of E-cadherin in mouse embryonic stem cells. Sci Rep, 7, 41827.

Abstract No. P113

Functions of mouse cortical long intervening ncRNAs in neuronal reprogramming of astrocytes

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The mammalian genome is profusely transcribed resulting in the generation of a variety of non-coding RNAs. A subclass of these, long non-coding RNAs (lncRNAs), has been implicated in regulating mammalian development including the central nervous system. One of the functions of lncRNAs exerts involvement in the recruitment of chromatin-modifying complexes through RNA-protein interactions and thereby regulates nearby coding-gene expression. In this study we have focussed on long intervening ncRNAs (lincRNAs) which are regulated during mouse cortical development in a similar manner as their nearest protein-coding gene. We identified significantly 344 upregulated lincRNAs in mouse embryonic cortex (analysed separately for expression in the ventricular zone, subventricular zone and cortical plate) compared to other tissues based on direct comparison of publically available RNA-sequencing data. We selected 6 lincRNAs, whose functions have not been studied. We first defined the respective orientations, full-length sequences, active promoter regions, transcribed exons, and precise neuroanatomical localizations. Some of them are differentially expressed during astroglia-to-neuron conversion following forced expression of Ascl1 or Neurog2 and might facilitate the cellular reprogramming. Intriguingly, knockdown of some of the lincRNAs in astrocytes affects neuronal reprogramming efficiency. Further functional studies will be carried out to better understand the physiological roles of these transcripts during the corticogenesis by utilizing in utero electroporation targeting lateral ventricle of mouse cortex.

Abstract No. P114

Development of a robust next generation feeder-free pluripotent stem cell medium

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Culture systems for pluripotent stem cell (PSC) expansion enable generation of a nearly unlimited pool of cells for downstream differentiation, disease modeling, drug discovery, and therapeutic applications. While a number of PSC feeder-free medium systems exist, there are many challenges encountered by stem cell scientists across the PSC workflow. Here we sought to improve the robustness and versatility of traditional PSC culture medium systems by utilizing several rounds of Definitive Screening and Custom Design DOEs to identify and optimize critical medium components. Through assessment of over 100 different formulations, an optimum medium composition was identified which provides compatibility across the PSC workflow from somatic cell reprogramming, PSC expansion, downstream differentiation, as well as providing support in gene editing applications. This system additionally provides versatility, allowing for every-other-day or weekend-free feed schedules and compatibility with a broad range of passaging reagents and matrices. We demonstrate that this system maintains normal PSC properties, including (1) expression of canonical pluripotency markers, including SOX2, SSEA4, Tra1-60, OCT4, and Nanog, (2) maintains trilineage differentiation potential, and (3) exhibit normal karyotype over long-term passaging. Together this system provides a robust next-generation stem cell medium system for today's PSC workflow needs.

Abstract No. P115

Standardized generation, purification and quality control of human pluripotent stem cell derived cardiomyocytes

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Fast and highly efficient generation of pure pluripotent stem cell derived cardiomyocytes (PSC-CMs) is a prerequisite for clinical applications, drug development and several research applications e.g. heart disease modeling. In recent years, various protocols to differentiate PSCs into cardiovascular cells such as cardiomyocytes (CMs) have been published. However, cardiovascular differentiations of human PSC cultures do not contain homogeneous cell populations, but are rather composed of a variety of CMs and non-CMs including different CM subtypes or subpopulations. The cell composition of each differentiation is currently depending on the stem cell clone, its passage, differentiation protocol used and additional experimental parameters. To circumvent these experimental variations and prepare for standardized processes suitable for automation and clinical scale up, we have established a new workflow ranging from controlled cardiac differentiation to CM harvesting, purification, analysis, replating and freezing. This work included the development of a robust, fast and highly reproducible differentiation protocol, yielding in cardiac differentiation efficiencies of 70% within less than 10 days of differentiation. To further purify PSC-derived CMs, we have developed a gentle harvesting method for PSC-derived cardiomyocyte monolayers and established both magnetic and flow cytometry-based purification protocols delivering PSC-CMs in purities of up to 97%. For quality control of differentiation and separation processes, we have engineered antibodies allowing for fast and easy characterization of PSC derived cardiomyocytes and their subpopulations in immunofluorescence and flow cytometry applications. Additionally, a novel cryopreservation method for PSC derived CMs allows for standardized freezing and thawing of PSC-CMs with high viabilities. Taken together, the newly established workflow solves several technical issues related to the generation of PSC-derived cardiomyocytes. In the next step, the process will be transferred into a scalable, automated closed system.

Abstract No. P116

The role of pluripotency in chimera formation between mouse or monkey iPS cells and porcine embryos

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The growth of human organs that are more or less entirely derived from human iPSCs in chimeric pigs, could be a new source for tissue therapy or organ replacement. In the present study, we analyzed the chimeric contribution of primed cynomolgous iPSCs and naïve mouse iPSCs to pig embryos.

In the first experiment, primed cynomolgous iPSCs transgenic with the Venus reporter (Venus cyiPSCs) were injected into porcine embryos. For embryo collection, donor sows were inseminated and 4 days later sacrificed. Embryos were injected with clusters of Venus cyiPSCs and transferred into two recipient sows and recollected after seven days. Out of 88 embryos 34 were recovered and 32 could be analysed on day 11. We could not detect any Venus cyiPSCs localised in the embryonic disc. Fifty three percent of the embryos showed only single Venus cyiPSCs in the trophectoderm. Thereafter, we analysed whether naïve stem cells can contribute to porcine embryos with higher efficiency and used Venus transgenic mouse iPSCs (Venus miPSCs). Donor sows were inseminated and 5 days later sacrificed. Embryos were injected with clusters of Venus miPSCs and transferred into two recipient sows. From 78 transferred embryos 50 (64%) were recollected and 40 could be analysed on day 11. Single Venus miPSCs were discovered in the trophectoderm of all embryos recovered from sow #823 (96 miPSCs per embryo) and from sow #827 (40 miPSCs per embryo). In two cases, Venus miPS cell clumps were localized in trophectoderm. Few embryos contained Venus miPS cell clumps in the porcine embryonic disc.

