GermanStemCellNetwork

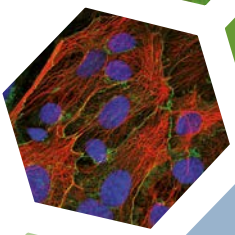
4th Annual Conference

of the German Stem Cell Network (GSCN)

12 – 14 September 2016

Hannover Medical School (MHH)

www.gscn.org



Program & Abstracts

Supported by

Federal Ministry of Education and Research

VDI Technologiezentrum

Niedersächsisches Ministerium für Wissenschaft und Kultur

DFG Deutsche Forschungsgemeinschaft

Collaboration with

MHH Hannover Medical School

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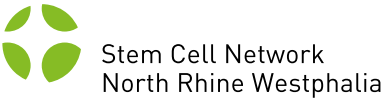
MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE IN THE HELMHOLTZ ASSOCIATION

Kompetenznetzwerk Stammzellforschung NRW

Gefördert aus Mitteln des Niedersächsischen Vorab



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Hannover Medical School (MHH)

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

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e-mail: gscn.office@mdc-berlin.de
web: www.gscn.org

Greetings from Stephan Weil, Minister-President of Lower Saxony

As Minister-President of Lower Saxony, it gives me great pleasure to welcome all attendants of the 4th International Annual Conference of the German Stem Cell Network (GSCN). Each year this event brings together experts in stem cell research and regenerative medicine to present their work and to discuss their latest research findings.

I am convinced that this year, Hannover Medical School and the Cluster of Excellence "REBIRTH" as host institutions will provide a very stimulating environment to discuss the latest developments in stem cell research and genome and tissue engineering. The State of Lower Saxony is at the forefront of research in these areas, not only with the Cluster of Excellence "REBIRTH", but also with other research institutions, innovation-minded professionals, and strong enterprises.

I am convinced that this conference represents an outstanding opportunity for national and international scientists to discuss new findings and emerging, cutting-edge advances in a stimulating and interdisciplinary atmosphere. I am proud that Lower Saxony is home to this conference.

During your visit to Hannover, I hope you will take the opportunity to enjoy everything that the capital of Lower Saxony has to offer – from its numerous cultural and scenic treasures, to its fine dining and gracious hospitality. From Hannover Medical School it is only a short trip to the City Center: home to our opera, museums and historic sites including the new and old town hall, and the famous baroque gardens at Herrenhausen.

Finally, I would like to extend a warm welcome to all participants on behalf of the State of Lower Saxony, in particular our international guests and speakers. I wish you lively discussions and active exchanges of experiences in all areas of stem cell research.

Hannover, September 2016

Stephan Weil
Minister-President of Lower Saxony

Welcome address

Dear Friends and Colleagues,

It is a great pleasure to welcome you to the **4th Annual Conference of the German Stem Cell Network (GSCN) in Hannover.** This conference is hosted by the Cluster of Excellence REBIRTH and Hannover Medical School.

This year, there will be a particular focus on genome engineering and on "translation" with various relevant aspects including disease modeling and drug development, relevant technologies, clinical trials, regulatory affairs and ethical aspects. The conference will be accompanied by a symposium of ethicists on pressing questions in gene, embryo and stem cell research, will foster the interaction between scientists working in different areas of stem cell research, and aims to bring together basic scientists and clinician scientists.

Supported by the Federal Ministry of Education and Research, the GSCN is now in its 4th year. Besides organization of the annual conference and the participation in the European UniStem Day, one key activity of the recent year was the composition and publication of the first White Paper on Stem Cell Research as an updated information source for the public and politics, also aiming at raising additional funds for the German stem cell community.

Meanwhile, the GSCN annual conferences are a "must-go" event for stem cell researchers in Germany and have substantially stimulated networking in the German and European stem cell scene. Remarkably, also the number of international GSCN members and attendees is continuously increasing.

Since promotion of junior scientists is a particular aim of the GSCN, most speakers are selected from the best abstracts that have been submitted. On the other hand, we invited internationally leading researchers to contribute a keynote lecture. We are happy that Alexander Meissner (Cambridge), Sean Morrison (Dallas), Hiroshi Nagashima (Tokyo), Thomas Eschenhagen (Hamburg), Peter Zandstra (Toronto) and Pete Coffey (London) agreed to join us here in Hannover. Again, awardees of the GSCN prizes will present their results during the Presidential Symposium. The final session of the conference will be a Joined Session with the Cluster of Excellence REBIRTH further highlighting translational aspects of stem cell research and providing an outlook by the former REBIRTH member and new president of the GSCN, Lenhard Rudolph. With best wishes for another great GSCN conference,

Yours sincerely,

Ulrich Martin (GSCN Acting president)

For the program committee

Daniel Besser (Berlin) • Thomas Braun (Bad Nauheim) • Tobias Cantz (Hannover) • Tilman Fabian (Hannover) • Ulrich Martin (Hannover) • Karl Lenhard Rudolph (Jena) • Claudia Waskow (Dresden)

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[1]Data on file.

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Conference Information

Venue

Hannover Medical School, Building J01
Carl-Neuberg-Str. 1 | 30625 Hannover, Germany

Date

Monday, 12 September to Wednesday, 14 September 2016

Registration

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

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

Internet

Internet access via Wireless LAN is free of charge. Please use this login data:
User: Hotspot 1; Password: mh-hannover

Posters exhibition

Posters will be displayed during the conference in two sessions on the ground floor (S0). 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 – 16:00 h and removed latest on Tuesday at 14:00 h. Posters in poster session II should be mounted on Tuesday, 14:00 – 16:30 h and removed latest on Wednesday at 16:00 h.

Poster session I (P001 – P077)
Monday, 12 September 2016, 17:00 – 19:00 h

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

- Pluripotency and reprograming (P001 – P017)
- Somatic stem cells and development (P018 – P039)
- Hematopoietic stem cells (P040 – P053)
- Stems cells and ageing, genome stability and epi-genetics (P054 – P060)
- Stem cells in diseases: cancer stem cells (P061 – P066)
- Computational stem cell biology and systems biology (P067 – P077)

The poster session I is supported by BioFroxx GmbH / Biological Industries.

Poster session II (P078 – P147)
Tuesday, 13 September 2016, 16:30 – 18:30 h

Even numbers will be presented 16:30 – 17:30 h and odd numbers 17:30 – 18:30 h.

- Tissue engineering and organoids (P078 – P091)
- Genome engineering and gene therapy (P092 – P097)
- Stem cells in regenerative therapies (P098 – P119)
- Stem cells in regenerative therapies: mesenchymal stem/ stroma cells (P120 – P124)
- Stem cells in disease modeling and drug development (P125 – P147)

The poster session II is supported by Thermo Fisher Scientific.

GSCN Awards

Travel awards

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

- *Birte Baudis,* University Hospital of Cologne
- *Christian Böhme,* University of Leipzig
- *Larisa Condurat,* University of Freiburg
- *Nora Freyer,* BCRT, Charité – Universitätsmedizin Berlin
- *Devy Garna,* Dental Institute King's College London, United Kingdom
- *Florian Murke,* University Hospital of Essen

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

eppendorf

Poster awards

There will be two poster awards for each poster session. Authors are asked to be present at the poster award ceremony, which will take place on Wednesday, 14 September 2016, 17:45 – 18:00 h in the lecture hall F.

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

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Scientific awards 2016

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, 13 September 2016, 14:00 – 16:00 h.

- **GSCN Young Investigator Award:** *Leo Kurian,* Center for Molecular Medicine Cologne (CMMC), University of Cologne
- **GSCN Female Scientist Award:** *Claudia Waskow,* Regeneration in hematopoiesis, TU Dresden
- **GSCN Publication of the Year Award** (July 2015 to June 2016): *Dr. Guangqi Song, Dr. Martin Pacher, Prof. Michael Ott* and *Dr. Amar Deep Sharma* of the REBIRTH Center and TWINCORE Center at Hannover Medical School. Their publication "Direct Reprogramming of Hepatic Myofibroblasts into Hepatocytes In Vivo Attenuates Liver Fibrosis" appeared in the journal *Cell Stem Cell* (Song, G. et al., 2016, Cell Stem Cell, 18, 797 – 808, doi: 10.1016/j.stem.2016.01.010.).

GermanStemCellNetwork

Social events

Get-together

Monday, 12 September 2016
19:45 – 21:00 h
Hannover Medical School

All participants and exhibitors are invited to a **Get-together** with dinner buffet at the foyer of the MHH (Ground floor).

Networking evening

Tuesday, 13 September 2016
19:00 – 01:00 h
Yukon Market Hall
Hannover Zoo

Experience an extraordinary evening with **polar bear feeding** in the unique **Yukon Market Hall** in the Hannover Zoo. In the 1920's flourishing fish trade was operated in the market hall. Today you can celebrate here like in Canada. Typical North American wood facades, salons, theaters and the Yukon Market Hall let dreams of the 'Wild West' come true. From the large terrace you have a great view to the polar bears and maybe you can hear the whine of the Timberwolves.

Shuttle buses will leave at 18:30 h from the main car entrance (Hauptein-fahrt) of MHH. The **Networking evening** with dinner buffet and DJ is included in the registration fee and open to all participants and exhibitors.

Address:
Hannover Zoo
Adenauerallee 3
30175 Hannover

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Cluster of Excellence | Hannover

Photos: © Erlebnis Zoo Hannover

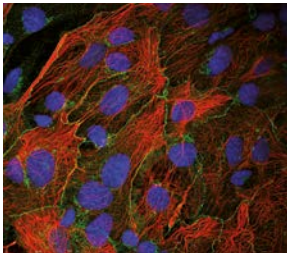
Public outreach event (in German)

Moderne Zelltherapien – Stammzellen bei Herz- und Lebererkrankungen

Podiumsdiskussion zur aktuellen Forschung in Labor und Klinik

Wann: **Mittwoch, 14. September, 19:30 – 21 Uhr**
Einlass: 19 Uhr, Posterausstellung zu Stammzellen

Wo: **HAZ Anzeiger-Hochhaus**
Goseriede 9
30159 Hannover



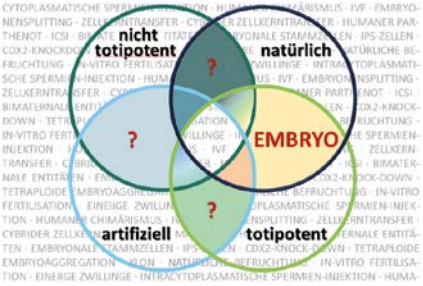
Patienten mit schwerwiegenden Erkrankungen der Leber und des Herzens kann bisher oft nur durch eine Organtransplantation das Leben gerettet werden. Moderne Zelltherapien mit Stammzellen könnten eine Alternative zur Organtransplantation bieten. Wie ist der Stand der Forschung? Welche Aussichten zeichnen sich für die Klinik und die Anwendung ab? Und wie ist eigentlich die ethische und rechtliche Situation beim Umgang mit Patientenzellen in der Forschung? Hochkarätig besetzt: In der öffentlichen Podiumsdiskussion stellen drei Ärzte und Wissenschaftler der Medizinischen Hochschule Hannover (MHH) und ein Medizinethiker der Leibniz Universität Hannover (LUH) den aktuellen Stand der Grundlagenforschung und den möglichen klinischen Einsatz vor. Kurze Filme veranschaulichen ihre Arbeit im Exzellenzcluster REBIRTH. Anschließend diskutieren sie Einsatzmöglichkeiten von Stammzellen bei Herz- und Lebererkrankungen und beantworten Fragen aus dem Publikum.

- *Prof Dr. Axel Haverich,* Klinik für Herz-, Thorax-, Transplantations- und Gefäßchirurgie, MHH,
- *Prof. Dr. Ulrich Martin,* Leibniz Forschungslaboratorien für Biotechnologie und künstliche Organe, MHH
- *Prof. Dr. Michael Manns,* Klinik für Gastroenterologie, Hepatologie und Endokrinologie, MHH
- *Prof. Dr. Nils Hoppe,* Centre for Ethics and Law in the Life Sciences Hannover, Leibniz Universität Hannover
- **Moderation:** *Dr. Stefanie Seltmann,* DKFZ, Heidelberg

Satellite event (in German)

ELSA-Forschungsverbundprojekt „Entwicklungsbiologische Totipotenz: Bestimmung als normatives Kriterium in Ethik und Recht unter Berücksichtigung neuer entwicklungsbiologischer Erkenntnisse“ (Verlängerung)

Totipotente Nicht-Embryonen und nicht-totipotente Embryonen Normative Herausforderungen durch artifizielle Entitäten



Im deutschen Recht existieren zwei unterschiedliche Legaldefinitionen des menschlichen Embryos: Nach § 8 Abs. 1 ESchG gilt als Embryo bereits die befruchtete, entwicklungsfähige menschliche Eizelle, ferner jede einem Embryo entnommene totipotente Zelle. Hingegen definiert § 3 Abs. 4 StZG den Embryo ausschließlich durch das Kriterium der Totipotenz. Im Hinblick auf artifiziell erzeugte totipotente Entitäten, die nicht durch Befruchtung entstehen, stellt sich damit die Frage, inwieweit diese unter den Begriff des Embryos des ESchG fallen. Da Totipotenz offenbar nicht den Grund für die Schutzwürdigkeit von Embryonen darstellt, sondern als biologisches Kriterium für die Zuschreibung von Schutzwürdigkeit dient, bedarf es für die Definition eines menschlichen Embryos weiterer normativer, ontologischer und lebensweltlicher Annahmen. Diesbezüglich lassen sich Unterschiede zwischen natürlichen Embryonen und artifiziell erzeugten totipotenten Entitäten erkennen, die die Fragen aufwerfen, ob und inwieweit artifizielle Entitäten anders zu bewerten sind als natürliche menschliche Embryonen und welchen Argumenten und Kriterien bei der ethischen und rechtlichen Beurteilung Bedeutung zukommt.

Sofern der Gesetzgeber am Kriterium der Totipotenz für die Schutzwürdigkeit entwicklungsfähiger Entitäten festhalten möchte, ist zu fragen, ob zwischen einer natürlichen und einer artifiziell erzeugten Totipotenz differenziert werden muss. Damit geraten Aspekte wie die Entstehung bzw. Erzeugung, die Finalität und die Eingriffstiefe der Manipulation in das Blickfeld und werfen die Frage auf, inwieweit sich diese als Kriterien einer konstitutiven Abweichung vom natürlichen Embryo qualifizieren lassen.

Teilprojekt Philosophie, Koordination
Thomas Heinemann, Barbara Advena-Regnery, Kathrin Rottländer
Lehrstuhl Ethik, Theorie und Geschichte der Medizin, Philosophisch-Theologische Hochschule Vallendar

PHILOSOPHISCH-THEOLOGISCHE HOCHSCHULE VALLENDAR
Kirchlich und staatlich anerkannte Wissenschaftliche Hochschule in freier Trägerschaft

Teilprojekt Rechtswissenschaft
Hans-Georg Dederer, Franziska Enghofer, Katharina Böhm
Lehrstuhl für Staats- und Verwaltungsrecht, Universität Passau

UNIVERSITÄT PASSAU

Teilprojekt Entwicklungsbiologie
Tobias Cantz, Susan Sgodda
Medizinische Hochschule Hannover

MHH Hannover Medical School

DIENSTAG, 13. September 2016

Hörsaal N

11:00 – 11:15	**Begrüßung** *Thomas Heinemann,* Philosophisch-Theologische Hochschule Vallendar
11:15 – 12:30	**Teilprojekt Entwicklungsbiologie** Moderation: *Hans-Georg Dederer* Die „Scoring"-Matrix – biologische Graduierung nach Natürlichkeit und Artifizialität *Susan Sgodda,* Medizinische Hochschule Hannover Kommentar: *Michael Ott,* Hannover
12.30 – 13:30	Mittagspause
13:30 – 15:15	**Teilprojekt Rechtswissenschaft** Moderation: Thomas Heinemann Rechtliche Kriterien für die Bewertung von „nicht-totipotenten Embryonen" und „totipotenten Nicht-Embryonen" *Franziska Enghofer* und *Katharina Böhm,* Universität Passau Kommentar: *Jens Kersten,* München
15:15 – 15:45	Kaffeepause
15:45 – 17:30	**Teilprojekt Philosophie** Moderation: *Tobias Cantz* Der Embryo zwischen Sein und Sollen. Zum Verhältnis von Naturwissenschaften und Normwissenschaften *Barbara Advena-Regnery,* Philosophisch-Theologische Hochschule Vallendar Artifizielle embryo-ähnliche Entitäten. Zwischen Statusdebatte und Handlungskontexten *Kathrin Rottländer,* Philosophisch-Theologische Hochschule Vallendar Kommentar: Markus Rothhaar, Eichstätt-Ingolstadt
17:30 – 18:15	**Abschlussdiskussion**

Gefördert vom

Bundesministerium für Bildung und Forschung

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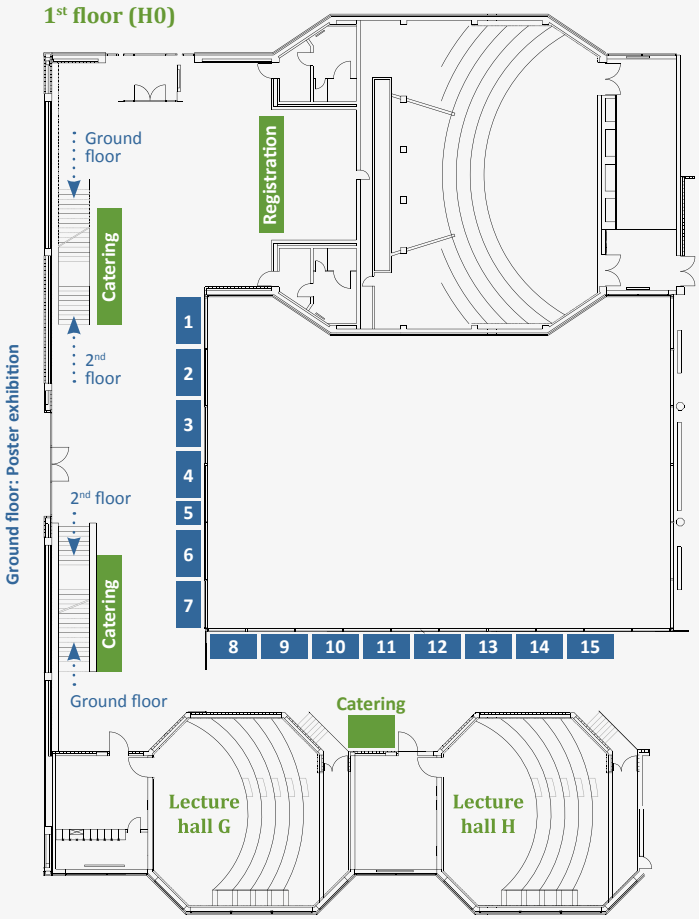
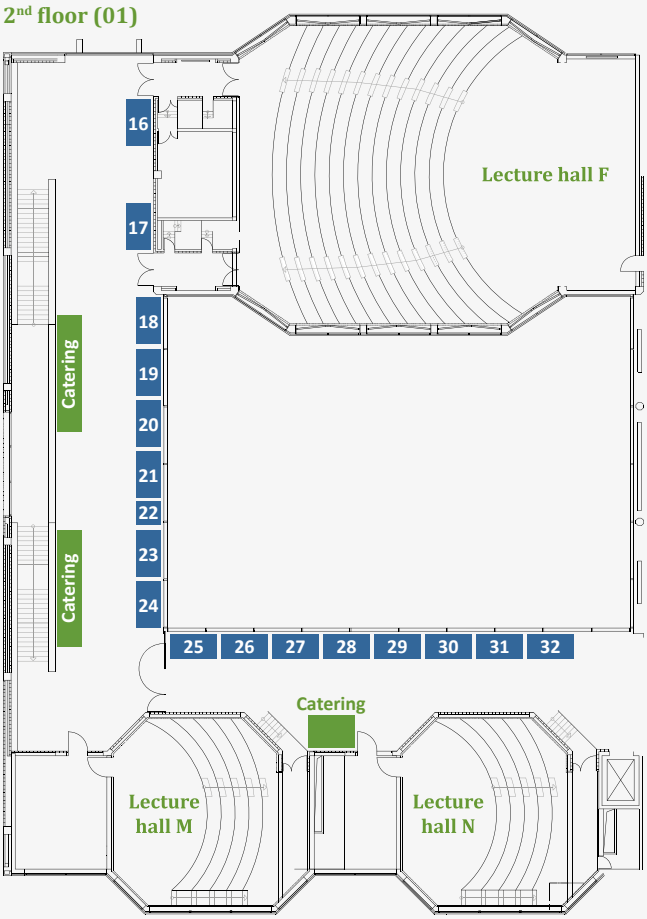
Floor plan

Legend

AMS Biotechnology (Europe) Limited	19	Cygenia GmbH	15
Becton Dickinson GmbH	25	eBioscience, an Affymetrix company	11
BioFroxx GmbH/Biological Industries	20	Eppendorf AG	17
BioTek Instruments GmbH	14	Gilson International Deutschland	9
BIOTREND Chemikalien GmbH	26	I&L Biosystems GmbH	8
CellTool GmbH	30	Labotect GmbH	6
Cenibra GmbH	29	LLS ROWIAK LaserLabSolutions GmbH	13
Cyagen Biosciences GmbH	28	Lonza Cologne GmbH	1



Macopharma International GmbH	23	PL BioScience GmbH	22
Miltenyi Biotec GmbH	21	PromoCell GmbH	27
NanoString Technologies Germany GmbH	2	ReproCell Europe Ltd	31
NEW ENGLAND BioLabs GmbH	4	SERVA Electrophoresis GmbH	12
OLS – OMNI Life Science GmbH & Co. KG	10	STEMCELL Technologies GMBH	7
OSPIN GmbH	32	Sysmex Suisse AG	5
PELOBIOTECH GmbH	3	Takara Bio Europe, SAS	24
PeproTech GmbH	18	Thermo Fisher Scientific	16



MONDAY, 12 September 2016

	Lecture hall F	Lecture hall H	Lecture hall G	Lecture hall M	Lecture hall N
10:00 – 11:00	Registration				
11:00 – 11:30	**Opening**				
11:30 – 12:15	**Keynote lecture I** *Alexander Meissner*				
12:15 – 13:00	**Keynote lecture II** *Sean Morrison*				
13:00 – 14:00	Lunch break / Industry exhibition				
14:00 – 15:30		**Concurrent scientific working group session I**			
		Stem cells in regenerative therapies I	Pluripotency and reprograming	Hematopoietic stem cells	Tissue engeneering and organoids
15:30 – 16:00	Coffee break / Industry exhibition				
16:00 – 17:00		**GSCN Members Meeting**			
17:00 – 19:00	**Poster Session I (P001 – P077)**				
17:00 – 18:00	posters with even numbers				
18:00 – 19:00	posters with odd numbers				
19:00 – 19:45	**Keynote lecture III** *Hiroshi Nagashima*				
19:45 – 21:00	**Informal GSCN Get-together** with dinner buffet at the ground floor (S0) of the conference venue				**Technology Exchange Workshop** (by invitation only)

TUESDAY, 13 September 2016

	Lecture hall F	Lecture hall H	Lecture hall G	Lecture hall M	Lecture hall N
09:00 – 10:30		**Concurrent scientific working group session II**			
		Stem cells in disease modeling and drug development	Stem cells and aging, genome stability and epigenetics	Computational stem cell biology and systems biology	Genome engineering and gene therapy
10:30 – 11:00	Coffee break / Industry exhibition				
11:00 – 12:35		**Industry session: "Technologies from GSCN industry partners"**			**Satellite event**
		PeproTech GmbH	Thermo Fisher	Apceth	**ELSA-Forschungsverbundprojekt**
		Eppendorf AG	Miltenyi Biotec	Nanostring Techn.	**Totipotente Nicht-Embryonen und nicht-totipotente Embryonen**
		Takara Bio Europe	Lonza	STEMCELL Techn.	Normative Herausforderungen durch artifizielle Entitäten
12:35 – 14:00	Lunch break / Industry exhibition / Poster viewing				Interdisziplinäres Symposium
12:45 – 13:45		**Meet-the-expert tables in the bistro/ground floor (S0)** Please register at registration desk (limited to 10 participants each).			S. 12 – 13
		Ingo Roeder	*Hans Schöler*	*Ana Martin-Villalba*	
14:00 – 16:00	**Presidential Symposium**				
14:00 – 14:30	*Oliver Brüstle*				
14:30 – 15:00	Young Investigator Award *Leo Kurian*				
15:00 – 15:30	Female Scientist Award *Claudia Waskow*				
15:30 – 16:00	Publication of the Year Award *Guanqgi Song*				
16:00 – 16:30	Coffee break / Industry exhibition				

	Lecture hall F	Lecture hall H	Lecture hall G	Lecture hall M	Lecture hall N
16:30 – 18:30	**Poster Session II (P078 – P147)**				**ELSA-Forschungs-verbundprojekt Session**
16:30 – 17:30	posters with even numbers				
17:30 – 18:30	posters with odd numbers				
18:30 – 19:00	Bus transfer to Networking evening				
19:00 – 01:00	**GSCN Networking evening** with dinner buffet and DJ at the Yukon Bay, Hannover Zoo				

WEDNESDAY, 14 September 2016

	Lecture hall F	Lecture hall H	Lecture hall G	Lecture hall N
09:00 – 10:30		**Concurrent scientific working group session III**		
		Somatic stem cells and development	Stem cells in regener-ative therapies II	Stem cells in diseases: cancer stem cells
10:30 – 11:00	Coffee break / Industry exhibition			
11:00 – 12:30		**Concurrent strategic working group session**		
		Technologies in sterm cell research	Career development and funding oppor-tunities	Clinical trials and regulatory affairs
12:30 – 14:00	Lunch break / Industry exhibition / Poster viewing			
12:45 – 13:45		**Meet-the-expert tables in the bistro/ground floor (S0)** Please register at registration desk (limited to 10 participants each).		
		Marisa Karow Cerebral organoids	*Henner Farin* Intestinal epithelial organoides	*Ina Gruh* 3D bioartificial cardiac tissue
14:00 – 17:35	**Joined Session with Rebirth/MHH**			
14:00 – 14:45	**Keynote lecture IV** *Peter Zandstra*			
14:45 – 15:30	**Keynote lecture V** *Peter Coffey*			
15:30 – 15:45	**GSCN Outlook 2017** *Karl Lenhard Rudolph*			
15:45 – 16:15	Coffee break / Industry exhibition			
16:15 – 17:35	**Rebirth session**			
17:35 – 17:50	**Poster award and closing ceremony**			
	End of conference			
19:00 – 21:00	**GSCN public outreach event with panelists**			

Legend

- Opening/Evening events/Members meeting/Rebirth session
- Keynote Lectures/GSCN Awardees/Outlook
- Concurrent scientific working group sessions/ Meet-the-expert-tables
- Concurrent strategic working group sessions/ Meet-the-expert-tables
- Industry session
- Technology Exchange Workshop/ Satellite event
- Poster Sessions and Poster award ceremony

Program

MONDAY, 12 September

10:00 – 11:00 Registration

Lecture hall F **Opening**

11:00 – 11:10 Welcome to Hannover Medical School (MHH)
Christopher Baum, President, Hannover Medical School

11:10 – 11:20 Welcome to Hannover and Lower Saxony
MPräs. Stephan Weil (Minister-President of Lower Saxony)

11:20 – 11:30 *Ulrich Martin,* Acting President, GSCN

11:30 – 12:15 **Keynote lecture I**
K1 – Mechanisms of epigenetic regulation in stem cells and development
Alexander Meissner, Harvard University, Cambridge, U.S.A. (Chair: *Ulrich Martin*)

12:15 – 13:00 **Keynote lecture II**
K2 – Identification of niches for hematopoietic stem cells and osteogenesis
Sean Morrison, UT Southwestern, Dallas, U.S.A. (Chair: *Claudia Waskow*)

13:00 – 14:00 Lunch break / industry exhibition

Concurrent scientific working group session I

Lecture hall H **Stem cells in regenerative therapies I**

(Chairs: *Wolfgang Wagner* / *Ulrich Martin*)

14:00 – 14:15 Overview
Wolfgang Wagner, RWTH Aachen

14:15 – 14:30 T01 – HLA-silenced platelets derived from induced pluripotent stem cells are protected against refractoriness in a platelet transfusion mouse model
Dorothee Eicke, Hannover Medical School

14:30 – 14:45 T02 – Immunogenicity of embryonic stem cell-derived cardiomyocytes in recipients mismatched for minor and major histocompatibility antigens
Birte Baudis, University of Cologne

14:45 – 15:00 T03 – BSA-free differentiation of hPSCs into cardiomyocytes
Hanna Möller, Hannover Medical School

15:00 – 15:15 T04 – A registry of pluripotent stem cells for clinical application
Andreas Kurtz, Berlin-Brandenburg Center for Regenerative Therapies, Charité, Berlin

15:15 – 15:30 Working group discussion

Lecture hall G **Pluripotency and reprograming**

(Chairs: *Micha Drukker* / *Mathias Treier*)

14:00 – 14:25 T05 – Pluripotency keynote – Trophoblast stem cells from murine fibroblasts – can the mouse serve as blueprint for the human situation?
Hubert Schorle, University of Bonn

14:25 – 14:40 T06 – Nanotopography guides morphology and spatial patterning of induced pluripotent stem cell colonies
Giulio Abagnale, RWTH Aachen

14:40 – 14:55 T07 – Propagation of the early murine inner cell mass state in cell culture
Xiushan Yin, Max Delbrück Center, Berlin

14:55 – 15:10 T08 – Contribution of cynomolgus monkey induced pluripotent stem cells to porcine embryos
Monika Nowak-Imialek, Friedrich Loeffler Institute, Mariensee

15:10 – 15:25 T09 – Characterization and potential immunomodulatory properties of human induced pluripotent stem cell (hiPSC)-derived trophoblast cells
Svitlana Malysheva, Hannover Medical School

15:25 – 15:30 Working group discussion

Lecture hall M **Hematopoietic stem cells** (Chairs: *Timm Schröder* / *Claudia Waskow*)

14:00 – 14:25 T10 – HSC keynote – Hematopoietic stem cell fate realized in vivo
Hans-Reimer Rodewald, German Cancer Research Center, Heidelberg

14:25 – 14:40 T11 – The bulk of the hematopoietic stem cell population is dispensable for murine steady-state and stress hematopoiesis
Kristina Schödel, TU Dresden

14:40 – 14:55 T12 – Heterogeneity and in vivo regulation of dormant hematopoietic stem cells
Nina Cabezas-Wallscheid, German Cancer Research Center, Heidelberg

14:55 – 15:10 T13 – Essential role for Setd1a-mediated histone methylation in adult hematopoietic stem cell function
Kathrin Arndt, TU Dresden

15:10 – 15:25 T14 – Biomimetic bone marrow analogs as artifical hematopoietic stem cell niches
Cornelia Lee-Thedieck, Karlsruhe Institute of Technology

15:25 – 15:30 Working group discussion

Lecture hall N **Tissue engineering and organoids** (Chairs: *Robert Zweigerdt* / *Benedikt Berninger*)

14:00 – 14:30 Overview
Robert Zweigerdt, Hannover Medical School
Benedikt Berninger, University Medical Center, Mainz

14:30 – 14:45 T15 – Stirred suspension culture for the scalable generation of billions of human induced pluripotent stem cells
Chee Keong Kwok, University of Würzburg

14:45 – 15:00 T16 – Biofabrication of a perfusable 3D liver tissue construct using organoids
Kerstin Schneeberger, Utrecht University, Netherlands

15:00 – 15:15 T17 – Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modeling
Alexander Kleger, University Medical Center, Ulm

15:15 – 15:30 T18 – Differentiation of pericytes from hiPSCs for the vascularization of bioartificial cardiac tissues
Mónika Szepes, Hannover Medical School

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

Lecture hall H

16:00 – 17:00 **GSCN General Membership Meeting**

17:00 – 19:00 **Poster session I: P001 – P077** supported by Biofroxx GmbH / Biological Industries

Pluripotency and reprograming (P001 – P017)
Somatic stem cells and development (P018 – P039)
Hematopoietic stem cells (P040 – P053)
Stems cells and ageing, genome stability and epigenetics (P054 – P060)
Stem cells in diseases: cancer stem cells (P061 – P066)
Computational stem cell biology and systems biology (P067 – P077)

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

Lecture hall F

19:00 – 19:45 **Keynote lecture III**
K3 – Generation of human organs in pigs
Hiroshi Nagashima, Meiji University, Tokyo, Japan (Chair: *Tobias Cantz,* in collaboration with ELSI project "induced totipotency")

19:45 – 21:00 **Informal GSCN Get-together for all participants**
with dinner buffet at the ground floor (S0) of the conference venue

TUESDAY, 13 September

Concurrent scientific working group session II

Lecture hall H **Stem cells in disease modeling and drug development** supported by Takara Bio Europe, SAS
(Chairs: *Karl-Ludwig Laugwitz* / *Oliver Brüstle*)

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

09:15 – 09:30 T19 – Stiff matrix induces switch to pure beta-cardiac myosin heavy chain expression in human embryonic stem cell-derived cardiomyocytes
Natalia Weber, Hannover Medical School

09:30 – 09:45 T20 – A new model to study neurotoxicity of drug metabolites based on chemical conversion to neurons-on-a-chip in tandem with liver-on-a-chip
Xinlai Cheng, Heidelberg University

09:45 – 10:00 T21 – Employing rapid phenotypic assays in SPG4 patient neurons for drug discovery and rescue
Kristina Rehbach, University of Bonn

10:00 – 10:15 T22 – Patient-specific iPS cell-based modeling of Transthyretin-Related Familial Amyloid Polyneuropathy
Jeannine Hoepfner, Hannover Medical School

10:15 – 10:30 Working group discussion

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

Lecture hall G **Stem cells and aging, genome stability and epigenetics**
(Chairs: *Hartmut Geiger* / *Karl Lenhard Rudolph*)

09:00 – 09:15 Overview
Hartmut Geiger, Ulm University

09:15 – 09:30 T23 – Dnmt3b-dependent intragenic DNA methylation prevents RNA Polymerase II spurious entry on gene bodies and cryptic transcription initiations
Francesco Neri, Leibniz Institute on Aging, Jena

09:30 – 09:45 T24 – Aging shifts the mode and outcome of the hematopoietic stem cell division
M. Carolina Florian, University of Ulm

09:45 – 10:00 T25 – Bone marrow niche and hematopoietic stem cell differentiation are regulated by the microbiota
Aline Bozec, University of Erlangen-Nuremberg

10:00 – 10:15 T26 – Restricted regeneration of hematopoietic stem cells in vivo following chronic inflammatory stress
Ruzhica Bogeska, German Cancer Reserach Center, Heidelberg

10:15 – 10:30 T27 – Hoxa9 induced developmental signals impair stem cells and regeneration of aging muscle
Simon Schwörer, Leibniz Institute on Aging, Jena

Lecture hall M **Computational stem cell biology and systems biology**
(Chairs: *Georg Fuellen* / *Ingo Roeder*)

09:00 – 09:15 Overview
Carsten Marr, Helmholtz Center Munich

09:15 – 09:30 T28 – Scoring cell identity from transcription profiles
Nancy Mah, Berlin-Brandenburg Center for Regenerative Therapies, Charite, Berlin

09:30 – 09:45 T29 – Understanding and predicting regulatory mechanisms in early differentiation of human pluripotent stem cells
Erika Gaspari, University of Bologna, Italy

09:45 – 10:00 T30 – FGF/MAPK signaling sets the switching threshold of a bistable circuit controling fate decisions in embryonic stem cells
Christian Schröter, Max Planck Institute of Molecular Physiology, Dortmund

10:00 – 10:15 T31 – Reconstructing lineage branching from single cell RNA-seq in adult haematopoiesis via diffusion pseudo time
Maren Büttner, Helmholtz Center Munich

10:15 – 10:30 Working group discussion

Lecture hall N **Genome engineering and gene therapy** (Chairs: *Axel Schambach* / *Hans Schöler*)
(in collaboration with ELSI project “induced totipotency”)

09:00 – 09:15 Overview – Germ Cell Differentiation
Hans Schöler, MPI f. Molecular Biomedicine, Münster

09:15 – 09:30 Overview – Gene Therapy & Genome Engineering
Axel Schambach, Hannover Medical School

09:30 – 09:45 T32 – Designer-nuclease mediated knockout of HIV co-receptor CCR5 – a promising gene-therapy approach to protect T cells from HIV infection
Boris Fehse, UKE Hamburg

09:45 – 10:00 T33 – Efficient introduction of homo- and heterozygous mutations with CRISPR/Cas9 and applications to disease modeling in stem cells
Dominik Paquet, The Rockefeller University, New York, U.S.A.

10:00 – 10:15 T34 – Efficient and accurate precision genome engineering of transcriptionally silent disease-related loci by CRISPR/Cas9 nickase
Reto Eggenschwiler, Hannover Medical School

10:15 – 10:30 T35 – Generation of HLA depleted human pluripotent stem cell lines for modulation of the immunogenicity of iPSC derivatives
Lena Engels, Hannover Medical School

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

Industry session: "Technologies from GSCN industry partners"

Lecture hall H **Main supporter** (Chair: *Michael Cross*)

11:00 – 11:05 Introduction

11:05 – 11:35 C1 – Simplified dopaminergic neuron and cardiac differentiation of single episome reprogrammed fibroblasts
Rick I. Cohen, Rutgers University, Piscataway, U.S.A., representing PeproTech GmbH

11:35 – 12:05 C2 – Expansion of Human Bone Marrow-Derived Mesenchymal Stem Cells in BioBLU® 0.3c Single-Use Bioreactors
Aurélie Tacheny, Eppendorf Application Technologies S.A., Namur, Belgium

12:05 – 12:35 C3 – A novel system to generate HPS cell-derived hepatocytes with potential application to drug discovery and metabolism, and hepatotoxicity studies
Barbara Küppers-Munther, Takara Bio Europe, Gothenburg, Sweden

Lecture hall G **Supporter** (Chair: *Dirk Strunk*)

11:00 – 11:05 Introduction

11:05 – 11:35 C4 – Generation of dopaminergic precursor cells and terminally differentiated neurons from human pluripotent cells for drug discovery and cell therapy
Mohan C Vemuri, Thermo Fisher Scientific, Frederick, U.S.A.