Here, we show that naïve miPSCs displayed a higher contribution to porcine embryos on day 11 than primed cyiPSCs. These results have led to the assumption that pluripotent state of cells used for the generation of interspecies chimera could be important for efficiency of chimera formation.

Abstract No. P117

EOMES is a context-dependent master regulator of cardiac induction in human ES cells

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Transcriptional master regulators are thought to induce specific cell lineages in gastrulation by orchestrating entire gene programmes. The T-box transcription factor EOMES is crucially required for the development of the heart – yet it is equally important for endoderm specification suggesting that it may act in a context-dependent manner. Using loss and gain-of-function approaches in human embryonic stem cells, we here uncover an unrecognised interplay between EOMES and the WNT signaling pathway in controlling cardiac induction. Dose-dependent EOMES induction alone could fully replace a cocktail of signaling molecules otherwise essential for the specification of cardiogenic mesoderm. Highly efficient cardiomyocyte programming by EOMES mechanistically involves autocrine activation of canonical WNT signaling via the WNT3 ligand, which necessitates a shutdown of this axis at a subsequent stage. Our findings provide novel insights into human germ layer induction and bear biotechnological potential for the robust production of cardiomyocytes from engineered stem cells.

J. Rao, M.J. Pfeiffer, S. Frank, K. Adachi, I. Piccini, R. Quaranta, M. Arauzo-Bravo, J. Schwarz, D. Schade, S. Leidel, H.R. Scholer, G. Seebohm, B. Greber, Cell Stem Cell, 18 (2016) 341-353.

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Abstract No. P118

Generation and screening of patient-derived induced pluripotent stem cells (iPS cells) obtained from schizophrenia patients

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Background and Aim
Schizophrenia is a frequent and severe neurodevelopmental psychiatric disease, but the aetology still remains mostly unknown. Copy number variations (CNVs) such as heterozygous deletions have been shown to increase the risk of schizophrenia, but the resulting effects on molecular and cellular mechanisms are poorly understood. The reprogramming of human somatic cells into induced pluripotent stem cells (iPS cells) provides an excellent approach to analyze disease mechanisms in affected neural cells. Our major goal is the identification of new and unknown Schizophrenia disease mechanisms.

Material and Methods
Reprogramming of B-lymphoblastoid cell lines (B-LCLs) was achieved using episomal plasmid vectors. Pluripotency of the generated iPS cells was verified by positive alkaline phosphatase (AP) staining and immune fluorescence (IF) staining of essential pluripotency factors. Patient-specific iPS cells were characterized by spontaneous differentiation and directed neuronal induction. Functional tests of obtained cortical neurons were performed by patch-clamp analysis.

Results and Conclusions
We have established a disease-specific in vitro model based on the reprogramming of B-LCLs for the analysis of schizophrenia. Morphology, growth characteristics, expression analysis, AP staining, IF staining, and the spontaneous differentiation verified successful generation of patient-derived iPS cells. The differentiation of iPS cells into patient-specific neural progenitors was characterized and quantified by the expression of crucial developmental marker genes.

In conclusion, successfully generated of patient-specific iPS cells provide a powerful tool for the functional analysis of CNVs associated with Schizophrenia.

Abstract No. P119

Reprogramming to pluripotency neither requires a transition through a primitive streak-like state nor follows reverse embryogenesis

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presented by *Meike Hohwieler*

Pluripotency can be induced *in vitro* from adult somatic mammalian cells by enforced expression of defined transcription factors regulating and initiating the pluripotency network. Despite the substantial advances over the last decade to improve the efficiency and understand the process of direct reprogramming, exact mechanisms underlying the conversion into the pluripotent stem cell state are still vaguely understood. Several studies suggested that induced pluripotency follows reversed embryonic development. For somatic cells of mesodermal and endodermal origin that would require the transition through a primitive-streak like state. We analyzed reprogramming in human and mouse cells of mesodermal as well as ectodermal origin by thorough marker-gene analyses in combination with genetic reporters and stable fate-labeling. We unambiguously demonstrate that induced pluripotency is not dependent on a transient primitive streak-like stage and thus does not represent reversal of embryonic mesendoderm development *in vivo*.

Abstract No. P120

Pluripotency induced by small molecules: *'Chemical impact on stem cell research'*

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Nucleic reprogramming from terminally differentiated somatic cells into induced pluripotent stem cells (iPSCs) achieved by ectopic expression of a core of transcription factors, provides a promising source for autologous organ transplantation, drug discovery and disease modeling, opening a new era in the field of regenerative medicine. In comparison to somatic cell nuclear trans¬fer, reprogramming of human fibroblasts is a time-consuming and inefficient process. Moreover, heterogeneity of resulting iPSCs often in company with random mutations, appearing during the reprogramming and cumulated in the maintenance, raised safety concerns of clinical application. In comparison to genetic manipulation, small molecules show distinct advantages in the application and control and thereby have been intensively investigated.

In an effort to find new chemical structures able to induce pluripotency, we performed high throughput screenings (HTSs) from a library containing ~250 000 chemicals based on cellular luciferase reporter assays and identified several hundreds of leading compounds for each reprogramming transcription factor. We are chemically modifying the leading structures and studying the structure-activity relationship. As results, we discovered novel promising compounds able to enhance human iPSCs viability by single cell expansion and support reprogramming resistant fibroblasts via epigenetic modulation, or replace Oct4 to generate hiPSCs probably due to activation of CDH1 and related-gene expression, or partially reprogram human fibroblasts to hiPSCs.

Abstract No. P121

Human L1 integrome libraries uncover insertion site preferences of reprogramming-triggered mutagenic L1 mobilization events in pluripotent stem cells

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Human induced pluripotent stem cells (hiPSCs) are used for disease modeling, drug discovery, and hold great promise for substitutive and regenerative cell therapies. Recently, we demonstrated that reprogramming-induced epigenome remodeling in hiPSCs resulted in the mobilization of endogenous retrotransposons LINE-1 [L1], Alu and SVA. Six out of eleven endogenous de novo retrotransposition events inserted into introns of protein-coding host genes. We showed that intronic L1 de novo insertions that occurred during reprogramming or hiPSC cultivation can interfere with host gene expression in hiPSCs which could affect biosafety of hiPSC-derived cell therapies [1].

Here, we report that reprogramming factors KLF4 and Nanog directly activate endogenous L1 promoters harbouring KLF4 and Nanog consensus binding motifs and thereby upregulate functional L1 expression and mobilization during the reprogramming process. To evaluate the potential extent of genomic destabilization caused by L1-mediated mobilization and identify potential L1 integration site preferences that could affect host gene function in hiPSCs and hESCs, we next generated marked L1 de novo insertion site libraries from 8 different pluripotent stem cell lines to establish genome-wide insertion site profiles. Analysis of 2280 marked L1 de novo insertions that occurred during cultivation of human pluripotent stem cells (hPSCs) uncovered that ~52% of all insertions accumulated in introns and exons of host genes, including 76 cancer-related genes. Data suggest that L1 retrotransposition events in hPSCs have an insertional bias for DNaseI-hypersensitive sites, active promoters and transcription start sites and are mediated by an interaction between host-encoded factors and L1 proteins. Considering that the latter insertions as well as intronic and exonic L1 insertions were demonstrated to affect host gene expression, these mutagenic L1-mediated retrotransposition events may affect the potential use of hiPSCs in cell therapy by increased tumorigenicity risk, their limited differentiation capacity, and potential functional deficiencies in the differentiated cells.

[1] Klawitter et al. (2016) Reprogramming triggers endogenous L1 and Alu retrotransposition in human induced pluripotent stem cells. Nat Commun. 7:10286

Funding by DFG Grants SCHU1014/8-1 and MA2331/11-1

Abstract No. P122

A stably self-renewing adult blood-derived induced neural stem cell exhibiting patternability and epigenetic rejuvenation

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Recent reports suggest that induced neurons (iNs), but not induced pluripotent stem cell (iPSC)-derived neurons largely preserve age-associated traits. Here we report on the extent of preserved epigenetic aging signatures in directly converted induced neural stem cells (iNSCs). Employing restricted and integration-free expression of SOX2 and c-MYC we generated a fully functional, bona fide NSC population from adult blood cells that remains highly responsive to regional patterning cues. The neuronal and glial differentiation potential of these iNSCs is also preserved upon transplantation into the adult mouse brain. Interestingly, newly converted low passage iNSCs display a profound loss of age-related DNA methylation signatures, which further erodes across extended passaging, thereby approximating the DNA methylation age of isogenic iPSC-derived neural precursors. In line with this epigenetic rejuvenation, long-term propagated iNSCs, when compared to isogenic iPSC-derived NSCs, do not show enhanced age-associated cellular hallmarks such as impaired autophagy activity or nuclear lamina disorganization. Despite loss of age-associated signatures, iNSCs lend themselves to modeling phenotypes of late-onset neurological disorders such as protein aggregation in Machado-Joseph disease. Our findings argue against an extensive preservation of age-related epigenetic alterations in iNSCs, which could make this cell population an interesting resource for both, disease modeling and transplant-based regeneration.

Abstract No. P123

A new synthetic ready-to-use FN1 motifs surface for human induced pluripotent stem cell expansion in an animal-component-free culture system

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Thanks to their extensive *in vitro* self-renewal property and their large differentiation potential, human pluripotent stem cells (hPSCs), constitute an exciting tool in research fields as regenerative medicine, pharmaceutical applications as well as disease modeling. hPSC expansion conditions including growth surface and culture medium have progressively evolved in the last decade from the traditional mouse embryonic fibroblast (MEF) feeder layer-based culture system towards more defined feeder-free cell culture systems. Optimal hPSC culture systems must combine a completely defined and animal-component-free composition and high consistency while ensuring robust pluripotent stem cell proliferation, routinely characterized by cell morphology, key pluripotency marker expression, genetic stability and differentiation potential maintenance. Based on a proprietary coating technology, a new ready-to-use surface, made up of synthetic fibronectin-derived motifs, was specifically designed to mimic the cell attachment site of native extracellular matrix proteins. Used in combination with well-defined culture medium and dissociation solution, this fully synthetic surface represents an effective animal- and human-component-free alternative to the conventional feeder layer-based culture system and to other biological coating-dependent hPSC culture systems. The present experimental work demonstrates the suitability of this ready-to-use surface for the long-term expansion of undifferentiated human induced pluripotent stem cells (hiPSCs). Throughout 25 successive passages cultured on this surface, hiPSCs maintain their typical cell morphology, a stable doubling time and karyotype as well as pluripotent marker expression. The *in vitro* trilineage differentiation capacity confirms finally the maintenance of their functional pluripotency. With its unique properties, this surface combines convenience with reliable high-quality hPSC performances.

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Abstract No. P124

Massive single cell targeted transcriptomics reveals subpopulations of human pluripotent stem cells

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Transcriptional heterogeneity among seemingly identical cells underlies tissue development, homoeostasis, and disease. High-throughput analysis of single cells is thus required to discover cell type compositions and cell-intrinsic gene networks. Different methods have been developed to dissect the single cell transcriptome, but majority of them are limited by relatively low sample numbers, ranging from dozens to hundreds, for meaningful representation of gene networks that govern cell fate. Targeted approaches can increase the sequencing coverage by lowering the number of analyzed genes, but current methods are not compatible with gene expression measurements of single cells, and are costly when applied to hundreds/thousands of samples. We developed a technique that removes these bottlenecks by a novel method enabling barcoding of gene-specific primer sets. The method is coined Barcode Assembly foR Targeted sequencing, BART-seq. We applied BART-seq to analyze thousands of single human pluripotent stem cells (hPSCs). We found differences in population distributions between hPSCs propagated in different culture media with respect to a pluripotency gene panel, which were averaged-out in bulk cell analysis. Importantly, we discovered a bimodal distribution of the single hPSCs in serum-free defined medium (mTeSR), which consisted of a *SOX2*-low population and a population with higher expression levels of the gene panel. Because an increase of pluripotency genes is implicated in transition to naïve state of hPSCs, we hypothesize that the mTeSR medium might poise the cells for naïve state. Additional variances of cell cycle genes *CCND1* and *CCNE1* were observed among single cells based on the culture media. Taken together, we developed BART-seq, a targeted cost-effective method that detects transcripts in numerous single cells across the entire physiological dynamic range of gene expression, an advance that will delineate cell states and gene networks in high resolution.