11:35 – 12:05 C5 – Enabling GMP-compliant iPSC expansion and differentiation on the CliniMACS® Prodigy platform
Sebastian Knöbel, Miltenyi Biotec GmbH, Bergisch Gladbach

12:05 – 12:35 C6 – Using Pluripotent Stem Cells in the Age of Genome Editing
Theresa Dsouza, R&T Lonza Bioscience, Cologne

Lecture hall M **Supporters** (Chair: *Michael Rieger*)

11:00 – 11:05 Introduction

11:05 – 11:35 C7 – Translation of cell-based gene therapy into clinical application
Elena Meurer, apceth GmbH & Co. KG, Munich

11:35 – 12:05 C8 – Simultaneous single-molecule quantification of DNA, RNAs & Proteins
Maik Pruess, Nanostring Technologies, Hamburg

12:05 – 12:35 C9 – STEMdiff™ Kits for Robust and Efficient Differentiation of Human Pluripotent Stem Cells
Katharina Debowski, Stem Cell Technologies SARL, Cologne

Lecture hall N **Satellitensymposium "Totipotente Nicht-Embryonen und nicht-totipotente Embryonen"**

11:00 – 18:15 open to GSCN participants (in German, program see page 12 – 13)

12:35 – 14:00 Lunch break / industry exhibition / poster viewing supported by Apceth GmbH & Co. KG

12:45 – 13:45 Meet-the-expert tables (Bistro/ground floor): Ingo Roeder (Bioinformatic analysis of biological data), *Hans Schöler* and *Ana Martin-Villalba*; please register at the registration desk (limited to 10 participants each)

Lecture hall F **Presidential Symposium** (Chair: *Ulrich Martin*)

14:00 – 14:30 PS1 – Engineered human heart muscles for disease modelling and cardiac repair
Thomas Eschenhagen, UKE, Hamburg

14:30 – 15:00 Young Investigator Award 2016
PS2 – Developmental programming by lncRNA-TF pairs during the induction of the embryonic heart
Leo Kurian, University of Cologne

15:00 – 15:30 Female Scientist Award 2016
PS3 – Generation and regeneration of human and murine hematopoietic stem cells
Claudia Waskow, TU Dresden

15:30 – 16:00 Publication of the Year 2016 Award (June 2015 – 2016)
PS4 – Direct reprogramming of hepatic myofibroblasts into hepatocytes in vivo attenuates liver fibrosis
Guanqgi Song, Hannover Medical School

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

16:30 – 18:30 **Poster session II: P078 – P147** supported by Thermo Fisher Scientific
Tissue engineering and organoids (P078 – P091)
Genome engineering and gene therapy (P092 – P097)
Stem cells in regenerative therapies (P098 – P119)
Stem cells in regenerative therapies: mesenchymal stem/stroma cells (P120 – P124)
Stem cells in disease modeling and drug development (P125 – P147)
Even numbers: please present your poster from 16:30 – 17:30
Odd numbers: 17:30 – 18:30

18:30 – 19:00 Bus transfer

19:00 – 01:00 **GSCN Networking evening for all participants**
with dinner buffet and DJ at the Yukon Bay, Hannover Zoo

WEDNESDAY, 14 September

Concurrent scientific working group session III

Lecture hall H **Somatic stem cells and development** (Chairs: *Ana Martin Villalba* / *Thomas Braun*)

09:00 – 09:15 Overview
Jan Lohmann, Centre of Organismal Studies, Heidelberg University

09:15 – 09:30 T36 – Fate-restriction precedes stemness during massive post-embryonic growth in the fish branchia
Lazaro Centanin, Centre of Organismal Studies, Heidelberg University

09:30 – 09:45 T37 – Visualization of stem cell induction and differentiation in real time
Rasmus Freter, University of Oxford

09:45 – 10:00 T38 – Embryo-derived macrophages regulate the dendritic cell pool size in the adult spleen
Gulce Percin, TU Dresden

10:00 – 10:15 T39 – A role for YAP and TAZ signaling in human neural crest development
Alexandra Larisa Condurat, Freiburg University

10:15 – 10:30 Working group discussion

Lecture hall G **Stem cells in regenerative therapies II: mesenchymal stem cells**
(Chairs: *Richard Schäfer* / *Dirk Strunk*)

09:00 – 09:15 Overview
Karen Bieback, Medical Faculty Mannheim, Heidelberg University

09:15 – 09:30 T40 – Stepwise maturation of human iPS cells into immunosuppressive mesenchymal stem/progenitor cells
Cornelia Scharler, Paracelsus Private Medical University of Salzburg, Austria

09:30 – 09:45 T41 – Cryopreserved or fresh mesenchymal stromal cells: only a matter of taste or key to unleash the full clinical potential of MSC therapy?
Guido Moll, Charité Berlin

09:45 – 10:00 T42 – Synthetic niche to modulate regenerative potential of mesenchymal stromal cells (MSCs) and enhance skeletal muscle regeneration
Sven Geißler, Charite Berlin

10:00 – 10:15 T43 – Extracellular vesicles – From bench to bedside
Verena Börger, University Hospital Essen

10:15 – 10:30 Working group discussion

Lecture hall N **Stem cells in diseases: cancer stem cells** (Chairs: *Thomas Brabletz* / *Andreas Trumpp*)

09:00 – 09:20 Overview
Andreas Trumpp, German Center Reserach Center, Heidelberg

09:20 – 09:40 T44 – ZEB1 turns into a transcriptional activator by interacting with YAP1 in aggressive cancer types
Julia Kleemann, University of Erlangen-Nuremberg

09:40 – 10:00 T45 – Heterotrimeric G-proteins are indispensable for FLT3-ITD autophosphorylation and oncogenic function
Michael Rieger, Goethe University Hospital and LOEWE Center Frankfurt

10:00 – 10:20 T46 – A mathematical model approach to study the immunological effects in CML patients during and after TKI treatment
Ingmar Glauche, TU Dresden

10:20 – 10:30 Working group discussion

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

Concurrent strategic working group session

Lecture hall H **Technologies in stem cell research** (Chairs: *Frank Emmrich* / *Andreas Bosio*)

11:00 – 11:25 S1 – Intestinal epithelial organoids – an accessible model for mammalian stem cell niche biology
Henner Farin, Georg-Speyer-Haus, Frankfurt

11:25 – 11:50 S2 – 3D bioartificial cardiac tissue from pluripotent stem cells
Ina Gruh, REBIRTH, Hannover Medical School

11:50 – 12:15 S3 – Using cerebral organoids for studying human disease modeling and lineage reprogramming
Marissa Karow, LMU Munich

12:15 – 12:30 Panel discussion

Lecture hall G **Career development and funding opportunities** (Chairs: Insa Schröder/ Hartmut Geiger)

11:05 – 11:40 S4 – Career paths for scientists – suitable application strategies
Anke Raloff, ZEIT Verlagsgruppe/ACADEMICS

11:40 – 12:15 S5 – tba
tbd

12:15 – 12:30 Panel discussion

Lecture hall N **Clinical trials and regulatory affairs** (Chairs: *Torsten Tonn* / *Andreas Kurtz*)

11:00 – 11:25 S6 – Investigator initiated trials (IIT) of advanced therapy medicinal products
Felipe Prosper, University Clinic Navarra, Pamplona, Spain

11:25 – 11:50 S7 – EU research perspective on advanced therapies
Arnd Hoeveler, European Commission, Brussels

11:50 – 12:15 S8 – Mapping the European landscape for patenting stem cell related inventions
Aliki Nichogiannopoulou, European Patent Office, Munich

12:15 – 12:30 Panel discussion

12:30 – 14:00 Lunch break / industry exhibition / poster viewing supported by STEMCELL Technologies SARL

12:45 – 13:45 Meet-the-expert tables (Bistro/ground floor): *Marisa Karow* (Cerebral organoids), *Henner Farin* (Intestinal epithelial organoids) and *Ina Gruh* (3D bioartificial cardiac tissue); register at the registration desk (limited to 10 participants each)

Lecture hall F **Joined session with REBIRTH/Hannover Medical School (MHH)**

14:00 – 14:45 **Keynote lecture IV**
K4 – Patterning mesoderm and blood development from human pluripotent stem cells
Peter Zandstra, University of Toronto, Canada (Chair: Karl Lenhard Rudolph)

14:45 – 15:30 **Keynote lecture V**
K5 – Stemming vision loss using stem cells – seeing is believing
Peter Coffey, University College London, U.K. (Chair: *Thomas Braun*)

15:30 – 15:45 GSCN Outlook 2017
Incoming president: *Karl Lenhard Rudolph*

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

Lecture hall F **REBIRTH Session** (Chair: *Ulrich Martin*)

16:15 – 16:35 RB1 – From cell to product: governance issues along the translational pathway
Nils Hoppe, CELLS – Centre for Ethics and Law in the Life Sciences, Leibniz University Hannover

16:35 – 16:55 RB2 – Emerging protein- and small molecule-based therapies for cardiac repair
Kai Wollert, Hans Borst Center for Heart and Stem Cell Research, Hannover Medical School

16:55 – 17:15 RB3 – Therapeutic noncoding RNA based approaches of heart failure
Thomas Thum, Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School

17:15 – 17:35 RB4 – Manufacturing human pluripotent stem cells and their progenies
Robert Zweigerdt, Hannover Medical School

Lecture hall F **Closing ceremony**

17:35 – 17:50 Poster award ceremony and announcement of industry quiz winners
Daniel Besser, GSCN

supported by PeproTech GmbH

End of GSCN conference 2016

Announcement (please infrom the registration for transfer)

19:00 – 21:00 **GSCN Public outreach event with panelists:**
Moderne Zelltherapien – Stammzellen bei Herz- und Lebererkrankungen
(in German, see page 11)

Speaker abstracts

4th Annual Conference
German Stem Cell Network
12 – 14 September 2016

Keynote lectures: K1 – K5

K1 Mechanisms of epigenetic regulation in stem cells and development
Alexander Meissner

K2 Identification of niches for hematopoietic stem cells and osteogenesis
Sean J. Morrison

K3 Generation of human organs in pigs
Hiroshi Nagashima

K4 Patterning mesoderm and blood development from human pluripotent stem cells
Peter Zanstra

K5 Stemming vision loss using stem cells - seeing is believing
Pete Coffey

Abstract No. K1

Mechanisms of epigenetic regulation in stem cells and development

Alexander Meissner

Harvard University, Cambridge, U.S.A.

In mammals, cytosine methylation is predominantly restricted to CpG dinucleotides and stably distributed across the genome, with local, cell-type-specific regulation directed by DNA binding factors. This comparatively static landscape is in marked contrast with the events of fertilization, where most of the genomic methylation is erased. We will present our latest advances in our understanding of how DNA methylation contributes to development and cellular differentiation.

Abstract No. K2

Identification of niches for hematopoietic stem cells and osteogenesis

Sean J. Morrison

Children's Research Institute at UT Southwestern Medical Center, Dallas, U.S.A.

We reported in 2005 that hematopoietic stem cells (HSCs) reside mainly around sinusoids in hematopoietic tissues and hypothesized that HSCs have a perivascular niche (Cell 121:1109[1]). To test this we made GFP or DsRed knockin alleles of the HSC niche factors, Scf and Cxcl12. Both are expressed mainly by endothelial cells and Leptin Receptor+ stromal cells associated with sinusoidal blood vessels throughout bone marrow (Nature 481:457; Nature 495:231). Conditional deletion of Scf or Cxcl12 in osteoblasts, and in most other bone marrow cells, does not affect HSC frequency or hematopoiesis; however, conditional deletion in endothelial cells or LepR+ cells depletes HSCs. The LepR+ cells are highly enriched for skeletal stem cells and are the main sources of bone and adipocytes in adult bone marrow (Cell Stem Cell 15:154). Conditional Scf deletion in both endothelial cells and LepR+ cells in Leprcre; Tie2cre; Scffl/- mice eliminates all quiescent and serially transplantable HSCs from bone marrow (Cell Stem Cell 13:102). This demonstrated that quiescent HSC maintenance depends upon a perivascular niche. By optically clearing the bone marrow and performing deep confocal imaging we found that most dividing and non-dividing HSCs reside adjacent to LepR+ cells (Nature 526:126). 80-90% of HSCs were most closely associated with sinusoidal blood vessels while 10-20% of HSCs were most closely associated with arterioles. We have also characterized the niche for extramedullary hematopoiesis (EMH) in the spleen. It is also perisinusoidal, created by endothelial cells and Tcf21+ perivascular stromal cells, which are the main sources of SCF and Cxcl12 for EMH (Nature 527:466[2]). This niche is necessary for recovery from diverse hematopoietic stresses, including myeloablation, blood loss, and pregnancy. We are in the process of identifying new factors by which these niche cells regulate HSC maintenance and osteogenesis.

1 *Kiel, MJ et al. (2005) SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells, Cell, 121, 1109-21*

2 *Inra, CN et al. (2015) A perisinusoidal niche for extramedullary haematopoiesis in the spleen, Nature, 527, 466 - 471*

Abstract No. K3

Generation of human organs in pigs

Hiroshi Nagashima

Meiji University International Institute for Bio-Resource Research, Tokyo, Japan

Generation of human organs from pluripotent stem cells is now one of the ultimate goals of regenerative medicine. We are currently conducting a study on blastocyst complementation where mechanisms involved in early embryogenesis and fetal development processes is used to generate organs and tissues in vivo in pigs from exogenous pluripotent cells. The strategy involved creating genetically modified pigs lacking a specific organ, and inducing cloned embryos of the organogenesis-disabled pig into a chimeric state, wherein the lacking organ is generated from the exogenous pluripotent cells. We have successfully created pancreatogenesis-disabled pigs through Pdx1-Hes1 overexpression and Pdx1 knockout. Chimeric pigs were created by blastocyst complementation, in which cloned embryos of the apancreatic pigs were used as host embryos, and cloned embryos of normal pigs were used as donors. The resulting chimeric pigs were confirmed to have formed pancreas from the exogenous cells, i.e. the donor cells. The chimeric pigs demonstrated normal physiological characteristics, including pancreatic functions, and developed into adults with normal reproductive ability. Thus, we showed that the formation of fully functional organs derived from allogenic exogenous cells could be achieved in vivo in apancreatic pigs by using blastocyst complementation technology. The next challenges include expanding the concept of blastocyst complementation to other organs such as kidney, and determining whether interspecies blastocyst complementation using the organogenesis-disabled pigs as the platform is possible. Multiple questions should be answered before attempting to generate chimeras between pigs and humans with distinct differences in the mechanisms of embryonic/fetal development, size and growth rate of the fetus, timing of implantation, placental structure, etc. Conceptus complementation where organ precursor cells or organoids are transplanted into the empty developmental niche of the organogenesis-disabled pig fetuses may be an alternative approach. The concept of xenoregeneration-based organ transplantation therapy including ethical issues will be discussed in this paper.

Abstract No. K4

Patterning mesoderm and blood development from human pluripotent stem cells

Peter Zanstra

University of Toronto, Canada

Functional tissue emerges from complex spatial-temporal interactions between heterogeneous cell populations. In this presentation I will review our efforts to establish an integrated understanding of the effects of cell type heterogeneity, spatial organization and multi-scale regulatory network engagement on defined and measurable stem cell fate transitions. Specific examples from pluripotent stem cell fate control will be highlighted and new data on modeling human pluripotent stem cell development into definitive blood cells by mimicking the hemogenic niche will be presented.

Abstract No. K5

Stemming vision loss using stem cells - seeing is believing

Pete Coffey

University College London, U.K.

The London Project to Cure Blindness is a collaboration between Pete Coffey and Lyndon da Cruz from University College London and Moorfields Eye Hospital. The project aims to use Stem Cell technology to restore sight, prevent progression and ultimately improve the quality of life for patients with Age-Related Macular Degeneration (AMD) and other retinal and macular disorders, combining cutting edge knowledge and technology from the laboratory, clinic and operating theatre.

Presidential Symposium lectures: PS1 – PS4

PS1 Engineered human heart muscles for disease modelling and cardiac repair
Thomas Eschenhagen

PS2 Developmental programming by lncRNA-TF pairs during the induction of the embryonic heart
Leo Kurian

PS3 Generation and regeneration of human and murine hematopoietic stem cells
Claudia Waskow

PS4/P116 Direct reprogramming of hepatic myofibroblasts into hepatocytes in vivo attenuates liver fibrosis
Guangqi Song

Abstract No. PS1

Engineered human heart muscles for disease modeling and cardiac repair

Thomas Eschenhagen

University Medical Center Hamburg Eppendorf

The discovery of human induced pluripotent stem cell (hiPSC) technology and improvements in protocols to differentiate cardiomyocytes from hiPSC-derived cardiomyocytes (hiPSC-CM) have opened new perspectives for cardiac biology. A current shortcoming is the limited maturity of hiPSC-CM. We have developed methods to generate 3-dimensional heart muscle strips from hiPSC-CM (engineered heart tissue, EHT) and showed that CM develop an advanced degree of cardiac maturity, both structurally and functionally. Human EHT show canonical responses to a variety of drugs with known effects on cardiac repolarization, force and contraction kinetics. Patient-derived EHTs reflect typical abnormalities in contractile function, suggesting that this approach will be useful to model complex cardiac diseases such as dilated and hypertrophic cardiomyopathies. Limitations such as clone-to-clone and intra-assay variability will be discussed. Human EHTs may also serve a patches for heart muscle repair after myocardial infarction. EHTs made from human induced hiPSC-CM and endothelial cells (hEHT) were sutured onto injured guinea pig hearts. In a large series of experiments we compared effects of hEHTs with that of constructs made from human endothelial cells only (hEET) or cell free patches. Transplantation was done 7 days after large cryo-injury inducing a mean transmural infarct size affecting 22% of the left ventricular wall. 28 days after transplantation and double immunosuppression, hEHT-transplanted hearts showed large human heart muscle grafts within the scar that showed cardiomyocyte proliferation, vascularization and partial electrical coupling. hEHT improved echocardiographically determined LV function, while hEET or cell-free patches had no effect. Thus, the study provides the first proof of efficacy of human 3D heart muscle constructs in repairing the injured heart and suggests that this approach is an attractive alternative to cell therapy.

Abstract No. PS2: Junior Investigator Award

Developmental programming by lncRNA-TF pairs during the induction of the embryonic heart

Stefan Frank[1], Gaurav Ahuja[1], Joseph Ku[2], Lilija Brant[1], Ivan Gesteira Costa Filho Gesteira Costa Filho [2], Argyris Papantonis[1], Chandra Shekhar Khanduri[3], and Leo Kurian[1,]*

[1]University of Cologne
[2]University of Aachen
[3]University of Gothenberg, Sweden
*Presenting author

Embryonic organogenesis requires precise timing, patterning and coordinated activity of core transcriptional networks that drive developmental progression. Understanding the mechanisms by which these transcriptional networks program developmental decisions is pivotal in devising ways to engineer specialized cell types or to re-engineer them for regenerative repair. Yet, our knowledge of the mechanisms that synchronize developmental progression of a tissue/organ is far from complete. We identified regulatory pairs of long non-coding RNAs (lncRNAs) and core transcription factors (TFs) at key developmental transitions during the formation of the embryonic heart. Our data indicate a significant proportion of developmental TFs are associated with lncRNAs. This phenomenon is conserved during mammalian development. Importantly, loss-of-function experiments confirm the necessity of these TF-associated lncRNAs in enabling the developmental transition at which they are expressed. Interestingly, these lncRNAs are enriched in actively transcribing regions in the genome. Our preliminary data indicate that mechanistically, they form RNA-DNA triple helical structures at the promoters of developmental genes. Importantly, this phenomenon is a characteristic of TFs and genes regulating cell-fate transitions during embryonic development. Based on our data, we hypothesize that these lncRNAs enable the timely expression of developmental genes by facilitating the essential chromatin environment allowing for precision in embryonic cell-fate decisions. Together, we describe a regulatory layer in embryogenesis where lncRNA-TF pairs shape the developmental transcriptional code that programs cell-fate decisions.

Abstract No. PS3: Female Scientist Award

Generation and regeneration of human and murine hematopoietic stem cells.

Claudia Waskow

Regeneration in Hematopoiesis, Institute for Immunology, TU Dresden

Stem cells are the natural unit of embryonic generation and adult regeneration and the regulation of their fate choice decisions is one of the oldest problems in stem cell research. Keeping the delicate balance between self renewal versus differentiation ensures self renewal of the stem cell pool and the continuous supply of mature cell types while preventing carcinogenic transformation. Hematopoietic stem cells are one of the most thoroughly studied adult stem cells that give rise to all mature blood cell lineages throughout life and they are the only adult stem cell type that is routinely used in the clinic for the replacement of diseased blood tissues. The aim of my studies is the identification of cell autonomous and -extrinsic factors and mechanisms regulating hematopoietic stem cell fate choices, including the understanding of their generation and homeostasis during steady-state, under inflammatory conditions, and in the context of tumor formation.

Abstract No. PS4/P116

Direct reprogramming of hepatic myofibroblasts into hepatocytes in vivo attenuates liver fibrosis

Guangqi Song[1,], Martin Pacher[1], Asha Balakrishnan[1], Qinggong Yuan[1], Hsin-Chieh Tsay[1], Dakai Yang[1], Julia Reetz[2], Sabine Brandes[1], Zhen Dai[1], Brigitte M. Pützner[2], Marcos J. Araújo-Bravo[3], Doris Steinemann[1], Tom Luedde[4], Robert F. Schwabe[5], Michael P. Manns[1], Hans R. Schöler[6], Axel Schambach[1], Tobias Cantz[1], Michael Ott[1], and Amar Deep Sharma[1]*

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Direct induction of induced hepatocytes (iHeps) from fibroblasts holds potential as a strategy for regenerative medicine but until now has only been shown in culture settings. Here, we describe in vivo iHep formation using transcription factor induction and genetic fate tracing in mouse models of chronic liver disease. We show that ectopic expression of the transcription factors FOXA3, GATA4, HNF1A, and HNF4A from a polycistronic lentiviral vector converts mouse myofibroblasts into cells with a hepatocyte phenotype. In vivo expression of the same set of transcription factors from a p75 neurotrophin receptor peptide (p75NTRp)-tagged adenovirus enabled the generation of hepatocyte-like cells from myofibroblasts in fibrotic mouse livers and reduced liver fibrosis. We have therefore been able to convert pro-fibrogenic myofibroblasts in the liver into hepatocyte-like cells with positive functional benefits. This direct in vivo reprogramming approach may open new avenues for the treatment of chronic liver disease.

REBIRTH lectures: RB1 – RB4

RB1 From cell to product: governance issues along the translational pathway
Nils Hoppe

RB2 Emerging protein- and small molecule-based therapies for cardiac repair
Kai Wollert

RB3 Therapeutic noncoding RNA based approaches of heart failure
Thomas Thum

RB4 Manufacturing human pluripotent stem cells and their progenies
Robert Zweigerdt

Abstract No. RB1

From cell to product: governance issues along the translational pathway

Nils Hoppe

CELLS – Centre for Ethics and Law in the Life Sciences, Leibniz University Hannover

Abstract not available

Abstract No. RB2

Emerging protein- and small molecule-based therapies for cardiac repair

Kai Wollert

Hans Borst Center for Heart and Stem Cell Research, Hannover Medical School

Inflammatory wound healing after myocardial infarction (MI) provides an unexploited therapeutic window of opportunity to improve patient outcomes and prevent the development of heart failure after MI. Wound healing involves multiple cell types that are interacting with each other in a highly orchestrated manner. This intercellular communication occurs primarily through secreted proteins. The importance of paracrine signaling in the infarcted heart suggests that individual secreted proteins might be developed as therapeutic agents. Moreover small molecule-based approaches may be used to modulate the inflammatory response and stimulate tissue repair after MI. Several examples for such emerging therapies will be discussed.

Abstract No. RB3

Therapeutic noncoding RNA based approaches of heart failure

Thomas Thum

Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School (MHH)

A variety of novel noncoding RNA based approaches of heart failure will be presented and discussed. This includes use of miRNAs and long non coding RNA inhibitors to prevent cardiac remodeling and to improve cardiac healing.

Abstract No. RB4

Manufacturing human pluripotent stem cells and their progenies

Robert Zweigerdt

REBIRTH, Hannover Medical School

The envisioned routine application of human Pluripotent Stem Cell (hPSC)-derived progenies in regenerative medicine, including tissue engineering and pre-clinical studies in large animal models, will require reliable, regulatory-compliant cell production processes. Given the complexity of the pluripotent state and of lineage-specific differentiation, process development and up-scaling faces a number of general as well as cell type-specific challenges. However, recent studies have enabled the transition of lab-scale protocols towards more controlled hPSC manufacturing in industry-compatible culture platforms. In this talk advanced strategies for the cultivation of hPSCs and their differentiation in mesodermal lineages will be discussed, including cardiomyocytes, endothelial cells and hematopoietic lineages, which are in the focus of the REBIRTH cluster of excellence. Progress and limitations will be highlighted in particular related to three dimensional (3D) hPSC suspension culture in fully controlled, stirred tank bioreactors.

Selected presentations

4th Annual Conference
German Stem Cell Network
12 – 14 September 2016

Selected presentations: T01 – T47

(the PXXX number indicates an accompanying poster presentation)

T01 HLA-silenced platelets derived from induced pluripotent stem cells are protected against refractoriness in a platelet transfusion mouse model
Dorothee Eicke

T02 Immunogenicity of embryonic stem cell-derived cardiomyocytes in recipients mismatched for minor and major histocompatibility antigens
Birte Baudis

T03/P111 BSA-free differentiation of hPSCs into cardiomyocytes
Hanna Möller

T04 A registry of pluripotent stem cells for clinical application
Andreas Kurtz

T05 Trophoblast stem cells from murine fibroblasts – can the mouse serve as blueprint for the human situation?
Hubert Schorle

T06/P001 Nanotopography guides morphology and spatial patterning of induced pluripotent stem cell colonies
Giulio Abagnale

T07 Propagation of the early murine inner cell mass state in cell culture
Xiushan Yin

T08 Contribution of cynomolgus monkey induced pluripotent stem cells to porcine embryos
Monika Nowak-Imialek

T09 Characterization and potential immunomodulatory properties of human induced pluripotent stem cell (hiPSC)-derived trophoblast cells
Svitlana Malysheva

T10 Hematopoietic stem cell fate realized in vivo
Hans-Reimer Rodewald

T11 The bulk of the hematopoietic stem cell population is dispensable for murine steady-state and stress hematopoiesis
Kristina B. Schoedel

T12 Heterogeneity and in vivo regulation of dormant hematopoietic stem cells
Nina Cabezas-Wallscheid

T13 Essential role for Setd1a-mediated histone methylation in adult hematopoietic stem cell function
Kathrin Arndt

T14 Biomimetic bone marrow analogs as artifical hematopoietic stem cell niches
Cornelia Lee-Thedieck

T15 Stirred suspension culture for the scalable generation of billions of human induced pluripotent stem cells
Chee Keong Kwok

T16 Biofabrication of a perfusable 3D liver tissue construct using organoids
Kerstin Schneeberger

T17/P083 Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modeling
Alexander Kleger

T18 Differentiation of pericytes from hiPSCs for the vascularization of bioartificial cardiac tissues
Mónika Szepes

T19/P145 Stiff matrix induces switch to pure β-cardiac myosin heavy chain expression in human embryonic stem cell-derived cardiomyocytes
Natalie Weber

T20/P126 A new model to study neurotoxicity of drug metabolites based on chemical conversion to neurons on a chip in tandem with liver-on-a-chip
Xinlai Cheng

T21 Employing rapid phenotypic assays in SPG4 patient neurons for drug discovery and rescue
Kristina Rehbach

T22 Patient-specific iPS cell-based modeling of Transthyretin-Related Familial Amyloid Polyneuropathy
Jeannine Hoepfner

T23 Dnmt3b-dependent intragenic DNA methylation prevents RNA Polymerase II spurious entry on gene bodies and cryptic transcription initiations
Francesco Neri

T24 Aging shifts the mode and outcome of the hematopoietic stem cell division
M. Carolina Florian

T25 Bone marrow niche and hematopoietic stem cell differentiation are regulated by the microbiota
Aline Bozec

T26 Restricted regeneration of hematopoietic stem cells in vivo following chronic inflammatory stress
Ruzhica Bogeska

T27/P059 Hoxa9 induced developmental signals impair stem cells and regeneration of aging muscle
Simon Schwörer

T28 Scoring cell identity from transcription profiles
Nancy Mah

T29 Understanding and predicting regulatory mechanisms in early differentiation of human pluripotent stem cells
Erika Gaspari

T30 FGF/MAPK signaling sets the switching threshold of a bistable circuit controling fate decisions in embryonic stem cells
Christian Schröter

T31 Reconstructing lineage branching from single cell RNA-seq in adult haematopoiesis via diffusion pseudotime
Maren Büttner

T32 Designer-nuclease mediated knockout of HIV co-receptor CCR5 – a promising gene-therapy approach to protect T cells from HIV infection
Boris Fehse

T33 Efficient introduction of homo- and heterozygous mutations with CRISPR/Cas9 and applications to disease modeling in stem cells
Dominik Paquet

T34 Efficient and accurate precision genome engineering of transcriptionally silent disease-related loci by CRISPR/Cas9 nickase
Reto Eggenschwiler

T35 Generation of HLA depleted human pluripotent stem cell lines for modulation of the immunogenicity of iPSC derivatives
Lena Engels

T36 Fate-restriction precedes stemness during massive post-embryonic growth in the fish branchia
Lazaro Centanin

T37/P023 Visualization of stem cell induction and differentiation in real time
Rasmus Freter

T38/P034 Embryo-derived macrophages regulate the dendritic cell pool size in the adult spleen
Gulce Percin

T39 A role for YAP and TAZ signaling in human neural crest development
Alexandra Larisa Condurat

T40 Stepwise maturation of human iPS cells into immunosuppressive mesenchymal stem/progenitor cells
Cornelia Scharler

T41 Cryopreserved or fresh mesenchymal stromal cells: only a matter of taste or key to unleash the full clinical potential of MSC therapy?
Guido Moll

T42 Synthetic niche to modulate regenerative potential of mesenchymal stromal cells (MSCs) and enhance skeletal muscle regeneration
Sven Geißler

T43 Extracellular vesicles – from bench to bedside
Verena Börger

T44/P064 ZEB1 turns into a transcriptional activator by interacting with YAP1 in aggressive cancer types
Julia Kleemann

T45 Heterotrimeric G-proteins are indispensable for FLT3-ITD autophosphorylation and oncogenic function
Michael Rieger

T46 A mathematical model approach to study the immunological effects in CML patients during and after TKI treatment
Ingmar Glauche

Abstract No. T01

HLA-silenced platelets derived from induced pluripotent stem cells are protected against refractoriness in a platelet transfusion mouse model

Dorothee Eicke[1,], Ann-Kathrin Börger[1], Christina Wolf[1], Carlos Guzman[2], Nico Lachmann[1], Thomas Moritz[1], Ulrich Martin[1], Rainer Blasczyk[1]*, and *Constanca Figueiredo[1]*

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The high variability of the human leukocyte antigen (HLA) remains the main immunological cause for platelet (PLT) transfusion refractoriness. Multiple transfused patients often develop antibodies against HLA class I, which mediate PLT depletion after transfusion. Here, we evaluated the feasibility to use HLA-silenced PLTs differentiated from induced pluripotent stem cells (iPSCs) to escape refractoriness conditions. A lentiviral vector encoding for a β2-microglobulin specific shRNA was used to transduce iPSCs typed as HLA-A*02. HLA-silenced iPSCs were differentiated into megakaryocytes (MKs) and PLTs using VEGF, BMP-4 and TPO. The capacity of anti-HLA antibodies to target HLA-silenced PLTs was evaluated in antibody-mediated complement-dependent (CDC) and antibody-mediated cellular-dependent (ADCC) cytotoxic assays. PLT production and survival was analysed after MK transfusion of NOD/SCID/IL-2Rγc–/– mice treated or not with an anti-HLA-A*02 antibody to mimic refractoriness conditions. A HLA silencing effect of 60% was observed in the differentiated MKs. In CDC assays, lysis rates of HLA-silenced MKs were significantly lower ($p<0.05$) in comparison to HLA-expressing MKs. In ADCC assays, MK lysis rates derived from HLA-expressing iPSCs exposed to anti-HLA-A*02 antibodies were significantly increased ($p<0.001$) in comparison to a non-specific antibody. In contrast, no significant changes in lysis rates were observed among HLA-silenced MKs incubated with the non-specific antibody and the specific anti-HLA-A*02 antibody. In absence of anti-HLA antibodies, transfusion of HLA-expressing or HLA-silenced MKs resulted in PLT production in the circulation. However, HLA-silenced MKs showed significantly lower lysis rates in comparison to HLA-expressing MKs under anti-HLA-A*02-mediated refractoriness conditions ($p<0.01$). HLA-silenced MKs were also able to produce significantly higher PLT rates in comparison to HLA-expressing MKs under refractoriness. In biodistribution assays, HLA-silenced MKs were not accumulated in any organ suggesting that PLT production occurs in the blood circulation. In conclusion, iPSC-derived HLA silenced PLTs showed to be a promising strategy to treat severe alloimmunized thrombocytopenic patients.

Abstract No. T02

Immunogenicity of embryonic stem cell-derived cardiomyocytes in recipients mismatched for minor and major histocompatibility antigens

Birte Baudis[1,*], *Carl-Philipp Hackstein*[2], *Marek Molcanyi*[1], *Jürgen Hescheler*[1], *Zeinab Abdullah*[2], and *Tomo Šarić*[1]

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*Presenting author

Pluripotent stem cell (PSC)-derived cardiomyocytes (CMs) represent a promising cell type for functional regeneration of infarcted heart. The long-term success of this therapeutic approach strongly depends on acceptance of the donor tissue by the host. In order to determine the immunogenicity of murine embryonic stem cell (ESC)-derived CMs in immunocompetent allogeneic mice mismatched for major histocompatibility complex (MHC) molecules we used a transgenic ESC line expressing an antibiotic resistance and a green fluorescence protein under the control of the cardiac α-myosin heavy chain gene promoter allowing purification and tracking of CMs. To monitor the specific cytotoxic T cell (CTL) response to a defined minor histocompatibility (miH) antigen expressed in ESC-CMs we constitutively expressed ovalbumin (OVA) in this cell line. To minimize cell loss due to the operation procedure known to occur during intramyocardial transplantation, CM clusters were transplanted heterotopically under the kidney capsule in all recipients. Using immunohistochemistry to assess CM survival and immune cell infiltration, we found that ESC-CMs survived for at least 8 weeks in immunodeficient and MHC-mismatched BALB/c mice. However, ESC-CMs transplanted into another MHC-mismatched mouse strain FVB/N were fully rejected within the same time period, which was accompanied by a significant leukocyte infiltration. miH-antigen mismatched OVA-expressing ESC-CMs survived for 8 weeks in naïve syngeneic mice without inducing an OVA-specific CTL response as determined by a SIINFEKL-H-2K^b-dextramer assay. Surprisingly, the immunization of syngeneic mice with OVA did not reduce the survival of OVA-expressing ESC-CMs transplanted under the kidney capsule although it induced OVA-specific CTLs which caused rejection of OVA-expressing melanoma cells. This data suggests that miH antigen disparity may not be sufficient to cause rejection of ESC-CMs in syngeneic recipients even in the presence of active antigen-specific CTLs and that immune response to MHC-mismatched cells strongly depends on the genetic background of allogeneic inbred mouse recipients.

Abstract No. T03/P111

BSA-free differentiation of hPSCs into cardiomyocytes

Hanna Möller[1,*], *Sarah Anna Konze*[1], *Anne Höfer*[1], and *Falk Fritz Buettner*[1]

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*Presenting author

Cardiovascular diseases cause a high number of deaths in the world and around 17 million people are affected every year. The heart has a very low regeneration capacity and donor organs for transplantation are scarce. Therefore regeneration of lost myocardium with stem cell-derived cardiomyocytes is an attractive strategy for regenerative medicine. hPSCs can be differentiated into cardiomyocytes but the molecular mechanism behind that differentiation are poorly understood. It is likely that differentiating cells secrete paracrine factors into the supernatant that function as important regulatory determinants. A recent and very effective protocol leading to high proportions of cardiomyocytes from hPSCs depends on modulation of Wnt signaling at early stages of differentiation. The differentiation medium according to Lian et al.[1] contains 2.5 mg/ml BSA, originating from the applied B27 supplement. Considering that secreted paracrine factors are found at concentrations in the range of ng/ml, the enormous excess of BSA in cell culture supernatants hampers analytics to identify little amounts of secreted proteins. Therefore the development of a BSA-free cardiomyocyte differentiation protocol is pivotal for the subsequent mass spectrometry-based analysis of secreted proteins. According to Roth et al., we generated a custom made B27 and omitted the BSA. hESCs and hiPSCs could be successfully differentiated under BSA-free conditions into cardiomyocytes and our results are in line with a recent publication describing the suitability of BSA-free conditions for cardiomyogenic differentiation[2]. For secretome analysis, seven time points of the differentiation process were analyzed. In total, more than 5,000 proteins could be identified and there relative levels at the different time points were quantitatively assessed by label-free quantification. Beyond the identified proteins there are many factors involved in signaling pathways that are related to cardiomyocyte development. We further identified several cytokines in the supernatants of differentiating cardiomyocytes with not yet know function on cardiomyogenesis.

1 Lian X, Zhang J, Azarin SM, Zhu K, Hazeltine LB, Bao X, Hsiao C, Kamp TJ, Palecek SP, (2012), Nature Protocols, 8(1):162-75

2 Lian X, Bao X, Zilberter M, Westman M, Fisahn A, Hsiao C, Hazeltine LB, Dunn KK, Kamp TJ, Palecek SP, (2015), Nature Methods, 12, 595–596

Abstract No. T04

A registry of pluripotent stem cells for clinical application

Andreas Kurtz

Charité, Berlin

Clinical application requires specific and strict quality assessment of pluripotent stem cells (PSC). This includes generation and manufacturing under good manufacturing practice conditions, extensive geno- and phenotyping and ethical procurement conditions that have to be met. Harmonization of these criteria at the onset of the expected expansion of the use of PSC for clinical application will prevent issues of reproducibility and comparability between clinical study results, as has been observed in the MSC field. The human pluripotent stem cell registry will therefore establish a global PSC registry for clinical application. The registry will define validation criteria for these cells in close collaboration with the Global Allience for iPSC Therapy and regulatory bodies, and disseminate these for standardization purposes. The registration criteria for clinical grade PSC, and the Registry expansion into PSC-derived clinically applied cells, and clinical trial result registration will be discussed. The goal is to establish a comprehensive data resource for clinical application of PSC from cell generation via preclinical data and clinical trial outcomes.

Abstract No. T05

Trophoblast stem cells from murine fibroblasts – can the mouse serve as blueprint for the human situation?

Hubert Schorle

University of Bonn

Trophoblast stem cells arise as result of the first cell fate decision and represent the stem cell population of the extra-embryonic lineage. A distinct epigenetic lineage barrier established at blastocyst stage, strictly separates mouse embryonic and extra-embryonic lineages. This epigenetic barrier cannot be fully overcome as the expression of TS-determining factors in embryonic stem cells lead to incomplete reprogramming. We recently demonstrated that transient expression of Tfap2c, Gata3, Eomes and Ets2 in fibroblasts suffices to generate cells, which resemble trophoblast stem cells in terms of morphology, expression and methylation pattern. These iTSC (induced trophoblast stem cells) display transgene independent self-renewal, differentiate along the extra-embryonic lineage and chimerize the placenta upon blastocyst injection. Our findings provide insights into the transcription factor networks governing trophoblast stem cell identity and raise the question whether such an approach would be feasible in the human situation, where the hTSC is still evasive.

Abstract No. T06/P001

Nanotopography guides morphology and spatial patterning of induced pluripotent stem cell colonies

Giulio Abagnale[1,], Antonio Sechi[1], Michael Steger[2], Gülcan Aydin[1], Gerhard Müller-Newen[3], Arnold Gillner[2]*, and *Wolfgang Wagner[1]*

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*Presenting author

Induced pluripotent stem cells (iPSCs) raise high hopes in regenerative medicine, but little is known about how they are affected by surface topography. iPSCs usually grow on tissue culture plastic as round colonies and it has been demonstrated that morphogen induced lineage-specification, e.g. in response to factors as BMP-4, is heterogeneous but spatially ordered within colonies. In this study, we analyzed how nanotopograhy impacts on the morphology of iPSC colonies and whether it modulates lineage-specific differentiation. To this end, we have structured polyimide (PI) by laser interference in a groove-ridge pattern with a periodicity of 650 nm (Abagnale et al., Biomaterials 2015). Individual iPSCs displayed an elongated morphology, parallel to these structures as well as modifications in the number and size of focal adhesions. Colonies of iPSCs acquired too an elongated shape along the nanopatterns, apparently through a modulation of cell division planes and the rearrangement of apical actin fibers. Treatment of iPSCs with BMP-4 led to a different spatial distribution of the pluripotency markers NANOG and E-cadherin in round versus elongated colonies. Notably, a similar pattern was observed in the intracellular localization of the transcriptional co-activators YAP and TAZ, which play a key role in the mechanotransduction, particularly with regards to proliferation and cell fate decisions. Interestingly, TAZ (but not YAP) was localized in filamentous structures similar to actin fibers, suggesting a direct link between the cytoskeleton and the mechanotransducer machinery in iPSCs. Taken together, our findings demonstrate that growth of iPSCs colonies is controlled by surface topography at nano-scale. This is also reflected by distinct cellular differences in mechanostransduction upon stimulation with morphogens.

Abstract No. T07

Propagation of the early murine inner cell mass state in cell culture

Xiushan Yin[1,], Christian Klasen[1], Guglielmo Roma[2], Franziska Block[1], Antje Brouwer-Lehmitz[1], Anna-Corina Treier[1], Mali Salmon-Divon[3], Martin Beibel[2], Konstantinos Anastassiadis[4], Tewis Bouwmeester[2]*, and *Mathias Treier[1]*

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[4]Technische Universität Dresden
*Presenting author

Mammalian reproduction out of cell culture would either require the propagation of functional germ cells, totipotent embryonic stem cells or the pre-implantation blastocyst state in vitro. Pluripotent murine embryonic stem cells (mESCs) however can only contribute to the embryo proper, extra-embryonic mesoderm and amnion of the mammalian embryo. In contrast cells of the inner cell mass are in addition able to contribute to primitive endoderm. We have previously shown that SALL4 is essential for epiblast formation and the establishment of mESCs in vitro. Delineation of the SALL4 proteomic interactome in ESCs has revealed beside established stem cell players functionally poorly characterized factors. To establish their expression pattern in vivo we generated GFP knock-in fusion proteins for most of them identifying one that is prominently expressed in the epiblast of the mature murine blastocyst. Gene knockout studies demonstrate that this factor is not essential for mouse development or reproduction. Unexpectedly, from this mouse strain it is possible to propagate the inner cell mass state in vitro which so far has not been possible. We furthermore show that conventional mESCs can acquire inner cell mass properties upon loss of this factor. Using an integrated omics approach we will present the molecular mechanism that explains why propagation of the inner cell mass state from this mouse strain is possible in vitro. Importantly, these ICM-like state cells can be co-cultured with trophoblast stem cells which for the first time allows now to co-culture all cell lineages of the pre-implantation embryo opening the door further for mammalian reproduction out of a dish.

Abstract No. T08

Contribution of cynomolgus monkey induced pluripotent stem cells to porcine embryos

Monika Nowak-Imialek[1,], Stephanie Wunderlich[2], Doris Herrmann[1], Sabine Klein[1], Ulrich Baulain[1], Andrea Lucas-Hahn[1], Stoyan Petkov[1], Eva Mall[1], Björn Petersen[1], Ulrich Martin[2]*, and *Heiner Niemann[1]*

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With the advent of induced pluripotent stem cells (iPSCs), it is possible to derive patient specific pluripotent stem cells. The production of chimeric piglets carrying organs that have a high contribution of human cells, could be used as a source for organ replacement.

Here, we optimized an interspecific chimera assay using cynomolgus monkey induced pluripotent stem cells (cyiPSCs) and porcine embryos to evaluate the feasibility of producing primate stem cell-derived organs in pigs. First, we identified an effective in vitro culture system for porcine parthenogenetic blastocysts and monkey cyiPSCs and compared porcine blastocyst rates in eight different stem cell media and porcine zygote medium 3 (PZM-3). The number of blastocysts on day 8 cultured in iPS 20% medium was significantly higher (91%) than in the commonly used porcine PZM-3 medium (65%). We found significantly fewer degenerated embryos on day 8 after culture in iPS 20% medium (9%) compared to PZM-3 (35%). Next, we injected clusters of Venus-marked cyiPSCs into porcine embryos from days 4 and 6 after parthenogenetic activation. Injection of cyiPSCs into porcine embryos from day 4 resulted in significant higher number of Venus-positive blastocysts (74.5%) compared to injection into blastocysts from day 6 (43%). We observed 26% blastocysts with Venus-expressing cyiPSCs exclusively in the porcine ICM, 53% in trophectoderm and 21% in both ICM and trophectoderm.

We investigated the potential for chimera formation between cyiPSCs and vivo derived porcine embryos from day 4. Chimeric embryos were transferred into recipients and recovered seven days later. Unfortunately, only single cyiPSCs were found in the trophectoderm of porcine embryos.

In summary, we optimized culture conditions for interspecies chimera in which monkey cyiPSCs were able to integrate and survive in porcine embryos in vitro. However, cyiPSCs injected into porcine in vivo embryos did not integrate into the epiblast of host embryos.