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Abstract No. P125

Effect of caloric restriction on hematopoietic stem and progenitor cells ageing

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Dietary status of organism is one of the essential factors that affect its health status and disease progression during ageing. It has been shown that caloric restriction improves the health status and delays the onset of age-related pathogenesis (Mihaylova, Sabatini, & Yilamz, 2014) . However, this effect of caloric restriction varies among species. On stem cell level it has been shown that caloric restriction preserves the functionality of stem cells in multiple tissues. In hematopoietic system we previously showed that short-term (1month) to intermediate-term (9 months) caloric restriction (CR) reduced age-associated increase in Hematopoietic stem cells (HSCs) number and improved the repopulation capacity of HSCs after transplantation. However, HSCs treated with caloric restriction have impaired differentiation capacity toward lymphoid progenitors. This resulted in increased susceptibility to bacterial infection (Tang et.al 2016). In our current study we addressed the effect of long term- caloric restriction on hematopoietic stem and progenitor cells (HSPCs). We observed that long-term caloric restriction caused a high decrease in the frequency of Multi-potent (MPP) and Lymphoid- primed progenitors (LMPP) in bone marrow of CR-treated. However, it didn't inhibit the age-associated increase in HSCs. Our results suggest that long-term caloric restriction cannot overcome age –associated changes that affect the ageing of HSCs. This might indicate that there are molecular alterations that happen during aging which account to the difference between long and short-term caloric restriction. They also indicates that caloric restriction has an additional influence on the ageing of Multi-potent progenitors. Our current focus is to delineate the molecular changes that mediate these effects and to study the functionality of HSPCs exposed to Long-term CR. Also we are interested to study the effect of exposure of aged mice to short period of caloric restriction.

Abstract No. P126

Dynamics of hematopoietic reconstitution: young versus old grafts in elderly recipients

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Introduction:
Human stem cell grafts are usually enriched for CD34 expression, even though it is well known that this marker is also frequently expressed on various progenitors. Thus, the distribution and influence of specific subsets within grafts in hematopoietic reconstitution still remains unknown. We took advantage of our coloured genetic barcode system to analyse the influence and clonal contribution of subpopulations within a murine model.

Methods:
We sorted common lymphoid, myeloid and multipotent progenitors (CLP, CMP, MPP), and stem cells (HSCs) from young (8 weeks) or old (18 months) murine donors. After lentiviral transduction with our barcoded vectors, populations were mixed and transplanted into, lethally irradiated old recipients (approx. 2x104 per HSC, MPP and CLP subset and up to ~150.000 CMPs per animal). 7, 21, 57 and 112 days later, hematopoietic organs were analysed via FACS and genomic DNA extracted for barcode analysis (ongoing).

Results:
FACS analysis (d7) shows contribution from three populations (no CLPs detectable). CMPs disappear after d21. In both groups, the contribution of MPP progeny in spleen and BM seems stronger at d21 compared to HSCs. Contribution of young HSCs equal or exceeds MPPs at d57, an effect not observed for aged HSCs. FACS analysis of selected myeloid and lymphoid subsets revealed similar patterns. On d21, we detected low marking for lymphoid populations arising from MPPs, on d57 HSC-descendent lymphoid cells were detectable. Contribution from MPPs and HSCs to CD11b+ myeloid populations was clearly detectable at d21, with a higher percentage for MPP progeny.

Conclusions:
This ongoing study allows for clonal tracking of four different subpopulations in parallel. Further analysis of the barcodes will also reveal the clonal composition of the blood, which then can be traced back to the originally transduced stem cell/progenitor population.

Abstract No. P127

Enhanced ex vivo erythropoiesis from hiPSCs by a simplified culture system

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To date, ex vivo generation of red blood cells (RBCs) from human induced pluripotent stem cells (iPSCs) is time- and cost- intensive due to complex cell culture systems and a high amount of cytokines. Furthermore, ex vivo erythropoiesis from iPSCs fails in terms of proliferation, hemoglobin switching and enucleation. Underlying reasons might be a rather primitive than definitive phenotype of iPSC-derived erythroid cells or a bypass of the high proliferative erythroid progenitor cell stage.

Here we describe a simplified ex vivo erythropoiesis assay, which is based on our previous work, focusing on the generation of myeloid cells from hiPSCs (Lachmann et al., Stem Cell Reports 2015).

Human iPSC-derived embryoid bodies (EBs) are cultured in a defined medium with minimal cytokine support (SCF, EPO, IL-3). Furthermore, EBs are allowed to adhere to the plastic surface and to develop a surrounding "stromal layer". After 3-4 weeks, supernatants containing CD43+ hematopoietic cells (~95%, n=4) can be harvested for several times (3-4 weeks). Collected CD43+ cells show a high colony-forming potential with the predominance of BFU-Es. During further ex-vivo erythropoiesis, CD43+ cells mature into a homogenous population of GPA+ erythroid cells (98% GPA+) of which near 50% undergo enucleation (n=4). This is in contrast to previous reports describing enucleation rates below 20% and low colony forming potential.

We developed an innovative erythroid differentiation protocol that allows for the generation of erythroid cells with near 50% enucleation over a prolonged time period. In comparison with other protocols in the field, our protocol combines simplified culture conditions, minimal cytokine support and a high output of terminal differentiated erythroid cells. These advantages might in part be explained by simulating the physiological niche due to the formation of a stromal cell compartment during EB differentiation.

Abstract No. P128

Differential glucose requirements of human hematopoietic lineages

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Hematopoiesis is organised spatially within the bone marrow with erythroid and myeloid lineages being produced in distinct regions. However, it is still unclear how the metabolic microenvironment of bone marrow is organised and whether different metabolic conditions influence survival and/or proliferation of specific lineages.

Although UCB CD34+ cells are unable to initiate colony formation in the complete absence of glucose, we show that a starting concentration of just 0.1mM is sufficient to generate normal, large colonies over 14 days. Accordingly, primary CD34+ cells induce gluconeogenesis genes in short term culture, while a perfusion-type colony assay shows that it is sufficient to supply very low levels of glucose for the first 3 days of colony initiation, subsequent growth being glucose-independent. At least some hematopoietic progenitor cells can therefore undergo extensive proliferation using glucose generated by gluconeogenesis.

FACS and RT-PCR analysis of cells recovered from perfusion-type colony assays show that very low glucose levels are sufficient to support the expansion of erythroid progenitors and the production of both eosinophils and basophils, while erythroid maturation and the generation of neutrophils are inhibited. This pattern of lineage and stage-specific glucose requirement suggests that glucose metabolism is organised spatially and perhaps even temporally in the marrow to optimise substrate usage. Interestingly, glucose independent growth appears to require a 3D environment and is not seen in liquid differentiation cultures.

Abstract No. P129

Targeting telomerase in human acute myeloid leukemia (AML)

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Replicative immortality depends on telomerase activation in the majority of cancers including acute myeloid leukemia (AML). AML is an aggressive and rapidly lethal blood cancer maintained by rare populations of leukemic stem cells (LSCs). We have previously shown that targeting telomerase eradicates LSCs in AML. Genetic depletion of Terc in retroviral mouse AML models induces cell cycle arrest and apoptosis of LSCs via p53-dependent mechanisms. Inactivation of telomerase in murine AML leads to widespread transcriptional changes that also predict outcome after chemotherapy in human AML.

Here we investigated the efficacy of imetelstat, a covalently lipidated 13-mer oligonucleotide that competitively inhibits telomerase activity, in human AML using a randomized trial in patient-derived xenografts (PDX).

To establish an AML PDX cohort, primary bone marrow (BM) or peripheral blood (PB) samples from 15 AML patients were transplanted into NOD.Cg Prkdcscid Il2rgtm1Wjl Tg (CMV-IL3,CSF2,KITLG)1Eav/Mlo (NSGS; n=12 per AML patient sample). Engraftment was defined by reconstitution of BM and spleen with CD33+ donor cells, PB circulating blasts, anemia or thrombocytopenia. For each experiment, mice were randomized and treated with imetelstat or control. AML patient samples were divided into 2 groups based on survival outcomes, “Sustained responders” (60%) or “Poor responders” (40%). Sustained responders were enriched for favorable cytogenetic risk and molecular signatures associated with DNA damage, cell cycle regulation and apoptosis. Poor responders showed a mutational profile of genes regulating growth factor independence and evasion of apoptosis. Imetelstat response was associated with induction of γ-H2AX and loss of LSC quiescence. The effects on normal human hematopoiesis were modest and predominantly seen in the B-lymphocyte lineage with relative preservation of myeloid and stem cell populations.

This work provides a preclinical template to understand the mechanism of in vivo response to telomerase inhibition and to guide the development of future planned clinical trials of imetelstat in AML.

Abstract No. P130

Vitamin A/ retinoic acid signaling regulates dormant hematopoietic stem cells

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Rare dormant hematopoietic stem cells (dHSCs) harboring the highest long-term reconstitution capacity define the top of the hematopoietic system. However, the molecular identity of dHSCs and immediate progeny as well as the mechanism regulating maintenance and exit from dormancy remain unknown. We now show by single-cell RNA-seq analysis that no discrete cell types exist immediately downstream of dHSCs. Instead, the transition from dormancy towards cell cycle entry is achieved by a continuous and coordinated up-regulation of all major biosynthetic processes including c-Myc expression, protein translation and levels of ROS. Using a novel transgenic reporter mouse that specifically labels dHSCs, we show that these processes are inhibited by vitamin A/ retinoic acid signaling which maintains dHSC properties and restricts their exit from dormancy upon stress stimulation. Importantly, a diet lacking vitamin A impairs stem cell homeostasis, highlighting the major impact of dietary habits on the correct function of the hematopoietic system.

Abstract No. P131

Reconstruction of the hematopoietic stem cell niche by creating a 3D-structure cultivation system

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Hematopoietic stem cells (HSCs) are capable to generate the whole range of human blood cells. Within the human body they reside in small numbers in a special location so called stem cell niche that is responsible for the maintenance of the stem cell state. As soon as HSCs enter the peripheral blood system they start to differentiate and lose their specific abilities essential for their therapeutic usage.

Our aim is the characterization of incubation conditions necessary for the development of an *ex vivo* culture and expansion system of previously isolated human CD34+ HSCs from peripheral blood of various healthy donors for a prospective application in stem cell based therapy. In collaboration with the Technical University of Ilmenau nanotechnologically produced 3D-structures, the interaction with stem cell related cells and reduced oxygen concentrations are examined.

The 3D-scaffold design is based on a human long bone cross section as a representative of the bone marrow, the natural HSC niche. Therefore, cells are incubated on the scaffolds for 14 days using a medium with a previously established composition of cytokines and promoting factors such as valproic acid. To evaluate HSC expansion and the maintenance of the stem cell state we determined the cell number, the expression of several stem cell surface markers such as CD34 or CD133, examined the cell vitality and applied colony forming unit (CFU) assays.

We found first evidence how to create a stem cell appropriate environment in vitro. We were able to observe a positive effect on CD34+ cell expansion for several donors when culturing HSCs on 3D-structures. Additionally, a co-culture system for HSCs and stem cell related cells in combination with 3D-structures was established. Other parameters, such as low surrounding oxygen concentration as well as other 3D-scaffold materials and structures, are under investigation.