Abstract No. T09

Characterization and potential immunomodulatory properties of human induced pluripotent stem cell (hiPSC)-derived trophoblast cells

Svitlana Malysheva[1,*], *Stephanie Wunderlich*[1], and *Ulrich Martin*[1]

[1]Hannover Medical School
*Presenting author

Pregnancy is a unique situation when mother and semi-allogeneic fetus coexist through mechanisms that induce and maintain peripheral immunological tolerance of mother towards fetus. Trophoblast cells play a key role in induction and maintenance of this tolerance. Recently it was shown that trophoblast cells can be generated from human embryonic and induced pluripotent stem cells (hiPSCs) *in vitro*. hiPSCs-derived trophoblast cells may provide a valuable basis for development of *in vitro* models for investigation of early fetal-maternal interactions.

In the current study we compared the efficiency of trophoblast differentiation from hiPSCs through formation of embryoid bodies (EBs), and by induction with BMP4. EB-based differentiation as well as targeted differentiation through BMP4 yielded cells with epithelial morphology that expressed trophoblast markers like cdx2, cytokeratin 7 (CK7), eomes and chorionic gonadotropin. BMP4 induction yielded 93,7 % of CK7-positive cells already at day 4, compared to 33,8 % in EB-mediated differentiation on day 18. Moreover, during BMP4-induced differentiation we detected expression of placental lactogen, which was not detected in the EB-based approach. However, during differentiation through EBs we observed the formation of CK7-positive cystic structures, obviously formed by fluid-pumping epithelium.

Finally, we have investigated expression of immunomodulatory molecules by hiPSCs-derived trophoblast cells. Indeed, various isoforms of non-classical class I histocompatibility antigen HLA-G could be detected. Indoleamine 2,3-dioxygenase (IDO) could not be detected, but was inducible by interferon γ (IFN-γ). Some other co-stimulatory molecules like TRAIL (TNF-related apoptosis-inducing ligand) and PD-L1 (programmed death-ligand 1) were also expressed in hiPSCs-derived trophoblast cells. Further studies concerning the targeted generation of defined trophoblasts subsets, upregulation of immunomodulatory molecules and functional analysis of the resulting cell lineages are ongoing.

The derivation of defined trophoblasts sublineages will enable the investigation of early fetal-maternal interactions including mechanisms of tolerance induction and maintenance in novel human *in vitro* models.

Medawar PB. Symp. Soc. Exp. Biol. 1953 7, 320-38.

Ezashi T et al. Cell Tissue Res. 2012 349(3), 809-24.

Abstract No. T10

Hematopoietic stem cell fate realized in vivo

Hans-Reimer Rodewald[1,*], *Weike Pei*[1], *Thorsten Feyerabend*[1], *Katrin Busch*[1], *Melania Barile*[1], *Jens Rössler*[1], and *Thomas Höfer*[1]

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Information on the hematopoietic tree has largely been based on analysis of hematopoietic stem (HSC) and progenitor cells in colony assays in vitro and by transplantation in vivo. In contrast, less is known about the physiology of hematopoiesis under normal conditions in the bone marrow in vivo. To this end, we have generated genetic tools to follow the output of HSC under non-perturbed or perturbed conditions in situ. These experiments yield a comprehensive view on the differentiation flow emerging from HSC and spreading through the system towards mature cells. We have, in addition, built an endogenous inducible DNA-based random generator that allows the heritable tagging of single cells in vivo without a need for prior cell isolation. We are using this experimental system to dissect at high resolution the HSC fate as it is realized in vivo. These experiments and the accompanying modeling aim at a description of the structure and function of the hematopoietic hierarchy, including its major routes of differentiation, and the kinship of lineages, under steady state, unperturbed conditions in vivo.

Abstract No. T11

The bulk of the hematopoietic stem cell population is dispensable for murine steady-state and stress hematopoiesis

Kristina B. Schoedel[1,], Mina N. F. Morcos[1], Thomas Zerjatke[1], Ingo Roeder[1], Tatyana Grinenko[1], David Voehringer[2], Joachim R. Göthert[3], Claudia Waskow[1], Axel Roers[1]*, and *Alexander Gerbaulet[1]*

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[2]University Hospital Erlangen
[3]University Hospital of Essen
[*]Presenting author

Long term repopulating (LT-) hematopoietic stem cells (HSCs) are the most undifferentiated cells at the top of the hematopoietic hierarchy. The regulation of HSC pool size and its contribution to hematopoiesis are incompletely understood. We depleted hematopoietic stem and progenitor cells (HSPCs) in adult mice in situ and found that LT-HSCs recovered from initially very low levels (<1%) to below 10% of normal numbers but not more, while progenitor cells substantially recovered shortly after depletion. In spite of the persistent and massive reduction of LT-HSCs, steady-state hematopoiesis was unaffected and residual HSCs remained quiescent. Hematopoietic stress, although reported to recruit quiescent HSCs into cycle, was well tolerated by HSPC-depleted mice and did not induce expansion of the small LT-HSC compartment. Only upon 5-Fluorouracil treatment, HSPC-depleted bone marrow was compromised in re-constituting hematopoiesis, demonstrating that HSCs and early progenitors are crucial to compensate myeloablation.

Hence, a contracted HSC compartment cannot recover in situ to its original size and normal steady-state blood cell generation is sustained with less than 10% of normal LT-HSC numbers without increased contribution of the few residual cells.

Abstract No. T12

Heterogeneity and in vivo regulation of dormant hematopoietic stem cells

Nina Cabezas-Wallscheid[1,], Florian Buettner[2], Daniel Klimmeck[1], Pia Sommerkamp[1], Frederic B. Thalheimer[3], Daniel Pastor-Flores[1], Leticia P. Roma[1], Simon Renders[1], Roberta Scognamiglio[1], Petra Zeisberger[1], Adriana Przybylla[1], Paul Collier[4], Dinko Pavlinik[4], Timm Schroeder[5], Vladimir Benes[4], Tobias P. Dick[1], Michael A. Rieger[3], Oliver Stegle[2]*, and *Andreas Trumpp[1]*

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Hematopoietic stem cells (HSCs) harbor the capacity to generate a series of multipotent progenitors (MPPs) that differentiate into lineage-committed progenitors and subsequently mature cells. To explore essential HSC features, we recently integrated quantitative proteome, transcriptome, and methylome analyses of five FACS-sorted HSCs and MPP populations (MPP1-4) and combined these OMICs analyses to their functional potential (Cabezas-Wallscheid et al., Cell Stem Cell 2014; Klimmeck et al., Stem Cell Reports 2014; Lipka et al., Cell Cycle 2014). We have now expanded this analysis to dormant HSCs (dHSCs) identified by label-retaining assays (Wilson et al., 2008). Rare dHSCs reside at the top of the blood hierarchy harboring the highest long-term reconstitution capacity. However, till the date the molecular identity of dHSCs, as well as the mechanism regulating maintenance and the transition out of dormancy remain unknown. We now show by single-cell RNA-seq analysis that the transition from dormancy towards cell cycle entry is achieved by a continuous and coordinated up-regulation of all major biosynthetic processes rather than a switch on/off mechanism. We generate a novel transgenic reporter mouse that specifically labels dHSCs avoiding label retention assays and we demonstrate by in vitro and in vivo approaches the relevance of retinoic acid signaling in keeping HSC dormancy.

Abstract No. T13

Essential role for Setd1a-mediated histone methylation in adult hematopoietic stem cell function

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Setd1a is one out of six histone methyltransferases (Mll1-4, Setd1a, Setd1b) placing methylation marks on histone 3 lysine 4 (H3K4), an epigenetic signature that is related to active transcription. A non-redundant regulatory role of Setd1a in B cell differentiation and erythropoiesis was recently shown but potential effects on the function of adult hematopoietic stem cells (HSCs) remain unknown. Due to early lethality of constitutive knock-out embryos (E7.5), we here took advantage of conditional knock-out mice to analyze the functional role for Setd1a in adult HSCs. Specific deletion in adult hematopoietic stem and progenitor cells (HSPCs) revealed that HSC maintenance is independent of Setd1a-mediated H3K4 methylation, but, instead, continuous hematopoietic differentiation is severely reduced evidenced by lack of Setd1a-deficient mature hematopoietic cells in the periphery. Consistently, the frequency of growth factor-responsive bone marrow progenitor cells was found decreased. HSC-restricted loss of Setd1a mediates a transition of HSCs into cell cycle where they accumulate in the G1 phase. However, also here, HSC numbers remain unaffected but the pool size of HPCs significantly increases despite the depletion of mature cells. Setd1a-deficient HPCs are functionally impaired because they can be outcompeted by Setd1a-proficient precursors upon transplantation of wild type donor HSPCs. Although, Setd1a-null HSCs efficiently home to the bone marrow after transplantation, they fail to proliferate to enlarge their pool size. Consistently, transcriptome analysis of Setd1a-deficient HSPCs revealed the down-regulation of genes involved in cell cycle progression, especially DNA damage- and cell cycle checkpoint control-associated genes. These data establish a non-redundant role for Setd1a in the regulation of specific gene regulatory networks important for cell proliferation and differentiation of HSPCs to maintain tissue homeostasis within the hematopoietic system without affecting the survival of HSCs.

Abstract No. T14

Biomimetic bone marrow analogs as artifical hematopoietic stem cell niches

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Blood is replenished with billions of fresh cells every day throughout the entire life. The source of these cells are hematopoietic stem cells (HSCs). Their ability to reconstitute the entire blood system makes them the key to the cure of many hematological diseases. Upon transplantation from a healthy donor, they are able to reconstitute the hematopoietic system of a patient. However, this treatment is restricted by the limited availability of HSCs. Gaining control over HSC behavior *in vitro*, would be one approach to overcome that limitation.

In vivo HSCs are controlled by a highly specialized microenvironment – the niche – within the bone marrow. In this niche HSCs are supported by mutual cell-cell as well as cell-matrix interactions. While it is clear that biological and/or chemical parameters play an important role in this interplay, surprisingly little attention was paid to physical signals that are transmitted by the niche microenvironment. During the last years, we found that these physical signals include matrix stiffness, nanostructure as well as the three-dimensional architecture. In reductionist approaches, in which we studied one parameter at a time, we could show that all of these parameters impact HSC behavior. We developed 3D bone marrow analogs that mimic crucial features of the niche and are suitable for culturing HSC.

In order to achieve the goal of a synthetic stem cell niche to guide HSC behavior, the complexity of the natural HSC niche, which combines a variety of different signals, has to be taken into account. For this purpose, we increased the complexity of our systems to study the synergistic effects of different biological and/or physical signals. With these experiments we hope to get one step closer towards a synthetic stem cell niche that is as simple as possible but as complex as necessary to instruct HSCs.

Abstract No. T15

Stirred suspension culture for the scalable generation of billions of human induced pluripotent stem cells

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The production of human induced pluripotent stem cells (hiPSCs) in quantities that are relevant for cell-based therapies and cell-loaded implants using standard adherent culture is hardly achievable and lacks process scalability. This has prompted the development of more scalable cell culture systems. A promising approach is the culture of hiPSCs in suspension as aggregates. In this study, stirred suspension culture vessels with capacities scalable between 100 and 1,000 mL were investigated for their suitability in the expansion of hiPSCs inoculated as a single cell suspension, without the use of microcarriers or additional substrata such as extracellular matrix. The process developed here first generates hiPSC aggregates of 324 ± 71 µm diameter in 7 days in 100 mL volume (spinners), then dissociates them into a single cell suspension for subsequent inoculation at the 1,000 mL scale (bioreactors). After an additional 7 days of cultivation, hiPSC aggregates of 198 ± 58 µm are generated. Spinner- and bioreactor-cultivated hiPSC aggregates maintain an undifferentiated state as assessed by expression of pluripotency-associated markers TRA-1-60, OCT4, and SSEA-4, and are capable of differentiating into cells of all three germ layers *in vitro*. Starting with 16×10^6 hiPSCs, in the first 7 days of cultivation, up to a 16-fold increase in hiPSC quantity at the 100 mL volume was achieved, corresponding to a fold increase per day (FIPD) of 2.29; after a further 7 days of cultivation at the 1,000 mL scale, up to 2×10^9 hiPSCs were harvested. Taken together, we achieved a 125-fold increase in cell quantity over 14 days, representing an FIPD of 8.93. This quantity of hiPSCs readily meets the requirements of cell-based therapies and brings their clinical potential closer to fruition.

Abstract No. T16

Biofabrication of a perfusable 3D liver tissue construct using organoids

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Current in vitro systems do not allow an accurate prediction of drug-induced liver injury (DILI). DILI still occurs in clinical phases of drug development or even post-marketing, which makes it a serious health concern. New in vitro models that can reliably predict DILI are thus necessary.

Human bipotent liver organoids are long-lived and can be differentiated into hepatocytes and as such represent an exciting new in vitro model. However, organoids are heterogeneous in size, differentiation potential and functionality. Combining organoid technology with biofabrication offers unmatched possibilities to create perfusable and vascularized adult stem cell based tissue constructs under highly controllable and reproducible conditions, which can be used for studies predicting DILI.

We here combined liver organoid cells and liver mesenchymal stem cells (LMSCs) with a photocrosslinkable gelatin methacryloyl (GelMA) hydrogel to create 3D liver tissue constructs. Cells were printed in 5% w/v GelMA and subsequently cross-linked by UV-A irradiation. The simultaneous deposition of pluronic F127 as a sacrificial support material allowed the formation of a perfusable porous construct. Cell aggregates remained viable for at least six days after 3D printing. The combination of liver organoids and LMSC aggregates in GelMA increased the albumin expression compared to single cell aggregates. We also confirmed that the constructs are suitable for acute toxicity studies, since we observed a four-fold increase in ATP levels after Triton X-100 treatment.

Furthermore, we designed a flow bioreactor that allowed printing of GelMA embedded cells directly into the bioreactor, and subsequent perfusion culture of the construct. Bioreactors were custom designed and produced by stereolithography technology. They ensure highly stable culture conditions, and functional in situ assays without compromising the liver tissue construct.

Our results indicate that perfusable 3D liver constructs in a bioreactor system have the potential to better predict DILI. We are currently further optimizing the system.

Abstract No. T17/P083

Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modeling

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The generation of acinar and ductal cells from human pluripotent stem cells is a poorly studied process, albeit various diseases arise from this compartment. We designed a straightforward approach to direct human pluripotent stem cells (PSC) toward pancreatic organoids resembling acinar and ductal progeny. Extensive phenotyping of the organoids not only shows the appropriate marker profile but also ultra-structural, global gene expression and functional hallmarks of the human pancreas in the dish. Upon orthotopic transplantation into immunodeficient mice, these organoids form normal pancreatic ducts and acinar tissue resembling fetal human pancreas without evidence of tumour formation or transformation. Finally, we implemented this unique phenotyping tool as a model to study the pancreatic facets of cystic fibrosis (CF). For the first time, we provide evidence that in vitro, but also in our xenograft transplantation assay, pancreatic commitment occurs generally unhindered in CF. Importantly, CFTR-activation in mutated pancreatic organoids not only mirrors the CF-phenotype in functional assays but also at a global expression level. We also conducted a scalable proof-of concept screen in CF-pancreatic organoids using a set of CFTR correctors and activators, and established an mRNA-mediated gene therapy approach in CF-organoids. Taken together, our platform provides novel opportunities to model pancreatic disease and development, screen for disease rescuing agents and to test therapeutic procedures.

Abstract No. T18

Differentiation of pericytes from hiPSCs for the vascularization of bioartificial cardiac tissues

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Introduction: Living grafts produced by combining stem cells and tissue engineering techniques are a promising therapeutic option for post-myocardial infarction patients. In order to generate functional heart tissue, vascularization is a critical aspect. Next to endothelial cells (EC), pericytes (PC) are also known to play an important role in vessel development and stabilization. PCs are identified by the co-expression of surface markers e.g. NG2, PDGFRβ, CD146 and contractile proteins e.g. α-smooth muscle actin (αSMA) and calponin (CNN1). Although recent protocols describe the generation of PCs from human induced pluripotent stem cells (hiPSCs), there is no directed differentiation strategy for the generation of pure PCs. Here we describe a modified PC differentiation and selection protocol and an *in vitro* model for the investigation of the role of PCs in vascular network formation in bioartificial cardiac tissues (BCT).

Methods: For the differentiation hiPSCs were cultivated in the presence of BMP-4, VEGF-A and CHIR99021 for three days (D). Afterwards, the culture medium was supplemented with VEGF-A and SB431542. On D10 the FACS-sorted $CD31^-/PDGFR\beta^+$ fraction was further maturated either in Pericyte medium (Promocell) or DMEM complemented with growth factors (TGFβ, PDGF-BB). PC marker expression was analysed by flow cytometry, qRT-PCR and immunofluorescence staining. BCTs were prepared as previously described with or without hiPSC-ECs and hiPSC-PCs. Contraction forces were measured and the spatial organization of different labelled cell types was examined using fluorescence microscopy.

Results and Conclusions: With our PC differentiation protocol up to 94% of the cells are $CD31^-/PDGFR\beta^+$ on D10 and after maturation they express the contractile proteins αSMA and CNN1. The expression of PDGFRβ (>90%) and proliferation capacity was maintained in PC medium, only. Addition of hiPSC-ECs and hiPSC-PCs resulted in spontaneously formed networks distributed throughout the spontaneously beating BCTs while maintaining mechanical properties compared to control tissues.

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

Abstract No. T19/P145

Stiff matrix induces switch to pure beta-cardiac myosin heavy chain expression in human embryonic stem cell-derived cardiomyocytes

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Cardiomyocytes generated from human pluripotent stem cells (hPSCs) represent a powerful tool for in vitro modelling of cardiac diseases such as hypertrophic and dilated cardiomyopathies. Yet, knowledge about the expression and functional impact of different sarcomeric protein isoforms like myosin heavy chain (MyHC) in hPSCs is poorly understood.

After differentiation, human embryonic cardiomyocytes (hESC-CMs) typically express high levels of the fast atrial myosin heavy chain (α-MyHC). In human ventricular cardiomyocytes, however, the slow β-MyHC predominates.

Here we aimed to generate hESC-CMs with exclusive β-MyHC protein expression in individual cardiomyocytes and to characterize the contractile properties of single cardiomyocytes with different MyHC-isoform composition.

MyHC-isoform composition and contractile properties of single cardiomyocytes were evaluated in prolonged in vitro culture in cardiac bodies (soft matrix) versus cardiomyocytes plated onto laminin coated glass coverslips (rigid matrix).

Using a specific antibody against ventricular β-MyHC and a newly generated anti-atrial α-MyHC-specific antibody we found that individual cardiomyocytes grown in cardiac bodies for a maximum of 110 days mostly contained a mixture of α- and β-MyHC. Only a minority of about 10% of cardiomyocytes expressed β-MyHC exclusively. However, cardiomyocytes plated on laminin-coated coverslips shifted MyHC-expression towards 66% and 87% of all cardiomyocytes expressing exclusively β-MyHC after 35 and 75 days, respectively. This isoform switch was accompanied by morphological changes towards more elongated cardiomyocytes with highly organized sarcomeres. Surprisingly, twitch kinetics and calcium transients were found unaffected by the MyHC-isoform in the sarcomeres while cardiomyocytes grown on laminin-coated coverslips in general displayed faster twitch kinetics and calcium transients.

In conclusion, stiff substrate induces pure β-MyHC-protein expression in hESC-CMs, with several contractile parameters close to ventricular cardiomyocytes, thus providing a well-defined in vitro system for modeling of cardiomyopathies and drug screening approaches.

Abstract No. T20/P126

A new model to study neurotoxicity of drug metabolites based on chemical conversion to Neurons on a chip in tandem with liver-on-a-chip

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Microscale engineering technology can mimic organ microenvironments through integration of multi-cellular and -functional devices and thereby offers a unique niche to study physiology and pathophysiology of human tissues. Recently, several models have been developed to investigate neuroregeneration and neurodegeneration towards personalized medicine, in which patient-specific iPSCs are exploited. However, this process including generation of patient-specific iPSCs and differentiation to neurons are very complicated and time-consuming. In an effort to establish a reliable and reproducible chemical reprogramming protocol, we used small molecules derived from high throughput screenings based on their ability to induce pluripotency-associated transcription factors involved in reprogramming and transdifferentiation. Surprisingly, we found one compound is sufficient to convert fibroblasts to Tuj1+/MAP2+ neuron-like cells with comparable efficiency to virus-mediated reprogramming (5%). Optimization by combination with other small molecules and growth factors led to discover a chemical cocktail able to directly generate neurons from patient fibroblasts with more than 80% efficiency. More intriguingly, Tuj1+ neuron-like cells can be observed within days and MAP2+/NeuN+/Tau+ within one week. In cooperation with ChipShop (Jena, Germany), we applied this cocktail to a microfluidic chip-based system and reproducibly achieved conversion of fibroblasts to Tuj1+/Chat+ neuron-like cells within days. Finally, we developed a microfluid-based model in a tandem neuron-on-a-chip, liver-on-a-chip design to study patient-specific neurotoxicity of drug metabolites, which can be potentially applied for personalized disease model and neurotoxicity study of drugs and drug metabolites via high throughput screening.

This work was supported by BMBF Grant FKZ 0315398B and FKZ 031A303E.

Cheng, X. et al. Identification of 2-[4-[(4-Methoxyphenyl)methoxy]-phenyl]acetonitrile and Derivatives as Potent Oct3/4 Inducers. J Med Chem 2015, 58, 4976-83

Cheng, X. et al. Chemically reprogramming human fibroblast into neuron-like cells by single small molecule. Submitted

Abstract No. T21

Employing rapid phenotypic assays in SPG4 patient neurons for drug discovery and rescue

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Hereditary spastic paraplegia (HSP) is characterized by progressive spasticity in the lower limbs caused by axonal degeneration of corticospinal motoneurons. Spastic paraplegia 4 (SPG4) makes up 40% of all HSP cases and is the most frequent, autosomal dominant subtype. Affected patients carry mutations in the SPAST gene encoding the microtubule-severing enzyme spastin. So far, no curative treatment for HSP is available. Thus we became interested in developing a SPG4 model enabling rapid phenotypic analyses within a few days. IPSCs from three patients carrying heterozygous SPAST nonsense mutations were differentiated into highly enriched neuronal cortical cultures comprising >80% glutamatergic neurons expressing the layer V/VI markers CTIP2 and TBR1 that can be cryopreserved to be readily available for downstream assays. We found that axonal swellings, a hallmark of HSP pathology, can be reliably detected already 5 days after plating of SPG4 iPSC-derived cortical neuronal progenitors. Swellings were 1-7 µm in diameter and stained positive for the axonal marker TAU1. In an in vitro regeneration assay SPG4 neurons exhibited a 40% reduction in overall neurite length compared to controls already 24 hours after plating. At that time point we also observed enlarged growth cones suggestive of cytoskeletal imbalance. To identify new potentially therapeutic compounds counteracting SPG4-associated neuronal phenotypes, we transferred all three fast phenotypic assays to a semi-automated 96-well setup. Several drugs rescued one or two phenotypes but only one compound positively modulated all three phenotypes in SPG4 neurons without affecting healthy controls. Overall, we expect the described rapid phenotypic assays on SPG4 neurons to accelerate the study of pathomechanisms underlying HSP as well as the drug development for the treatment of this disease.

Abstract No. T22

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

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

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

We generated iPSCs from heterozygous Valin30Methionin and Glycin47Alanin patients and differentiated these towards hepatocyte like cells (HLCs). Investigating the gene expression of hepatic markers (Albumin, AFP and TTR), we detected comparable expression levels in TTR-FAP-HLCs and in control HLCs. The TTR-FAP-HLCs also showed functional characteristics like secretion of TTR and Albumin as well as cytochrome P450 1A1 activity. By mass spectrometry TTR protein with wildtype sequence as well as protein with Valin30Methionin or Glycin47Alanin substitution, respectively, was found in supernatants of TTR-FAP-HLCs. Most importantly for the disease modeling approach, those supernatants contained significant amounts of TTR in an insoluble form. This insoluble TTR could be identified as amyloid fibrils by staining with intercalating dyes such as Congo Red and Thioflavin T. Importantly, amyloid fibril formation was also detectable in multiwell plate cell culture formats as prerequisite for potential drug screening approaches.

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

Abstract No. T23

Dnmt3b-dependent intragenic DNA methylation prevents RNA Polymerase II spurious entry on gene bodies and cryptic transcription initiations

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DNA methylation is a heritable epigenetic modification required for embryonic development and it causes transcriptional repression, when established on gene promoters. Recent studies reported that Dnmt3b binds preferentially to the gene bodies by interacting with the histone modification H3K36me3. The molecular and biological functions of intragenic DNA methylation are still unknown, although deregulation of this epigenetic feature has been associated with several diseases.

The hypothesis of its function in several reviews and recent papers mainly concerned regulation of splicing, inhibition of expression of repetitive elements, compacting chromatin. We analyzed all the hypotheses, but our experiments unveil the actual function of intragenic DNA methylation, which is to inhibit the entry of RNA polymerase within the gene body.

Here we show that the Dnmt3b-dependent intragenic DNA methylation protects the gene body from RNA Polymerase II (RNA Pol II) spurious entry and cryptic transcription initiations. By using different genome-wide approaches, we demonstrate that loss of Dnmt3b leads to an increase of the RNA Pol II engagement within gene bodies resulting in the onset of spurious intragenic transcription initiations. We specifically demonstrated that inhibition of RNA Pol II spurious entry depends on the enzymatic activity of the Dnmt3b recruited by H3K36me3.

Our results elucidate the functional role of the Dnmt3b-dependent 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 in embryonic stem cells.

Abstract No. T24

Aging shifts the mode and outcome of the hematopoietic stem cell division

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Hematopoietic stem cells (HSCs) balance self-renewal and differentiation to maintain the homeostasis of the hematopoietic system throughout the lifespan of an organism. With ageing their functional activity decrease and they expand in number resulting in impaired tissue homeostasis, reduced engraftment following transplantation and increased susceptibility to disease. Many drivers of HSC ageing have been proposed but mechanisms explaining why HSC function degrades with age remain unknown. We hypothesize that the aging phenotype is a consequence of the imbalance between symmetric and asymmetric cell divisions driven by the age-depended loss of cell polarity. We use different functional and transcriptional assays to analyze the fate of individual daughter cells after division. Our data shows that aged HSCs, which are mainly apolar for the distribution of Cdc42 and H4K16ac, undergo more frequently symmetric divisions. We also show that the asymmetric/symmetric mode of distribution of Cdc42 and H4K16ac in daughter cells in vitro correlates with an asymmetric/symmetric colony forming ability and expression program (transcriptome) in vitro and an asymmetric/symmetric outcome in vivo. Restoring proper cell polarity in aged HSCs by in vitro CASIN (Cdc42-activity inhibitor) treatment increases the number of asymmetric divisions to a frequency similar to young untreated HSCs, while an increased Cdc42 activity after Wnt5a treatment in young HSCs is paralleled by an increased propensity to undergo symmetric division. Our findings are supported by a mathematical model, which indicates that a loss in the ability to regulate cell polarity prior to division is a potential molecular mechanism to convey the aging phenotype. We reason that the capability to direct asymmetric cell division is a key feature of HSCs to robustly adjust the balance between self-renewal and differentiation, which is progressively lost during aging.

Abstract No. T25

Bone marrow niche and hematopoietic stem cell differentiation are regulated by the microbiota

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Obesity has become a worldwide health problem, associated with pre- mature death and reduced life quality. The molecular and cellular mechanisms explaining how obesity negatively affects the function of multiple organs are not fully understood. Changes in the function of the hematopoietic and immune cell compartment are an attractive concept to explain the deleterious effects of obesity on organ function. We showed that high fat diet (HFD) decreased long-term Lin-Sca-1+c-Kit+ (LSK) stem cells and shifted lymphoid to myeloid cell differentiation. Functionally, these changes induced by HFD led to a lethal failure of the hematopoietic reconstitution after 5-fluorouracil treatment as well as a coagulation defect likely due to low number of platelets in obese mice. Mechanistically, HFD led to robust activation of PPARγ2, which impaired osteoblastogenesis while enhancing bone marrow adipogenesis. At the same time expression of genes, such as Jag-1, SDF-1 and IL-7, forming the bone marrow niche was highly suppressed after HFD. Moreover, structural changes of microbiota were associated to HFD-induced bone marrow changes. Antibiotic treatment partially rescued HFD-mediated effects on the bone marrow niche, while transplantation of stools from HFD mice could transfer the effect to normal mice. These insights will open new possibilities to impact BM and immune cell function by modulation of microbiota.

Abstract No. T26

Restricted regeneration of hematopoietic stem cells in vivo following chronic inflammatory stress

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Ageing of the hematopoietic system in humans is characterized by peripheral blood cytopenias and bone marrow aplasia (termed anemia of ageing) as well as increased incidence of hematologic malignancies. These phenomena are likely caused by gradual attrition of the hematopoietic stem cell (HSC) compartment. Unfortunately, the standard experimental mouse model of hematopoiesis does not recapitulate these important clinical features of ageing.

We have recently shown that prolonged exposure to inflammatory stress leads to premature hematologic ageing in wild type mice, caused by depletion of the HSC pool (Walter et al. Nature, 2015). In order to further dissect this phenotype, we exposed wild type mice to a dose escalation of 1, 2 or 3 rounds of treatment with polyinosinic:polycytidylic acid (pI:pC) in order to mimic viral infection and promote a type I interferon response. We observed a progressive depletion of the HSC compartment, with each round of pI:pC treatment provoking an approximate 2-fold reduction in HSCs/femur, as assessed by competitive repopulation assay. Inflammatory stress could be demonstrated to vacate HSC niches within the bone marrow, since treated mice could establish a donor graft of up to 65% when injected with purified lineage-, Sca1+, c-Kit+ cells in the absence of any conditioning regimen. Critically, when mice were allowed to recover for up to 20 weeks following treatment, there was absolutely no evidence of HSC regeneration, demonstrating that HSCs fail to engage a program of extensive self-renewal in vivo following inflammation-induced depletion.

Our data contradict the canonical view that HSCs demonstrate extensive self-regenerative capacity following injury, but instead are progressively depleted by hematologic stress in a manner consistent with an age-associated decline in function. We would propose that the absence of profound hematologic ageing phenotypes in laboratory mice could be explained by their lack of exposure to environmental stress agonists, including infection.

Stress-induced exit from dormancy provokes de novo DNA damage in hematopoietic stem cells leading to cellular attrition and eventual bone marrow failure. Walter D. et al. Nature (2015)

Abstract No. T27/P059

Hoxa9 induced developmental signals impair stem cells and regeneration of aging muscle

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Aging induced impairments in muscle stem cells, also known as satellite cells (SCs), contribute to the onset of sarcopenia during aging. Alterations in several pathways have been demonstrated to lead to the functional decline of SCs during aging, but master regulators mediating these gene regulatory defects have yet to be identified. There is an emerging discussion that developmental pathways may contribute to the inhibition of stem cell function and tissue maintenance in aging tissue. Hox genes are master regulators of stem cells and tissue patterning during embryogenesis with an unknown role in aging. Here we analyzed the functional role of Hox genes in aging induced impairments in SC function and skeletal muscle regeneration. From all genes of the 4 Hox gene clusters (A-D), specifically Hoxa9 is strongly overexpressed in activated SCs in response to muscle injury in aged mice. The study provides experimental evidence that Hoxa9 hyperinduction represents a main cause for alterations in key pathways that impair SC self-renewal and muscle regeneration in aging mice including Bmp-, JAK/STAT-, and Wnt-signaling as well p16 induction. Mll1 dependent histone modifications and chromatin decompaction of the HoxA gene cluster are identified as the cause of aging associated Hoxa9 hyperinduction in activated SCs. Of note, the knockdown of Hoxa9 by itself rejuvenates the regenerative capacity of SCs to repair injury of skeletal muscle in aging mice. Together, these data delineate a dynamically regulated, epigenetic loop that is induced upon activation of aged SCs limiting SC function and muscle regeneration by aberrant activation of developmental pathways.

Abstract No. T28

Scoring Cell Identity from Transcription Profiles

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Induced pluripotent stem cell (iPS) somatic cell reprogramming and direct lineage programming offer promising autologous and patient-specific sources of cells for personalized drug-testing and cell-based therapy. However, it is important to evaluate the extent to which the derived cells resemble their desired target cell types before the derived cell types can be widely used. We have developed a method to generate a cell identity score, which can be used to evaluate the success of a derived/reprogrammed cell type in relation to its donor and desired target cell type. We show that in the case of reprogramming to iPS cells, the method is able to distinguish partially and fully reprogrammed iPS cells. Meta-analysis of a number of studies also highlights "outlier" cell lines in the public domain. The method is based on microarray expression data, but in principle, the method can be applied to any transcriptome data in which reference data is available for the donor and target cell, including RNA-seq data.

Abstract No. T29

Understanding and predicting regulatory mechanisms in early differentiation of human pluripotent stem cells

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With their capability to self-renew and to differentiate into derivatives of all three germ layers, human pluripotent stem cells (hPSCs) offer a unique cell source for regenerative therapies and to study human development. Mesendodermal differentiation in vitro is a complex process, recapitulating transition through a primitive streak (PS)-like stage. PS induction and patterning into definitive endoderm, cardiac mesoderm and presomitic mesoderm is initiated by the concentration-dependent supplementation of the chemical compound CHIR99021, a GSK3 inhibitor resulting in activation of the WNT pathway. Another experimental parameter that we have recently identified to direct PS patterning, is the bulk cell density (BCD). Our data suggest that modulation of the BCD can dominantly shape earliest steps of differentiation, which seems to be mediated by paracrine factors. However, the identity and mechanistic consequences of these factors are currently unknown.

To quantitatively study the dynamics of these regulatory processes and to reveal their interplay, we applied a mathematical modeling approach that is based on ordinary differential equations. We compared time-course data of early hPSC differentiation to increasingly complex model structures, incorporating a rising number of paracrine factors. Model simulations demonstrate the importance of CHIR99021 as the driving factor in PS-like patterning. Furthermore, our approach suggests that at least three paracrine factors are required to recapitulate the experimentally observed dynamics. In particular, feedback mechanisms from both, differentiated as well as undifferentiated cells, turned out to be crucial. Evidence from double knock-down experiments and secreted protein enrichment allowed us to hypothesize the identity of two of the three factors predicted by the computational approach. Moreover, the mathematical model suggests optimal settings for directing lineage-specific differentiation protocols, which are subsequently validated experimentally.

Abstract No. T30

FGF/MAPK signaling sets the switching threshold of a bistable circuit controlling fate decisions in embryonic stem cells.

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Differentiating cells take fate decisions based on the activities of transcriptional regulators and signaling pathways. How these activities are integrated to ensure that different lineages are reliably populated from heterogeneous precursor populations is not well understood. The emergence of the epiblast (Epi) and primitive endoderm (PrE) lineage from cells of the inner cell mass of the mammalian preimplantation embryo is a simple example for this problem. PrE differentiation requires FGF/MAPK signaling and the activity of GATA transcription factors, but how these inputs are integrated to specify the appropriate number of PrE cells is not known. To address this question, we have developed an embryonic stem (ES) cell model for the Epi-vs-PrE fate decision using doxycycline-inducible expression of mCherry-tagged GATA factors. In combination with live reporters for the respective fates, this system allows us to manipulate and track the inputs into this fate decision at the level of single cells, and to follow the dynamics of fate choice with high temporal resolution. We find that a threshold dose of GATA factors is required to induce PrE-like differentiation in individual ES cells, and that the level of FGF/MAPK signaling determines the quantity of this threshold dose. Our dynamic experimental data can be recapitulated by a simple toggle-switch model for the decision-making circuit, which defines basins of attraction for each fate. The position and depth of these basins is a function of the FGF/MAPK signaling level in the system, thereby controlling the proportion of differentiating cells for a given distribution of transcription factor inputs. In the future we will apply a similar combination of live-cell imaging, quantitative measurements and mathematical modeling to test if this is a generic mechanism through which signaling controls cell fate decisions beyond those of preimplantation development.

Abstract No. T31

Reconstructing lineage branching from single cell RNA-seq in adult haematopoiesis via diffusion pseudotime

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Single-cell transcriptomic sequencing allows determining subtle changes in gene expression profiles as they appear in continuous differentiation processes. However, there is often substantial asynchrony in cell fate decisions contributing amongst other factors (such as cell cycle) to the observed cellular heterogeneity in such data. Still, single-cell gene expression profiles of differentiating cells encode their intrinsic latent temporal order, which can be computed from similarities in the gene expression profiles among single cells.

We describe an efficient way to estimate this intrinsic order according to a *diffusion pseudotime*, which measures transitions between cells using diffusion-like random walks. Particularly, *diffusion pseudotime* exploits the information of the full dimensional gene expression space without preceding dimension reduction. This allows us to order cells by the degree of differentiation. Using this synchronized view, we can identify cells that undergo branching decisions or are in metastable states, and recover genes being differentially regulated at these states.

We apply *diffusion pseudotime* to a recently published single-cell RNAseq study of myeloid progenitors in adult haematopoiesis (Paul et al 2015). Here, bone marrow cells are FACS sorted for the c-Kit+Sca1-Lin- population and 2730 cells are sequenced via MARS-seq protocol. Without complex pre-processing, *diffusion pseudotime* identifies branching into different myeloid lineages and a subpopulation of lymphoid outliers as well as a graded transition reflecting erythroid differentiation that dissents from previously stated clusters. In contrast to clustering approaches, *diffusion pseudotime* allows for metastable states and enables to distinct whether differences in gene expression arise from cell fate decisions or differentiation along a lineage encoded as branches and paths. In each lineage path in adult haematopoiesis, we observe a sequential rise in gene expression for lineage specific genes.

Thus, our approach serves as basis to identify regulatory events and interactions and can help to resolve molecular interactions during differentiation and lineage decisions.

Transcriptional Heterogeneity and Lineage Commitment in Myeloid Progenitors, Paul et al (2015), Cell 163, 1663–1677

Abstract No. T32

Designer-nuclease mediated knockout of HIV co-receptor CCR5 – a promising gene-therapy approach to protect T cells from HIV infection

Ulrike Mock[1], *Rafał Machowicz*[1], *Ilona Hauber*[2], *Alena Shakirova*[1], *Stefan Horn*[1], *Pierre Abramowski*[1], *Belinda Berdien*[1], *Joachim Hauber*[2], and *Boris Fehse*[1,*]

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Besides its physiological role, the chemokine receptor CCR5 plays an essential part during HIV infection, acting as the co-receptor for so-called R5-tropic strains that usually mediate initial infection. App. 1% of Caucasians are homozygous for a natural deletion variant of CCR5 (CCR5Δ32) and, based thereon, resistant towards infection with R5-tropic HIV strains. The "Berlin patient" provided evidence that resistance can be acquired even in case of established HIV infection by transfusion of CCR5-negative cells. Consequently, CCR5 has become an interesting target for gene-editing strategies using designer nucleases. We have recently introduced a new transcription activator-like effector nuclease, CCR5-Uco-TALEN for highly specific CCR5 knockout. Long-term and/or high-level expression of designer nucleases increases the risk of unwanted off-target activity. Therefore, we established an expedient mRNA-electroporation protocol for primary T lymphocytes – a gentle and truly transient method for efficient TALEN delivery. The latter enabled us to regularly obtain high-rate CCR5 knockout in T cells combined with low off-target activity, as demonstrated by flow cytometry and next-generation sequencing. More recently, we have gained evidence that efficient CCR5 knockout can be achieved in CD34-positive haematopoietic stem cells, too. We also devised and validated a novel, very handy digital-PCR technique that facilitates easy and fast detection of gene-knockout frequencies with a sensitivity of at least 0.2%. Using functional assays, we finally showed that CCR5-edited T cells were not only resistant towards HIV-derived lentiviral vectors, but also protected from infection with the replication-competent CCR5-tropic HIV-1BaL strain. Indeed, exposure to HIV-1BaL resulted in a profound selective advantage of CCR5-gene edited T cells in vitro and in vivo. In summary, we have introduced a novel TALEN for high-efficiency knockout of CCR5. We propose that our new CCR5-Uco-TALEN in conjunction with the mRNA-based transfection protocol has a strong potential for immediate clinical translation.

Abstract No. T33

Efficient introduction of homo- and heterozygous mutations with CRISPR/Cas9 and applications to disease modeling in stem cells

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CRISPR/Cas9 is a promising tool to generate human disease models, e.g. in human induced pluripotent stem cells (iPSCs). CRISPR/Cas9 introduces double stranded breaks (DSBs) with high efficiency and locus specificity, which are typically repaired by non-homologous end-joining (NHEJ) resulting in nonspecific indel mutations, useful for generating knockouts. DSBs can also be repaired by homology-directed repair (HDR) using a DNA template, such as an single-stranded oligo DNA nucleotide (ssODN), allowing knock-in of specific mutations. Although CRISPR/Cas9 is used extensively to engineer gene knock-outs through NHEJ, editing by HDR remains inefficient and can be corrupted by additional indels, preventing its widespread use for modeling genetic disorders by introducing disease-associated mutations. Furthermore, targeted mutation knock-in at single alleles, to model diseases caused by heterozygous mutations, such as early-onset Alzheimer's disease (EOAD), has not been reported.

We developed an efficient CRISPR/Cas9-based genome-editing framework that allows selective introduction of mono- and bi-allelic sequence changes with high efficiency and accuracy into iPSCs.

We show that HDR accuracy is dramatically increased by incorporating silent CRISPR/Cas-blocking mutations together with pathogenic mutations, and establish a method we termed "CORRECT" for scarless editing. Furthermore, by exploiting a stereotyped inverse relationship between the incorporation rate of a mutation and its distance to the DSB, we achieve predictable control of zygosity. Homozygous introduction require targeting a guide RNA close to the intended mutation, whereas heterozygous introduction can be achieved by distance-dependent suboptimal mutation incorporation or by using mixed repair templates. Using this approach, we establish human induced pluripotent stem cells (iPSCs) with homozygous and heterozygous dominant EOAD mutations in amyloid precursor protein (APPSwe) and presenilin 1 (PSEN1M146V) and derive cortical neurons, which display genotype-dependent disease-associated phenotypes.

Our findings enable efficient introduction of homo- and heterozygous disease-associated mutations with CRISPR/Cas9, facilitating the study of molecular mechanisms of dementia in human in vitro models.

Abstract No. T34

Efficient and accurate precision genome engineering of transcriptionally silent disease-related loci by CRISPR/Cas9 nickase

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We have used Cas9_D10A nickase in combination with specific sgRNAs for the precise modification of various disease-related and transcriptionally silent genomic loci in patient-derived iPSC. To this end, we targeted the SERPINA1-locus in iPSC from a patient with severe-α1-antitrypsin-deficiency (ATD), the TTR-locus of a familial-amyloid-polyneuropathy (FAP) patient and the SOD1-locus of individuals with amyotrophic-lateral-sclerosis (ALS). We employed a highly sensitive lentiviral vector-based reporter-assay for the in cellam evaluation of gRNA efficiencies at their respective on- and off-target loci. This system allowed us to identify highly efficient sgRNAs which can be applied in concert with Cas9_D10A at genomic target loci. Moreover, we could distinguish differences in HDR efficiencies on target-sequences which differ by a single nucleotide, allowing for the identification of allele-specific sgRNAs. Efficient combinations of opposite strand-binding sgRNAs were identified for disease-specific loci in ATD-, FAP- and ALS-iPSC and were applied for efficient gene editing. To this end, we used two sgRNAs together with a ssODN-donor for a one-step bi-allelic correction of ALS-iPSC. For ATD- and FAP-iPSC we have used a selectable 'long-flanking-arms' plasmid-donor and we found bi-allelic integration in up to 40% of selected clones. Moreover, we have designed an improved version of a piggyBac-flanked puroΔtk selection/counter-selection cassette, which allowed us to pre-select potentially bi-allelic targeted clones by flow cytometry. Using the silencing-resistant CAG promoter, we achieved 100% counter-selection efficiency in puro-sensitive FIAU-resistant clones after excision of the puroΔtk cassette by piggyBac transposase. Moreover, none of the corrected clones showed any detectable sequence changes at the top five off-target sites predicted by http://crispr.mit.edu/ for each sgRNA used.

Together, we present a toolbox for efficient and accurate CRISPR/Cas9-based gene editing at transcriptionally silent loci in patient-derived iPSC. Using double-nicking and an improved selection/counter-selection cassette, we have established a system which allows for fast and high-throughput identification of precisely edited cells.

Abstract No. T35

Generation of HLA depleted human pluripotent stem cell lines for modulation of the immunogenicity of iPSC derivatives.

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Regenerative medicine offers great potential for emerging cell or tissue replacement strategies, but a major problem in the development of such therapies is the provision of clinically relevant cell sources, especially regarding their quality and quantity. Human pluripotent stem cells (hPSCs) could serve as an adequate cell source due to their unlimited potential for proliferation and differentiation into all different cell types of the human body. Using autologous induced pluripotent stem cells (iPSCs) for each patient would be associated with high medical costs and an exceeding timeframe for iPSC generation, correction, differentiation and accompanying quality control. In consequence, more realistic is the collection of iPSC stocks derived from various donors and the establishment of both human embryonic stem cell (hESC) and iPSC banks to reduce the risk of rejection. Immunological tolerance of transplanted cells or tissue is mainly dependent on the human leukocyte antigens (HLAs), discriminating "self" from "non-self". A solution to circumvent immunological rejection might be the generation of universal HLA depleted iPSCs which should be clinically applicable for multiple allogeneic recipients without or with very limited pharmacological immunosuppression. For this reason, we excised complete or individual regions of HLA class I and/or class II on chromosome 6 in hiPSCs via genome editing with TALENs. After we successfully excised HLA-A, we generated a full HLA knockout iPSC clone to use for further analysis. These cell lines were first differentiated into endothelial cells to compare between undifferentiated and differentiated cells. We also performed first immunological in vitro assays, e.g. IFN-γ EliSpot, to analyze their rejection potential.

Beside the immune response triggered by T cells, also NK cell-mediated cell lysis has to be addressed, which will be achieved via the integration of a constitutively expressed artificial HLA-E molecule in the safe harbor AAVS1 site of the respective cell lines.

Abstract No. T36

Fate-restriction precedes stemness during massive post-embryonic growth in the fish branchia

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In animals that reach a final fixed size - like mammals -, adult stem cells maintain organ homeostasis by generating cells that replace the ones that are regularly lost. Adult fish increase their size constantly and therefore stem cells have an additional task, i.e. to drive organ growth besides maintaining organ homeostasis. This feature places fish as a fascinating model to explore whether these two tasks - homeostatic replacement and growth - are performed by the same or by different individual cells within a stem cell population. We have developed a CRE/LoxP toolkit that allows tackling this and other issues, including how new stem cells are generated during post-embryonic stages, and how the proper number of stem cells per organ is regulated in an intact organism.

The fish branchia has a fairly modular organization. A branchia is divided in branchial arches, branchial arches are composed of filaments and filaments contain lamellae. By using short and long-term linage analysis we have identified fate-restricted stem cells in all these compartments that either maintain homeostasis or sustain organ growth. Using the exquisite temporal organization of the organ, we mapped the cell-of-origin of post-embryonic lamella to the tip of a filament, and the cell-of-origin of post-embryonic filaments to peripheral extreme of a branchial arch. We could track back clones to embryonic stages to conclude that in the fish branchia, fate-restriction precedes stemness. Additionally, we used mathematical modeling to prove that the entire system can be generated and maintained with a very reduced number of stem cells that work coordinately to sustain the constant generation of functional units to the organ.

Abstract No. T37/P023

Visualization of stem cell induction and differentiation in real time

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Tissue homeostasis depends on self-renewing cells while developmental cues or stress conditions, such as injury or transplantation, activate dormant stem cells. Regardless of cell lineage and signaling pathways, the common signature of cellular activation is induction of mRNA transcription.

We showed previously that dormant adult stem cells of many different lineages, including intestinal, haematopoietic, neural, muscle, spermatogonial, skin and melanocyte lineages, display inactive RNA polymerase II (RNApII) during tissue homeostasis, suggesting a lower rate of global mRNA transcription.

We have now developed a specific reporter for detection of dormant stem cells derived by insertion of a short RNApII kinase target peptide directly into the yellow fluorescent protein Venus. This reporter, called OSCAR for Optical Stem Cell Activity Reporter, is phosphorylated and dim in activated cells and shows up to 9-fold induction of fluorescence in dormant cells, enabling isolation of adult stem cells by FACS and examination of stem cell dynamics by timelapse microscopy.

Analysis of small intestine crypt culture using OSCAR shows significant enrichment of colony formation in OSCAR^{high} cells, comparable to LGR5-GFP positive crypt stem cells. Using this reporter, dynamics of crypt stem cell formation and differentiation are visualized in real time.

Furthermore, examination of haematopoietic stem cells reveals strong heterogeneity of global mRNA transcription activity in this population. We also show that dormant stem cell-like cells can easily be detected in other primary cell populations, such as human melanoma biopsies.

Taken together, OSCAR is invaluable for identification, isolation and characterization of dormant adult stem cells from many different lineages and enables detection of pure stem cell-like cells in heterogeneous tissues, including cancer.

Abstract No. T38/P034

Embryo-derived macrophages regulate the dendritic cell pool size in the adult spleen

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Dendritic cells (DCs) are potent antigen presenting cells that depend on Flk2-mediated signals for their differentiation. In Flk2-deficient mice, however, a small but functionally active fraction of DCs remains. We show here that the combined deficiency for Flk2 and Csf1r, another member of the same family of receptor tyrosine kinases, results in natural DC null mice. In contrast to Flk2, Csf1r-mediated signals affect DC differentiation by a cell extrinsic, non-hematopoietic mechanism that fails to require Csf1r-mediated signals within the entire adult mouse. During embryonic development Csf1r signaling is crucial for Csf1r and F4/80 expressing macrophages which emerge from erythro-myeloid progenitors in the yolk sac before the presence of hematopoietic stem cells (HSCs). EMP-derived RPMps differ from definitive-HSC-derived macrophages by their site of emergence, the required transcription factors. We could show that Csf1r signaling is required for the development of specialized embryonic macrophages, the tissue-resident red pulp macrophages (RPMp) in the spleen of adult mice. RPMps are ontogenetically heterogeneous cells that are long-lived and have been implicated in the maintenance of tissue hemostasis, removal of pathogens and clearance of cellular debris. We assign here a novel important role to RPMps in the maintenance of tissue homeostasis by regulating the DC pool size in the spleen. In the absence of Flk2-mediated signals, loss of Csf1r expression results in complete ablation of spleen DCs due to the depletion of embryonic macrophages in vivo.

Abstract No. T39

A role for YAP and TAZ signaling in human neural crest development

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The Hippo tumor suppressor pathway has emerged as a major regulator of stem cell niches in ontogenesis, tissue homeostasis and cancer. Its downstream effectors YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ binding motif; WWTR1) mediate key developmental processes such as self-renewal, differentiation and survival. Neural crest cells represent a transient embryonic cell population with multipotent and migratory character that can generate diverse cellular progeny including melanocytes, chondrocytes, peripheral neurons and glial cells. Using multiple *in vitro* models of human neural development (pluripotent stem cell-derived neural stem cells; neuroblastoma cell lines), we set out to investigate the regulatory mechanisms linking Hippo signaling to neural crest development. YAP/TAZ overexpression and knockdown coupled with detailed signaling molecule analysis further corroborated a role for both factors in neural crest migration. Moreover, detailed flow cytometric subpopulation analysis enabled the identification of small molecule modulators of retinoic acid and BMP signaling in neural crest and neuroblastoma model systems. These data highlight the functional relevance of both YAP and TAZ signaling at early as well as late stages of neural crest differentiation and in cellular models of neuroblastoma.

Hindley CJ, Condurat AL, Menon V, Thomas R, Azmitia LM, Davis JA, Pruszak J: The Hippo pathway member YAP enhances human neural crest cell fate and migration. Sci Rep. 2016 6:23208.

Abstract No. T40

Stepwise maturation of human iPS cells into immunosuppressive mesenchymal stem/progenitor cells

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

In this study induced pluripotent stem cell (iPSC) lines were generated from healthy parental bone marrow (BM)-MSPCs and umbilical cord blood (UCB)-MSPCs to optimize GMP-compliant iPSC differentiation and study mechanisms of iPSC-derived mesenchymal progenitor cells (i-MPCs) function.

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

Under feeder-free conditions, iPSCs differentiated into CD73+/CD105+/Tra-1-81- MSPC-like cells lacking immune suppressive competence. Small molecules further modified the MSPC-like phenotype of i-MPCs. Additional passaging was required for reaching full immunosuppressive competence comparable to their parental MSPCs. Our data extend published knowledge by showing that the complete immune phenotype and functional repertoire of i-MPCs can be established in a stepwise order under animal serum-free conditions to comply with the functionality of their parental MSPCs. This strategy builds the basis for GMP-compliant iPSC-derived functional MSPC propagation.

Abstract No. T41

Cryopreserved or fresh mesenchymal stromal cells: only a matter of taste or key to unleash the full clinical potential of MSC therapy?

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Mesenchymal stromal cells (MSCs) harbor great therapeutic potential for numerous diseases. From early clinical trials, success and failure analysis, bench-to-bedside and back-to-bench approaches, there has been a great gain in knowledge, still leaving a number of questions to be answered regarding optimal manufacturing and quality of MSCs for clinical application. For treatment of many acute indications, cryobanking may remain a prerequisite, but great uncertainty exists considering the therapeutic value of freshly thawed (thawed) and continuously cultured (fresh) MSCs. The field has seen an explosion of new literature lately, outlining the relevance of the topic. MSCs appear to have compromised immunomodulatory activity directly after thawing for clinical application. This may provide a possible explanation for failure of early clinical trials. It is not clear if and how quickly MSCs recover their full therapeutic activity, and if the "cryo stun effect" is relevant for clinical success. Here, we will share our latest insights into the relevance of these observations for clinical practice that will be discussed in the context of the published literature. We argue that the differences of fresh and thawed MSCs are limited but significant. A key issue in evaluating potency differences is the time point of analysis after thawing. To date, prospective double-blinded randomized clinical studies to evaluate potency of both products are lacking, although recent progress was made with preclinical assessment. We suggest refocusing therapeutic MSC development on potency and safety assays with close resemblance of the clinical reality.

Moll G et al. Do cryopreserved mesenchymal stromal cells display impaired immunomodulatory and therapeutic properties? Stem Cells 2014; 32:2430-2442

Moll G and Le Blanc K: Engineering more efficient multipotent mesenchymal stromal (stem) cells for systemic delivery as cellular therapy. ISBT Science Series 2015; 10:357-365

Abstract No. T42

Synthetic niche to modulate regenerative potential of Mesenchymal Stromal Cells (MSCs) and enhance skeletal muscle regeneration.

Sven Geißler[1,], Taimoor H. Qazi[1], Matthias Pumberger[1], Carsten Perka[1], David J. Mooney[2]*, and *Georg N. Duda [1]*

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Skeletal muscles have an inherent capacity to regenerate after injury, but severe trauma often leads to fatty degeneration and fibrotic scar tissue formation, compromising muscle function and its structural integrity. Traditionally, tissue engineering strategies for muscle repair have focused on substrates that promote myogenic differentiation of transplanted cells. Since the failure of direct myoblast delivery in earlier clinical trials, current efforts include strategies aiming at the local delivery of progenitor cells, growth factors, or a combination thereof to support endogenous regeneration.

Here, we report that MSCs transplanted via multifunctional cryogels promote muscle regeneration by secreting bioactive factors that modulate the function of muscle progenitor cells. Perhaps most importantly, we present an innovative approach to prolong viability of transplanted MSCs while simultaneously boosting their secretory function via local stimulation by IGF-1 and $VEGF_{165}$. We engineered a porous multifunctional alginate scaffold that acts as a *synthetic niche* for MSCs, supports their adhesion and spreading, protects them during transplantation thereby prolonging viability, and enhances their paracrine effects via the local release of incorporated human growth factors. Remarkably, the conditioned media of growth factor stimulated MSCs was found to substantially influence myoblast proliferation, survival, migration, and differentiation under in vitro culture conditions. Accordingly, transplantation of this *synthetic niche* in a clinically relevant crush muscle trauma model significantly improved muscle strength, reduced fibrosis, and increased muscle fiber density and the number of regenerated fibers.

In summary, we showed that MSC-mediated paracrine signaling can support endogenous muscle healing processes in a robust manner, and that the functional and structural outcomes of regeneration can be further improved by enhancing the secretion pattern of MSCs via local growth factor stimulation. The *synthetic niche* approach described in this study is an important example of enabling in situ regeneration of tissues without physical engraftment of transplanted cells.

Pumberger et al. Synthetic niche to modulate regenerative potential of MSCs and enhance skeletal muscle regeneration. Biomaterials. 2016 May 10;99:95-108. (http://authors.elsevier.com/a/1T5zdWWN0Wwh4).

Qazi TH, Mooney DJ, Pumberger M, Geissler S, Duda GN. Biomaterials based strategies for skeletal muscle tissue engineering: existing technologies and future trends. Biomaterials. 2015;53:502-21. (Review)

Abstract No. T43

Extracellular Vesicles – From Bench to Bedside

Verena Börger[1,], Michel Bremer[1], Rita Ferrer Tur[1], Amina Becic[1], Peter A. Horn [1]*, and *Bernd Giebel[1]*

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*Presenting author

Human mesenchymal stem/stromal cells (MSCs) represent a promising tool in regenerative medicine. Up to now, more than 600 NIH-registered clinical trials investigated their therapeutic potential in various diseases. Despite controversial reports regarding the efficacy of MSC-treatments, MSCs seem to exert their beneficial effects rather in a paracrine manner than by cell replacement. In this context, extracellular vesicles (EVs), such as exosomes and microvesicles, are discussed to execute the MSCs' therapeutic effects.

In collaboration with different project-partners, human MSC-derived EVs were tested for their therapeutic impacts in various disease models including a human steroid-refractory graft-versus-host disease (GvHD) patient. In a murine stroke model MSC-EV administration reduced ischemic stroke-induced symptoms to the same extend than MSCs themselves. Upon MSC-EV application hypoxia-ischemia induced brain damages were reduced in a foetal sheep model. In a neonatal rat model the degree of inflammation-induced preterm-brain-injury was successfully suppressed following MSC-EV injection. The symptoms of the GvHD patient declined significantly following a two weeks lasting MSC-EV therapy. Thus, our data confirm therapeutic activities of the prepared MSC-EV fractions. Since in all of these models MSC-EV treatment reduced elevated immune responses, we consider that MSC-EVs – as part of their "mode of action" machinery – can modulate immune responses.

Aiming to qualify MSC-EVs as a novel therapeutic agent to initially treat additional steroid-refractory GvHD patients, we have optimized the large scale production of clinical grade MSC-EVs. Furthermore, for the functional testing an in vitro assay was set up, allowing us to study the immune-modulatory activities of prepared MSC-EV samples on different immune cell types. So far, in a MSC-line specific manner this assay allows us to reproducibly detect differences in the immuno-modulatory activities of prepared MSC-EV fractions. Currently, this assay gets qualified and validated to serve as potency assay for clinical grade MSC-EV preparations in the future.

Abstract No. T44/P064

ZEB1 turns into a transcriptional activator by interacting with YAP1 in aggressive cancer types

Waltraut Lehmann[1], Dirk Mossmann[2], Julia Kleemann[3,], Kerstin Mock[1], Chris Meisinger[2], Tilmann Brummer[4], Ricarda Herr[5], Simone Brabletz[3], Marc P. Stemmler[3]*, and *Thomas Brabletz[3]*

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[5]Institute for Molecular Medicine and Cell Research, Albert-Ludwigs-University Freiburg, Germany
*Presenting author

Metastasis formation and therapy resistance, central hallmarks of aggressive cancer types, are major clinical problems and the leading cause of cancer-associated deaths. Dissemination of tumor cells is enabled by an aberrant activation of the epithelial to mesenchymal transition (EMT) program. A key inducer of EMT is the transcription factor ZEB1, which is highly expressed in many aggressive tumor types and couples cellular motility with stemness and survival properties. The potent effects of ZEB1 however, cannot be solely explained by its well-established role as a transcriptional repressor of epithelial genes.

EMT has also been linked to the inactivation of the Hippo tumor suppressor pathway, resulting in nuclear shuttling of its key downstream effector YAP. As shown for ZEB1, expression of the oncogenic transcriptional co-activator YAP is associated with aggressive behavior, metastasis and poor prognosis in various tumor types.

We recently discovered a functional link between ZEB1 and YAP and found ZEB1 to be an activator of a set of classical YAP target genes. ZEB1- mediated activation of these genes was thereby not due to regulation of YAP activity but caused by simultaneous binding of ZEB1 and YAP to the promotors of these genes. We could further demonstrate a direct interaction of ZEB1 and the hippo pathway effector YAP. Intriguingly such interaction was not found between ZEB1 and TAZ, a YAP paralogue. This underlines the highly specific nature of the ZEB1/YAP interaction upon which ZEB1 switches its function to a transcriptional co-activator. The identification of the ZEB1/YAP interaction directly links two cancer promoting pathways and provides insight into how ZEB1 is driving tumor progression beyond EMT induction. The ZEB1/YAP common target gene set is a predictor of poor survival, therapy resistance and increased metastatic risk in breast cancer, indicating the clinical relevance of our findings.

Abstract No. T45

Heterotrimeric G-proteins are indispensable for FLT3-ITD autophosphorylation and oncogenic function

Maike Rehage[1], Susanne Wingert[1], Nadine Haetscher[1], Hubert Serve[1], Manuel Grez[2], Joachim Schwaeble[1], and *Michael Rieger[1,*]*

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*Presenting author

Heterotrimeric G-proteins transmit signals of G-protein coupled receptors and regulate many basic cellular functions. However, their role in normal and malignant hematopoietic stem cells remains obscure. Activating mutations in the heterotrimeric G-protein Gaq were found in various cancers and its expression is enhanced in diffuse large B-cell lymphoma and T-ALL. Our previous data suggested the involvement of heterotrimeric G-proteins in Flt3-ITD-mediated leukemic transformation. FMS-like tyrosine kinase 3 with internal tandem duplication (FLT3-ITD) is a frequent oncoprotein in acute myeloid leukemia causing constitutive active STAT5 signaling. Here, we investigated the role of Gaq in Flt3-ITD-induced leukemic transformation.

We could show that Gaq is indispensable for aberrant FLT3-ITD activation and oncogenic function as Gaq activity is necessary to maintain the autophosphorylation of FLT3-ITD. Gaq abrogation resulted in diminished cell proliferation and colony formation as well as delayed leukemogenesis in vivo of Flt3-ITD leukemic cells. Importantly, the growth inhibition could be rescued by addition of IL3 and did not occur in the presence of FLT3 ligand-activated FLT3 wildtype receptor, demonstrating the specificity of Gaq requirement for FLT3-ITD oncogenic signaling. Interestingly, co-immunoprecipitations revealed a direct physical interaction between FLT3-ITD and Gaq which did not require phosphorylation of the receptor tyrosine kinase. Hence, FLT3-ITD hyperphosphorylation seems to be rather a consequence of the interaction than a prerequisite. Flt3-ITD-induced transformation of murine hematopoietic stem/progenitor cells (HSPCs) strictly depended on the presence of Gaq, and the ablation of Gaq/11 in transplanted Flt3-ITD-transduced HSPCs from conditional Gaq/11 double knock-out mice delayed leukemic burden. These results place Gaq as an important target for antileukemic strategies.

Abstract No. T46

A mathematical model approach to study the immunological effects in CML patients during and after TKI treatment

Tom Hähnel[1], *Christoph Baldow*[1], *Ingo Roeder*[1], and *Ingmar Glauche*[1,*]

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*Presenting author

Molecular monitoring of BCR-ABL levels in the peripheral blood of patients with CML under continuous tyrosine kinase inhibitor therapy revealed that the majority respond well to the treatment and achieve sustained molecular remission. This encouraged the initiation of treatment cessation trails, in which approximately 50% of the patients relapsed within the first 12 months after therapy stop. The rare observation of relapses later then 12 months after cessation indicates that the residual leukemic disease is either completely eradicated in the non-relapsing patients or that other factors such as immunological effects constrain leukemia regrowth.

Previously, we developed a mathematical model to functionally describe CML as a competition phenomenon between healthy and leukemic stem cells. By fitting our model to time course data of BCR-ABL levels under TKI-treatment from the peripheral blood, we can estimate residual disease levels and derive estimates on leukemia reoccurrence after therapy cessation. Now, we explicitly integrate a population of immune cells within our model to account for the particular role of immunological effects and to critically discuss the role of anti-cancer immune competence in the treatment-free maintenance of CML patients. In brief, a high abundance of CML cells represses the immunological population while a therapy induced decline in tumor load releases this limitation and supports anti-cancer immune competence, which in case of treatment cessation can lead to a sustainable control of the leukemic cell population.

We identify suitable factors and feedback mechanisms that account for the observed heterogeneity between relapsing and non-relapsing patients. We further aim to use this approach to speculate about accessible clinical parameters that can potentially support clinical decision making for therapy cessation trails. Our model represents a further step towards deriving a more holistic view on leukemia occurrence and treatment in the context of multiple, interacting tissues and cell types.

Company presentations

4th Annual Conference
German Stem Cell Network
12 – 14 September 2016

Company presentations: C1 – C9

C1 Simplified dopaminergic neuron and cardiac differentiation of single episome reprogrammed fibroblasts
Rick I. Cohen

C2 Expansion of human bone marrow-derived mesenchymal stem cells in BioBLU® 0.3c single-use bioreactors
Aurélie Tacheny

C3 A novel system to generate HPS cell-derived hepatocytes with potential application to drug discovery and metabolism, and hepatotoxicity studies
Barbara Küppers-Munther

C4 Generation of dopaminergic precursor cells and terminally differentiated neurons from human pluripotent cells for drug discovery and cell therapy
Mohan C Vemuri

C5 Enabling GMP-compliant iPSC expansion and differentiation on the CliniMACS® Prodigy platform
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C6 Using pluripotent stem cells in the age of genome editing
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C7 Translation of cell-based gene therapy into clinical application
Elena Meurer

C8 Simultaneous single-molecule quantification of DNA, RNAs & proteins
Maik Pruess

C9 STEMdiff™ kits for robust and efficient differentiation of human pluripotent stem cells
Katharina Debowski

Abstract No. C1

Simplified dopaminergic neuron and cardiac differentiation of single episome reprogrammed fibroblasts

Nicola Francis[1], *Astha Saini*[1], *Jared Sterneckert*[2], *Lydia Reinhardt*[2], *Peter Reinhardt*[3], and *Rick I. Cohen*[1,*]

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*Presenting author

representing Peprotech GmbH

Four Factor pluripotency reprogramming of somatic cells and subsequent multi-lineage directed differentiation can be most easily accomplished by robust genetically modifying/manipulating methods. More recently nongenetically modifying methods for induction have gained popularity as they lend themselves to translational and eventually clinically related research paradigms. Further, genetic-directed differentiation of somatic cells into other specialized cell types has been somewhat successful, however often limited in the amounts of material that can be produced from starting materials. In this study we developed an optimized single Episomal vector bearing multiple genes interspersed using using foot and mouth disease type “2A” motifs; Oct4-Myo-Dtransactivating domain fusion; Sox2; KLF4; L-Myc; RFP Blasticidin-S-Deaminase fusion. This single entity plasmid electroporated into fibroblasts and cultured with optimized xeno-free media and accompanying small molecule mixtures leads to reproducible reprogramming. Simplifications of xeno-free neuronal and cardiac differentiation methods relies on a 2 step procedure; for neuronal, IPSCs first transition into highly expandable FOXA2+ floor plate type neuro-epithelial cells (FPNE) using a cocktail of small molecule inhibitors; and secondly FPNE are differentiated into multiple neuronal cell types, specifically dopaminergic neurons in the presence of a cocktail of growth factors and small molecules. These cells become TH+/MAP2+, and secrete dopamine as measured by HPLC. For cardiac differentiation, a robust upregulation of the TCF pathway using GSK-3-β inhibitor followed by a second step using Wnt inhibition leads to efficient appearance of beating clusters within 8-10 days of intitiating the protocol. In order to ready methods for translational and clinical type research, it is our goal in this study to optimize and simplify the regimine of medias, growth factors, and small molecules in order to streamline these multiple differentiation pathways using nonanimal components.

eppendorf

Abstract No. C2

Expansion of human bone marrow-derived mesenchymal stem cells in BioBLU® 0.3c single-use bioreactors

Aurélie Tacheny

Eppendorf AG

The use of adult stem cells holds great promise for new cell-based therapies and drug discovery. For their routine application, large numbers of cells have to be produced with consistently high quality. Two-dimensional cultivation systems, such as T-flasks, are widely used, but they are limited in terms of control and scalability. Stem cell expansion in rigid-wall, stirred-tank bioreactors, however, facilitates the precise control of critical process parameters like pH and dissolved oxygen, and allows a more straightforward scale-up to larger process dimensions. We tested the suitability of Eppendorf BioBLU 0.3c Single-Use Vessels controlled by a DASbox® Mini Bioreactor System for the expansion of human bone marrow-derived mesenchymal stem cells on microcarriers, and obtained 1 x 108 cells in a working volume of 250 mL. The cells were able to differentiate into osteocytes and chondrocytes, respectively, demonstrating that their expansion in stirred-tank bioreactors did not affect multipotency. BioBLU Single-Use Vessels with maximum working volumes of 250 mL and 3.75 L were previously used for the expansion of human induced pluripotent stem cells as cell-only aggregates [1] and of adipose-derived mesenchymal stem cells on microcarriers [2]. These results, and our current data, suggest that Eppendorf BioBLU Single-Use Vessels are widely applicable for the expansion of different stem cell types at various scales.

Abstract No. C3

A novel system to generate HPS cell-derived hepatocytes with potential application to drug discovery and metabolism, and hepatotoxicity studies

Barbara Küppers-Munther[1,*], *Annika Asplund*[2], *Jane Synnergren*[3], and *Josefina Edsbagge*[1]

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[3]School for Biosciences, Skövde University, Skövde, Sweden
*Presenting author

Human induced pluripotent stem (hiPS) cell-derived hepatocytes have the potential to serve as predictive human in vitro model systems for drug discovery, drug metabolism research, and hepatotoxicity studies provided that they possess relevant hepatic function. Until recently, however, the functionality of hiPS cell-derived hepatocytes has been insufficient for applications that demand high expression of multiple drug metabolizing enzymes. We have recently developed a novel, robust differentiation system to generate hepatocytes from human induced pluripotent stem cells. These resulting hiPS cell-derived hepatocytes have substantial CYP1A, 2C9, 2C19, 2D6, and 3A4 enzyme activities and important adult hepatic features, such as low expression of fetal genes (e.g., CYP3A7 and alpha-fetoprotein) and high expression of adult genes (e.g., CYP2C9, 2C19, and 3A4). In this study, we illustrate the system's differentiation protocol that begins with culturing any hiPS cell line, continues with directed differentiation into definitive endoderm (DE) cells, and ends with further differentiation into hepatocytes, thereby mimicking normal embryonic development. Morphological images and immunostaining data demonstrate the robustness and reproducibility of the system to generate hepatocytes from several different cell lines. Of 28 different hPSCs tested, all 28 lines were efficiently differentiated into hepatocytes that exhibit substantial CYP1A, 2C9, 2C19, 2D6, and 3A4 enzyme activity and important adult hepatic features. More importantly, these hepatocytes generated from multiple hiPSC lines show diverse CYP activity profiles, indicative of the inter-individual variation present in the human population. hiPS cell-derived hepatocytes are potentially suitable for toxicity assays: they correctly identify known hepatotoxins and respond similarly to human primary hepatocytes when exposed to test compounds. The new Cellartis® iPS Cell to Hepatocyte Differentiation System can reliably generate a virtually inexhaustible source of human hepatocytes from different genetic backgrounds for use in in vitro drug discovery, drug metabolism research, toxicology-related studies, and disease modeling.

Abstract No. C4

Generation of dopaminergic precursor cells and terminally differentiated neurons from human pluripotent cells for drug discovery and cell therapy

Mohan C Vemuri[1,], and Soojung Shin[1]*

[1]Thermo Fisher Scientific
*Presenting author

Stem cell derived midbrain dopaminergic (DA) neurons provide an excellent cell source for disease modeling and drug screening for Parkinson's disease. To expedite this effort, we have developed culture system which can make authentic midbrain DA neurons starting from human pluripotent stem cells. Our system was designed to simplify and standardize the whole differentiation process while compressing timelines and adding increased flexibility in to this complex differentiation workflow. The process has 3 distinct steps: (1) specification of hPSC to midbrain floor plate (mFP) cells, (2) expansion and cryopreservation of derived mFP cells, and (3) maturation to DA neurons. Our system has the unique feature of enabling the generation of expanded banks at the midpoint, with mFP cells expanded at least 200 fold (2 passages) prior their cryopreservation (optional step). However, we were challenged to further increase the size of the cell bank to facilitate certain applications requiring large numbers of cells and standardization, such as high through put drug screening. To achieve this goal we developed an alternative workflow enabling stable growth of mFP cells up to passage 10 (p10). The cryopreserved mFPp10 were able to be directly recovered on assay format and after 7 days in maturation, phenotype marker of TH was examined using high throughput imaging system. The standardized work flow resulted in consistent differentiation of our reference PSC line over time. However we have noticed significant variation in maturation efficiency can occur across PSC lines and looked for ways to reduce this variability. To improve the differentiation efficiency, we tested a number of conditions and ultimately incorporated an extended differentiation procedure for low performing lines. As a result, we could get comparable differentiation efficiency from this line. Thus, we could use our system successfully across multiple cell lines.

Abstract No. C5

Enabling GMP-compliant iPSC expansion and differentiation on the CliniMACS® Prodigy platform

Sebastian Knöbel

Miltenyi Biotec GmbH

When translating research protocols into clinical applications, regulatory requirements call for rigorously characterized cell lines and highest quality reagents, such as GMP-grade media, cytokines, and antibodies. We will introduce iPS-Brew GMP Medium, a GMP-compliant version of StemMACS™ iPS-Brew XF, for the expansion and maintenance of pluripotent stem cells along with StemMACS™ Cryo-Brew, a chemically defined cryo-preservation medium, and compelling assays for phenotypic and functional quality control. Purification of cellular products through cell sorting and standardized, automated processing play central roles in the manufacturing of hPSC-derived cellular products. We present a closed system process for the expansion and differentiation of pluripotent stem cells on the CliniMACS® Prodigy platform. Specifically, we have translated the differentiation to mesencephalic dopaminergic (mesDA) progenitor cells to the device. Adaption of an embryonic body based to a fully adherent differentiation paradigm enabled straight forward upscaling of a lab protocol to a medium-scale production process suited for 150-250 patient doses.

Lonza

Abstract No. C6

Using pluripotent stem cells in the age of genome editing

Theresa Dsouza

Lonza Bioscience

In light of the decline of drug candidates in Pharma R&D output, there is an increasing demand for cell-based in vitro-systems that provide a better model for the physiological situation in vivo. The hope is that new cell-based models will better reflect physiological disease mechanisms, which will lead the way to identifying new types of pharmaceuticals. The challenge for advanced cell-based models is that they need to be robust, scalable, and compatible with assays that generate reproducible data. Induced pluripotent stem cells (iPSCs) represent one solution to the challenge of robustness, in that they provide a means of generating large numbers of theoretically homogeneous differentiated cells for use in cell-based assays. New strategies for genetic engineering of these cells - CRISPR/Cas9 genome editing for example - are helping advance the capability of generating cells with specific disease features. Additionally, 3D cell culture methods promise another level of biological relevance for cell-based models. Finding the right strategy to incorporate new technologies that enable higher-value in vitro-models will be important for advancing drug discovery and pre-clinical development.

Abstract No. C7

Translation of cell-based gene therapy into clinical application

Elena Meurer

apceth GmbH & Co. KG

apceth is a pioneering clinical stage biopharmaceutical company in the field of cell and gene therapy. apceth develops genetically engineered cell products aimed for the treatment of solid cancers and non-malignant diseases for which no satisfactory treatment options are available today. To this end apceth has created a modular platform that combines mesenchymal stem cells (MSCs) with different therapeutics genes. The therapeutic genes are chosen in such a way to target the specific cellular mechanisms of a chosen disease. Currently apceth is completing the first-in-class, first-in-men clinical phase I/II study with genetically modified autologous MSC-therapeutic Agenmestencel-T for cancer treatment. It is the worldwide first clinical application of genetically modified MSCs and the first report that MSCs have been used in oncology. Recently, apceth has also established an allogeneic platform for MSC manufacturing, based on MSCs derived from healthy volunteers. The switch to a bioreactor-based manufacturing and upscaling of the manufacturing process further contribute to the availability of off-the-shelf products based on genetically modified MSC.

Abstract No. C8

Simultaneous single-molecule quantification of DNA, RNAs & proteins

Maik Pruess

Nanostring Technologies

NanoString technology uses unique color-coded molecular barcodes that can hybridize directly to many different types of biomarker molecules, making it ideal for a range of discovery, validation and translational research applications. Our portfolio of application-specific solutions currently includes kits for gene expression, lncRNA and fusion gene analysis, ChIP-String, copy number variation, miRNA and protein expression analysis. - Multiplex up to hundreds of biomarker targets in a single reaction - High sensitivity (<1 copy per cell) - Fully-automated system - Exceptionally easy-to-use - No enzymes required to perform expression assay - Multiplex up to 800 targets from as little as 300ng of DNA or 50 ng of RNA The new 3D Biology technology enables the measurement of any combination of DNA (SNVs and mutations), RNA & Protein simultaneously on a single system. With the capability to multiplex up to 800 different targets using as few as 150,000 cells.

Abstract No. C9

STEMdiff™ kits for robust and efficient differentiation of human pluripotent stem cells

Katharina Debowski

STEMCELL Technologies SARL

Human pluripotent stem cells (hPSCs) have many applications in developmental biology studies, disease modeling, drug screening and regenerative medicine. To support this research, we have developed standardized reagents that are rigorously optimized for efficient and reproducible differentiation to specific cell types derived from each of the three germ layers: ectoderm, mesoderm and endoderm. The most recent developments will be highlighted in this presentation. The STEMdiff™ Trilineage Differentiation Kit is a simple culture assay for rapid assessment of the pluripotency of newly derived or established hPSC lines. Using monolayer-based directed differentiation protocols, ectoderm, mesoderm and endoderm differentiation capacity was demonstrated within one week, and analyzed using flow cytometry, immunocytochemistry and transcriptome analysis. STEMdiff™ Hematopoietic Kit generates hematopoietic progenitor cells from hPSC under defined, serum-free and feeder-free conditions. Cells harvested at day 12 of differentiation were on average 35% CD34+CD45+ and contained 75 colony-forming units (CFU) per 10,000 cells. STEMdiff™ Mesenchymal Progenitor Kit differentiates hPSC to mesenchymal progenitor cells (MPCs) under defined, animal component-free conditions. More than 90% of MPC-like cells expressed CD73, CD105, CD90 and CD146, and lacked expression of hematopoietic (CD34, CD45) and endothelial (CD144) cell markers after 21 days of differentiation. The hPSCs-derived MPCs showed an average >10-fold expansion per passage and were able to differentiate into adipogenic, osteogenic and chondrogenic cells in vitro. Finally, STEMdiff™ Pancreatic Progenitor Kit supports the generation of PDX1+NKX6.1+ cells from hPSC in less than 2 weeks. Pancreatic progenitor cells generated with this kit showed appropriate upregulation of pancreatic transcription factors, and were capable of maturing to uni-hormonal endocrine cells and ductal tissue in vivo. In summary, STEMdiff™ directed differentiation media are optimized to provide reproducible and efficient differentiation across multiple human embryonic stem cell and induced pluripotent stem cell lines to generate specific cell types of interest.

Presentations strategic sessions

4th Annual Conference
German Stem Cell Network
12 – 14 September 2016

Presentations strategic sessions: S1 – S8

S1 Intestinal epithelial organoids – an accessible model for mammalian stem cell niche biology
Henner Farin

S2 3D bioartificial cardiac tissue from pluripotent stem cells
Ina Gruh

S3 Using cerebral organoids for studying human disease modeling and lineage reprogramming
Marisa Karow

S4 Career paths for scientists – suitable application strategies
Anke Raloff

S5 tbd

S6 Investigator initiated trials (IIT) of advanced therapy medicinal products
Felipe Prosper

S7 EU research perspective on advanced therapies
Arnd Hoeveler

S8 Mapping the European landscape for patenting stem cell related inventions
Aliki Nichogiannopoulou

Abstract No. S1

Intestinal epithelial organoids – an accessible model for mammalian stem cell niche biology

Henner Farin

Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt

Exogenous signals from multiple sources such as epithelial cells and surrounding mesenchyme confine stem cells and proliferation to the intestinal crypt. Recently our molecular knowledge on the signaling activities involved, in particular Wnt-signaling, has provided the rationale for development of the 'organoid' culture system that allows expansion of gastrointestinal stem cells over long periods ex vivo. In a 3-D extracellular matrix pure epithelial structures are formed that undergo continuous self-renewal and differentiation, recapitulating normal crypt-villus architecture. In this talk I will summarize our research and highlight features that predestine organoids as a tool for stem cell biology: First, the defined growth conditions preserve cells in a primary, non-transformed state. This dependency on exogenic niche factors has allowed the establishment of pharmacologic protocols to direct efficient stem cell expansion and cell lineage differentiation. Another unique feature of mouse small intestinal organoids is the presence of dedicated niche cells, the so-called Paneth cells that produce Wnt3 and thereby locally instruct a stem cell fate. We have used this accessible model to address the molecular and cellular mechanisms how Wnt signaling gradients are set up to self-organize the crypt compartment. Finally, organoids can be established from endoscopic patient biopsies of normal and tumor tissue and are amenable to genetic engineering. This permits us – for the first time – to use human intestinal epithelia as a genetic model to e.g. modulate stem cell niche signaling by defined oncogenic mutations.

Abstract No. S2

3D bioartificial cardiac tissue from pluripotent stem cells

Ina Gruh

REBIRTH, Hannover Medical School (MHH)

Abstract not available

Abstract No. S3

Using cerebral organoids for studying human disease modeling and lineage reprogramming

Marisa Karow

LMU Munich

With the advent of the cerebral organoid technology a new era of studying human neural cells within a three-dimensional context has begun. As previously reported (Lancaster et al., 2013; Kadoshima et al., 2013), different developmental stages of human brain development can be discerned within cerebral organoids including the ventricular/subventricular-like germinative zones as well as different phases of neurogenesis. Further modifications of the experimental setup has led to the development of specialized protocols that allow for the controlled generation of brain-region specific cerebral organoids, enriched for progenitors and neurons of either midbrain, hindbrain, or forebrain identity (Qian et al., 2016). I will discuss the current status and limitations of existing protocols and provide some examples of how these cerebral organoids can be used for different research applications such as neuronal reprogramming and disease modeling. Work from our lab has shown that pericytes derived from the adult human cerebral cortex can be successfully reprogrammed into induced neurons in vitro via overexpression of the transcription factors Sox2 and Ascl1, thus identifying brain pericytes as a new target cell population for in vivo conversion (Karow et al., 2012). We are now using cerebral organoids as an experimental platform to assay the lineage conversion of these pericyte-derived cells within the context of a human tissue-like environment. Also, I will present work on using the cerebral organoid technology to assess putative patho-physiological consequences of a gene mutation present in patients suffering from intellectual disability on human neurogenesis.

Karow et al. Cell Stem Cell. 2012 Oct 5; 11(4): 471-6

Lancaster et al. Nature. 2013 Sep 19; 501(7467): 373-9

Kadoshima et al. Proc Natl Acad Sci U S A. 2013 Dec 10; 110(50): 20284-9

Qian et al. Cell. 2016 May 19; 165(5): 1238-54

Abstract No. S4

Career paths for scientists – suitable application strategies

Anke Raloff

ZEIT Verlagsgruppe/ACADEMICS

Abstract not available

Abstract No. S6

Investigator initiated trials (IIT) of advanced therapy medicinal products

Felipe Prosper

University of Navarra, Pamplona, Spain

Advanced therapy medicinal products (ATMPs), including gene therapy medicinal products, somatic cell therapy medicinal products and tissue-engineered products, represent new therapeutic opportunities for a broad spectrum of human diseases and will likely contribute to the forefront of innovation. After the advent of biologics (recombinant hormones, soluble receptors, and antibody-based drugs) the pharmaceutical industry and the society is facing a new revolution base on the development of (stem) cell therapeutics. Unlike previous strategies, cell therapeutics represent a paradigm change not only in the regulatory strategies but also in modification of business models. Research and development once monopolize by large pharmaceutical industry requires significantly more participation from academics and small biotech companies. In my presentation, I will summarize the model established in our institution based on the development of a combination of a strong basic research program, the accreditation of a GMP facility authorized for the production of several ATMP products and the collaboration with industrial partners that may bridge the gap between preclinical research and clinical application. I will present the rationale and results of clinical trials with ATMPs in patients with cardiovascular diseases (1), vitiligo (2), GVHD (3), osteoarthritis or cancer (dendritic cell vaccines) (4) with a particular emphases on the difficulties to develop investigator initiate trials, securing funding and valorizing clinical translation.