Abstract No. P132

Epi-Blood-Count: Leukocyte differential counts based on DNA methylation levels at individual CpG sites

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The cellular composition of blood is usually determined based on histomorphological analysis and flow cytometric measurements, including immunophenotypic classification of lymphocyte subsets. Alternatively, leukocyte differential counts (LDCs) can be estimated with deconvolution algorithms based on genome-wide DNA methylation (DNAm) profiles. However, deconvolution analysis of microarray data or deep sequencing is not applicable for blood counts in clinical routine. Here, we report that even DNAm levels at individual CG dinucleotides (CpGs) facilitate relative quantification of granulocytes, CD4+ T cells, CD8+ T cells, B cells, NK cells, and monocytes. Candidate CpGs were selected from genome-wide DNAm profiles of purified leukocyte subsets. DNAm levels were analyzed by pyrosequencing in 60 whole blood samples and implemented into a non-negative least-squares approach. This method was subsequently applied to 193 independent blood samples to compare results with manual blood counts, automated analyzers, immunophenotypic analysis, and deconvolution algorithms for DNAm profiles. Site-specific analysis of DNAm levels provides similar precision as conventional LDC methods, with Pearson correlation coefficient R = 0.98 across all cell types and a mean absolute deviation (MAD) of only 3.1%. Furthermore, we describe a new method for absolute quantification of cell numbers that is based on a non-methylated reference DNA. Our “Epi-Blood-Count” is applicable to frozen samples, it yields robust results even after long-term storage, and analysis requires only very small volumes of blood. This approach may revolutionize leukocyte differential counts and facilitate more standardized and cost effective analysis of blood counts in clinical application.

Abstract No. P133

Functional identification of longevity and aging associated genes that control self-renewal and function of hematopoietic stem cells during aging

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Aging is a complex process, which is controlled by various genetic pathways. Gerontogenes are genetic elements, which are associated with the regulation of aging and life span. In the past gerontogenes were mainly identified and analyzed in vertebrates such as worms, fly and yeast; despite proven life-extension properties their function remains largely unknown in mammals, therefore we are utilizing the murine model system to analyze the function of gerontogenes during stem cell aging.

Hematopoietic stem cells (HSCs) are able to restore the entire hematopoietic system in vertebrates and offer constant immune protection. During aging, the self-renewal capacity of HSCs increases, while also accompanied by a skewing towards myeloid differentiation. To what extent gerontogenes play a role in HSC aging remains unknown. In order to address this, 1075 gerontogenes from different species were collected from various publications and databases. These genes served as a basis for the construction of a short hairpin RNA (shRNA) library in order to target the identified gerontogenes in transplanted murine HSCs.

After two rounds of transplantation, the shRNAs targeting six different ribosomal protein genes were lost. This indicated, the ribosomal protein genes are essential for hematopoietic stem cells.

The following in vitro experiments showed, that knockdown of these ribosomal protein genes inhibits HSCs proliferation and differentiation, meanwhile induce apoptosis. Proteomic analysis and functional experiments are planned to verify the function of the six candidates as actual genetic factors controlling aging of murine HSCs.

Abstract No. P134

Immortalization of erythroid precursor cells by c-myc and BCL-XL using antibiotic inducible lentiviral vector system.

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Blood pharming using embryonic, bone marrow or induced stem cells represents a new and fascinating option to warrant the blood supply in the future. However, little is known about the mechanisms and factors that would allow the most efficient in vitro generation of enucleated blood cells. Since the differentiation from stem cells and/or IPS cells into the erythroid lineage would involve considerable amount of cytokines and purification steps, we aimed to immortalized cells already determined to the erythroid lineage. Using an antibiotic inducible lentiviral vector system we transduced erythroid precursor cells derived from CD34+ stem cells from mobilized peripheral blood (blood group O RhD) with five different oncogenes (c-myc, BCL-XL, SV40 LargeT, Bmi-1, LhX-2) and combinations of each two. 48h after transduction gene expression was induced by the addition of doxycycline. In the absence of doxycycline oncogene expression is turned-off. Using single cell printing technology single cell colonies were obtained, expanded and analysed. The transduction of each oncogene alone does not lead to immortalization of the cells. Only combined transduction of c-myc and BCL-XL leads to immortalization of erythroid precursor cells and continuously proliferation for more than 2 years. Furthermore we obtained more than 270 single cell colonies from these cells with different morphology and expression pattern for different surface marker (CD71, CD45, CD34, CD235a). After removal of doxycycline a part of these cells were differentiated into normoblasts and reticulocytes within 7 days with an enucleation rate of 0.5 – 1%. Immortalization of erythroid precursor cells is feasible using stable inducible lentiviral gene transfer for c-myc and BCL-XL. Withdrawal of dox allows differentiation in to further differentiated red blood cells. The conditions to obtain high yields of enucleated red blood cells, which then would also not harbour the risk attributed to the proto-oncogenes, has to be further optimized.

Abstract No. P135

AGEs in hematopoietic development

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Advanced glycation end products (AGEs) are posttranslational protein modifications that emerge from glycation, a non-enzymatic reaction between the protein and a sugar. AGEs are reported to have negative effects on proteins, cells, tissues and organisms and are linked to several age-related diseases such as Glycation is a non-enzymatic reaction between a reducing sugar and a protein, which leads to the formation of advanced glycation end products (AGEs). These posttranslational modifications are often seen as protein damage as they can lead to aggregation, degradation or loss of function. AGEs are linked to several age-related pathologies such as Alzheimer's disease, diabetes, inflammation signaling and cardiovascular diseases among others. We investigate AGEs in the murine hematopoietic system, a tissue strongly relying on stem cell function and differentiation which is compromised in older individuals. We find that throughout hematopoietic development, populations show different AGE levels, depending on their lineage and differentiation stage. We also see that depending on their differentiation commitment, cells actively regulate their enzymatic capacities to deal with AGE formation. Lastly we see a correlation between AGE formation and differentiation of hematopoietic stem and progenitor cells. These early data point towards a relationship between AGEs and differentiation within the hematopoietic system. The question to be answered is if differentiation drives AGE levels or if AGEs could actually contribute to differentiation.