1 M. Arana et al., Epicardial delivery of collagen patches with adipose-derived stem cells in rat and minipig models of chronic myocardial infarction. Biomaterials 35, 143-151 (2014)

2 P. Redondo et al., Efficacy of Autologous Melanocyte Transplantation on Amniotic Membrane in Patients With Stable Leukoderma: A Randomized Clinical Trial. JAMA Dermatol, (2015)

3 F. Sanchez-Guijo et al., Sequential third-party mesenchymal stromal cell therapy for refractory acute graft-versus-host disease. Biol Blood Marrow Transplant 20, 1580-1585 (2014)

4 R. D. Valle et al., Dendritic cell vaccination in glioblastoma after fluorescence-guided resection. World J Clin Oncol 3, 142-149 (2012)

Abstract No. S7

EU research perspective on advanced therapies

Arnd Hoeveler

European Commission, Brussels

Abstract not available

Abstract No. S8

Mapping the European landscape for patenting stem cell related inventions

Aliki Nichogiannopoulou

European Patent Office

The European Patent Office (EPO) is the patent granting authority for Europe. Patents are granted under the European Patent Convention (EPC) which lays down in its Article 53(a) that no European patents shall be granted in respect of inventions the commercial exploitation of which would be contrary to “ordre public” or morality. An additional rule of this convention exemplifies as inventions offending morality, all uses of human embryos for industrial or commercial purposes as well as processes for cloning human beings and processes for modifying the germ lone genetic identity of human beings. Conventional human ES cells originate from human embryos and this very fact presents an obstacle to their patentability as well as to the patentability of inventions relating thereto. Technological advances may however pose solutions to this moral dilemma. iPS cells for instance, share the unlimited potential of ES cells but do not originate from an embryo and are as such patentable under the provisions of the EPC. Similarly, parthenogenetically derived human ES cells also share the unlimited potential of conventional ES cells without being derived from a human embryo. Patentability of such “entities” as well as their modification through CRISPR/cas technology is the subject of a dynamic and hotly debated stage. The EPO has some answers and many more questions which will be discussed in the presentation.

Poster presentations

4th Annual Conference
German Stem Cell Network
12 – 14 September 2016

Poster session I: P001 – P077

P001 – P017: Pluripotency and reprograming
P018 – P039: Somatic stem cells and development
P040 – P053: Hematopoietic stem cells
P054 – P060: Stem cells and ageing, genome stability and epigenetics
P061 – P066: Stem cells in disease: cancer stem cells
P067 – P077: Computational stem cell biology and systems biology

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

Poster session II: P078 – P147

P078 – P091: Tissue engineering and organoids
P092 – P097: Genome engineering and gene therapy
P098 – P119: Stem cells in regenerative therapies
P120 – P124: Stem cells in regenerative therapies:
Mesenchymal stem/stroma cells
P125 – P147: Stem cells in disease modeling and drug development

Even numbers: Tuesday, 13 September from 16:30 - 17:30
Odd numbers: 17:30 - 18:30

Poster session I: P001 – P077

P001 – P017: Pluripotency and reprograming

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Giulio Abagnale

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Abstract No. P001/T06

Nanotopography guides morphology and spatial patterning of induced pluripotent stem cell colonies

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Induced pluripotent stem cells (iPSCs) raise high hopes in regenerative medicine, but little is known about how they are affected by surface topography. iPSCs usually grow on tissue culture plastic as round colonies and it has been demonstrated that morphogen induced lineage-specification, e.g. in response to factors as BMP-4, is heterogeneous but spatially ordered within colonies. In this study, we analyzed how nanotopograhy impacts on the morphology of iPSC colonies and whether it modulates lineage-specific differentiation. To this end, we have structured polyimide (PI) by laser interference in a groove-ridge pattern with a periodicity of 650 nm (Abagnale et al., Biomaterials 2015). Individual iPSCs displayed an elongated morphology, parallel to these structures as well as modifications in the number and size of focal adhesions. Colonies of iPSCs acquired too an elongated shape along the nanopatterns, apparently through a modulation of cell division planes and the rearrangement of apical actin fibers. Treatment of iPSCs with BMP-4 led to a different spatial distribution of the pluripotency markers NANOG and E-cadherin in round versus elongated colonies. Notably, a similar pattern was observed in the intracellular localization of the transcriptional co-activators YAP and TAZ, which play a key role in the mechanotransduction, particularly with regards to proliferation and cell fate decisions. Interestingly, TAZ (but not YAP) was localized in filamentous structures similar to actin fibers, suggesting a direct link between the cytoskeleton and the mechanotransducer machinery in iPSCs. Taken together, our findings demonstrate that growth of iPSCs colonies is controlled by surface topography at nano-scale. This is also reflected by distinct cellular differences in mechanostransduction upon stimulation with morphogens.

Abstract No. P002

Dissecting the complexity of cell types present in urine, identifies renal progenitor cells with regenerative potential

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Urine is increasingly becoming the choice for isolating cells for deriving induced pluripotent stem cells for the simple reason that urine is easily accessible, non-invasive, safe, and ethically unproblematic. Given the massive tubular network within the kidney, it is estimated that approximately 2000-7000 cells are flushed out in urine. Amongst these are podocytes, renal, parietal, and renal tubule epithelial cells. Based on this, it is most likely that the majority of urine cell derived iPSCs are of a mixed cell origin. The aim of our study was to identify the numerous cell types shed into urine, identify progenitor/stem cells amenable for reprogramming into iPSCs. We obtained urine samples from 13 healthy individuals of both genders with ages 19 to 62 years. Bulk urine cells (BUCs) were selected using MSCs expansion medium, Urine Renal Progenitor Cells (URPCs) were selected with a high proliferation-supporting medium. Like BM-MSCs, URPCs are Vimentin-positive, express CD73, CD105 and not CD14, CD20, CD34 and CD45. URPCs differentiate into adipose, osteoblasts and secrete trophic factors such as SDF-1, IL6, IL8, GDF-15, SERPINE-1, Angiogenin, VEGF, and Thrombospondin-1. Like pluripotent stem cells, URPCs express SSEA4, TRA-181, NANOG, CD117, CD133, C-MYC. The transcriptomes of BUCs, URPCs, BM-MSCs and pluripotent stem cells were compared. Pathway analysis identified active WNT, NOTCH, ERRB, FGF and TGF-Beta. We uncovered a 140-gene signature specific to URPCs, amongst these are kidney and renal-associated genes- PKHD1, ZBTB16, AQP4, SOX9, SLCO4C1, PAX2 and PAX8. Over-represented pathways and Gene Ontologies include kidney development, renal and urogenital system development. Finally, we could induce pluripotency in URPCs using episomal-based plasmids, colonies appeared as early as 11 days post nucleofection. To summarize, we have optimized a selection protocol for Urine Renal Progenitor Cells with regenerative potential for genitourinary tissue repair, drug discovery and studying nephrogenesis and banking HLA homozygous iPS cells.

Abstract No. P003

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

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Pluripotent stem cells (PSCs) are powerful tools for developmental biology, regenerative medicine, and the study of debilitating human diseases. While the development of feeder-free culture systems has significantly simplified routine PSC culture, additional hurdles continue to challenge today's stem cell scientists. Prominent among these is the daily media exchange required to maintain healthy PSC cultures. This daily feeding not only necessitates seven-day workweeks for scientists but also increases the potential for contamination and introduces variability resulting from multiple users processing a single culture. The requirement for daily feeding is driven largely by the heat sensitivity of key factors crucial to the maintenance of pluripotency in culture, with activity loss beginning immediately upon warming of growth medium and deteriorating upwards of 80-90% after 24 h at 37°C. In this work we have developed a new Essential 8 (E8)-based cell culture media that allows for long-term, feeder-free, healthy PSC culture without the need for weekend feeding. In this new culture medium virtually no loss of bioactivity is observed in key heat-labile components, including FGF2, at 37°C for periods of 72 h or greater. The medium has been used with equal success in embryonic and induced pluripotent stem cell culture and does not require low cell densities or other significant changes to current culture workflows. Furthermore, our data indicate that PSCs cultured in this formulation using a weekend-free culture schedule (1) maintain expression of canonical pluripotency markers, including Nanog and TRA-1-60, (2) retain the capacity for trilineage differentiation, and (3) exhibit a normal karyotype over long-term passaging. In addition to simplifying and securing day-to-day PSC culture, enhanced stability of key factors will have added benefits in large-scale cultures for regenerative medicine, where large volume media exchanges are impractical and consistent performance is critical.

Abstract No. P004

Metrology-data driven non-invasive QC and scheduling of human iPSC subcultivation on the StemCellFactory

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The increasing demand for human induced pluripotent stem cells (hiPSCs) has created an urgent need for standardized and automated production of hiPSC lines. This demand is met by the StemCellFactory (www.stemcellfactory.de), a large system integration that provides automation for all required cell culture steps, ranging from adult human dermal fibroblast (HF) expansion via feeder-free, Sendai virus-based reprogramming to clonal selection and enzyme-free expansion of the obtained hiPSC clones and lines. The implementation of on-line measurement technologies is key for the establishment of a fully automated production process on the StemCellFactory. This is realized by evaluation of in-process generated data by the novel programmed control level software, which controls, triggers and allows used defined workflow assembly. Here we report the realization and biological validation of non-invasive measurement technologies for QC of hiPSC cultures and the scheduling of the fully automated splitting processes. For on-line assessment of metabolic activity and detection of potential bacterial contamination we implemented a plate reader and established instrument settings that enabled us to detect a shift of pH and/or turbidity. In addition, an automated high-speed microscopy platform was implemented to schedule and perform fully automated long-term cultivation of hiPSCs. To that end we developed a confluence detection assay that enables dynamic feedback by computing confluence-based splitting ratios. For parallel and fully automated hiPSC generation and cultivation, well-based and plate-based automated splitting protocols were developed, which enable clonal expansion of individual hiPSC clones in 24-well plates as well as scaled production of hiPSCs in 6-well plates. Validation by immunocytochemical and in vitro differentiation assays confirmed that automatically expanded hiPSCs remain pluripotent for at least 10 passages. Our data show that dynamic feedback via generation and analysis of in-process data can be used by the control level software to facilitate automation of highly dynamic cell culture processes.

Abstract No. P005

Generating an iPSC Cohort for the Study of Tau Pathology in Sporadic Alzheimer's Disease

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the presence of intracellular aggregates of the microtubule associated protein Tau and extracellular plaques of the amyloid-b peptide. The pathology is complex and poorly understood, in great parts due to the lack of relevant disease model systems. Here, we generate a cohort of induced pluripotent stem cells (iPSCs) from patients with sporadic AD and healthy donors by ReproRNA reprogramming of fibroblasts. The iPSCs are quality controlled by expression analysis of pluripotency markers, in vitro differentiation into the three germ layers and karyotype analysis. Moreover, high resolution analysis of genomic integrity after reprogramming is planned using array-CGH. iPSC-derived neurons generated from this cohort constitute a powerful human model system of AD and will be used to study cell biological aspects of Tau pathology.

Abstract No. P006

Use of non-modified RNAs for the derivation of clinically relevant iPS cell lines from human blood, urine and skin cells using GMP-compliant reagents

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Human fibroblasts can be reprogrammed with a cocktail of mRNAs into integration-free human induced pluripotent (iPS) cells. Human blood provides easy access to adult human cell types for reprogramming purposes. Notably, blood-outgrowth endothelial progenitor cells (EPCs) can be clonally isolated from only 10 mL of fresh or frozen mononuclear cell (MNC) preparations from both human peripheral and cord blood. The adherent nature and high proliferative capacity of EPCs makes them highly desirable for repeated transfection with RNA when compared to commonly isolated hematopoietic suspension cell types. In 2015 we published the unique application of non-modified RNA technology to the reprogramming of human blood-derived EPCs and adult fibroblasts. Since then we could extend this novel reprogramming technology to urine-derived epithelial cells (UDCs) which can be highly reproducibly isolated from only 30 mL of urine (the most non-invasive form of cell procurement). Here we present a flexible, yet powerful, RNA-based reprogramming method that combines a novel cocktail of synthetic, non-modified reprogramming [OCT4, SOX2, KLF4, cMYC, NANOG and LIN28 (OSKMNL)] and immune evasion mRNAs [E3, K3, B18-R] with reprogramming-enhancing mature, double-stranded microRNAs from the 302/367 cluster. This unique combination of different RNAs results in a highly efficient (up to 4%) and robust reprogramming protocol using only GMP-compliant substrates (iMatrix-511 and vitronectin), media compositions (xeno-free or human serum), and RNA to produce clinically relevant iPS cells from blood-derived EPCs, neonatal as well as adult fibroblasts, and for the first time from cells derived from human urine.

Abstract No. P007

A differentiation-defective human iPSC line reveals the strengths and limitations of commonly used in vitro and in vivo pluripotency assays

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The pluripotency of human pluripotent stem cells (hPSCs) is characterized by the expression of the core transcription factors OCT3/4, Sox-2 and Nanog and the ability to differentiate into all three germ layers. Currently the teratoma assay is regarded as the most stringent assay to assess functional pluripotency of hPSCs in vivo. However the assay is animal-dependent and not quantitative and therefore unable to reveal donor-dependent differences in the differentiation propensity of human PSCs. Recently the microarray-based TeratScore algorithm has been developed for quantitative analysis of teratomas. In addition the PluriTest algorithm and the Scorecard assay have been proposed as in vitro alternatives. We used various hPSCs for a side-by-side comparison of the different assays: a differentiation-defective hiPSC line with inducible transgenes (LU07+Dox), the validated H9 hESC line with a known differentiation bias towards ectoderm, a H9Hybrid PSC line with a differentiation bias towards mesendoderm and the nullipotent 2102Ep embryonal carcinoma line (EC). All hPSC lines were cultured on vitronection in TESR-E8 whereas EC cells were cultured in DMEM/10%FCS. When analysed by the PluriTest algorithm all lines were similar to normal human PSCs in terms of pluripotency and novelty score with the exception of the EC cells which showed an elevated novelty score. In vitro differentiation towards endoderm revealed failure of differentiation for LU07+Dox and EC cells, whereas all other hPSC lines efficiently differentiated. Using TeratoScore confirmed failure of differentiation of EC cells and for two out of three LU07+Dox teratomas. The histological analysis revealed the presence of a major embryonal carcinoma-like component in EC and LU07+Dox tumors. Taken together PluriTest can confirm pluripotency of undifferentiated cells based on gene expression whereas the Scorecard quantifies the in vitro differentiation potential. TeratoScore detects the differentiation potential of hPSCs in vivo whereas histological analysis can reveal additional features such as potential malignancy.

Abstract No. P008

Identification of an X-linked inhibitor of the MAPK/Erk pathway in mouse embryonic stem cells

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Double X-chromosome dosage in mouse embryonic stem cells augments expression levels of stem cell factors and interferes with differentiation, potentially by inhibiting the MAPK signaling pathway [1]. The relevance of the later in the maintenance of mESCs in vitro has been exploited in the so called 2i culture conditions, where its inhibition leads to an enhancement of their pluripotent state. Our goal is the identification of the X-linked factor that leads to the observed decrease in the levels of MAPK/Erk signaling in mouse embryonic stem cells. We will achieve this by using the CRISPRi technology to carry out a screen that knocks-down candidate genes on the X-chromosome. For this, we will generate a MAPK/Erk pathway GFP-reporter system in mESCs that enables us to visualize the dynamics of this pathway in female and male mESCs. During the screen, sgRNAs targeting the different genes on the X chromosome will integrate into the cells genome. The ones that are able to reduce the expression of the X-linked MAPK inhibitor(s) will hopefully lead to an increase GFP-reporter expression, which will allow the sorting, expansion and subsequent sequencing of this population. So far we have managed to generate a variety of readout systems that are sensitive to x-dosage as well as external cellular stimuli. Once we have the top hits from the screen, some of the candidates will be validated by targeting just one the alleles in female mESCs. By doing this we will see if the mutant female cells recapitulate the phenotype observed in male cells.

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Abstract No. P009

Development of standard operating procedures for generation of transgene-free GMP-compatible hiPS cell lines

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Recent advances in iPSC technology have enabled the efficient generation of transgene-free hiPSCs even from easily accessible cell sources such as blood. The developed reprogramming protocols have now to be adapted to GMP compliant conditions including application of fully defined animal-derived components and feeder cell- free culture conditions. Using Sendai virus-based reprogramming vectors we have developed protocols for the generation of non-transgenic iPS cells from CD34+ human hematopoietic stem cells. All procedures necessary for reprogramming including isolation of source cells, transduction with non-integrating sendai virus vectors, picking of clones, and establishment and cultivation of resulting iPS cell lines are performed under animal-derived component-free conditions. These protocols now represent the basis for development of standard operating procedures and future production of clinically applicable iPSC lines under GMP-compliant conditions.

Abstract No. P010

Evaluation of miR-302/367-containing polycistronic reprogramming cassettes for the generation of human iPSCs

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Since the discovery of the generation of iPS cells, considerable effort has been put into research about the exact mechanisms of cellular reprogramming and how its efficiencies can be improved. The inclusion of c-MYC in the cocktail of reprogramming factors, for example, entails several disadvantages, such as possible oncogenic transformation upon transgene reactivation. However, omission of c-MYC substantially delays reprogramming and impairs efficiency. Inevitably, the question arises whether or not c-MYC can possibly be substituted with miR-302/367 to achieve robust epigenetic reprogramming towards a pluripotent state that is not biased for specific germ layer derivatives. Therefore, we constructed polycistronic reprogramming cassettes containing codon-optimised versions of the Yamanaka factors and the miR-302/367 cluster and evaluated their characteristics. For this, the constructs were introduced in human fetal liver fibroblasts using 3rd generation self-inactivating lentiviral vectors. Indeed, reprogramming efficiency was found to be slightly higher in OKSM+miR302/367- compared to OKSM-transduced cells, with both of them showing considerably higher numbers of colonies than OKS- as well as OKS+miR-302/367-transduced cells. From each of these four reprogramming cassettes, six stable iPS cell lines were established. In order to evaluate the pluripotency status of these cell lines, we compared the differentiation propensities *in vitro* by means of the hPSC Scorecard Assay. iPSC clones of all of the four reprogramming strategies were able to spontaneously differentiate into all three germ layers, although OKS+miR-transduced cells interestingly exhibited the greatest potential to do so – especially in the ectodermal and endodermal lineages, which are otherwise difficult to obtain. With this work, we established that overexpression of the miR-302/367 cluster from a polycistronic construct cannot replace c-MYC in terms of iPS cell colony yield. However, cells reprogrammed with an OKS+miR-302/367 vector (lacking c-MYC) appear to have superior differentiation potential suggesting a more robust reprogramming system.

Abstract No. P011

Controlled Human Pluripotent Stem Cell Culture in Perfused Stirred Tank Bioreactors

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Human pluripotent stem cells (hPSCs), including human induced pluripotent stem cells and embryonic stem cells, are a unique source for the, in principle, unlimited production of functional human cell types in vitro, which are of high value for therapeutic and industrial applications. All these therapeutic and industrial applications will require the constant supply of billions of lineage-specific cells generated by robust, economically viable and well defined processes. The expansion of hPSCs as aggregates in 3D suspension provides a feasible and potentially superior solution for achieving the cell numbers needed for their envisioned applications. Stirred tank bioreactors are well monitored and controlled systems for the expansion of 3D suspension cultures and are widely used in pharmaceutical biotechnology for the production of recombinant proteins with mammalian cell lines. Recently, the expansion of single cell inoculated hPSCs as cell-only-aggregates (Olmer et al., Tissue Eng Part C, 2012) as well as their subsequent cardiac differentiation (Kempf and Kropp, Nature Prot, 2015) in this bioreactor type was shown by us. Using a chemically defined and xeno-free culture medium we recently demonstrated a novel, more effective and homogenous perfusion process yielding up to 3.8 x 10^6 cells/ mL in a working volume of 100 mL in 7 days, supporting clinical translation of hPSCs at reduced cost (Kropp et al., Stem Cells Transl Med, in press). Interestingly, physiological and gene expression assessment indicated distinct changes of the cells' energy metabolism providing clear physiological and molecular targets for process monitoring and further development. In perfusion processes, better automation and consequently improved control of the culture environment including parameters such as dissolved oxygen, pH, and the concentration of nutrients is possible. Here we present how stirred tank bioreactor based hPSC expansion processes can be further optimized resulting in more controlled process characteristics and substantially elevated cell yields.

Kropp C, Kempf H, Halloin C, Robles-Diaz D, Franke A, Scheper T, et al. Impact of feeding strategies on the scalable expansion of human pluripotent stem cells in single-use stirred tank bioreactors. Stem Cells Translational Medicine, in press.

Kempf H and Kropp C, Olmer R, Martin U, Zweigerdt R. Cardiac differentiation of human pluripotent stem cells in scalable suspension culture. Nature Protocols 2015;10:1345-1361

Abstract No. P012

Defining data requirements: What is a pluripotent stem cell?

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A cell is commonly defined as pluripotent if it has been shown that it can differentiate into alls cells of the three germ layers. This is usually shown experimentally by providing supportive evidence, first the presence of markers that are associated with stem cells and second the experimental proof that the cells can indeed differentiate into cells of all germ layers. Both tests provide only circumstantial evidence for the claim and the required stringency especially of the second test has been frequently questioned. In addition, there are some principal problems associated with assigning a cell the term 'pluripotent stem cell' For example, a pluripotent stem cell with a induced or sporadic mutation, thet has been derived from an embryo or generated by induction may not be able to differentiate into all cell types of the three germ layers. hence, this cell is trictly not pluripotent. Moreover, detection of markers of the three germ layers in different experimental assays is transient and failure of detection may not justify the conclusion that the cell lacks pluripotency. The ambiguity to extends to transcriptome data, which are alone not sufficient to define pluripotency. This provides a dilemma to pluripotent stem cell registries in terms of validating data as sufficient evidence to show that a cell is pluripotent. We propose a more practical approach by allowing ambiguity but provide transparency as to the tests applied to characterize a cell line. We predict that users will chose the line based on required performance and not necessarily on formal criteria of pluripotency.

Abstract No. P013

GMP-Compliant, stable, and efficient expansion of pluripotent stem cells in a closed cultivation system

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Human pluripotent stem cells (hPSCs) hold great promise for clinical use and cell therapy applications. To ensure highest quality and consistency of the resulting cellular products, suitable hPSCs lines have to be maintained under standardized cultivation conditions and procedures. In this context, we have developed the xeno-free iPS-Brew GMP Medium following the recommendations of USP 1043 on ancillary materials, thus enabling expansion of hPSC for clinical research use. For qualification of the iPS-Brew GMP Medium, hiPSC were expanded for 20 passages on recombinant Laminin-521 using a pilot lot of this medium. Resulting cells displayed a normal karyotype and were highly pluripotent as assessed by multi-color flow cytometry and in vitro differentiation assays. To increase the level of process standardization and product consistency we developed a procedure for cultivation of hPSCs using the integrated cell-processing platform CliniMACS Prodigy®. PSCs could be expanded by a factor of 25 to 60 in a single passage using automated coating, feeding and harvesting procedures in a closed, single use tubing set (TS730) under adherent culture conditions. Subsequently, these cells could be replated and differentiated into neuroectodermal progenitor cells within the closed system, illustrating the feasibility of an automated cell production for future clinical cell manufacture.

Abstract No. P014

Severe reduction of hPSC vitality is mediated by insulin stability in culture media: impact of physicochemical conditions on bioprocess development

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For the envisioned clinical translation of human pluripotent stem cells (hPSCs), controlled large-scale production represents an indispensable key step. In this perspective, stable chemically defined culture media play a critical role for the development of GMP/GCP-compliant production processes, both in terms of quantity and quality. Culture media for hPSC maintenance contain insulin, a protein essential for stem cell survival and proliferation. Under certain chemical and physical conditions, such as oxidation, hydrophobic surfaces, and shear stresses, insulin tends to precipitate and to form aggregates/fibrils [1]. Insulin is thereby depleted from the medium reducing its biological activity. In this study, we investigated the effect of physicochemical conditions on insulin stability in different hPSC culture media, using a peristaltic pump-based recirculation circuit. StemMACS™ iPS-Brew XF (Miltenyi Biotec Inc.), mTeSR®1, TeSR®-E8 (STEMCELL Technologies Inc.), and self-made E8 culture medium were systematically tested i) imposing several flow rates, ii) adopting different tubing materials, iii) adding stabilizing excipients such as glycerol and human serum albumin (HSA). Subsequently, the media were analysed in a bioassay regarding their efficacy in supporting aggregate-based hPSC suspension culture [2]. In all recirculated media, even when supplemented with excipients, micro-sized precipitates were detected. LC-MS analysis revealed that only ~10% of dissolved insulin remained in exposed E8 medium compared to non-recirculated control. Moreover, only recirculated culture media containing albumin (mTeSR®1, StemMACS™ iPS-Brew XF, and E8 supplemented with HSA) largely maintained their potency to support hPSC propagation and pluripotency. Our results show that physicochemical conditions, which are typical for specific bioreactor designs and can occur in bioprocess development, can induce unexpected “cytotoxic” effects related to insulin precipitation. Such effects are limited and somewhat masked in the presence of albumin, which might act as a molecular chaperone, protecting and stabilizing dissolved insulin [1]. Our results have substantial consequences for culture media, bioreactor and bioprocess development.

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Abstract No. P015

Highly efficient purification of hPSC derived cardiomyocytes by cell sorting

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Pure cardiomyocytes derived from human pluripotent stem cells (hPSCs) are of high interest for heart disease modeling, drug safety studies, and development of cellular therapies. Although several protocols for cardiac differentiation of hPSCs have been developed, major limitations are high variability in differentiation efficacies due to clone-to-clone, experiment-to-experiment variations and heterogeneity of generated cardiomyocyte (CM) populations. Therefore, we have developed a workflow covering cardiomyocyte differentiation, monolayer dissociation and purification of hPSC-derived CMs as well as flow and immunofluorescence based analysis of CMs and cardiac subtypes. hPSCs were maintained in StemMACS iPS-Brew medium and differentiated using a monolayer protocol including the timely regulated activation and inhibition of Wnt signaling. Based on a surface marker screen with >400 antibodies several markers suitable for CM enrichment or depletion of non-myocytes were identified. They were used for the development of a novel magnetic cell separation procedure consistently delivering CM purities of up to 98%, independent of the differentiation protocol and the hPSC line used or the time point and efficacy of differentiation. Interestingly, the same surface marker combination could be used for flow sorting using the MACSQuant Tyto, allowing for high-speed microchip-based cell sorting, thereby providing a gentle approach for CM purification. Both methods yield highly pure and viable CMs that plated well, initiated contractions after 24-48h in culture and showed typical morphological features. Additionally, purified CMs could be cryopreserved in StemMACS CryoBrew medium, thawed with high viabilities and successfully be maintained in culture. In order to address potential CM subtype heterogeneity in hPSC-CM cultures, recombinant antibodies against general CM markers, α-Actinin, Myosin Heavy Chain, cardiac Troponin T and subtype specific markers MLC2a and MLC2v, were generated. Taken together, we have developed novel tools supporting the workflow for efficient generation, magnetic and fluorescence based purification, and flow cytometry or immunofluorescence-based characterization of PSC-derived cardiomyocytes.

Abstract No. P016

Principles of Cardiac Induction in Human Pluripotent Stem Cells

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Cardiac induction from human pluripotent stem cells (hPSCs) requires the stepwise integration of extracellular cues by the BMP and WNT pathways. We show that these signals, to a large extent, drive cardiac specification by sequentially removing regulatory roadblocks which otherwise redirect hESC differentiation towards competing fates - rather than activating a cardiac program per se. As a first step, BMP and WNT signals dose-dependently pattern mesendoderm and repress SOX2 which acts as a potent mesoderm antagonist. Interestingly, BMP and WNT antagonize SOX2 in a cooperative manner but via distict mechanisms - one involving miRNA-877 maturation and one involving EOMES induction, respectively. In a broader sense, our data suggest that various longer term mesendodermal differentiation fates are encoded by an initial combined BMP/WNT pulse in a dose-dependent manner (proposed corridor model of early lineage induction). As a second step, WNT inactivation is crucial for subsequent specification of a cardiomyocyte fate. This requirement is evolutionally conserved but it was poorly understood until now. Our results demonstrate that WNT inhibition serves to diminish another set of cardiac repressors - MSX and CDX transcription factors: Accordingly, for instance, their simultaneous disruption partially abrogates the requirement for WNT inhibition at the cardiac specification stage. Collectively, these results reveal key mechanistic insights into the complex process of hESCs converting into spontaneously contracting heart muscle cells. In ongoing work, we now focus on intrinsic factors orchestrating cardiac specification. For instance, we have recently identified an intrinsic master regulator of cardiac induction in the human system. Moreover, we are currently investigating extrinsic as well as intrinsic regulators of the human cardiovascular precursor cell state. An up-to-date view on these results will be presented on the meeting.

Rao, J. et al. (2016) Stepwise Clearance of Repressive Roadblocks Drives Cardiac Induction in Human ESCs. Cell Stem Cell, 18, 341 - 353

Abstract No. P017

miR29 family members overexpression increases PAX2 and LRP2 gene levels in human embryonic stem cells

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Human embryonic stem cells (hESC) are pluripotent stem cells characterized by their capacity of self-renewal and maintenance of the pool of stem cells, as well as differentiate into the three germs layers (mesoderm, endoderm and ectoderm) that will further generate specialized cell types with distinct functions. However, the molecular pathways and biological mechanisms that drive pluripotent stem cells towards specialized cell types are still not totally uncovered. MicroRNAs (miRs) are small non-coding RNAs known to modulate multiple genes simultaneously, thereby regulating multiple signaling pathways. Previously, increased levels of miR29 family members have been shown in hESC undergoing early differentiation. In the present work, we emphasize the use of synthetic miRs, to explore their potential roles in early differentiation of pluripotent cells. Therefore, we independently transfected mimics of miR29a/b/c and control in human ESC. The transfection efficiency was validated by qRT-PCR. Next, we focused on the analysis of genes expressed in human epithelial cells, in particular kidney progenitor cells. We show that overexpression of miR29 family members in hES pluripotent cells increases PAX2 and LRP2 gene levels. Moreover, mir29 family members overexpression induced morphological differentiation of hESC. Pax2 is a transcription factor active in kidney progenitor epithelial cells critical for the early kidney development, whereas LRP2 is expressed in epithelial cells such as proximal tubule cells and mediates endocytosis of numerous proteins. Our preliminary findings add to the understanding of the molecular mechanisms by which miR29 drives early differentiation of human pluripotent cells, and may help in the future development of miR-mediated differentiation protocols towards epithelial cells.

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Abstract No. P018

Controlling Cardiovascular Progenitor Cells culture from human Pluripotent Stem Cells by chemical compounds

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Cardiovascular progenitor cells (CPCs) may represent a favourable cell type for cell-based heart repair and a valuable model to investigate heart development. Human pluripotent stem cell (hPSC)-derived CPCs might be expandable whilst maintaining their multi-lineage differentiation potential, comprising cardiomyocytes, endothelial cells and smooth muscle cells. In this study we aim at characterizing markers and pathways of hPSCs' cardiovascular differentiation to ultimately establish maintained CPCs propagation in vitro. Directed cardiac differentiation of hPSCs was achieved by means of chemical Wnt pathway modulators CHIR99021 and IWP1. Mesendodermal induction was monitored using a MIXL1-eGFP reporter line and expression of endogenous MESP1, marking formation of cardiac mesoderm. In our differentiation protocol the second heart field multipotent progenitors' marker ISL1 is upregulated shortly before NKX2.5, which marks the appearance of functional cardiomyocytes. A close interplay between ISL1 expression and Wnt pathway activity was reported in the mouse embryo, in which ISL1 expressing cardiac progenitor cells depend on Wnt signalling for proliferation and survival. We therefore hypothesize that Wnt pathway control could allow propagation and expansion of hPSC-derived CPCs. Monitoring endogenous ISL1 and transgenic NKX2.5-eGFP expression we have screened for small molecules supporting maintained ISL1 expression, thereby avoiding progression of differentiation into NKX2.5-positive cardiomyocytes. Presented results provide a better characterization of in vitro-derived human CPCs, support the understanding of pathways involved in their commitment and maintenance, and might enable the long term<in vitro > expansion of CPCs in larger scale.

Birket et al. 2015 Expansion and patterning of cardiovascular progenitors derived from human pluripotent stem cells. Nature Biotechnology

Kempf et al. 2014 Controlling expansion and cardiomyogenic differentiation of human pluripotent stem cells in scalable suspension culture. Stem Cells Reports

Abstract No. P019

Identification of early cardiovascular precursors in naïve mouse embryonic stem cells

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Nitric oxide (NO) negatively regulates the zinc-finger E-box binding homeobox 1 and 2 (Zeb1, 2) factors via upregulation of miR-200 family members. Although Zeb1, 2 are well known inducers of epithelial-to-mesenchymal transition, an important step to heart formation, their role in mesendoderm differentiation is still poorly understood. In this study, we provide evidence that, in mES, the Zeb factors are downregulated by NO with important consequences on the expression of crucial mesendodermal genes and on a subset of NO-dependent lncRNAs. mES, classically cultured in the presence of serum and LIF (L), synthesize NO only late during differentiation. Instead, mES kept in the presence of three inhibitors (3i), namely L, GSK3i and ERKi realize the so called “naïve state”, express rapidly the eNOS and generate detectable NO levels very early after release from stemness. In fact, as early as 2 hours of release from stemness, a NO-positive mES subpopulation (ESNO+) has been identified. Sorted ESNO+ cells expressed high levels of mesendodermal markers and rapidly behaved as cardiovascular precursors. Mechanistically, the endogenous NO production determined a rapid S-nitrosylation of HDAC2 and the reduction of its association with the transcription repression factor Zeb1. This condition significantly destabilized the Zeb1/chromatin interaction with its genomic targets. The effect of NO was further explored by an integrated full RNA-seq and genome wide ChIP-seq approach aimed at the identification of relevant Zeb1 targets. The results showed the induction of a number of Zeb1-dependent lineage specific mesendodermal markers and the concurrent down modulation of neuroectodermal markers. Among them, we identified 17 lncRNAs unknown to be NO-dependent or transcriptionally targeted by Zeb1. Although some lncRNAs have been previously associated to mesendodermal differentiation, our finding provides unprecedented mechanistic information about their transcriptional regulation in mES. The eNOS-HDAC2-Zeb1 pathway is important for gene expression and lncRNAs regulation in early mesendoderm precursors.

Abstract No. P020

CRISPR-Cas mediated knockout of a putative C-mannosyltransferase in human induced pluripotent stem cells

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C-mannosylation is a unique type of protein glycosylation, where a mannose is covalently attached to a tryptophan residue via a C-C bond. C-mannosylation typically can be found on type I cytokine receptors as well as on secreted proteins and cell surface receptors containing thrombospondin type 1 repeats. Recently, C-mannosylation of R-spondin1 was shown to influence its secretion and downstream Wnt signalling in human cells (Niwa et al. 2016). The discovery of DPY-19 as the C-mannosyltransferase of *Caenorhabditis elegans* (Buettner et al. 2013) allowed the identification of its human homologues, named DPY19L1 to DPY19L4 with DPY19L1 being the closest homologue. To unravel whether DPY19L1 represents a vertebrate C-mannosyltransferase and to study the relevance of C-mannosylation for developmental biology, the aim of our studies is to generate a functional knock-out (KO) of DPY19L1 in human induced pluripotent stem cells (hiPSCs) applying the CRISPR-Cas system. We applied two different sgRNAs to mediate the excision of a complete exon within the DPY19L1 gene. Of 724 generated single cell clones there were 255 clones with a monoallelic deletion (35%) but no clones with a biallelic deletion, suggesting that a KO of DPY19L1 is lethal. As a proof of concept we knocked out DPY19L2, a putative C-mannosyltransferase that has been shown to be exclusively expressed in sperm cells. Applying the same strategy we obtained two clones out of 41 (5 %) harbouring a biallelic deletion. This result further underlines the lethality of the DPY19L1 KO. To proof this hypothesis and to study the effect of a DPY19L1 KO in differentiated cell types, a conditional KO of DPY19L1 applying the Flp/FRT system is currently under construction. To still study the function of DPY19L1 in hiPSCs, a stable knock down was already generated and will be analysed in the near future.

Buettner, F.F. et al., 2013. C. elegans DPY-19 is a C-mannosyltransferase glycosylating thrombospondin repeats. Molecular cell, 50(2), pp.295–302.

Niwa, Y. et al., 2016. Identification of DPY19L3 as the C-mannosyltransferase of R-spondin1 in human cells. Molecular biology of the cell, 27(5), pp.744–56.

Abstract No. P021

LATS-independent control mechanisms of the Hippo pathway transducer YAP

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During the last years, the Hippo pathway and its downstream transducers, the coactivators Yes-associated protein (YAP) and Transcriptional coactivator with PDZ-binding motif (TAZ), have emerged as crucial regulators of adult stem cells in several tissues. Here, YAP and TAZ convey essential stem cell-related traits to these kind of cells and fulfil essential functions during lineage commitment, tissue regeneration and tissue homeostasis. The canonical regulation of YAP/TAZ happens via the so-called LATS kinases integrating the information of many upstream pathways. Previously, we could identify a new LATS-independent mechanism of YAP regulation. Here, the transcription factor c-MYC induces metabolic changes via altered mitochondrial dynamics, ultimately leading to activation of AMP-activated protein kinase (AMPK). Activated AMPK in turn phosphorylates a conserved serine residue in YAP resulting in the disruption of the interaction between YAP and TEAD, the transcription factor family mainly responsible for the recruitment of YAP to target promoters. Importantly, this pathway functions in mammary stem cells and breast cancer to switch off YAP activity. To identify new LATS-independent regulators of YAP we performed genome-wide CRISPR screen yielding several unknown regulators of YAP/TAZ activity. Currently, we are determining the role of these new regulators for YAP's role in lineage commitment for several adult stem cell types.

von Eyss, B. et al. (2015). A MYC-Driven Change in Mitochondrial Dynamics Limits YAP/TAZ Function in Mammary Epithelial Cells and Breast Cancer. Cancer Cell 28, 743–757.

Abstract No. P022

Axin2+ cells are the early precursors of intestinal stem cells

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Various types of adult stem cells, including intestinal stem cells (ISCs), are marked by the expression of *Lgr5*, the target gene and the regulator of Wnt signaling pathway. In the embryonic small intestine $Lgr5^+$ cells only appear at embryonic day 13.5 (E13.5), and their developmental origin is unknown. Moreover, it is unclear whether other cell populations, appearing before $Lgr5^+$ cell population, can contribute to the adult ISC pool. In our study we show that at E9.5, four days prior to the onset of *Lgr5* expression, a subset of the intestinal epithelium expresses *Axin2*, another member of Wnt signaling. Using genetic cell fate mapping and RNA-seq analyses, we found that these early $Axin2^+$ cells give rise to the embryonic $Lgr5^+$ cells in the small intestinal epithelium at E13.5. Importantly, the progenies of embryonic $Axin2^+$ cells contribute to the adult intestinal tissue, and thus serve as precursors of adult $Lgr5^+$ ISCs. Furthermore, we show that Lgr5+ cells derived from Axin2 progenitors have higher levels of Wnt signaling compared to the rest of $Axin2^+$ cells. Together, this study identifies the novel progenitor population of $Lgr5^+$ ISCs and shows that during early embryogenesis *Lgr5* is a better marker for Wnt-responsive cells than *Axin2*.

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Abstract No. P023/T37

Visualization of stem cell induction and differentiation in real time

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Tissue homeostasis depends on self-renewing cells while developmental cues or stress conditions, such as injury or transplantation, activate dormant stem cells. Regardless of cell lineage and signaling pathways, the common signature of cellular activation is induction of mRNA transcription. We showed previously that dormant adult stem cells of many different lineages, including intestinal, haematopoietic, neural, muscle, spermatogonial, skin and melanocyte lineages, display inactive RNA polymerase II (RNApII) during tissue homeostasis, suggesting a lower rate of global mRNA transcription. We have now developed a specific reporter for detection of dormant stem cells derived by insertion of a short RNApII kinase target peptide directly into the yellow fluorescent protein Venus. This reporter, called OSCAR for Optical Stem Cell Activity Reporter, is phosphorylated and dim in activated cells and shows up to 9-fold induction of fluorescence in dormant cells, enabling isolation of adult stem cells by FACS and examination of stem cell dynamics by timelapse microscopy. Analysis of small intestine crypt culture using OSCAR shows significant enrichment of colony formation in OSCAR^{high} cells, comparable to LGR5-GFP positive crypt stem cells. Using this reporter, dynamics of crypt stem cell formation and differentiation are visualized in real time. Furthermore, examination of haematopoietic stem cells reveals strong heterogeneity of global mRNA transcription activity in this population. We also show that dormant stem cell-like cells can easily be detected in other primary cell populations, such as human melanoma biopsies. Taken together, OSCAR is invaluable for identification, isolation and characterization of dormant adult stem cells from many different lineages and enables detection of pure stem cell-like cells in heterogeneous tissues, including cancer.

Abstract No. P024

The interaction between gingival gibroblast and periodontal ligment stem cells on expression of periodontal markers and osteogenic capacity in vitro

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Objectives: Periodontal ligament stem cells (PDLSCs) have the characteristics of mesenchymal stem cells and are a potential source of cells for regeneration of the periodontal tissues. Periodontal regeneration may depend on the interaction of adjacent cell populations within the tissues such as gingival fibroblasts (GFs). The aims of this study were to investigate the effects of PDLSC on the expression of periodontal markers and osteogenic differentiation of GFs. Materials and methods: Primary human PDLSCs isolated from extracted third molars were co-cultured with primary GF cultures, by direct co-culture with subsequent FACS sorting, indirect co-culture using transwell cultures and PDLSC conditioned medium. The expressions of periodontal markers PLAP, Nestin and Periostin were assessed by qPCR. Alkaline phosphatase activity was assessed by para-nitrophenol enzymatic assay. Single cultures of PDLSC and GF were used as controls Results: PDLSC induced expression of PDL markers in GFs in both direct and indirect coculture methods (EG increases of 6.05 and 59.48 fold of PLAP expression $p<0.05$). PDLSC co- cultures,at a ratio of 1PDLSC: 3GF also resulted in increased ALP activity in GF when compared with single GF cultures ($p<0.001$). Similar results were seen using conditioned medium isolated from PDLSC cultures. Conclusions: PDLSCs stimulate expression of periodontal markers and osteogenic capacity of gingival fibroblasts via a mechanism involving paracrine signalling. The results demonstrate that GF contains MSC-like cells which may be recruited for periodontal regeneration by the action of PDLSC cells. Further studies are required to identify specific secreted factors responsible for this activity.

Keywords: periodontal stem cells, co-culture, cell signalling

Abstract No. P025

Role of MicroRNA in Mesenchymal Stem Cell Fate Decisions

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Mesenchymal stem cells (MSC), are adult stem cells which show self-renewal and differentiation capacity to three mesenchymal lineages: osteoblast, adipocyte and chondrocyte. MicroRNAs (miRs) are a class of small non-coding RNAs of 20-22 nucleotides molecules which suppress protein synthesis and may regulate many aspects of cell function. Therefore the aim of this study was to investigate the hypothesis that miRNAs may regulate cell fate and differentiation of MSCs. First, MSC differentiation capacity into osteoblast, chondrocyte and adipocyte lineages were validated by qRT-PCR for lineage specific genes. Using a miRNA PCR-array, 5 miRNA were selected for further investigation, based on their significant up/down-regulation in array, which according to previous studies are unknown as being involved in hMSC differentiation. We validated their expression by qRT-PCR. Finally, to determine the role of the miR-302 family during MSC differentiation, their functional activity was tested by knockdown of miR-302 family, using miR-302 family inhibitor. MSCs showed osteoblast, chondrocyte and adipocyte differentiation on induction with specific culture media. In the PCR array members of the miR-302 family were down regulated during MSC differentiation, and this was confirmed by qRT-PCR. Knockdown of miR-302 with miRNA family inhibitor resulted in an 80% and 50% decrease in miR-302 b and a expression respectively. Knock down of miR-302 family in MSCs resulted in increased osteocalcin (BGP) and alkaline phosphatase (ALP) expression during osteogenesis and increase in FAB, PPARg and CEBP during adipogenesis The results suggest that miR-302 may be an inhibitor of osteogenic and adipogenic differentiation of MSCs and may maintain stem cell properties. Further investigations are in progress on the effect of miR-302 a and b overexpression on MSC differentiation into osteoblast and adipocyte.

Abstract No. P026

Glucocorticoid-mediated stress impacts on neurogenesis and synaptogenesis of hiPSC-derived neurons

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Antenatal glucocorticoid treatment of women at risk of preterm labor is a common method to reduce complications of premature birth. Application of dexamethasone (Dex), a synthetic glucocorticoid (sGC), can significantly reduce the child's risk of respiratory distress syndrome or intraventricular hemorrhage and promote organ maturation. However, recent findings suggest a major drawback to the benefits provided by sGCs: reduced birth size and body weight of infants, decreased size of all brain structures, especially the hippocampus, and an increased risk for developing psychiatric disorders like schizophrenia later on in life. Furthermore, not only sGC treatment of expectant mothers, but also increased maternal stress during pregnancy, mediated by elevated endogenous glucocorticoids (eGC), seems to have an impact on the child's later physical and mental health.Due to their resemblance to the fetal developing brain, neuronal cells generated from human induced pluripotent stem cells (hiPSC) provide an optimal tool to study in vitro the impact of sGC, as well as elevated eGC, on different aspects of differentiation in the premature fetus.In this study, neuronal precursor cell lines derived from hiPSCs were differentiated into glutamatergic neurons according to a standardized protocol and used to analyze glucocorticoid-mediated long term stress response. Terminal neuronal differentiation was triggered by growth factor withdrawal and from then on cells were treated either with Dex or Hydrocortisone (HDC), or a combination of GC and the glucocorticoid receptor (GR) antagonist RU-486. The long term effect of stress was assessed by analyzing expression levels of the GR and mineralocorticoid receptor (MR), neuronal outgrowth, and proliferation. Moreover, the influence of stress on neurogenesis and synaptogenesis during early neurodevelopment was assessed until day50 of terminal differentiation. We show that sGC-mediated stress leads to reduced neurogenesis and synaptogenesis. This effect was not observed in the presence of RU-486 which indicates that the stress response is GR-mediated.

Abstract No. P027

Elucidation of the role of Airn in murine cardiomyocytes

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The current understanding in the field of transcriptomics is that a majority of the human genome is transcribed, which only a few percentages of them encode for proteins. Those RNAs that do not encode for proteins are collectively called as “non-coding RNAs (ncRNAs)”, which includes well-known ribosomal and transfer RNAs as well as microRNAs (miRs). For those ncRNAs whose lengths are longer than 200 nucleotides (nt) are currently classified under a general category called “long non-coding RNAs (lncRNAs)”. Airn is one of the first lncRNAs to be characterized to date. Airn (antisense Igf2r RNA) is an imprinted gene transcribed from the paternal chromosome and is in antisense orientation to the imprinted but maternally expressed Igf2r gene, which Airn exerts its regulation in cis. Although both Airn and its isoforms are expressed highly in the heart, their functions are not known besides imprinting. Here, we aim to elucidate the functions of Airn isoforms in the heart using murine cardiomyocytes cell line HL-1 by employing loss-of-function experiments followed by molecular profiling. To understand the mechanism of action, we performed RNA pull-down assay followed by mass spectrometry (MS) in order to detect potential binding protein binding candidates. RNA immunoprecipitation (RIP) experiment was utilized to confirm the candidates obtained from pull-down that binds to Airn isoforms. After confirmation of binding candidates, we are currently investigating the molecular mechanism in murine cardiomyocytes HL1.

Abstract No. P028

Integration-free iPSCs as a tool for modeling hepatogenesis and maturation

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We have generated episomal-derived and integration-free iPSCs (E-iPSCs) from human fetal foreskin fibroblast cells (HFF1). We used the E-iPSC line to model hepatogenesis in vitro. The differentiation of hepatocyte-like cells (HLCs) involves a three-step process, from the undifferentiated E-iPSC to definitive endoderm (DE), to hepatic endoderm (HE) and ultimately HLCs. The HLCs were fully characterized biochemically, i.e. glycogen storage, ICG uptake and release, UREA production, and CYP3A4 activity. Ultra-structure analysis by electron microscopy revealed the presence of lipid and glycogen storage, tight junctions and bile canaliculi- all typical features of hepatocytes. Furthermore, the transcriptome of undifferentiated E-iPSC, DE, HE and HLCs were compared to that of fetal liver and primary human hepatocytes (PHH). K-means clustering identified 100 clusters which include developmental stage-specific groups of genes, e.g. OCT4 expression at the undifferentiated stage, SOX17 marking DE stage, AFP and ALB at the HLC stage. The NOTCH and HIPPO pathways were over represented in HLC, fetal liver and PHH. Furthermore, we uncovered a gene regulatory network which uncovered the presence of bipotential progenitor populations in HE and HLC stage. Additionally, MYC was identified as a prominent regulator of bipotential hepatoblast-related genes expression. Furthermore, we are on to improve the maturation of HLC in vitro by using small molecules and expansion of cultivation. It is still a big challenge to maturate HLCs. In summary, we have generated episomal-derived iPSCs (E-iPSCs) and demonstrated that they are pluripotent both in vitro and in vivo. These E-iPSCs are able to mimic hepatogenesis and represent a tool for studying human gastrulation at the molecular and cellular levels in vitro. Additionally, small molecules can help to HLC maturation.

Abstract No. P029

Identifying CD surface markers of human dopaminergic neuronal differentiation

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Pluripotent stem cell-derived dopaminergic neurons represent a valuable option for disease modeling and pharmacological screens in vitro and may provide novel cell therapeutic avenues in the future. Their specific and safe application depends on criteria and markers enabling the selection of the most vulnerable dopaminergic neuronal subtypes. By conducting an exhaustive analysis of cluster of differentiation (CD) molecules expressed on the human midbrain-derived LUHMES cell line, as well as on human induced-pluripotent stem cell-derived dopaminergic neuronal in vitro cultures, we provide a comprehensive analysis of neural surface antigen patterns as well as new surface marker candidates for enriching dopaminergic neurons from these model systems. Moreover, combinatorial flow cytometric analysis allows for quantitative readout and assessment of the dopaminergic subset in heterogeneous cultures in multiwell plate-based toxicity assays.

Abstract No. P030

Proliferation Mechanism in Human Amnion Derived Mesenchymal Stromal Cells Under Normoglycemic and Hyperglycemic Conditions

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Objective: Glucose is vital for the proliferation and differentiation of mesenchymal stromal cells (MSCs). The clarification of the relationship between glucose amount and proliferation is necessary for clinical applications of stem cells and for optimization of culture conditions. In this study we aimed to investigate how proliferation mechanisms regulated in amnion derived mesenchymal stromal cells in response to glucose concentration. Methods: Mesenchymal stromal cells were isolated from amniotic membrane of human term placentas. Characterization of isolation was done by flow cytometry and cells were cultivated under normoglycemic and hyperglycemic conditions for 24 hours and for 48 hours. Then, PCNA, p57, Cyclin E protein amounts were examined by using Western Blotting method. Results: Isolated cells were positive for mesenchymal stromal cell markers which are CD44, CD73, CD90 and CD105. They were also negative for hematopoetic markers which are CD34, CD11b, CD19, CD45, CD14 and HLA-DR. In both 24-hour and 48-hour hyperglycemic cultivation (25 mM D-glucose) PCNA expression was elevated. Oppositely, p57 protein amounts were decreased in the hyperglycemic groups in time independent manner. p27 protein amounts were also decreased in time dependent manner. Cyclin E expression remained unchanged among the groups. Conclusion: Glucose is vital for the proliferation and survival of the cells. Hence, examination of the relation between glucose and mesechymal stromal cells may enhance the understanding of stem cell cultivation conditions to maximize the cell amplification and the understanding of glucose-related pathologies during pregnancy. Cell cycle takes place differently in stem cells and it shows glucose dependent variations. More work is required to enlighten this mechanism.

Abstract No. P031

Establishment of ES-, TS- and XEN-Cells from Mouse Blastocysts

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Beside the typical jobs (rederivation, cryopreservation, ES-cell culture to produce new gene modified organism) in our Transgenic Core Facility of the Charité-Universitätsmedizin Berlin, we were asked to generate 3 cell lines from mature mouse blastocysts (ES-, TS- and XEN-cells). In the future our plan is to establish these cell types from a single blastocyst. We modified the existing protocols (Nagy and Nichols 2011, Golding 2012 and Niakan 2013) to exclude loss of cell types due to culture conditions. From previous establishments of ES-cells we know that there are strain specific differences in the potential to establish ES-cells. Therefore we started with genetic backgrounds (129/Sv and C57BL/6J x129S6/SvEvTac/F1-Hybrids) which are often used for producing ES-cells. With these genetic backgrounds we were able to generate all 3 cell types from multiple blastocysts, which confirms our experience with the establishment of ES-cells. The next step was to test these adapted culture conditions with blastocysts from C57BL/6N (a strain that is known to be complicate for the generation of ES-cells). We were able to establish XEN-cells but failed to generate ES- and TS-cells from this inbred strain.

Abstract No. P032

Gap junction dependent cell-to-cell communication during transdifferentiation of human mesenchymal stem cells

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The capacity of mesenchymal stem cells (MSCs) to transdifferentiate into non-mesodermal cell types like neurons is a very promising property in the field of stem cell therapy due to their accessibility for isolation and the promise of generating patient-specific cells for autologous cell therapy. In the field of fundamental research, transdifferentiation offers an opportunity to understand the molecular and cellular mechanisms governing cell differentiation. The aim of our project is to analyse the involvement of gap junction dependent direct cell-to-cell communication in the development of cerebral cells such as neurons and astrocytes. Using gold nanoparticle-mediated laser perforation / dye transfer (GNOME) and single cell dye injection, we showed that human bone marrow-derived MSCs form functional gap junction channels. With qPCR and western blot experiments we found high expression of a variety of connexins, the subunits of the gap junction channels, in undifferentiated MSCs. Application of all-trans retinoic acid in combination with a neuronal induction medium for 48 hours induced differentiation into cerebral cells as showed by morphological changes in brightfield and after phalloidin staining as well as reduced expression of MSC characteristic markers (CD73/CD90/CD166). With respect to connexins, qPCR revealed a strong reduction in expression of the MSC typical connexins, correspondingly, dye transfer experiments showed a reduction of the gap junction coupling, suggesting a possible switch in expression pattern of connexins even on protein level.

Abstract No. P033

Impact of cardiac differentiation methods on human embryonic stem cells and their response to ionizing irradiation

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Human embryonic stem cell (hESC)-derived cardiomyocytes (CM) hold great potential to evaluate the effects of medical treatments during early embryogenesis when the heart develops. We compared the impact of ionizing radiation on H9 hESCs differentiated into cardiomyocytes using the protocols by Lian et al. [1] and Kadari et al. [2] to ensure that the observed effects are attributable to the irradiation but not the differentiation method chosen. Both protocols are based on WNT-signaling modulation, but in addition the Kadari protocol uses BMP4 and ascorbic acid leading to embryoid body-like beating clusters with a homogeneous layer of cuboid supporting cells and a high beating frequency. In contrast, the Lian protocol results in more fibrous clusters with lower beating frequency. qPCR analyses of early mesoderm markers (MESP1, T) indicated that the protocol according to Kadari leads to a prolonged mesoderm formation. After 15 days of culture, there is a marked difference in the expression of MLC2v, a mature CM marker: unlike in the Lian protocol, MLC2v is highly expressed in cardiac cells generated by the Kadari protocol. Thus, the Kadari method seems to lead to more ventricular-like cells. Preliminary data show that these cells contain less miR-1 than those resulting from the Lian protocol. Upon irradiation with 1Gy X-rays, the majority of cells differentiated according to the Lian protocol did not develop beating clusters. In contrast, the cells differentiated with the Kadari method still developed beating clusters, but their number was reduced. In conclusion, the two protocols differ in the preferred generated cardiac cell type and in their response to irradiation. Thus, the differentiation strategy chosen influences the radiation response and has to be taken into account in radiation safety assessments.

[1] Lian et al. (2013) Nat Protoc 8, 162-175

[2] Kadari et al. (2015) Stem Cell Rev and Rep 11:560-569

Abstract No. P034/T38

Embryo-derived macrophages regulate the dendritic cell pool size in the adult spleen

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Dendritic cells (DCs) are potent antigen presenting cells that depend on Flk2-mediated signals for their differentiation. In Flk2-deficient mice, however, a small but functionally active fraction of DCs remains. We show here that the combined deficiency for Flk2 and Csf1r, another member of the same family of receptor tyrosine kinases, results in natural DC null mice. In contrast to Flk2, Csf1r-mediated signals affect DC differentiation by a cell extrinsic, non-hematopoietic mechanism that fails to require Csf1r-mediated signals within the entire adult mouse. During embryonic development Csf1r signaling is crucial for Csf1r and F4/80 expressing macrophages which emerge from erythro-myeloid progenitors in the yolk sac before the presence of hematopoietic stem cells (HSCs). EMP-derived RPMps differ from definitive-HSC-derived macrophages by their site of emergence, the required transcription factors. We could show that Csf1r signaling is required for the development of specialized embryonic macrophages, the tissue-resident red pulp macrophages (RPMp) in the spleen of adult mice. RPMps are ontogenetically heterogeneous cells that are long-lived and have been implicated in the maintenance of tissue hemostasis, removal of pathogens and clearance of cellular debris. We assign here a novel important role to RPMps in the maintenance of tissue homeostasis by regulating the DC pool size in the spleen. In the absence of Flk2-mediated signals, loss of Csf1r expression results in complete ablation of spleen DCs due to the depletion of embryonic macrophages in vivo.

Abstract No. P035

Differential contribution of proneural factors Neurogenin1 and Olig2 to dopaminergic neurogenesis

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Neurogenesis designates the formation of neurons from neural stem and precursor cells. Neurogenesis is easily observed in zebrafish as the embryo is translucent, develops outside of its mother and genetic analysis is advanced. In zebrafish, during primary neurogenesis cells of the embryonic neuroepithelium differentiate into the earliest neurons. Secondary neurogenesis is characterized by differentiation of proliferating neural progenitor cells into mature neurons, which occurs initially broadly in the neuroepithelium, but also continues lifelong in selected neural stem cell niches. Proneural transcription factors contribute to all phases of neurogenesis in higher metazoans, but their differential contributions to the different phases of neurogenesis and differentiation are not fully understood. Here we report the differential contributions of two proneural factors, Neurog1 and Olig2, to dopaminergic neurogenesis. Expression analysis of both genes revealed coexpression in some regions during embryonic and larval stages of development. The analysis of *neurog1* and *olig2* mutant alleles reveals that both *neurog1* and *olig2* are required for the specification and differentiation of subgroups of ventral diencephalic dopaminergic neurons. *Neurog1* and *olig2* double mutant embryos develop a combinatorial if not synergistic phenotype, suggesting parallel or shared pathways. The overexpression of Neurog1 and Olig2 each causes formation of supernumerary or ectopic dopaminergic neurons. Further experiments show that perturbation of Notch signaling affects the phenotype of *olig2* mutants, but not *neurog1* mutants. These results suggest that in dopaminergic differentiation Notch signaling acts downstream or in parallel of *olig2*, but upstream of *neurog1*. This assumption is further confirmed by the fact that the expression of *neurog1* is reduced in *olig2* mutants, but not vice versa. Future directions will be the analysis of *neurog1* and *olig2* during neural stem cell niche formation and their role in the maintenance and differentiation of specific neural stem/progenitor cell lineages.

Abstract No. P036

Molecular mechanisms underlying pancreatic identity and plasticity in mammalian species

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Reprogramming of lineage-restricted somatic cells into pancreatic beta-cell equivalents has emerged in recent years as a promising strategy in cell-based therapy to treat diabetes. The liver is an ideal tissue source for new beta-cells, due to its close developmental origin with the pancreas, both deriving from adjacent regions within the definitive endoderm, and its regenerative ability. We previously identified the TALE homeoprotein TG-interacting factor 2 (TGIF2) as a molecular regulator of the liver *versus* pancreas cell fate decision in the mouse. Moreover, stable lentiviral expression of TGIF2 in adult mouse liver cells has been shown to repress the hepatic transcriptional program and to initiate the pancreatic progenitor transcriptional program, in a step-wise manner. In the present study, we address the conservation of TGIF2 reprogramming activity in human hepatocytes by using the non-integrative Adeno-associated viral (AAV) delivery system. Producing human pancreatic cells from liver cells would be extremely valuable toward the development of personalized cell therapies for diabetic patients. Moreover, to answer the question whether TGIF2 is able to modulate the identity of cell types different from hepatocytes, we have examined its role when ectopically expressed in a non-endodermal context, such as fibroblast cells. These results suggest a general cell lineage reprogramming activity of this transcription factor. Finally, the *ex vivo* lineage reprogramming observations are completed by an *in vivo* loss-of-function approach in the mouse embryo, using a *Tgif2* floxed allele. These complementary approaches will allow us to dissect the requirements of Tgif2 and to elucidate the molecular mechanisms underlying pancreatic identity and plasticity in mammalian species.

Abstract No. P037

3-D time lapse analysis of the growth of cultured embryonic stem cell colony and the expression of a pluripotency regulator protein

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Embryonic stem cells (ES cells) have been a fundamental resource in gene engineering and regenerative medicine. As ES cells are increasingly essential tools in life science, there are growing demands for 3-dimensional analysis of the dynamic regulation of physiological functions in living ES cells. The CQ1 is an all-in-one confocal quantitative imaging cytometer based on the confocal scanner unit (CSU) from Yokogawa Electric Corporation. The core of the CSU is a microlens-enhanced dual Nipkow disk confocal optical system. In this system, laser beams with moderate power scan the sample multiple times during image acquisition. This scanning method results in drastically lower-phototoxicity and lower-photobleaching compared to conventional single beam scanning instruments. Therefore, the CSU has long been an ideal solution for live cell imaging since the first model was launched in 1996. In this presentation we will report the results of 3D time lapse analysis of the growth of cultured mouse ES cell colony. Time-lapse imaging was conducted with CQ1 equipped with an internal incubation chamber to control culture environment. We successfully tracked the proliferation of the cells and consequent increase of the volume of the colony for over two days. In addition, we were able to quantify the change of the expression of a pluripotency regulator protein Nanog as well.

Abstract No. P038

Fast and label-free tracking of stem cell behavior – enabling their further utilization

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Introduction Raman spectroscopy (RS) is a highly sensitive analytical method for marker-free and non-invasive identification and characterization of single cells. Here, we present RS as a novel tool for gentle yet highly precise stem cell analysis in three independent experiments, providing an overview about the large versatility of this method. We could provide evidence that RS is an easy tool to discriminate cell types, observe cell differentiation and monitor treatment effects. Materials and Methods In a first experiment, neuronal progenitor cells (NPCs) were isolated from spinal cord of rats and treated with 10 ng/ml transforming growth factor (TGF)-β1 on days 1, 4 and 7. Secondly, RS was used to investigate stem cells isolated from adipose tissue and exposed to standard (21% O2) or hypoxic (5% O2) oxygen conditions. In a last experiment, mesenchymal stem cells (MSCs) were treated with erythropoetin (EPO) for certain time points and subsequently analyzed using Raman analysis. Results Using NPCs, RS was able to discriminate TGF-β1-treated neurons from vehicle-treated ones. Thereby, TGF-β1-induced changes in Raman spectra could be associated with different amounts of nucleic acids. In hypoxia experiments, Raman spectra differed between hypoxic and normoxic cells, whereupon RNA expression level was found to be the main difference. In the last experiment, RS could monitor differentiation of EPO treated MSCs and found that around 35% of treated cells showed fibroblast like profiles. Discussion RS is a photonic marker for gentle yet highly specific cell analysis, allowing discrimination of cells, monitoring of treatment reactions and observation of differentiation. As label-free method, it provides highly specific molecular information about the entire metabolome of single cells. The huge versatility of this non-invasive approach allows new insights into almost every biological question without impairing cell viability.

Acknowledgement This Project received funding from the EU 7th Health Program grant agreement No 279288.

Abstract No. P039

The Role of Hes/Her Genes in Regulating Neural Stem/Progenitor Cell Properties in Zebrafish

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While mammalian adult neurogenesis is limited, zebrafish are able to generate new neurons and repair brain damage lifelong. The *in vivo* mechanisms underlying neural stem cell proliferation and precursor pool expansion as well as the specific differentiation of stem-cell derived precursors are poorly understood. Maintenance of stem cell properties is regulated by a fine-tuned gene network involving *Hairy and enhancer of split (Hes)* family genes, which inhibit neurogenesis to allow stem cell pool expansion and maintenance, and thus differentiation of neurons also at later stages. Most *Hes* genes are still expressed in stem cell zones of adult zebrafish, but it is unknown how these niches form. Therefore, zebrafish *Hes* gene knockout models may provide additional information on the function of these genes and their roles during the establishment of stem cell niches. Since loss of *Hes1* and *Hes5* in mice causes premature differentiation followed by loss of radial glia, we focus on the zebrafish *Hes1* homologs *her6* and *her9* as well as on the *Hes5* homologs. There are nine zebrafish *Hes5* homologs located in only two gene clusters, which enabled enables us to create deletions of all *Hes5* type genes using the CRISPR/Cas9 system. We analyze phenotypes of the deletion alleles for changes in expression of her genes, proneural genes and stem cell markers. To better understand *her* gene functions during neural stem cell niche formation, we characterize and compare the expression of *her* and proneural genes using double-fluorescent *in-situ* hybridizations at larval stages (3-5 days old). We also correlate *her* gene expression with stem and progenitor cell properties by staining for stem cell markers in wild-type and *her* gene mutant fish. Together this will help to elucidate with high spatio-temporal resolution the function of *her* genes during the establishment of stem cell zones.

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Abstract No. P040

Interleukin-3 favors endothelial to hematopoietic transition (EHT) of human iPSC-derived hemogenic endothelium

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Hematopoietic differentiation of hPSCs holds great promise for innovative cell based therapies, and represents a valuable system to gain novel insights into human embryonic hematopoietic development. Ontogeny of hematopoietic cells represents a finely balanced process in which different in- and extrinsic factors act synergistically in a spatiotemporal fashion. As little is known about early human hematopoietic development, we have examined the role of cytokines during early hematopoietic specification. Thus, we have directed PSCs towards mesoderm by embryoid body formation and induced hematopoiesis in the presence of IL-3/M-CSF to generate macrophages (MΦ) or IL-3/G-CSF for granulocytes. Hematopoietic differentiation employing IL-3 only gave rise to immature $CD45^+/CD11b^+/CD14^-/CD163^-$ myeloid cells, able to further differentiate into macrophages, granulocytes or erythrocytes, in the presence of G-CSF, M-CSF or EPO/SCF respectively. Analysis of hematopoietic specification in the presence of IL-3 revealed time-dependent up-regulation of transcription factors MIXL1, T, SOX17, KDR1, GATA2, TAL1 or RUNX1, and a transition of $CD34^+/CD45^-/CD144^+$ hemato-endothelial progenitors (HEP) to $CD34^+/CD45^+/CD144^+$ hematopoietic progenitors (HP) and finally $CD34^-/CD45^+/CD144^-$ mature hematopoietic cells. Of note, impaired endothelial to hematopoietic transition (EHT) of $IL3Ra^+$ HEP was observed in the absence of IL-3. Altered EHT was accompanied by insufficient downregulation of SOX17 and induction of GATA2, known as key regulator of EHT. In contrast, presence of IL-3 resulted in normal EHT characterized by decreased SOX17 and increased GATA2 expression, confirmed by microarray analysis. Similarly, IL-3 dependent EHT was demonstrated when HEP were sorted from early differentiation cultures and propagated on OP9 stroma cells. Here, only differentiation in the presence of IL-3 resulted in $CD34^+/CD45^+$ cells with clonogenic potential in methylcellulose based assays. In summary, we provide evidence that IL-3 plays a crucial role not only in adult hematopoiesis but also constitutes one key cytokine for early hematopoietic specification of human PSC.

Abstract No. P041

Identification of novel Hox target genes with essential roles in hematopoietic stem cell self renewal and differentiation

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Hematopoietic stem cells (HSCs) are able to restore the entire hematopoietic system in vertebrates. It is of key importance to understand how the equilibrium of self-renewal and differentiation is maintained in HSCs. Members of the Hox gene have been shown to be involved in this process. For example, Hoxa9 and Hoxb4 overexpression increase hematopoietic stem cell renewal and enhance murine HSCs repopulation. In order to understand the function of Hox genes in HSCs we dissected the downstream effect of Hox genes. For this purpose we applied both in vitro and in vivo approaches to investigate the role of Hox genes and their downstream pathways in HSCs. We conducted an unbiased in vivo screen using a focused shRNA pool targeting all Hox gene members in HSCs in serial transplanted mice by 4 shRNAs per gene. This screen revealed a comprehensive map of Hox genes that are required for the maintenance of HSCs including Hox genes that have not been implicated in HSCs control. The functional role of Hox genes in HSC self renewal was validated by transplantation experiments. Since Hox genes have very similar DNA binding motifs and show similar phenotype in our study, we investigated whether they share common molecular targets. Using microarray-based transcription profiling on single Hox gene knock-downs that affect HSCs maintenance, novel target genes were identified in hematopoietic cells that are regulated by 2 or multiple Hox genes. Of note, the knockdown or overexpression of individual target genes phenocopies the known effects of Hox gene overexpression or knockdown on HSCs self renewal and differentiation. Together, this study identifies novel essential regulators of HSCs that require a combinational transcriptional input of Hox genes for their proper expression and function in HSCs.

Abstract No. P042

Haematopoietic Colony Formation in the Absence of Added Glucose

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Haematopoietic bone-marrow maintains high-level production of neutrophils and erythrocytes in distinct regions of the space between nutritionally rich arterioles and sinusoid venules, which are probably nutritionally-poor. It is unclear how the metabolic environment is organised to achieve rapid proliferation of progenitors at different distances from the arterioles, although the metabolism of mature neutrophils and erythrocytes is clearly very different. We have modified the methylcellulose colony-forming assay to permit regular exchange of medium throughout the period of colony-development, using dialysed serum depleted for small metabolites, and in this way have investigated the metabolic requirements for colony-formation from progenitor cell populations purified from human umbilical cord blood. Surprisingly, we find near-normal colony formation using media containing no detectable glucose. To achieve this, however, it is necessary to refresh the medium over the first 3 days of culture, suggesting that early stages of colony formation require a medium component that is present at limiting-concentration. Since glucose-derived metabolites are essential for cell growth and division, we hypothesise that at least a subset of cells in developing colonies can perform gluconeogenesis – a process normally attributed to cells of the liver and kidney. In support of this, we show that the cytoplasmic isoform of phosphoenolpyruvate-carboxykinase (PEPCK, an enzyme specific to gluconeogenesis) is expressed in the developing colonies and increased in colonies forming in the absence of glucose. Our results suggest that the maintenance of high proliferation rates at various depths in the marrow may be achieved independently of arterial blood glucose, relying instead on gluconeogenesis from amino acids. These findings raise questions about the interplay between lineage-specific signalling and metabolism in haematopoiesis *in vivo*, but also have consequences for the cost-effective *in vitro* production of haematopoietic cells for cell therapy, transfusion medicine and for the identification of metabolic-distinctions between normal progenitors and leukemic cells.

Abstract No. P043

Leukotriene receptor inhibition mobilizes human stem cells in vivo

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Stem/progenitor cell mobilization is an alternative to local and systemic stem cell transplantation in regenerative medicine. Leukotrienes are important biological response mediators and their receptors are expressed on a variety of cell types including immune cells, hematopoietic stem/progenitor cells (HSPCs) and mesenchymal stem/progenitor cells (MSPCs) and endothelial progenitors. Leukotriene receptor antagonists are used for asthma treatment but were also shown to modulate HSPC homing and promote neurogenesis by a so far unknown mechanism. In a project aiming to better understand injury-induced and drug-induced stem/progenitor cell mobilization, we asked whether cysteinyl-leukotriene receptor (cysLTR1) antagonism influences circulating stem cells. Blood samples from five healthy adults taking an LTD4 antagonist daily for one week were analyzed at four different time points defining baseline, early (4-6h after 1st medication), intermediate (day 3-4) and late (day 7) mobilization by complete blood count and high-resolution 10-color flow cytometry focusing on critical HSPCs, MSPCs and endothelial colony forming progenitor cells (ECFCs). Human bone marrow aspirate and umbilical cord blood served as technical positive controls. We found a significant mean 1.9-fold mobilization of lineage^{NEG}/CD34^{+}/CD38^{-}/CD45RA^{-}/CD90^{+} hematopoietic stem cells (HSCs; baseline=79±18; day seven=147±36/mL; mean±SEM) after seven days of drug intake. There were no significant differences in total CD34^{+} and various hematopoietic progenitor cell types analyzed (multipotent progenitors, common myeloid progenitors, megakaryocyte/erythrocyte progenitors, granulocyte/macrophage progenitors, common lymphoid progenitors and lymphoid-primed multipotent progenitors) before day seven. We observed no significant changes in leukocyte count, subsets (neutrophils, monocytes, lymphocytes) or lymphocyte subsets (T cells, B cells, NK cells). No detectable levels of MSPCs or ECFCs were mobilized by leukotriene inhibition (detection threshold 10^{-6}). While inhibition of leukotrienes induced no significant changes in the composition of mature blood cells and their progenitors, it may have a previously unobserved mobilizing effect on the most immature pluripotent HSCs.

Abstract No. P044

MCMV infection modulates cell cycle state and functionality of long-term hematopoietic stem cells in vivo

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Quiescent long-term hematopoietic stem cells (LT-HSCs) are efficiently activated by type I interferon (IFN-I). However, this effect remains poorly investigated in the context of IFN-I-inducing virus infections. We report here that murine cytomegalovirus (MCMV) evokes LT-HSC activation that differs substantially from effects triggered by administration of synthetic IFN-I-inducing agents. Upon infection, BM cellularity was drastically reduced, and distinct inflammatory cytokine and chemokine responses exceeded local thresholds within the bone marrow (BM) to confer LT-HSC activation. Interestingly, even though IFN-I receptor signaling was not critical, it was contributing to this effect. After resolution of acute virus infection, LT-HSCs returned to phenotypic quiescence. However, persistent peripheral MCMV infection induced a sustained inflammatory milieu in the BM and changes in LT-HSC gene expression that coincided with long-lasting impairment of LT-HSC reconstitution potential. In conclusion, our results show that MCMV infection fundamentally affects LT-HSC phenotype and function, and that subclinical, persistent inflammatory stimuli in BM donors can affect the reconstitution potential of BM transplants.

Abstract No. P045

Nitric Oxide-Releasing Thin Films for Hematopoietic Stem Cell Culture

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Nitric Oxide (NO) is a key signaling molecule involved in cell survival, proliferation and differentiation in various cultured cell types. In hematopoietic stem and progenitor cell (HSPC) research, inhibition of NO generation increased the number of stem cells compared to controls in mice and impaired the mobilization of bone marrow stem and progenitor cells. Furthermore, NO has been shown to alter the metabolic state of HSPCs [1] and regulate the transition between HSPC proliferation and differentiation [2]. These findings render NO an interesting molecule for the *in vitro* expansion of HSCs, one of the most relevant challenges in stem cell biology, because today these are the only stem cells used routinely in the clinic to treat patients and their number is limited. However, *in vitro* multiplication of hematopoietic stem cells cannot be achieved without differentiation and loss of stem cell potential. Since the biological effects of NO are highly dependent on its concentration, release systems that allow tight spatiotemporal control over NO concentration have to be developed in order to investigate the potential of NO for culturing HSPCs *in vitro*. SURGELs as "ideal network polymer" thin films, derived from Metal-Organic Frameworks, offer chemical versatility, high porosity and biocompatibility. Thus, they fulfill the requirements for such a system—controlled release due to the homogenous distribution of NO donating moieties and unhindered diffusion of the gas – making them promising candidates as platforms for HSPC culture. Functionalization of SURGELs with photo- or pH-sensitive donating moieties should yield a drug-release platform which is responsive to external stimuli like light and allow for precise spatiotemporal control of NO release *in vitro*.

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Abstract No. P046

Transcriptional profiling of in vitro generated red blood cells from human adult HSCs and pluripotent stem cells

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We have reported a donor-independent hematopoietic differentiation potential of human induced pluripotent stem cells from different somatic cells of origin. Although displaying an epigenetic memory, all tested iPSC-lines performed similarly regarding hematopoietic induction potential and erythroid differentiation (Dorn, Klich et al., 2015). Despite this positive outcome erythroid maturation from human iPSCs is less efficient than from adult-derived CD34+ stem/progenitor cells with regard to expansion rates and terminal maturation, especially enucleation and globin switching. Thus the attempt to use iPSC-derived RBCs for transfusion purposes remains challenging. In order to investigate potential differences on the transcriptome level between adult, cord blood and ESC- as well as iPSC-derived RBCs, we collected samples on carefully chosen time points during in vitro erythroid differentiation and conducted a comparative gene expression study using microarray followed by quantitative RT-PCR and QuantiGene Plex Assay for single gene analysis. Our results reveal a high similarity of iPSC-derived RBCs with those of embryonic and fetal origin rather than with cells of adult origin. When comparing terminal maturation stages of iPSC-derived erythroid cells with those of adult origin especially apoptosis and protein biosynthesis related genes are differentially expressed. Adult β-globin and ε-globin were differentially expressed between adult- and iPSC-derived RBCs whereas the putative switch factors BCL11A and Klf1 revealed no differences on the gene expression level. Taken together our studies provide for a better understanding of iPSC-derived erythropoiesis and pave the way towards patient-specific RBCs, generated in vitro.

Abstract No. P047

Cross-communication between hematopoietic stem cells and the niche

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Hematopoietic stem cells (HSCs) reside in a specialized niche microenvironment within the bone marrow (BM). Therein, HSC function is regulated by direct cell-cell-interactions, secreted cytokines and extracellular matrix molecules. Known cellular components of the HSC niche include endothelial cells and different mesenchymal cells types, which secrete HSC-supporting factors including SCF and CXCL12. However, a detailed understanding of the HSPC-niche cell interactome and its alterations in the context of transplantation is still missing. Therefore, we applied a FACS-based strategy to identify non-hematopoietic components of the murine endosteal and central BM, and revealed a high overlap of our isolated niche cells with VE-Cadherin$^+$ endothelial cells and LepR$^+$, Nestin$^+$ and CXCL12$^+$ mesenchymal stromal cell populations. All central BM niche cells were found to be sensitive to transplantation conditioning, and rapidly declined following sublethal irradiation. Conversely, the endosteal fraction resisted radiation treatment and might therefore serve as stem cell niche following BM transplantation into conditioned recipient mice. Moreover, we could observe that the niche of irradiation-conditioned, as well as of unconditioned Kit-deficient recipients with a functionally impaired endogenous HSC compartment, could respond to transplanted murine and human donor cells, affecting absolute cell numbers and the transcriptional network of mesenchymal progenitors. This suggests a tight cross-talk of the niche with endogenous but also transplanted hematopoietic cell types. To identify further molecular regulators of HSC function, we established an extensive database of soluble and membrane-bound ligand-receptor interactions and used it to predict potential HSC-niche interactions based on ranked expression values from deep transcriptome sequencing of sorter purified niche cells and HSPCs. This analysis confirmed well-known interactions between HSCs, endothelial cells and mesenchymal progenitors, including regulators like Vcam1, SCF and E-Selectin. Moreover, we predicted strong novel interactions that will serve as basis for further analysis of HSC regulation in steady-state and post-transplantation conditioning.

Abstract No. P048

Dissecting the origin of dendritic cell and macrophage subsets in human hematopoiesis

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According to our recent findings multipotent progenitors (MPPs) do not create common lymphocyte (CLP) and common myeloid progenitors (CMP) as suggested by the classical model of hematopoiesis. Instead, they create lymphoid-primed multipotent (LMPP) and erythro-myeloid progenitors (EMP). Thus, subsets of myeloid cells derive from both branches. In this context, we previously showed that neutrophils are derivatives of LMPPs and eosinophils and basophils of EMPs. Monocytes/Macrophages arise from progenitors of both branches. Without dissecting their concrete origin, dendritic cells (DCs) have been classicaly discriminated into lymphoid [plasmacytoid DC (pDC)] and myeloid DCs [monocyte-related DC (MoDC), myeloid DC1 (mDC1), myeloid DC2 (mDC2)] Now, the novel hematopoietic lineage relationships raise the question whether myeloid DCs derive from LMPPs and/or EMPs. Consequently, in our ongoing work, we aim to unravel the exact origin of both, the different human macrophage subsets as well as of the different DC subtypes. By comparing different growth conditions we have established and optimized *in vitro* differentiation assays allowing for the generation and quantification of all current DC subtypes from umbilical cord blood-derived MPPs. Upon characterizing obtained cells by multicolor flow cytometry (12 colors), we were able to increase the phenotypic resolution currently used for *in vitro* generated DC-subtypes. Upon adding HLA-DR and CD11c as markers, we identify pDCs as HLA-DR^{dim}CD14^{-}CD1c^{-}CD303^{+}CD123^{+}, mDC1s as HLA-DR$^{dim/+}$CD14^{-}CD1c^{+}CD11c^{+}CD141^{dim}, and mDC2s as HLA-DR$^{dim/+}$CD14^{-}CD1c^{+}CD11c^{low}CD141^{+} CLEC9a^{+} cells, now. According to the literature MoDCs are defined as HLA-DR^{+}CD14$^{-/+}$CD1c^{+}CD11c^{+} cells. Although we obtain cells with this phenotype in our assays, the CD1c^{+} cell fraction is still a very heterogeneous cell population which needs to be dissected any further. After defining the MoDC containing cell population in more detail, defined progenitors of the lymphomyeloid and the erythromyeloid branches will be analyzed for their exact DC potentials, finally allowing us to further unravel the model of the human hematopoietic tree.

Abstract No. P049

Human hematopoiesis is influenced by co-injected cell types and Notch-ligand Delta-like1 in a teratoma-based model

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Background Hematopoietic stem cells (HSC) transplantation is successfully applied to treat a variety of blood-borne diseases. To overcome actual limitation of donor cell sources, researchers are investigating alternative sources and developing protocols for efficient *de novo* generation of HSC. The highly sophisticated microenvironment of HSC is challenging to mimic in culture. Hence, human induced pluripotent stem cell (hiPSC) differentiation protocols to generate transplantable HSC are still missing. Interestingly, functional human HSC evolve during teratoma formation in immunodeficient mice (1,2). We employ teratoma formation assay with hiPSC as an *in vivo* hematopoiesis model to study cell type compositions, Notch signaling and cytokine environment necessary for *bona fide* HSC development. In this line we evaluated different cell types for co-injection to support teratoma-linked hematopoiesis and the reported supportive effect of Notch ligand Delta-like-1 (1). **Methods** $CD34^+$-derived hiPSC, with or without supporter cell types, were subcutaneously injected into flanks of immunodeficient mice. Teratoma samples were examined by immunolabeling of specific surface markers via flow cytometry and immunohistochemistry. **Results** Differentiation of hiPSC during teratoma formation gave rise to low percentage of hematopoietic cells. Localized close to mesodermal descendants, we detected $CD90^+$ and $CD34^+$ cells representing potential hematopoietic cells in early differentiation stages. To improve hematopoietic cell development we co-injected the murine stromal cell line OP9. In this setting, the amount of teratoma-derived $CD45^+$ cells was elevated from 0.2% to 0.7%. Co-injection of human umbilical vein endothelial cells or murine embryonic fibroblasts did not result in higher proportions of $CD45^+$ cells compared to OP9. Hematopoietic differentiation was best when OP9 expressing Delta-like-1 were co-injected. We detected a small but distinct hematopoietic progenitor population of 10^7 cells ($CD34^+/CD45^+/CD43^+$) which was not present when unmodified OP9 or other cell types were co-injected. In immunohistochemical staining these progenitors were located close to $CD34^+$ structures.