Abstract No. P136

Epigenetic control of hematopoiesis by RUNX1/PRMT6

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Hematopoietic differentiation is driven by transcription factors, which orchestrate a fine tuned transcriptional network. At bipotential branching points lineage decisions are made, where key transcription factors initiate cell type specific gene expression programs. These programs are stabilized by the epigenetic activity of recruited chromatin modifying cofactors. An example gives the association of the transcription factor RUNX1 with the protein arginine methyltransferase 6 (PRMT6). We could show that PRMT6 is differentially recruited to RUNX1 target genes during differentiation. Here PRMT6 mediates a repressive chromatin environment by establishment of a histone modification pattern with high H3R2me2a and low H3K4me3. Interestingly, the repressive RUNX1/PRMT6 complex is formed cell-type and promoter dependent. This way RUNX1 is able to initiate a specific cell type dependent gene expression program, while actively repressing the competing program. Importantly, inhibition of PRMT6 by shRNA or small molecule inhibitor leads to growth inhibition and a promotion of erythropoiesis. Our data reveal that the RUNX1/PRMT6 axis could be a molecular target to facilitate enhanced erythropoiesis for regenerative medicine and may suppress cell growth in a therapeutic setting for the treatment of leukemia.

Kuvardina, O.N.; Herglotz, J.; ..... Lausen, J.: RUNX1 represses the erythroid gene expression program during megakaryocytic differentiation. Blood, 125(23): 3570-9. 2015.

Abstract No. P137

Sca-1 expression level identifies quiescent hematopoietic stem and progenitor cells

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The hematopoietic system is a highly regenerative system responsible for the continuous production of all different lineages of mature blood cells. This continuous blood supply is known to be driven by the proliferation and differentiation of the hematopoietic stem cells (HSCs) residing at the top of the hierarchy of the hematopoietic system, which then give rise to multipotent progenitor populations, lineage committed progenitors and finally terminally differentiated blood cells. Early hematopoietic stem and progenitor cells (HSPCs) express the surface molecule Stem cell antigen-1 (Sca-1/LY6A), a glycosyl phosphatidylinositol-anchored cell surface protein, and constitute the Lineage$^-$Sca-1$^+$Kit$^+$ (LSK) population of the murine bone marrow. Analysis of Histone-2B red fluorescent fusion protein (H2B-RFP) retention revealed a positive correlation between Sca-1 expression and quiescence of HSCs as well as hematopoietic progenitor cells. This finding was further confirmed by analyzing Ki67-RFP cell cycle reporter mice, in which actively cycling HSPCs displayed lower levels of Sca-1 expression while their quiescent counterparts showed higher Sca-1 expression. Purification and transplantation of HSPCs according to their Sca-1 expression level revealed higher repopulation activity of various Sca-1^{hi} LSK subpopulations. Although the function of Sca-1 is poorly understood, it is known to be strongly up-regulated by type I interferon (IFN). We show however that the link between high Sca-1 expression and quiescence as well as repopulation activity among HSPCs is type I interferon independent. Our finding that quiescent subpopulations of HSPCs are identified by differential Sca-1 expression easily allows for refined purification and analysis strategies. We are currently investigating the lineal relationship and differentiation pattern of LSK subpopulation with diverse Sca-1 expression by lineage tracing experiments.

Morcos, M.N., Schoedel, K.B., Hoppe, A., Behrendt, R., Basak, O., Clevers, H.C., Roers, A. and Gerbaulet, A., 2017. SCA-1 Expression Level Identifies Quiescent Hematopoietic Stem and Progenitor Cells. Stem Cell Reports, 8(6), pp.1472-1478.

Abstract No. P138

The metabolic environment of hematopoietic progenitor cells influences their signaling response to hematopoietic growth factors

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Haematopoietic bone marrow is functionally divided into areas supporting the maintenance and self-renewal of stem cells on the one hand and the proliferative differentiation of progenitor cells on the other, with erythropoiesis and granulopoiesis occupying most of the space. We propose that this functional organization is determined not just by the signaling environment provided by the local presentation of growth factors, but also by the metabolic environment, which is likely to vary extensively with the position relative to arterioles and sinusoids. A key issue here is whether or not variations in the metabolic environment of a progenitor cell alter the response of that cell to growth factors.

To investigate this, we have used Phosflow analysis of the multipotent progenitor FDCP-Mix cells to compare the short term response of the RAS/MAPK and JAK/STAT pathways to growth factor signaling at 1mM and 5mM glucose. We find that a simple increase in glucose concentration within this physiological range increases the degree of growth factor-dependent ERK1/2 phosphorylation while decreasing that of STAT5 phosphorylation.

We also confirmed that the FDCP-Mix cells undergo myeloid differentiation in the absence of myeloid growth factors in response to nicotinamide, as has been previously reported for primary CD34+ cells. In the long term, this has been shown to involve a sirtuin-dependent induction of G-CSF and G-CSFR expression. However, we find that short term exposure to nicotinamide is also sufficient to increase the ERK1/2 signal in the presence of 5mM glucose, suggesting that NAM-derived NAD may also be acting directly to increase glycolysis and support the G-CSF response.

Taken together, these results suggest that the relative activities of the RAS/MAPK and JAK/STAT pathways may be influenced by glycolytic flux over a physiological range of glucose concentration, providing an example of how the metabolic environment could influence growth factor responses during haematopoiesis.