(1) Amabile et al., 2013 (In vivo generation of transplantable human hematopoietic cells from induced pluripotent stem cells. Blood 2013, 121 8, 1255-64)

(2) Suzuki et al., 2013 (Generation of engraftable hematopoietic stem cells from induced pluripotent stem cells by way of teratoma formation. Molecular Therapy 2013; 21 7, 1424–31)

Abstract No. P050

The homeobox transcription factor hlx1 is essential for hematopoietic development

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Upregulation of the non-clustered homeobox transcription factor HLX (H2.0-Like Homeobox) is frequently observed in patients with acute myeloid leukemia. Since developmental pathways are often reactivated in cancer, we asked whether hlx1 plays a critical role during hematopoietic formation and differentiation in zebrafish. Zebrafish hlx1 morphants have normal primitive hematopoietic specification, but show a decline in erythrocytes and concomitant increase in myeloid cells at 48 hpf, suggesting a crucial role of hlx1 during the formation of intermediate erythromyeloid precursors (EMPs). Since EMPs arise from endothelial precursor cells, where hlx1 is primarily expressed, we performed expression analysis of sorted endothelial cells. Interestingly, we observed that many genes involved in erythropoiesis are expressed in wild-type endothelial cells but the whole erythroid program and heme biosynthesis related genes are massively downregulated in hlx1 morphants. Chromatin immunopreciptation assays in human cell lines show that HLX binds to putative enhancers and regulatory elements of erythropoietic regulators like TAL1, establishing HLX as a direct regulator of the erythroid program. hlx1 morphants have also diminished numbers of definitive hematopoietic stem and progenitor cells (HSPCs), resulting in fewer thymocytes. Overexpression of HLX in endothelial cells leads to rescue of the morphant phenotype and results in aberrant production of HSPCs. Surprisingly, hlx1 morphants exhibit diminished ribosomal protein production, significantly downregulated glycolysis and attenuation of metabolic and unfolded protein response pathways that account, at least partially, for the HSPCs phenotype. Together, our data suggest that hlx1 controls the erythro-myeloid decision during EMP formation and positively regulates HSPCs formation by fine-tuning metabolic pathways.

Abstract No. P051

Mechanism of long-term robust human hematopoietic stem cell engraftment in Kit-deficient NSGW41 mice

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Xenotransplantation models enable the in-depth analysis of human hematopoietic stem cell (HSC) function in vivo [1]. However, stable long-term engraftment and self-renewal of human HSCs is limited in xenotransplantation settings. We aimed at generating a mouse model that supports improved stable HSC engraftment, which we considered a prerequisite for the continuous differentiation of all hematopoietic lineages. By introducing a loss-of-function Kit receptor into NOD/SCID Il2rg-/- (NSG) mice we recently generated a novel mouse strain, NOD/SCID Il2rg-/- KitW41/W41 (NSGW41) [2]. NSGW41 mice combine an impaired endogenous HSC compartment with immunodeficiency and efficiently support stable long-term engraftment of human HSCs without the need for conditioning. Multilineage engraftment, especially the differentiation of cells of the myeloid megakaryocyte-erythroid lineages, is highly improved in NSGW41 mice. To investigate whether self-renewal of engrafted human HSCs is improved in NSGW41 mice over conventional Kit-proficient NSG mice we transplanted titrated numbers of human HSCs and could show improved pick-up and expansion of human HSCs in vivo. Secondary transplantation experiments revealed an increased frequency of functional human donor HSCs in NSGW41 mice, leading to robust levels of human engraftment in secondary recipients. We hypothesized that endogenous HSCs with a defective Kit receptor are largely replaced by human Kit-proficient HSCs. In fact, in NSGW41 mice the endogenous HSC pool is significantly decreased after engraftment of human HSPCs, supporting our hypothesis that endogenous HSCs are actively replaced from their niche. Analysis of the murine bone marrow niche before and after transplantation revealed a significant increase of the SCF-producing CXCL12-abundant reticular (CAR) cells. CAR cell increase strictly correlates with the number of engrafted human HSCs, suggesting that the donor HSCs contribute to the expansion of CAR cells. We suggest that CAR cells provide a niche for human HSCs in mice and that human HSCs modify the murine bone marrow niche after engraftment.

[1] Mende, N., et al., CCND1-CDK4-mediated cell cycle progression provides a competitive advantage for human hematopoietic stem cells in vivo. The Journal of experimental medicine, 2015. 212(8): p. 1171-83.

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Abstract No. P052

Development of a functional humanized immune system using different sources of human hematopoetic stem cells

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Objective: In previous experiments, we have demonstrated, that hematopoietic stem cells (HSC) from cord blood can proliferate and differentiate in vivo and can be used to generate humanized mice (HuMic) for preclinical immuno-oncology models. However, it needs to be evaluated whether other HSC sources can be used to generate HuMic. We compared the differentiation and function of HSC from three different sources: cord blood, buffy coat and iPSC-derived.

Methods: HSC, isolated from cord blood and buffy coat were transplanted intravenously into three week old irradiated immunodeficient mice. Every 4 weeks, blood was screened by FACS for human leukocytes (huCD45+). For iPSC-derived cells we compared hematopoetic differentiation of iPSC in vitro induced by cytokines with in vivo differentiation capacity using teratoma assay. After successful engraftment of HSC, bone marrow of HuMic was retransplanted to determine the engraftment capacity of huCD34+ cells after growing 12 weeks in mice.

Results: Up to 50% of leukocytes in the blood were huCD45+ cells 12 weeks after transplantation of HSC from cord blood, and T cell subpopulation reached up to 20% in the blood. Using HSC from buffy coat a significant reduced engraftment potential could be observed (under 5% huCD45+ cells). After s.c. transplantation of iPSC into mice teratoma growth could be determined. FACS analysis revealed huCD34+ stem cells in 1/5 teratomas from iPSC alone (up to 2%), 5/5 teratomas from iPSC in combination with MSC (up to 6%) and 4/5 teratomas from iPSC in combination with bone marrow of HuMic (up to 2%).

Conclusion: In summary, HSC from cord blood represent the best source to induce the engraftment and differentiation of human immune cells in vivo. Differentiation protocol of iPSC into huCD34+ cells has to be improved, but seems to be a suitable source to generate and enrich HSC from iPSC as an unlimited source.

Abstract No. P053

Terminal differentiation as stem cell specific DNA damage response

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The accumulation of cellular DNA damage is considered to be a central driver of organism ageing and cancer development. Specific cell intrinsic DNA repair mechanisms aim to preserve genome integrity. Most repair processes are closely coupled to cell cycle checkpoints that may promote cell cycle arrest at different cell cycle phases to allow time-consuming DNA repair. In case of unrepairable damage most somatic cells preferentially undergo apoptosis to avoid fatal consequences for the organism. Dealing with DNA damage in the stem cell compartments raises additional challenges compared to committed cells due to their longevity and importance for organ regeneration, which is supported by stem cell-specific DNA damage response mechanisms. We focus on hematopoietic stem cells (HSCs) as a model to study the consequences of DNA damage on adult stem cell behavior with special interest on the tightly regulated balance between HSC self-renewal and differentiation. We and others described the induction of terminal differentiation upon DNA damage as a stem cell-specific stress response effectively eliminating damaged HSCs from the system. Following this concept, we aim to dissect each HSC fate upon the introduction of several types of DNA damage at the single cell level in real-time. Time-lapse microscopy-based tracking of individual HSCs and their progeny for many days allows the continuous observation of cell fate decision control and behavior upon DNA-damage. The exact timing of the entry into cell cycle and of the cell cycle progression can be linked to future cellular fates such as differentiation or death/survival in real-time. With this approach established HSC specific DNA damage response mechanisms, e.g. radioresistance, are revisited and novel concepts are elucidated that circumvent accumulating damage in the HSC compartment.

P054 – P060: Stems cells and ageing, genome stability and epigenetics

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Chao Sheng

Abstract No. P054

The Functional Relevance of Specific DNMT3A Transcripts in Hematopoietic Stem and Progenitor Cells

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DNA methyltransferase 3A (DNMT3A) plays pivotal roles in *de novo* DNA methylation (DNAm) during development. It undergoes alternative splicing in a tissue- and disease-specific manner and is frequently mutated in patients with acute myeloid leukemia (AML). Notably, mutations in *DNMT3A* may affect expression of alternative transcripts. In this study we addressed the functional role of specific splice variants of *DNMT3A* in hematopoietic stem and progenitor cells (HSPCs). Specific transcripts (Tr.1+3, Tr.2, and Tr.4) were knocked down by lentiviral expression of shRNAs in $CD34^+$ cells isolated from cord blood. Downregulation of either Tr.2 or Tr.4 reduced the proliferation rate of HSPCs significantly ($n=3$, $p<0.05$). HSPCs maintained CD34 expression for a higher number of cell divisions upon knockdown of Tr.2 ($n=3$; $p<0.05$). In colony forming unit assays downregulation of Tr.4 resulted in a clear bias towards erythroid colonies ($n=3$, $p<0.05$). To address the question whether these functional effects are caused by specific epigenetic modifications we used Illumina 450k BeadChip technology: DNAm patterns revealed significant differential methylation of several CpG sites upon knockdown of Tr.2 and Tr.1+3 (8,905 and 352 CpGs, respectively; adjusted p-value<0.05). In contrast, knockdown of Tr.4, which does not exhibit methyltransferase activity, did not reveal any significant changes. In analogy, we observed similar changes in gene expression profiles. For example, CpG sites in the promoter region of CD34 were significantly hypomethylated in HSPCs upon knockdown of Tr.2 and Tr.1+3 – and this gene was then upregulated on gene expression level.

Our results demonstrate that specific *DNMT3A* transcripts have different effects on DNAm patterns, gene expression patterns, and differentiation of HSPCs. Distinct effects of alternative DNMT3A isoforms might also be the reason why this gene is frequently mutated in AML and clonal hematopoiesis.

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Abstract No. P055

Intestine-specific Sirt1 deletion improves aging phenotypes in telomere dysfunctional mice

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Sirt1, a member of sirtuin family, is an NAD-dependent deacetylase and known as a longevity gene. Sirt1 deacetylates many targets that are involved in cell resistance to stress, metabolism, differentiation, aging and tumor suppression. It is also reported that Sirt1 contributes to telomere maintenance. However, the functional consequences of inhibition of Sirt1 in intestinal organ in response to telomere dysfunction during aging are largely unknown. Here we crossed late-generation telomerase knockout mice (G3 mTerc−/−) carrying a heterozygous floxed allele of Sirt1 with Terc+/− Sirt1fl/+ mice carrying an inducible Cre-recombinase transgene under control of the villin promoter, which is specifically active in the intestinal epithelium. The crosses generated intercross F1 Terc+/- (iF1), iF1 Sirt1fl/fl, intercross G4 mTerc-/- (iG4) or iG4 Sirt1fl/fl mice. In the study we analyzed effects of Sirt1 deletion on intestinal organ in telomere-dysfunctional mice. iG4 mice show premature aging phenotypes predominantly affecting proliferative organs, such as intestinal stem cells and Hematopoietic stem cells. In contrast, iG4 Sirt1fl/fl mice show improvement of lifespan, intestinal stem cell maintenance. We have found that the Sirt1 deletion attenuates cell apoptosis and chromosome end fusion, resulted from impaired DNA damage response (DDR), which lead to better maintenance of number of intestinal stem cells in mice carrying critically shorten telomeres. Furthermore histone proteins involved in DDR are hyper-acetylated by Sirt1 deletion, indicating that impairment of histone deacetylation by Sirt1 deletion attenuate initiation of DDR. Our results demonstrate that sirt1 plays an important role for DDR in intestinal organ during aging. Moreover impairment of DDR may rescue intestinal organ from apoptosis in response to telomere shortening.

Abstract No. P056

Reprogramming enriches for subchromosomal variants that pre-exist at low frequency in parental cells

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It is believed that the observed high mutational load in induced pluripotent stem cells (iPSCs) is partly derived from their parental cells, while other mutations are generated *de novo* during reprogramming, further impacting the clinical value of iPSCs. We have derived 37 human iPSC lines from neonatal and aged individuals under comparable conditions. High accuracy exome and amplicon sequencing showed that iPSCs from aged donors do not contain a substantially increased numbers of single nucleotide polymorphisms (SNPs) or small-scale insertions and deletions (INDELs). Moreover, we demonstrate that all analyzed 'true' SNPs and INDELs pre-existed in their parental cells and that individual mutations became enriched during reprogramming. Comparative analysis of a total of twelve iPSC lines and their corresponding four parental cell populations (three lines per donor) allowed to identify a collection of genetic variants that were enriched during reprogramming. Enrichment of a substantial number of these variants was confirmed by highly sensitive amplicon assays. Interestingly, some of those mutations were found to be enriched even in iPSC lines from different donors. These findings question the significance of reprogramming-induced *de novo* mutations in iPSCs, and the assumption that iPSCs from aged patients are of lower biological quality than other cell sources. On the other hand, our data suggest that individual genetic variations that become enriched during reprogramming may not only confer a selection advantage during reprogramming but may also increase the risk for potential malignant transformation of iPSC-derivatives.

Abstract No. P057

Disruption of the TFAP2A locus regulatory topology in Branchio-Oculo-Facial Syndrome (BOFS) as a novel mechanism for human congenital disease

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BOFS is a rare autosomal dominant congenital disease characterised by branchial skin, facial and ears defects. All BOFS cases reported to date are caused by heterozygous mutations in TFAP2A gene, a master regulator of neural crest cells (NCC) and craniofacial development that is also involved in the aetiology of orofacial clefting, a common congenital abnormality. Here we report a unique BOFS patient carrying a de novo 80 Mb heterozygous inversion in chromosome 6 in which the TFAP2A gene is intact. Combining our epigenomic data from human NCC with topologically associated domain (TAD) maps, we observed that TFAP2A is part of a large 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 regulatory topology of the TFAP2A locus and enhancer-gene interactions, resulting in abnormal expression of this gene during embryogenesis and ultimately BOFS. Hence, we performed Circular Chromosome Conformation Capture sequencing (4C-seq) experiments in WT NCC, which demonstrated strong physical interaction between the TFAP2A promoter and some NCC enhancers. Using CRISPRs/Cas9 technology, we engineered human induced pluripotency stem cells (hiPSC) with a heterozygous 0.1Mb inversion that mechanistically and functionally resembles the patient's genomic rearrangement and with deletion of the TFAP2A gene or of several of its putative NCC enhancers. In vitro differentiation of those hiPSC showed a significant delay in NCC development and decreased TFAP2A expression. We are currently generating hiPSC lines with the 80 Mb inversion as found in our patient and studying the functional consequences of all genomic rearrangements upon NCC differentiation by RNA-seq, 4C-seq and TFAP2A ChIP-seq analysis. The results should reveal the major regulatory networks controlled by this transcription factor, thus providing important mechanistic insights into how defects in TFAP2A contribute to various congenital abnormalities, including BOFS.

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Abstract No. P058

Somatic increase of CCT8 mimics proteostasis of embryonic stem cells and extends organismal longevity in C. elegans

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Human embryonic stem cells (hESCs) can replicate indefinitely while maintaining their undifferentiated state and, therefore, are immortal in culture. This capacity may demand avoidance of any imbalance in protein homeostasis (proteostasis) that would otherwise compromise hESC identity. Here we show that hESCs exhibit enhanced assembly of the TRiC/CCT complex, a chaperonin that facilitates the folding of 10% of the proteome. By using hESCs as a model to study proteostasis, we found that ectopic expression of a single subunit (CCT8) is sufficient to increase TRiC/CCT assembly. Moreover, increased TRiC/CCT complex is required for the remarkable ability of stem cells to avoid mutant Huntingtin aggregation, the hallmark of Huntington's disease (HD). Thus, mimicking proteostasis of hESCs in post-mitotic somatic tissues could protect from damaged proteins. By using *Caenorhabditis elegans* as a model organism, we found that somatic increased expression of CCT8 extends lifespan in a TRiC/CCT-dependent manner. Furthermore, ectopic expression of CCT8 ameliorates the age-associated demise of proteostasis and corrects proteostatic deficiencies in HD models. Our results establish a novel regulation of proteostasis that links organismal longevity with hESC immortality.

Abstract No. P059/T27

Hoxa9 induced developmental signals impair stem cells and regeneration of aging muscle

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Aging induced impairments in muscle stem cells, also known as satellite cells (SCs), contribute to the onset of sarcopenia during aging. Alterations in several pathways have been demonstrated to lead to the functional decline of SCs during aging, but master regulators mediating these gene regulatory defects have yet to be identified. There is an emerging discussion that developmental pathways may contribute to the inhibition of stem cell function and tissue maintenance in aging tissue. Hox genes are master regulators of stem cells and tissue patterning during embryogenesis with an unknown role in aging. Here we analyzed the functional role of Hox genes in aging induced impairments in SC function and skeletal muscle regeneration. From all genes of the 4 Hox gene clusters (A-D), specifically Hoxa9 is strongly overexpressed in activated SCs in response to muscle injury in aged mice. The study provides experimental evidence that Hoxa9 hyperinduction represents a main cause for alterations in key pathways that impair SC self-renewal and muscle regeneration in aging mice including Bmp-, JAK/STAT-, and Wnt-signaling as well p16 induction. Mll1 dependent histone modifications and chromatin decompaction of the HoxA gene cluster are identified as the cause of aging associated Hoxa9 hyperinduction in activated SCs. Of note, the knockdown of Hoxa9 by itself rejuvenates the regenerative capacity of SCs to repair injury of skeletal muscle in aging mice. Together, these data delineate a dynamically regulated, epigenetic loop that is induced upon activation of aged SCs limiting SC function and muscle regeneration by aberrant activation of developmental pathways

Abstract No. P060

Blood-derived stable induced neural stem cells: Preservation of age-related DNA methylation patterns and applicability to disease modelling

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Reprogramming of human somatic cells into induced pluripotent stem cells (iPSCs) represents an attractive approach to generate donor cells for disease modelling, drug development, and neuro-restorative approaches. However, generation and subsequent differentiation of human iPSCs is time-consuming, and the extended cultivation periods may result in acquisition of mutations. Here, we present a robust approach for the direct conversion of human peripheral blood cells into homogenous and transgene-free induced neural stem cells (iNSCs). After transduction of the blood cells with non-integrating Sendai viruses expressing SOX2 and c-MYC, neuroepithelium-like iNSC colonies emerge within a week in chemically defined conditions. iNSCs express early neuroectodermal markers including PAX6, SOX2, DACH1, NESTIN, PLZF and ZO-1 as well as neural crest markers AP2α and HNK1, and are stably self-renewing and multipotent at single cell level. iNSCs efficiently respond to instructive patterning and differentiation cues promoting, e.g., specification of various neuronal and glial subtypes including spinal motoneurons, midbrain dopamine-like neurons and oligodendrocytes. iNSC-derived neurons exhibit mature electrophysiological properties including spontaneous postsynaptic currents indicating functional synapse formation. iNSCs display similar global gene expression and DNA methylation profiles with PSC-derived neural cells. However, unlike the embryonic-like age signatures of PSCs and PSC-derived neural cells, iNSCs at least partially preserve age-associated epigenetic signatures and can be used for modelling pathological aggregate formation in late-onset neurodegenerative diseases such as spinocerebellar ataxia type 3 (SCA3; Machado–Joseph disease). Human peripheral blood cell-derived iNSCs thus may represent a promising alternative patient-specific cellular resource for disease modelling, regenerative medicine and other biomedical applications.

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P061 – P066: Stem cells in diseases: cancer stem cells

P061 Flow-cytometric identification and small molecule-based modulation of neuroblastoma subpopulations in vitro
Fraua Christina Ferlemann

P062 Cell fusion as a possible mechanism for cancer stem cells origin
Darja Gauck

P063 Neural retina differentiation of hESCs as in vitro model for retinoblastoma
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Abstract No. P061

Flow-cytometric identification and small molecule-based modulation of neuroblastoma subpopulations in vitro

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Neuroblastoma (NB) is the most common extra-cranial solid tumor in children with a broad spectrum of clinical outcomes due to its heterogeneous histology. Beyond the known genetic risk factors, there is a need to identify markers to further resolve cellular heterogeneity and to identify cancer (stem) cell populations. Using several *in vitro* models of NB (SH-SY5Y, SH-EP, BE(2)-M17, Kelly) subjected to a detailed flow cytometric surface molecule analysis, we aimed to investigate specific cellular subsets in our model systems and associated signaling networks. For visualization of combinatorial flow cytometric data, an established clustering algorithm (spanning tree progression of density normalized events; SPADE) was applied. The combinatorial detection of glycoprotein epitopes (CD15, CD24, CD44, CD57, CD171), integrins (CD29, CD49c, CD49e) or the chemokine receptor CXCR4 (CD184) enabled the quantitative identification of cellular subsets differentially responding to small molecule modulators in a multiwell-based screen. Our approach may provide tools for an improved prognostic analysis of NB as well as for pharmacological screens towards developing novel avenues of NB diagnosis and treatment.

Abstract No. P062

Cell fusion as a possible mechanism for cancer stem cells origin

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Cancer stem cells (CSCs) represent a small cell population in a bulk tumor mass, which are able to self-renew and to differentiate. According to these characteristics CSCs are proposed to be responsible for maintenance of cancer, initiation of metastasis, tumor heterogeneity and cancer relapse. Several hypotheses dealing with CSCs origin has been described, such as crucial mutations in progenitor cells and cell fusion events. In our lab we investigate the theory of CSCs formation originating in cell fusion events. Therefore, hybrid cells (M13HS) were generated due to spontaneous cell fusion events between HS578T Hyg human breast cancer cells and M13SV1 EGFR Neo human breast epithelial cells exhibiting stem cell properties. Initially the hybrid cells were characterized referring to their expression of stemness factors like SOX9 and SLUG as well as EMT factors like N-cadherin, TWIST, ZEB1 and ZEB2, respectively. All examined hybrid cell lines displayed SOX9 and SLUG co-expression pattern as well as EMT-phenotype. Conjointly the hybrid cells exhibited both an increased clonogenic and mammosphere formation capacity. Additionally we explore the impact of interleukin-6 (IL-6) and interleukin-8 (IL-8) on CSCs state initiation and maintenance. Preliminary results indicated constant expression of both CXCR1/2 and hIL-6R receptors in all hybrid cells. Interestingly, two of analyzed hybrids expressed IL-6 and IL-8 simultaneously, directing to the presumption of an autocrine loop presence, which could increase CSC activity. In conclusion, these observations lead to the assumption that cell fusion events between breast cancer cells and breast epithelial cells with stem cell like character can originate hybrid cells with a cancer stem-like phenotype and behavior.

Abstract No. P063

Neural retina differentiation of hESCs as in vitro model for retinoblastoma

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Retinoblastoma is the most common eye tumor of early childhood. For retinoblastoma to develop, inactivation of both alleles of the retinoblastoma gene (RB1) is necessary and sufficient. Our aim is to establish a human cell-based model for retinoblastoma. We employed the CRISPR/Cas9 system to generate hESCs carrying either heterozygous or homozygous mutations in the RB1 gene and we will subsequently differentiate those cell lines into neural retina, the tissue of origin for retinoblastoma. We designed guide RNAs to target the 3'-end of RB1 exons 3 and introduced them transiently into H1 hESCs. We isolated 192 clones and screened for aberrations in fragment length by Genescan analysis. We observed aberrations in 66 clones, resulting in an efficiency of CRISPR mutagenesis of 34%. 48 clones were selected for expansion and cryopreservation. For identification of the specific mutations in the cells, these clones were subjected to high-throughput amplicon sequencing on the Roche Junior 454 platform. We chose 2 homozygous clones and 3 heterozygous clones (constituting 7 mutant RB1 alleles) for further analysis on DNA, RNA and protein level. The splice site of exon 3 is preserved on 4 of the 7 mutant alleles and accordingly we observed skipping of exon 3 in 2 clones. On 6 of the 7 mutant alleles, the deletions result in a frameshift and premature stop codon in exon 4, on the remaining allele we observed a frameshift but no premature stop codon, resulting in a new artificial protein. Absence of RB1 protein was confirmed in the two homozygous lines by western blot. We also started comparative differentiation of wildtype H1 hESCs and RB1 null derivatives into neural retina. However, analyses are ongoing and results will be presented.

Abstract No. P064/T44

ZEB1 turns into a transcriptional activator by interacting with YAP1 in aggressive cancer types

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Metastasis formation and therapy resistance, central hallmarks of aggressive cancer types, are major clinical problems and the leading cause of cancer-associated deaths. Dissemination of tumor cells is enabled by an aberrant activation of the epithelial to mesenchymal transition (EMT) program. A key inducer of EMT is the transcription factor ZEB1, which is highly expressed in many aggressive tumor types and couples cellular motility with stemness and survival properties. The potent effects of ZEB1 however, cannot be solely explained by its well-established role as a transcriptional repressor of epithelial genes. EMT has also been linked to the inactivation of the Hippo tumor suppressor pathway, resulting in nuclear shuttling of its key downstream effector YAP. As shown for ZEB1, expression of the oncogenic transcriptional co-activator YAP is associated with aggressive behavior, metastasis and poor prognosis in various tumor types. We recently discovered a functional link between ZEB1 and YAP and found ZEB1 to be an activator of a set of classical YAP target genes. ZEB1- mediated activation of these genes was thereby not due to regulation of YAP activity but caused by simultaneous binding of ZEB1 and YAP to the promotors of these genes. We could further demonstrate a direct interaction of ZEB1 and the hippo pathway effector YAP. Intriguingly such interaction was not found between ZEB1 and TAZ, a YAP paralogue. This underlines the highly specific nature of the ZEB1/YAP interaction upon which ZEB1 switches its function to a transcriptional co-activator. The identification of the ZEB1/YAP interaction directly links two cancer promoting pathways and provides insight into how ZEB1 is driving tumor progression beyond EMT induction. The ZEB1/YAP common target gene set is a predictor of poor survival, therapy resistance and increased metastatic risk in breast cancer, indicating the clinical relevance of our findings.

Abstract No. P065

Inducing stemness and sphere formation in primary cancer culture

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According to the today's view of tumour biology, cancer stem cells (CSC) are a subpopulation of tumour cells that possess the ability to self-renew and serve as an ongoing source for differentiated tumour cells. With their stem cell-like properties, they play a major role in therapeutic resistance and the metastatic process. The CSC phenotype is an unstable feature not only in cell culture experiments but it is also fluid in primary cancer tissues meaning that it can be inhibited or induced by a permissive environment. As we have shown in previous experiments, a spherical growth pattern retains the stem-like phenotype of CSCs as it reduces cell culture artefacts by mirroring some stem cell niche characteristics and promotes self-renewal of undifferentiated cells. Furthermore, the (cancer) stem cell niche is dependent on secreted factors. Activators of the WNT-signalling pathway and its associated agonists like R-Spondin 3 (RSPO3) are described as being essential for maintaining primary tumour cells in culture in an undifferentiated state. We derived a RSPO3-secreting colon cancer cell line by plasmid transfection of a CMV-driven flag tagged RSPO3 expression plasmid. Conditioned media of this cell line was used to stimulate primary cancer tissue obtained from colorectal cancer as well as squamous head and neck cancer showing functional relevance like increased sphere formation and proliferation. In order to understand the underlying mechanisms of increased proliferation and self-renewal we analysed the transcriptome of RSPO3 expressing cancer cell lines showing a significant up regulation not only of the WNT-pathway and its closest connected pathways such as oncogenic MAPK and TGF-β Pathway but also numerous cancer-associated cascades such as down regulation of p53 signalling and an increased insulin response were found. Having this tool, we plan to analyse both the transcriptome and epigenome of primary tumour cells cultured as cancer spheroids.

Abstract No. P066

Inhibition of potential cell fusion factors caused by TNF-α suppress cell fusion between human breast cancer cells and breast epithelial cells

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Cell fusion plays an important role in several physiological events and is also involved in tumor progression. Less is still known about the fundamental underlying mechanisms as well as the driving factors, which could promote such a cell fusion event. Previous studies revealed that inflammation lead to a tumor-promoting microenvironment, which could facilitate cell-fusion. To identify factors/components that trigger cell fusion a Cre-LoxP double fluorescence reporter system was established to quantify fusion events between M13SV1-Cre_Puro breast epithelial cells and MDA-MB-435-pFDR.1 breast cancer cells. Here, it was observed that cell fusion was increased by stimulation with the pro-inflammatory cytokine TNF-α. A screening of cell-fusion relevant factors by RT-PCR and Western Blot indicate that TNF-α increased the expression of several potential cell fusion factors, such as surface-receptors (ILR7, ICAM-1), chemokines (CCL-2, LTB, CSF) and proteinases (MMP-9). To further confirm the pro-fusiogenic role of TNF-α, minocycline, a potent anti-inflammatory substance, was applicated to the cells and cell-fusion experience was performed. The results implicated that minocycline can abolished the pro-fusiogenic effect of TNF-α and even reduced the upregulation of cell fusion-relevant factors. Furthermore, the target-gene expression of relevant factors, which may affect the fusion process, was examined by using specific inhibitor. The results show that inhibition of this potential fusion factors lead to a constitutive reduction of cell fusion events. The present study gives first insights in the characterisation and identification of potential factors, which are involved in the cell fusion process. The data suggest that TNF-α is a strong cell fusion-inducer, which regulate the expression of potential cell fusion target genes. The inhibition of specific cell-fusion factors constitutively reduces the TNF-α promoting effect of cell fusion. This understanding could be helpful for the developing of new cancer therapies and finally for the prevention of tumor progression caused by TNF-α.

P067 – P077: Computational stem cell biology and systems biology

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Abstract No. P067

Modelling Temporal HSC Lineage Tracing Data

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HSC contribute to several lineages of the peripheral blood. The decision process, in which the commitment occurs, is generally depicted as a hierarchical decision tree with several intermediate, multipotent progenitor states. The tracing of individual cell clones, either using integrations site analysis or cellular barcoding, revealed further details about the clonal identity of HSCs that contribute to distinct lineages. In a seminal study, Perie and colleagues revealed details of the decision process downstream of multipotent progenitor cells. As their approach is limited to transient cell types that are accessed at a single point in time, the method cannot easily be extended to temporal data that becomes available from lineage tracing studies using persistent hematopoietic stem cells in mouse and human. In this work we present a framework, which can describe the contribution of different HSC clones towards distinct lineages over time. We use different methods, such as probability generating functions (pgf) and single cell based monte carlo simulations, to investigate the likelihood for different, nested and competing models of the hierarchical decision process given a specific experimental observation. We show for the example of a lineage tracing experiment in mice (provided by Martijn Brugman) that a common TB-compartment is not necessarily but most likely involved in the decision making process. Our approach allows to derive strategies for the reconstruction of cellular decision processes based on clonal contributions. In particular, the availability of temporal contributions towards different lineages is a data source that will allow unpreceded insight into the organization of the blood forming system and other regenerative tissues.

Abstract No. P068

Data Driven Computational Modeling of Cell Division and Differentiation Mechanisms in Adult Murine Neurogenesis

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Neural stem cells continue to produce neurons in the adult mammalian brain via several intermediate cell types. Whether there are cell type specific division and differentiation mechanisms, how fast the different intermediate cell types divide and which features change during aging remains to be uncovered. Data from a neuronal clonal labeling experiment is used to computationally model different neurogenic cell division and differentiation mechanisms using moment equations. We estimate model parameters and determine the most reasonable cell division and differentiation mechanisms by performing model selection based on the Bayesian Information Criterion. The ensemble of plausible models is then filtered with independent population level data. For the selected models, data of young adult and mid aged adult mice are compared and differences identified. We thus combine different data types and perform a model comparison of hundreds of models in order to find the cell division and differentiation mechanism most likely occurring in adult neurogenesis. The approach aims at a better understanding of dynamical changes in neurogenesis during aging. Results might also help to understand and treat neurodegenerative diseases.

Abstract No. P069

Mathematical modeling of the population equilibrium of hair follicle stem cells and their progeny in an ex vivo niche

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In the classical model of lineage commitment differentiated cells arise from multipotent stem cells via a uni-directional, hierarchical process. We have recapitulated the minimal essential components of the mouse hair follicle stem cell (HFSC) niche *ex vivo*, and show that this system allows expansion and long-term maintenance of HFSCs in the absence of heterologous cell types without loss of multipotency. Strikingly, the *ex vivo* niche promotes de novo generation of HFSCs from epidermal non-HFSCs cells, and that bi-directional interconversion of HFSCs and progeny drives the system into a dynamic equilibrium state of HFSCs and non-HFSCs. To understand how the system would self-evolve into equilibrium despite differential proliferation rates, we modeled the steady state dynamics of the cultures. We used a mathematical model based on ordinary differential equations (ODE) to describe the behavior of both primary and purified HFSC and non-HFSC populations. The adapted model reflects the observed growth dynamics under the assumption that HFSCs divide more frequently than non-HFSCs. By applying a maximum likelihood approach, we estimate both interconversion rates to be in a similar range at about one transition per cell within two days. Moreover, the model predicts that purified populations of either HFSCs or non-HFSCs are able to establish the observed population equilibrium containing both subpopulations.

Abstract No. P070

Dissecting the Underlying Mechanism of Resetting Primed Pluripotency to Naïve Pluripotency

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Reprogramming to induced pluripotency is a process that can be achieved from diverse cell types in multiple steps. The final step of all conversions is the establishment of an embryonic stem cell (ESC) like state, which is safeguarded by a network of transcription factors. Activating this network is required to attain the naïve, pluripotent state, but how this is achieved during reprogramming remains elusive. Epiblast-derived stem cells (EpiSC) are the primed counterparts of naïve ESC, expressing key pluripotency factors and capable of multi-lineage differentiation. They can be reset to the naïve state through exposure to the ground state culture conditions, 2i+LIF. However, this transition has limited efficiency, and while the enhancement effect of a few transcription factors is known, the process by which conversion occurs has not been characterised. These cells provide a useful experimental model to examine the reprogramming phenomenon, being well characterised, functionally distinct, yet developmentally close to the naïve state. Using EpiSC to ESC reprogramming as a cellular model, we sought to derive mechanistic insight into the process of naïve network activation. Our previous work enabled us to characterise the transcriptional program governing naïve pluripotency. Utilising this understanding, and our formal reasoning methodology encapsulated in the tool RE:IN*, we investigated the dynamics of naïve network activation. Our approach allowed us to predict accurately the relative impact of single and double factor overexpression on conversion efficiency, in addition to the requirement of factors for the conversion to occur. Most importantly, we derived a mechanistic explanation of these results, and the process by which resetting proceeds, which is amenable to analysis. The understanding and methodology we bring to this domain is anticipated to have impact beyond this specific transition and could be applied to the study of reprogramming and direct conversion of murine and human cells.

**www.research.microsoft.com/rein*

Dunn, Martello, Yordanov et al. Defining an essential transcription factor program for naive pluripotency, Science (2014).

Yordanov and Dunn et al. A method to identify and analyze biological programs through automated reasoning, npj Systems Biology and Applications (2016).

Abstract No. P071

Prediction of disease-gene-drug relationships following a differential network analysis

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Great efforts are being devoted to get a deeper understanding of disease-related dysregulations, which is central for introducing novel and more effective therapeutics in the clinics. However, most human diseases are highly multifactorial at the molecular level, involving dysregulation of multiple genes and interactions in gene regulatory networks. This issue hinders the elucidation of disease mechanism, including the identification of disease-causing genes and regulatory interactions. Most of current network-based approaches for the study of disease mechanisms do not take into account significant differences in gene regulatory network topology between healthy and disease phenotypes. Moreover, these approaches are not able to efficiently guide database search for connections between drugs, genes and diseases. We propose a differential network-based methodology for identifying candidate target genes and chemical compounds for reverting disease phenotypes. Our method relies on transcriptomics data to reconstruct gene regulatory networks corresponding to healthy and disease states separately. Further, it identifies candidate genes essential for triggering the reversion of the disease phenotype based on network stability determinants underlying differential gene expression. In addition, our method selects and ranks chemical compounds targeting these genes, which could be used as therapeutic interventions for complex diseases.

Zickenrott, S. et al. (2016) Prediction of disease-gene-drug relationships following a differential network analysis. Cell Death Dis. 7:e2040. doi: 10.1038/cddis.2015.393.

Abstract No. P072

Modeling Dynamic of Stressed Functional Hematopoietic Stem Cells

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Lifelong maintenance, self-renewal and the potency to generate multi-lineage progeny are the main characteristics of functional hematopoietic stem cells (HSCs). During aging, HSCs lose these potentials, most likely due to an accumulation of stress-induced DNA damage. It is known from hematopoietic disorders like Fanconi anemia that the pool of functional HSCs is exhausted due to insufficient DNA repair mechanisms, which results in accelerated accumulation of DNA damage. Therefore, comparing wild-type (WT) mice and Fanca-deficient (FA) mice is a suitable experimental design to investigate the impact of stress on functional HSCs and to identify underlying regulatory principles. We apply mathematical models to describe the dynamics of DNA damage accumulation in functional HSCs in both WT and FA mice and study its impact on the tissue level. For this purpose, we developed several compartment-based ODE-models to investigate a minimal set of structural and regulatory interactions of HSCs and their progenitors. This set of interactions integrates the DNA damage accumulation on the cellular level with a range of observed phenomena on the tissue level. In particular, we use experimental findings on cell cycle behavior and repopulation ability in stressed wild-type and FA mice to qualitatively adapt and validate our models. Based on these findings, we are able to simulate and predict HSC survival and competitive repopulation scenarios for both WT and FA mice. We demonstrate the general ability of our modeling approach to reflect the ageing-related functional decline of HSCs. Moreover, our models are suited to derive testable predictions how functional HSCs decline under specific stress conditions and how this increases the susceptibility for the acquisition of secondary diseases.

Abstract No. P073

Educing a molecular signature of human bone marrow-derived mesenchymal stem/progenitor cells through meta-analysis

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Currently there are >600 clinical trials registered (www.clinicaltrials.gov) using mesenchymal stem/progenitor cells (MSPCs) for various trophic/immunomodulatory and regenerative applications. So far organ source selection for therapeutic MSPC propagation has largely been based on the supposed *in-vitro*-multipotentiality despite lack of engraftment and sustained *in vivo* differentiation of most MSPCs in many studies. Based on our recent observation that bone marrow-derived (BM)-MSCPs compared to MSPC from umbilical cord (UC), white adipose tissue (WAT) and fibroblasts (n=11) displayed a particular epigenomic and transcriptional profile linked to chondrogenesis *in vitro* and stable endochondral bone formation *in vivo* (Blood 125:249), we asked whether a unique organotypic MSPC transcriptome signature exists irrespective of donor variations and culture conditions. We performed a meta-analysis on data acquired from 17 independent studies including 173 different samples to identify differentially expressed genes in BM-MSPCs. The gene expression data were obtained from MSPCs derived from BM, WAT, UC, cord blood, placenta, amniotic fluid, skin, olfactory ectomesenchymal cells, neural progenitors, tubular and glomerular progenitors. Using robust statistical methods including *RankProd*, *ROC*, recursive partitioning and regressive trees (*rpart*) we identified and evaluated a signature of fifteen genes that can efficiently distinguish between BM-MSPCs (94.9%) and MSPCs obtained from other sources (92.2%). Further, these classifiers were experimentally validated at transcriptomic, protein and functional levels using qPCR, flow cytometry and 3-D-chondrogenesis, respectively. In addition, through functional analysis of differentially regulated genes using GO, KEGG and Reactome databases we identified a significant enrichment of biological processes and pathways related to the skeletal system development in BM-MSPCs. Further, phenotype analysis revealed mouse knockouts of 3 genes exhibit defects in skeletal/bone development. These meta-analysis results combined with *in silico* functional analysis aid better characterization of BM-MSPCs and also indicate that the selection of MSPC source can be influential in shaping the therapeutic potential of these cells.

Reinisch A et al. Epigenetic and in vivo comparison of diverse MSC sources reveals an endochondral signature for human hematopoietic niche formation. Blood. 2015 Jan 8;125(2):249-60.

R: The R Project for Statistical Computing (https://www.r-project.org/); Bioconductor (https://www.bioconductor.org/)

Abstract No. P074

Neural stem cell division patterns in the zebrafish brain

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Neural stem cells (NSCs) are maintained in a few niches of the adult brain and sporadically generate neural progenitors that give rise to new neurons and glial cells. The recruitment mechanisms within the NSC population and the modalities of the division processes are not fully understood. We thus analyze the spatial and temporal distribution of dividing NSCs in a neurogenic area of the adult zebrafish brain to assess the mechanisms governing the limited recruitment within the cell population. To that end, we developed a single cell identification pipeline using prior biological knowledge, which identifies GFP expressing NSCs in three-dimensional image stacks of zebrafish brains from whole-mount preparations. On these results we compare several spatial influence models that give rise to distinct division patterns. Specifically, we consider attractive and repulsive cell-cell interactions, which we evaluate on a metric and a topological distance measure, respectively. While the metric distance can be interpreted as the influence on the environment via secretion of messenger molecules, the topological distance can be seen as direct cell-to-cell interaction via cell-cell contacts. We validate our inference approach on simulated data, where we are able to identify the correct influence models. Finally, we apply our method to four adult zebrafish hemispheres having two stained sets of dividing stem cells, which are 24h apart. We show that NSCs tend to inhibit their neighbors in terms of the division probability and estimate the interaction range.