Abstract No. P139

Conditional p21 knockout mice: quiescence of HSCs and liver regeneration

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p21^{Waf1}, a well-known cell cycle inhibitor and senescence inducer, is now appreciated as a much more complex and broader regulator of different cellular programs that acts not always advantageously for the organism's health. Importantly, there are many *in vitro* and *in vivo* studies demonstrating that the deletion of p21 could be beneficial in anti-aging, pro-regeneration and even anti-cancer medicine. That is why experiments on inducible conditional p21 knockout mice seem to represent a particular interest. Such mouse was created in our lab for the first time, and due to the chosen Cre recombinase under *Mx* promoter, we were able to induce p21 deletion in liver and bone marrow. As initial experiments on this system, we investigated an effect of p21 loss on aged liver regeneration after 2/3 partial hepatectomy and on maintaining of quiescent status, proliferation and differentiation of HSCs (hematopoietic stem cells). Summarizing, we developed a mouse model, which can be used for answering intriguing questions of p21 role and its downregulation in cancer, aging and regeneration studies.

Abstract No. P140

Molecular immortalization signature of murine HSPC

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Safety assessment of integrating viral vectors is an integral part of the preclinical work package for new gene therapy trials. The number of reliable assays to screen for the effects of insertional mutagenesis in vitro is very limited, which primarily focus on the detection of integration sites. To analyze the underlying functional consequences of insertional transformation, we recently developed the surrogate assay for genotoxicity assessment (SAGA). This test determines a gene expression signature, which documents deregulation of genes in the course of in vitro immortalization of murine hematopoietic stem and progenitor cells (HSPC). We performed a series of microarray experiments (n=116) and RNA-sequencing runs to study genes involved in the transformation of HSPC in vitro. We used several different machine learning algorithms to determine a specific core set of genes, allowing us to classify the mutagenic effect of different vector architectures. We transplanted mice with cells from vector associated leukemia and found a subset of these genes also deregulated in vivo. To assess SAGA in clinically relevant settings in which insertional mutagenesis caused severe adverse events, we could show that vectors used in clinical immunodeficiency trials (MFG.yc, CMMP.WASP) deregulated our core set genes in vitro. Importantly, safety optimized SIN-lentiviral vectors did not show this molecular immortalization signature indicating a more favorable safety profile. To further improve the assay, we developed a NanoString approach to screen for the expression status of the core set after retroviral transduction. Here we show the basic principle of SAGA, explain the bioinformatic strategies to get meaningful results and introduce the NanoString panel to screen for insertional mutants.

Abstract No. P141

The niche limits hematopoietic stem cell activation by secreting factors which reduce DNA damage-dependent senescence

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The niche is required for the lifetime maintenance of hematopoietic stem cells (HSCs). HSCs restore the hematopoietic hierarchy after hematopoietic stress, such as infections, toxic insult, or transplantation. Importantly, leukemogenesis and aging have been associated with disfunctional niches. In gene expression studies, we determined there is a dynamic exchange of signals from stromal cells and HSCs during hematopoietic stress and vice versa. In a culture model of hematopoietic stress, we found that a deletion of different secreted factors: Ctgf, Sfrp1, or Sfrp2 in stromal cells, leads to an irreversible inability to maintain repopulating HSCs in vitro. Consistent with the idea that these factors are involved in regulating hematopoietic stress, deletion of Sfrp1, or Sfrp2 in vivo does not affect steady-state hematopoeisis. However, in a transplantation model (regenerative stress) recipients deficient in Sfrp1 or Sfrp2 show a defective restoration of the HSC pool. Since successful regeneration requires cell division, we examined clonal cell division of HSCs in media from stromal cells deficient in Ctgf or Sfrp1. In both cases, we found that cell division of HSCs was impaired, which rescued by addition of CTGF and SFRP1, respectively. Closer examination demonstrated that HSC grown in CTGF- or SFRP1-deficient media show similar accumulation of gH2AX+ heterochromatin foci, H4K16 acetylation and upregulation of p53. In HSCs grown with Ctgf-deficient stromal cells we further found activation of SMAD2/3 and PTEN. In vivo, HSCs from Sfrp1 or Sfrp2 deficient environment show similar changes in gH2AX and acetylated H4K16 after transplantation, or 5-fluorouracil treatment. All of these markers are frequently found in cellular senescence, strongly suggesting that CTGF, SFRP1, and SFRP2 are commonly required to prevent loss of stem cell function by preventing cellular senescence. In summary, our studies offer new targets in CTGF- and SFRP1/SFRP2-dependent niche signaling to prevent cellular senescence and stem cell exhaustion.

Abstract No. P142

MSC-derived extracellular vesicles modulate CD34+ HSPCs

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Intercellular communication in the bone marrow microenvironment, e.g. between hematopoietic stem and progenitor (HSPCs) and mesenchymal stromal (MSCs) cells, is facilitated either by direct cell-cell contact or by soluble factors. Recently, non-contact communication processes were described on the basis of secretion of extracellular vesicles (EVs) that were able to modify functional properties of recipient cells by the transfer of bioactive molecules. However, detailed mechanisms and a potential diagnostic or therapeutic relevance remain to be defined.

Therefore, EVs were isolated from serum-free culture supernatants of MSCs of healthy donors (HD) and myelodysplastic syndrome (MDS) patients. Nanoparticle tracking analysis and transmission electron microscopy revealed a heterogeneous morphology of the population with an average size of 148nm for HD MSC-derived EVs and 156nm for MDS MSC-derived EVs. The concentration was higher in HD than in MDS samples (5.9E+08 vs. 2.1E+08 particles/ml). EV isolation preparations expressed typical exosomal markers as CD9, CD63 and CD81. Flow cytometry analysis of the isolated EVs and the MSCs from which they were secreted revealed that EVs expressed some common MSC markers as CD90 (MDS only), CD105 and CD146. Moreover, we could demonstrate expression of Wnt5A mRNA and accumulation of miRNA-23A in MSC-derived EVs. The transfer of bioactive molecules containing EVs was visualized by immunofluorescence confocal imaging and modulation of downstream signaling pathways like β-catenin/Wnt was suggested. Proliferation of HSPCs was inhibited especially by EVs isolated from malignant MSCs and the phenotype was modulated with higher expression of CD34 and CD90.

In summary, we provide evidence for an active intercellular communication between MSCs and HSPCs mediated by EVs which modulates important characteristics of HSPCs and may serve as potential target for therapeutic response prediction but also for regenerative approaches.

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