Abstract No. P075

Application of Stem Cell Therapy by Nanotechnology Approach

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Stem cells are cells that become the initial growth of other cells that make up the whole body of an organism, such as nerve, heart muscle, and blood cells. The stem cell's ability in doing proliferation and differentiation gives a promising hope to treat degenerative diseases that up until recently is difficult to find a cure. The application of stem cell transplantation requires a marker substance capable of showing the migration and homing of the stem cells after being transplanted into the body, making it possible to control. Nanotechnology is a molecular material engineering with a scale of less than 100 nm, thereby controlling the synthesis of molecules within this scale enables the nano materials gaining outstanding properties as can be inserted into the body tissue. These attractive properties can be used for the translation of stem cell research because it allows a controlled application. This review summarizes the potential applications of nanotechnology for applying stem cell therapy in treating degenerative diseases.

Keywords: Stem cell, nanotechnology, transplantation, degenerative diseases.

Abstract No. P076

Optimization of barcode constructs: the longer, the better

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Introduction: Genetic barcodes (BC) are a promising tool for tracking cell clones in vitro and in vivo. The uniform barcode length allows for a more evenly amplification and for a quantitative assessment of clonal contributions in a marked cell population. The quantification of clonal contribution depends entirely on counting barcode-containing reads after next-generation sequencing (NGS). To our knowledge, data about detailed and systematic analyses of this procedure is limited. We used our recently published BC16 system (16 random nucleotides) to analyse recurrent amplification bias and possible error-sources. To overcome observed limitations of the BC16 system, we doubled its length and improved our BC design.

Methods: Four or five different barcoded and sanger-sequenced 293T cell clones were evenly mixed for systematic and repeated analysis. We reduced the number of PCR steps to analyse the influence of the PCR and used additionally restriction digest and artificially generated 1kb fragments as templates to study the impact of genomic accessibility. Furthermore, we prolonged our BC16 construct to 32 nucleotides (BC32) and additionally equipped the vector backbone with truncated Illumina-Adaptor sequences to facilitate amplification and PCR step reduction.

Results: The BC16 construct resulted in heavily uneven BC abundances after the standard protocol for PCR and NGS. Surprisingly, two of the five BC16 barcodes, carried by one particular cell-clone (due to double integration), also resulted in a final uneven read-count distribution. This was not improved by a reduction of PCR cycles or enhanced genomic accessibility. In contrast to these results, our newly developed BC32 construct showed much more balanced read-count abundances for the same experimental settings.

Conclusion: Our new BC32 construct shows superior performance in terms of BC quantification after amplification from genomic DNA. Finally, we conclude that validation experiments for BC quantification are ultimately required before BCs are used in a particular experimental setting.

Abstract No. P077

Analysing the impact of errors in single-cell tracking experiments

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Time-lapse video microscopy is an increasingly popular method to study the temporal and spatial behaviour of single cells. It is used for a broad range of applications, e.g. analysing cell migration, proliferation properties, or clonal composition, or reconstructing the complete divisional history of cells that is represented by cellular genealogies. A large number of automated methods have been developed for segmenting and tracking single cells. Although these methods are increasingly sophisticated to cope with a broad spectrum of situations they inevitably produce errors in the reconstruction of cellular tracks. Using post-processing tools for the manual correction of automatically created tracks can reduce the number of errors. However, ambiguous situations can still occur that lead to different subjective decisions of individual raters in the assignment of cellular objects. The number of these ambiguous situations and hence the number of differences in the reconstructed cellular tracks depends on cell type specific properties like migration speed or proliferation rate, as well as on specific properties of the experimental setting like the spatial and temporal resolution of the image sequence or the density of seeded cells. To study the inter-rater variability in single-cell tracking we exemplarily use time-lapse movie experiments of in vitro cultured haematopoietic stem and progenitor cells. We analyse the impact of the observed variability on the reconstruction of cellular genealogies and consequently the reliability of statistical measures defined on these data structures. This analysis is complemented by computer simulations of cell cultures that allow us to comprehensively mimic a broad range of cell type specific and experimental properties. Specifically, we aim to quantify maximum error rates that are admissible to reliably measure particular statistical outcomes. These maximum admissible error rates can then be accounted for in the design of the experimental set-up and the choice of the cell tracking procedure.

Poster session II: P078 – P147

P078 – P091: Tissue engineering and organoids

P078 Establishment of a human blood-brain barrier co-culture model based on human multipotent and pluripotent stem cells mimicking the neurovascular unit
Antje Appelt-Menzel

P079 3D cardiomyogenic microtissues for in vitro assays and heart repair
Michelle Coffee

P080 Establishment of a co-culture system of hiPSC with endothelial cells during hepatic differentiation
Nora Freyer

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Abstract No. P078

Establishment of a human blood-brain barrier co-culture model based on human multipotent and pluripotent stem cells mimicking the neurovascular unit

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In vitro models of the blood-brain barrier (BBB) are very strong tools in drug development and to study physiological as well as pathophysiological mechanisms. In order to closely mimic the BBB in vivo and to optimize model characteristics, we analyzed a set of different BBB co-culture models based on primary cells, human induced pluripotent stem cells (hiPSCs) and multipotent fetal neural stem cells (NSCs). The use of hiPSCs allows a very standardized, robust and reproducible model establishment. Further, they pose an effective cell source to generate functional brain cells and have the advantage to be independent of postnatal brain tissue biopsies, including their variations and limitations during in vitro culture. The aim of this study was to systematically investigate the individual impact of different cell types (astrocytes, pericytes, NSCs) on hiPSC-derived BBB endothelial cell (EC) function and gene expression. Differentiation protocols to generate brain endothelial cells as well as human astrocytes from iPSCs and NSCs mimicking the in vivo embryogenesis are performed as described recently (Lippmann et al. 2014, Wilson et al. 2015, Yan et al. 2013, Reinhardt 2013). With these cells in hand, ten different BBB-(co)-culture setups were performed and characterized by proving the cell morphology and functionality, analyzing the gene and protein expression, ultrastructural analyses, measurement of the trans-endothelial electrical resistance (TEER) and drug permeability using reference substances. Finally, we were able to present a quadruple BBB culture model with improved BBB characteristics compared to the monoculture and most of the co-cultures. TEER up to 2500 Ω*cm2 was achieved and at least 1.5-fold up regulation of characteristic BBB genes. Further, the important BBB related TJ proteins, CLDN1, CLDN3, CLDN4, CLDN5, could be analyzed at protein and gene level as well as by freeze fracturing. Moreover, the functionality of these proteins was investigated.

Abstract No. P079

3D cardiomyogenic microtissues for in vitro assays and heart repair

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The use of human pluripotent stem cell derived cardiomyocytes (hPSC-CMs) has advanced cardiac research. But, in contrast to native tissue, most of the *in vitro* models used to date have been largely two dimensional. Recent studies have indicated that 3D models might be more physiologically relevant and should therefore aid in creating a more accurate representation of *in vivo* conditions[1]. Therefore, the goal of this study is to establish and characterise 3D cardiomyogenic microtissues, either homotypic (consisting of hPSC-CMs only) or heterotypic (consisting of hPSC-CMs combined with other cardiac cell types such as fibroblasts, endothelial cells and/or mesenchymal stem cells). 96U-well low attachment plates have been used to create homotypic and heterotypic cardiac microtissues. Homotypic microtissues consisted of hESC-CMs, differentiated using a previously established protocol[2] (with a purity of >96% based on flow cytometry for selected cardiac markers) and combined with human foreskin derived fibroblasts (HFFs; either irradiated or non-irradiated). Combination of these two cell types were tested in ratios of 1:3, 1:1, 2:1, 3:1, 9:1 hESC-CMs:HFFs. In all cases the microtissues formed within 24h and started to contract within 3-5 days. Furthermore, the inclusion of fibroblasts seemed to aid in the formation and structure of the microtissues, yet an increase in the percentage of included fibroblasts caused a decrease in the beating frequencies of the microtissues. Based on preliminary metabolic analyses, the 3:1 CMs:HFFs microtissues had the closest respiration profile to the homotypic microtissues. Additionally, the beating frequencies of the 3:1 CMs:HFFs microtissues (~26 beats/min) were also closer to that of the homotypic microtissues (~28 beats/min), which altogether had increased over a 3 week period. Future work will require further characterization of the microtissues and the incorporation of additional relevant cell types in order to provide a more functionally relevant *in vitro* model and better understanding of cell interactions.

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Abstract No. P080

Establishment of a co-culture system of hiPSC with endothelial cells during hepatic differentiation

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The hepatic differentiation of human induced pluripotent stem cells (hiPSC) holds great potential for applications in regenerative medicine, pharmacological drug screening and toxicity testing. However, when compared to primary human hepatocytes, full maturation of hiPSC-derived hepatocytes has not yet been achieved. Previous studies reported that co-cultures with different cells including endothelial cells might enhance hepatic differentiation. Therefore, in this study a co-culture system of human umbilical endothelial cells (HUVEC) with hiPSC-derived hepatic cells was established with the aim to improve hepatic differentiation. The hiPSC line DF6-9-9T was differentiated into definitive endodermal (DE) cells by adding activin A and Wnt3a to the culture medium. Afterwards the HUVEC were added to the culture and an optimized co-culture medium was applied. This approach was performed in conventional 2D cultures and in perfused 3D bioreactors based on hollow-fibre capillaries. Interestingly, the results showed a distinct positive effect of the optimized co-culture medium on hepatic differentiation of hiPSC with respect to mRNA-expression and secretion of alpha-fetoprotein and albumin. Activities of the cytochrome P450 (CYP) enzymes CYP2B6 and CYP3A4 were 5- to 30-fold increased compared to the control medium. Furthermore, it was shown that co-cultivation of HUVEC with hiPSC-derived DE cells was feasible in both, 2D cultures and 3D bioreactors showing expression of hepatic markers such as albumin and cytokeratin 18 as well as activity of different CYP enzymes. In addition this study emphasizes the value of dynamic 3D culture systems in stem cell differentiation especially regarding the formation of tissue like structures.

Abstract No. P081

Investigation of defined coating materials for expansion of human induced pluripotent stem cells (hiPSC)

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For application of human induced pluripotent stem cells (hiPSC) in drug testing or in regenerative medicine it is essential to generate hiPSC preparations of constant quality and quantity. Currently the most often used coating material for stem cells is Matrigel, a not fully defined substance from animal origin. Therefore, two alternative completely defined coating materials were evaluated with respect to the growth behavior of hiPSC. The hiPSC line DF6-9-9T (WiCell) was seeded onto Synthemax, a synthetic peptide substrate, or Vitronectin, a recombinant fusion protein. Matrigel served as a positive control. The cell activity and proliferation was evaluated by cell counting, CellTiterBlue-assay and analysis of glucose uptake and lactate production. In addition, markers for the three germ layers and for pluripotency were analyzed on mRNA level. Cells were cultured in mTeSR1 over five passages. Microscopic evaluation showed a partly different appearance of hiPSC grown on the two defined materials compared to Matrigel. The nucleus-to-cytoplasm ratio decreased in cells grown on Vitronectin, and those cells also appeared less homogeneous than those cultured on Matrigel. Cells grown on Synthemax showed a more homogeneous morphology. The results of cell counting, CellTiterBlue analysis and glucose and lactate measurement indicated a similarly stable and efficient proliferation on both Matrigel and Synthemax, whereas Vitronectin coating resulted in varying proliferation rates. Expression of pluripotency markers was detected for all three materials.

In conclusion, the choice of coating material for hiPSC expansion influenced the culture morphology and homogeneity, especially in case of Vitronectin, while there was a minor effect on the proliferation rate and viability. To evaluate the long-term growth behavior of hiPSC on plates coated by Synthemax, further experiments with prolonged culture duration are needed. These findings are of importance for future upscale of hiPSC expansion in fully defined conditions in 3D cultures.

Abstract No. P082

Optogenetic control of human induced pluripotent stem cell-derived bioartificial cardiac tissue

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In the field of cardiac tissue engineering electrical stimulation is widely used to achieve tissue maturation. However faradaic reactions might occur during (long-term) electrical treatment, in the end leading to tissue damage. The limitations might be overcome through light-based stimulation of cardiac tissue. Therefore, we aimed at the generation of human induced pluripotent stem cell (hiPSC-) derived cardiomyocytes expressing channelrhodopsin, a light-activated cation channel. Using TALEN technology, we were able to heterozygously target the AAVS1 locus of human CBiPS2 cell line with channelrhodopsin coupled with an EYFP expression cassette (CAG-ChR2-EYFP) and a cardiomyocyte selection cassette (αMHC-zeocin-resistance). Integrations of transgenes into the "safe-harbour" locus were successfully proven by PCR- and southern blot analysis. Stable EYFP expression could be monitored in pluripotent and differentiated cells. By application of small-molecule based, biphasic WNT pathway modulation in 2D- or 3D-format cells were differentiated towards cardiomyocytes achieving efficiencies of 30-70%. Finally selection by antibiotic resistance enabled harvest of pure iPSC-derived cardiomyocytes (single cells or aggregates termed "cardiac bodies") for tissue production. Respective cells were mixed with a collagen-Matrigel matrix and human foreskin fibroblasts for production of Bioartificial Cardiac Tissue (BCT)[1]. Cardiomyocytes, cardiac bodies and BCTs react to royal blue (470nm) high power LED stimulation with induced contractions. Light stimulation of BCTs with 3 mW/mm^2 light intensity and 10 ms illumination time allowed us to evoke synchronous beating and to increase beating frequency up to 2-3 Hz displaying a 1:1 response. Comparing long-term light and electrical stimulation (1 Hz) for 14 days, first results showed lower forces in electrically stimulated BCTs but not for long-term light pacing (compared to the non-stimulated controls). Therefore, light-stimulation may be used as safe alternative approach to electrical stimulation of cardiac tissue constructs. Further analyses concerning potentially light-induced improvement of tissue maturation and/or damage are currently ongoing.

1. *Kensah et al. Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in vitro. European Heart Journal 2013; Apr; 34(15):1134-46*

Abstract No. P083/T17

Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modelling

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The generation of acinar and ductal cells from human pluripotent stem cells is a poorly studied process, albeit various diseases arise from this compartment. We designed a straightforward approach to direct human pluripotent stem cells (PSC) toward pancreatic organoids resembling acinar and ductal progeny. Extensive phenotyping of the organoids not only shows the appropriate marker profile but also ultra-structural, global gene expression and functional hallmarks of the human pancreas in the dish. Upon orthotopic transplantation into immunodeficient mice, these organoids form normal pancreatic ducts and acinar tissue resembling fetal human pancreas without evidence of tumour formation or transformation. Finally, we implemented this unique phenotyping tool as a model to study the pancreatic facets of cystic fibrosis (CF). For the first time, we provide evidence that in vitro, but also in our xenograft transplantation assay, pancreatic commitment occurs generally unhindered in CF. Importantly, CFTR-activation in mutated pancreatic organoids not only mirrors the CF-phenotype in functional assays but also at a global expression level. We also conducted a scalable proof-of concept screen in CF-pancreatic organoids using a set of CFTR correctors and activators, and established an mRNA-mediated gene therapy approach in CF-organoids. Taken together, our platform provides novel opportunities to model pancreatic disease and development, screen for disease rescuing agents and to test therapeutic procedures.

Abstract No. P084

Surface marker expression dynamics during in vitro human stem cell-derived renal cell differentiation

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Induced pluripotent stem (iPS) cell-derived models of renal differentiation have recently emerged as a novel option for modeling human kidney disease and development. Both 2D and 3D (organoid) model systems suffer from batch-to-batch variability and partially low efficiency during in vitro patterning and differentiation. Here, we provide a detailed flow cytometric characterization of the surface marker expression changes accompanying the differentiation from pluripotency over nephron progenitor cells toward the formation of tubular ("nephron-like") structures. Our analysis provides marker candidates for prospective identification and isolation of wanted vs. unwanted cells in renal differentiation paradigms.

Abstract No. P085

Fibrin based Matrix for Stem Cell-Derived Bioartificial Cardiac Tissue for Reconstructive Therapy

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Cardiovascular diseases are among the first three most frequent causes of death worldwide. Cardiac Tissue Engineering aims to replace the injured tissue; nevertheless, the use of xenogeneic scaffolds hinders the step forward into the in-vivo approaches. Therefore, to overcome this drawback more immuno-compatible sources should be used. Fibrin can be isolated and used in an autologous fashion. We generate Bioartificial cardiac tissue (BCT) from so-called “cardiac bodies” (CBs), produced from human and murine pluripotent stem cells which were genetically modified to express an antibiotic resistance gene controlled by a cardiac specific promotor. Non-dissociated beating CBs are mixed with human foreskin fibroblasts and a non-defined matrix of Matrigel® and rat Collagen type I. By the replacement of this matrix source with a haemostatic fibrin glue (human use approved), we were able to produce autonomous contracting BCTs with concentrations of 2.3 mg/mL (Fibrinlow) and 4.5 mg/mL (fibrinhigh). Fibrin matrices stiffness without cells was tested by rheological experiments and the tissue morphology and maturation over time was analyzed by microscopy pictures; contraction and passive forces were measured in a multimodal bioreactor. Fibrin BCTs were compared with our above mentioned standard xenogeneic matrix BCTs. Tissue morphology was similar but shrinkage and “CB” fusion was slower. Contraction forces were: Fibrinlow 1.8 mN, fibrinhigh 2.5 mN, and controls 2 mN. Passive forces were: Fibrinlow 0.3 mN, fibrinhigh 1.7 mN, and controls 6.2 mN. Stiffness at 30 min polymerization time showed values of: Fibrinlow 6.3 Pa, fibrinhigh 73 Pa, and controls 149 Pa. Contraction forces were similar or even higher, increasing according to the fibrin concentration, but passive forces were lower; probably due to the low fibrin matrix stiffness. In conclusion, we were able to show the use of clinical-grade fibrin as an alternative scaffold to produce bioartificial cardiac patches with similar properties to our standards.

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

Abstract No. P086

Evaluation of Three-Dimensional Cell Culture Systems for Improved Hepatic Differentiation of Human Pluripotent Stem Cells

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Human pluripotent stem cell (hPSC) -derived hepatic cells are broadly used as cell source for disease modeling and pharmacotoxicology studies. So far, hepatic differentiation of hPSCs applying conventional monolayer cell culture systems is well studied, but efficiencies and enrichment of functional active hepatic derivatives might be supported by three-dimensional cell culture systems. In this study, we evaluated gene expression levels and characteristics of hPSCs grown in three-dimensional aggregates in direct comparison to an established monolayer differentiation. In our first experiments, we analyzed the capability of fragments, obtained from the hepatoblast stage of the monolayer protocol to form hepatic aggregates in 6-well plates applying suspension culture conditions. Here, we detected a strong increase of hepatic marker gene expression in the hepatic aggregates, which provided clear evidence for an enhanced hepatic maturation in the three-dimensional culture system. Subsequently, we switched to a scalable Erlenmeyer flask-based suspension culture system to investigate critical parameters (rotation speed, inoculation density, fragment size) for a standardized generation of hepatic aggregates. Paraffin-embedded aggregates depicted solid-tissue like structures surrounding some cysts and support the notion of spontaneous formation of liver organoids. Interestingly, the size of the inoculated monolayer fragments interfered with the hepatic lineage commitment and larger fragments yielded more cholangiocyte features but small fragments provided more homogenous hepatocyte features with significant activity of Cytochrome P450 enzymes. Finally, we tested different dissociation enzymes to digest the organoids for subsequent re-plating on a collagen-matrix based monolayer culture. Gene expression profiles of these cells revealed that the acquired hepatic features was maintained in this culture setting with only minor changes in the hepatic marker gene expression levels. In conclusion, we evaluated three-dimensional cultivation conditions for growing hPSC-derived hepatic cells and establish an organoid culture system, which enhanced the maturation of hepatic hPSC-derivatives for down-stream use in disease-modeling or pharmacotoxicology applications.

Abstract No. P087

Derivation of liver organoids by self-condensation of human iPSC-derived MSCs, HLCs and endothelial cells

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Introduction: *In vitro* models of the human liver are wrought with limitations. Liver-biopsy derived primary hepatocytes have several limitations: They (i) are rare with a low number of healthy donors, (ii) have high inter-donor variability, (iii) show limited expansion in culture and (iv) rapid decline in function. Thus, the generation of hepatocyte like cells (HLCs) from induced pluripotent stem cells (iPSCs) can provide an alternative cell source. So far these cells lack full maturity even though they express ALBUMIN and cytochrome P450 family members. Mature HLCs are needed to maximize the relevance of the experimental outcome and applicability of these cells for toxicology and drug screening. Improved maturity and functionality of human iPSC-derived HLCs has been achieved employing three-dimensional (3D) approaches incorporating MSCs and endothelial cells.

Methods: Our preliminary proof of principle experiments involved mixing of iPSC-derived mesenchymal stem cells (iMSCs) with human umbilical vein endothelial cells (HUVECs) and HepG2 cells to generate 3D *in vitro* liver organoids. Furthermore it is planned to generate MSCs, HLCs and endothelial cells from the same iPSC line (same genetic background). Furthermore spinner flasks are used to provide better medium flow and to improve liver bud growth.

Results: Within three weeks these cells aggregated and formed vascularized liver buds when cultured on artificial extracellular matrices. These buds express ALBUMIN, VIMENTIN and CD31 - an endothelial specific marker.

Discussion/Conclusion: These iPSC-derived liver organoids have the added advantage of having present mesenchymal and endothelial cells from the same individual. Further studies are underway to better characterize these liver buds both molecular and biochemically for liver associated genes, pathways and functions.

Abstract No. P088

Metabolic maturation of human induced pluripotent stem cell derived cardiomyocytes in engineered heart tissue

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Human cardiomyocytes differentiated from induced pluripotent stem cells (hiPSC-CMs) serve as an unlimited source of patient-specific cardiomyocytes and may be useful for preclinical drug testing and disease modeling. An important limitation of these cells is their relative immaturity when compared to adult human cardiomyocytes. Evidence suggests hiPSC-CM acquire a higher degree of maturity when grown in a more physiological 3D engineered heart tissue (EHT) environment. Here, we compared the metabolic properties of hiPSC-CMs from an established control cell line cultured in 3D EHTs versus standard 2D monolayer culture. Quantitative proteomics revealed that 3D-cultured CMs contain more mitochondrial proteins and fatty acid metabolizing enzymes than 2D cultured CMs. Stainings with mitotracker suggested a higher content of mitochondria in 3D CM. Functional assays showed that 3D-cultured CMs utilize more mitochondrial oxidative metabolism of glucose, lactate and fatty acids than 2D cultured CM, a switch similarly seen during human cardiac development after birth. Overall glucose consumption was higher in 2D CM than in 3D CM, reflecting the lower energetic efficiency of anaerobic glucose metabolism. The results suggest that culturing hiPSC-CMs as EHT improves metabolic maturation and improves their usefulness for drug testing and disease modeling, particularly of diseases associated with mitochondrial abnormalities.

Abstract No. P089

Completely defined set up of an adipose tissue model based on human primary ASCs for disease modeling and drug development

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White adipose tissue (WAT) comprises about one fourth of the human body, interacts with many organs via endocrine signals and impacts levels of drugs through sequestration[1]. Additionally WAT is origin to different diseases and thus target of several drugs addressing those[2]. Thereby WAT represents a key element for drug screening and safety assessment and WAT-models are highly needed for in vitro evaluation. For this application artificial adipose tissue has to fulfill several requirements. First, the model has to show long-term stability to enable maturation and time-dependent investigations. Second, culture conditions should be free of animal-derived components or preferably completely defined to exclude possible impairments through unknown constituents. In the current approach human primary adipose-derived stem cells (ASCs) were differentiated to adipocytes for 14 days via a completely defined medium free of animal-derived components. As serum replacement the medium was supplemented by additional growth factors, vitamins, hormones and small molecules associated to cell survival and adipogenesis. Additionally, differentiated adipocytes were cultured in an analogously developed defined maintenance medium for 28 days. Further a 3D adipose tissue model was built with differentiated adipocytes and collagen type I. Adipocyte characteristics were analyzed via accumulated lipids, immune fluorescence staining of specific markers like perilipin and the glycerol- and leptin release. With this approach a functional adipose tissue model was established and maintained in the long-term under completely defined conditions. In combination with defined culture conditions the adipose tissue model represents a promising candidate to analyze drug sequestration and WATs` impact on drug distribution. As a further development the expansion to a disease model like inflamed adipose tissue via the addition of immunological cell components as well as the transfer to other tissue models is conceivable. Concluding our defined stem cell-based tissue model embodies an elementary tool in disease modeling and drug development.

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Abstract No. P090

Transferring an in vitro model of pathological cardiac hypertrophy from rat to human engineered heart tissue

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Background: Previously, we established an in vitro hypertrophy model based on rat engineered heart tissue (rEHT). In this system, afterload enhancement (AE) was induced by mechanical reinforcement of the silicone posts to which the EHTs were attached. We found that AE resulted in diminished contractile function, cardiomyocyte enlargement, increased fibrosis, and activation of the fetal (hypertrophic) gene program. The aim of the current study was to perform similar experiments on EHTs, made from human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), in order to more closely model the situation in patients.

Methods: Three different hiPS-cell lines from healthy donors were differentiated to cardiomyocytes using a growth factor-based two-step protocol, which yields up to 90% α-actinin-positive cells. Following the common mesodermal induction, cardiomyocytes were generated via inhibition of wnt-signaling and in parallel hiPSC-fibroblasts were differentiated from the same cell lines using wnt-activation and FGF-treatment. The cardiomyocytes were then used to generate fibrin-based human EHTs (hEHTs), or were supplemented with 5% hiPSC-fibroblasts to get isogenic multicellular constructs. After three weeks of cultivation, the spontaneously beating hEHTs were subjected to AE for 7 days.

Results: Video-optical analysis of the AE-hEHTs (n=47) revealed lower contractile forces (-24%) and longer relaxation times (+10%) compared to control hEHTs (n=51), but, in contrast to rat EHTs, no cardiomyocyte hypertrophy, fibrosis or fetal gene program. Fibroblast-containing multicellular hEHTs showed faster development, higher maximal forces (+35%, n=23) and more pronounced AE-induced dysfunction (35% lower forces, n=11). However, results were not completely consistent across different cell lines or different hypertrophic stimuli (AE or 50 nM endothelin-1).

Conclusion: AE in pure hiPSC-CM EHTs leads to contractile dysfunction, but without consistent hypertrophy. AE-induced functional impairment was more pronounced in fibroblast-supplemented multicellular EHTs. Future experiments are required to investigate their hypertrophic and fibrotic response and causes of inconsistencies between cell lines and stimuli.

Abstract No. P091

Generation of human mechanoreceptive neurons to study mechanical pain

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Sensory neurons in the dorsal root ganglia (DRG), including mechanoreceptors and nociceptors, belong to the peripheral nervous system (PNS) and govern the recognition of mechanical stimuli, such as touch, temperature, nociception and proprioception. Several publications reported the generation of human embryonic- or inducible pluripotent stem cells-derived sensory neurons, including mechanoreceptive ones, which constitute a highly heterogeneous population in vivo. To study mechanoreception and mechanical pain with human neurons, we developed a novel differentiation method and generated human peripheral neurons from small molecule-derived neural precursor cells. The differentiated cells express pan neuronal markers such as β-3-tubulin and microtubule-associated protein 2, and markers characteristics of peripheral neurons, including peripherin and Brn3a. They are functionally active, firing spontaneous and evoked action potentials. Patch clamp assay showed that the differentiated neurons express myelinated-neuron enriched sodium channels Nav1.1 and Nav1.7 but not unmyelinated-neuron enriched Nav1.8 and Nav1.9. Further characterization shows that the differentiated neurons express several markers specific to myelinated neurons in DRG, such as brain derived growth factor (BDNF) receptor TrkB, Neurofilament heavy (NFH) subunit, mechanoreceptor Piezo2, and schwann cell marker S100. These results collectively indicate that our neurons have a mechanoreceptive phenotype. Long-term differentiation resulted in DRG organoid-like structures. Most of these structures anatomically connect each other through axons fibres which also co-express tyrosine hydroxylase (TH), a marker for C-type low threshold mechanoreceptors in rodents. We also demonstrated that our derived neurons are both dopamine and norepinephrine transporters negative, but contain high levels of vesicle glutamate transporters, suggesting a glutamatergic phenotype, which is the predominant in DRG. We will perform functional assays to determine the role of our neurons in mechanoreception and mechanical pain.

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Katrin Schrenk-Siemens, et al. PIEZO2 is required for mechanotransduction in human stem cell–derived touch receptors. Nature Neurosci, 2015

P092 – P097: Genome engineering and gene therapy

P092 Tet-inducible vectors for regulated expression during megakaryocyte differentiation from pluripotent stem cells
Katharina Cullmann

P093 Hematopoietic stem cell gene therapy of Ifnγr1 deficiency repairs the cellular phenotype of Mendelian Susceptibility to Mycobacterial Disease
Miriam Hetzel

P094 The CpG-sites of the A2 Ubiquitous Chromatin Opening Element (A2UCOE) represent important components of its anti-silencing function
Jessica Kunkiel

P095 Analysis of the Foxg1 knockout phenotype in murine iPSC using neuronal differentiation
Eva Mall

P096 Effect of IL-3 on the engraftment and clonality of hematopoietic stem cells in gene therapy
Michael Rothe

P097 Modeling IRF8 Deficient Dendritic Cell Development and Function with Engineered Human Induced Pluripotent Stem Cells
Stephanie Sontag

Abstract No. P092

Tet-inducible vectors for regulated expression during megakaryocyte differentiation from pluripotent stem cells

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Induced pluripotent stem (iPS) cells are of great interest for the generation of differentiated cell types for various purposes including cellular therapy. As platelets are able to store proteins in their granules and release them after activation, engineered iPS cell-derived platelets may represent a novel system for the delivery of therapeutics. Successful generation of platelets from murine pluripotent cells was accomplished by an embryoid body (EB)-based differentiation protocol. Supplementation of IL-3 and SCF at EB day 5 induced hematopoietic specification. After EB-dissociation at day 7, megakaryocyte differentiation was achieved by co-cultivation on OP-9 feeder cells with 50ng/ml Thpo. Megakaryocyte differentiation, maturation and platelet generation could be demonstrated by cytomorphology and cell surface phenotype. However, the overall recovered platelets number was low. Therefore, to improve the differentiation, supporting elements such as essential transcription factors may be expressed in iPS cells during the culture time. However, these factors have to be expressed at defined time points and durations, and at specific levels. To achieve this, a well-controlled expression system in iPS cells is essential. We aimed to establish a tet-inducible vector system based on retroviral transduction but encountered problems due to vector silencing. To overcome this, we followed two arms: (1) we inserted different sized ubiquitous chromatin opening elements (UCOE) into the all-in-one tet-inducible vector, and (2) generated iPS cells reprogrammed from ROSA26 rtTA M2 mice which carry the transactivator in a safe harbor locus. Insertion of UCOE elements hampered vector titers for some of the elements. Although the UCOE vectors expressed with different intensities, tet-regulation was not impaired. The chromobox homolog 3 (CBX3) element was the most effective in preventing silencing of expression when tested in P19 and pluripotent cells. Transduction of rtTA M2 iPS cells with tet-responsive vectors resulted in faithfully regulated expression also at the polyclonal level.

Abstract No. P093

Hematopoietic stem cell gene therapy of Ifnγr1 deficiency repairs the cellular phenotype of Mendelian Susceptibility to Mycobacterial Disease

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Mendelian Susceptibility to Mycobacterial Disease (MSMD) is a rare primary immunodeficiency characterized by recurrent severe infections caused by otherwise only weakly virulent mycobacteria. Specifically, mutations in the interferon-gamma (IFNγ)-receptor-1 or-2 (*IFNγR1/2*) genes result in a life-threatening disease phenotype with most patients dying in early childhood. Thus, we here introduce a novel hematopoietic stem cell gene therapy approach for IFNγR1 deficiency and have designed a SIN lentiviral vector expressing Ifnγr1 from a SFFV promoter (Lv.SFFV.Ifnγr1.iGFP). Transduction of hematopoietic stem/progenitor cells from Ifnγr1$^{-/-}$ mice showed expression of Ifnγr1 by FACS and qRT-PCR with no abnormalities in clonogenic growth when compared to WT cells. Moreover, differentiation of transduced cells towards macrophages (MΦ) by M-CSF was normal as determined by morphology on cytospins and surface marker expression of CD11b/CD200R/CD115/CD45 and F4/80. When subjecting MΦ to IFNγ, corrected cells were able to clear IFNγ from the medium as efficiently as WT cells. Moreover, Lv.SFFV.Ifnγr1.iGFP transduced MΦ revealed restored up-regulation of HLA-DR and CD86 (B7.2) comparable to WT-MΦ. With respect to IFNγ-dependent T-cell activation WT and corrected MΦ were unable to activate T-cells in the presence of IFNγ and ovalbumin, whereas MΦ from Ifnγr1$^{-/-}$ mice induced significantly stronger proliferation of T cells. This observation was accompanied by the induction of indoleamine 2,3-dioxygenase (IDO) in WT and corrected MΦ, suggesting IDO-induced tryptophan depletion from the medium interfering with T-cell proliferation. In addition, Ifnγr1 downstream signaling showed restored phosphorylation of STAT1 in corrected MΦ, consistent with induction of *iNos* and *Irf1* upon stimulation with IFNγ. Most importantly, corrected MΦ showed significantly improved anti-mycobacterial activity as measured by comparable killing of *Mycobacterium Avium* and *Bacille Calmette Guérin* (BCG) in corrected and WT MΦ. Thus, we here introduce a novel gene therapy approach for MSMD in the context of Ifnγr1 deficiency.

Abstract No. P094

The CpG-sites of the A2 Ubiquitous Chromatin Opening Element (A2UCOE) represent important components of its anti-silencing function

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The 1.5kb A2 ubiquitous chromatin opening element (A2UCOE) as well as a 679bp region thereof, that comprises primarily the CBX3 promoter (CBX3-UCOE), protect retroviral transgenes from epigenetic silencing in pluripotent stem cells and their progeny. Mechanistically, the UCOE generates an active chromatin environment extending to the adjacent promoter. As the molecular basis for this epigenetic influence is still largely unknown, we here investigated several subfragments of the CBX3-UCOE with regard to their functional properties. Lentiviral vectors harboring an eGFP-cDNA driven by the viral SFFV promoter alone or in combination with different UCOE-fragments were employed. Expression of the vectors was assessed in pluripotent as well as differentiated murine embryonic stem cells (mESC) and induced pluripotent stem cells (miPSC). Analysis of the CBX3-UCOE subfragments indicated marked anti-silencing activity for subfragments comprising the base pairs 1-508, 85-508 and 170-508 but also 503-679. None of the subfragments, however, reached full activity, indicating that more than one subregion plays a role in the anti-silencing properties of the CBX3-UCOE. Interestingly, the subfragments with the highest anti-silencing activity also displayed the highest endogenous promoter activity, suggesting a link between these two features. Moreover, by scrambling the CBX3-UCOE sequence while maintaining the positions of the CpG-sites, we could show that the CpG-sites are important for the anti-silencing function. Finally, to investigate the anti-silencing ability of the CBX3-UCOE at a defined chromosomal site, we integrated the (CBX3-UCOE)-SFFV-eGFP reporter cassettes into the Rosa26 and the Tigre locus. In both loci the CBX3-UCOE alleviated the silencing of the SFFV promoter although it could not prevent silencing completely. Thus, while we were unable to map the anti-silencing activity of the CBX3-UCOE to specific subfragments, our findings highlight the crucial role of the CpG-sites in this context. Identification of relevant CpG-binding factors may therefore help to uncover the underlying molecular mechanism.

Abstract No. P095

Analysis of the Foxg1 knockout phenotype in murine iPSC using neuronal differentiation

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Foxg1 is a transcription factor critical for the development of the mammalian telencephalon. It is responsible for proliferation of the dorsal telencephalon progenitors and specification of the ventral telencephalon. A homozygous knockout of Foxg1 in mice leads to a significantly reduced size of the cortical structures and complete loss of the ventral telencephalon, like the basal ganglia. The goal of this study was to unravel the effects of a homozygous Foxg1 knockout on *in vitro* differentiation potential to cortical progenitors, using a serum free embryoid body culture system with quick reaggregation (SFEBq) technology. The ultimate phenotype will be verified using in vivo chimera formation.

Mouse iPSC were differentiated using the SFEBq method and collected after 10-25 days to analyze mRNA expression of several telencephalic marker genes. Quantitative real-time PCR revealed that Foxg1 and telencephalic markers were upregulated starting after 15 days of differentiation, whereas ventral telencephalic markers were expressed at very low levels.

The Foxg1 knockout was established using the CRISPR/Cas9 system in murine iPSC. After two rounds of limited dilution and clonal expansion, different lines with mutations in the Foxg1 coding exon were identified; all of which were associated with a premature stop codon with loss of the functional DNA binding domain. The three analyzed Foxg1-/- lines showed significant lower expression of Foxg1 and the cortical marker Emx1 after differentiation to cortical progenitors, but no common differences were found in the expression of other telencephalic markers. These results will be verified with additional Foxg1 knockout mouse PSC lines to analyze, if this phenotype is shared in differentially generated PSC with different genetic background.

The *in vivo* contribution of Foxg1-/- miPSC to chimeras was tested, but and preliminary data showed only very low contribution, so differences in the contribution to telencephalic progeny could not yet be analyzed.

Abstract No. P096

Effect of IL-3 on the engraftment and clonality of hematopoietic stem cells in gene therapy

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Ex vivo gene therapy of hematopoietic stem cells (HSC) is efficiently used to treat rare inherited disorders. During the culture phase, several cytokines are employed to support and stimulate HSC during gene transfer. Media usually contain SCF, FLT-3L, TPO and IL-3 in most clinical trial protocols. The role of cytokines on engraftment of cultured HSC has partially been studied in the past, but little is known about their effects on clonal diversity of the transplanted stem cell pool. The aim to generate a highly polyclonal graft might be influenced by the choice of the culture conditions. Early gene therapy protocols showed the crucial role of IL-3 on proliferation of the cells, facilitating the efficient transduction by gammaretroviral vectors. Today, more and more lentiviral vectors enter the clinical arena, able to transduce non-dividing cells. Hence, the necessity of IL-3 should be reconsidered. We monitored repopulation kinetics of lethally irradiated mice (n=65) by flow cytometry and found the early engraftment capabilities of HSC to be reduced in IL-3 containing cytokine conditions. We further marked the transplanted cells with a lentiviral barcode technology (16-N library) and analyzed the diversity of gene modified cells with Ion Torrent sequencing. Bioinformatic analysis revealed a reduction of clonal diversity in the stem cell pool when IL-3 was used during transduction. Lower numbers of repopulating stem cells ultimately increase the proliferative stress for the individual cell and hence the chance for secondary mutations. This might increase the risk for insertional mutants to succeed. We further found that the effect of enhancer mediated transformation of murine HSC *in vitro* was strictly linked to the presence of IL-3 in the medium. Together, our results show the impact of the early steps of gene therapy protocols on stem cell clonality and treatment safety.

Abstract No. P097

Modeling IRF8 Deficient Dendritic Cell Development and Function with Engineered Human Induced Pluripotent Stem Cells

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Human induced pluripotent stem (iPS) cells can differentiate into cells of all three germlayers, including hematopoietic stem cells and their progeny. Additionally, iPS cells are efficiently modified by CRISPR/Cas technology and thus offer a particularly appealing approach for studying gene function during human development. Interferon regulatory factor 8 (IRF8), also known as interferon consensus sequence-binding protein (ICSBP), is a transcription factor, which acts as lineage determining factor for myeloid cells, including dendritic cells (DC). Autosomal recessive or dominant IRF8 mutations in patients cause severe monocytic and DC immundeficiencies. To study IRF8 in human immundeficiencies we generated human IRF8-/- iPS cells and IRF8-/- embryonic stem (ES) cells using RNA guided CRISPR/Cas9n genome editing. Upon induction of hematopoietic differentiation, we demonstrate that IRF8 is dispensable for the development of hematopoietic progenitors from iPS or ES cells. We developed protocols to differentiate iPS and ES cell-derived hematopoietic progenitors into CD1c+ classical DC (cDC), CD141+ cross-presenting cDC and CD303+ plasmacytoid DC (pDC). We found that IRF8 deficiency compromised CD141+ cDC and pDC development while CD1c+ cDC development was largely unaffected. Additionally, in a non-directed differentiation regimen, IRF8-/- iPS and ES cells exhibited a clear bias towards granulocytes at the expense of monocytes. Functional analyses revealed that IRF8-/- DC lack HLA-DR expression and were impaired in cytokine responses upon activation.

Taken together, we engineered a human IRF8 knockout model that allows studying molecular mechanisms of human immundeficiencies *in vitro*, including the pathophysiology of IRF8 deficient DC.

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Abstract No. P098

Characterization of the regenerative potential of human induced pluripotent stem cell derived Megakaryocytes

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Platelet (PLT) lysates have demonstrated powerful regenerative properties in the field of tissue remodeling and angiogenesis. Also, PLT lysates were shown to be an effective alternative to FBS in the development of xeno-free culture conditions. So far, the production of PLT lysates is completely dependent on PLT donation. Induced pluripotent stem cells (iPSCs) constitute an unlimited cell source for blood pharming such as for the production of megakaryocytes (MKs), the precursors of PLTs. Here, the regenerative potential of in vitro differentiated megakaryocytes (MKs) was characterized by defining their content. In particular, the levels of typical PLT growth factors and micro-RNAs (miRNA) were profiled. MKs were differentiated from iPSCs using VEGF, BMP-4, and TPO in xeno-free conditions. Standard protocols for the generation of PLT lysates were used to produce MK lysates. Levels of epithelial growth factor (EGF), insulin growth factor-1 (IGF-1), platelet-derived growth factor (PDGF)-A and B, transforming growth factor-β (TGF-β), and vascular endothelial growth factor (VEGF) were evaluated by real-time PCR and ELISA. Also, the expression of specific miRNA levels was detected by real-time PCR. In comparison to iPSCs, in differentiated MK a significant upregulation in transcript levels of EGF (200-fold), IGF-1 (3500-fold), PDGF-A (3-fold), PDGF-B (20-fold), TGF-β (200-fold), and VEGF (4-fold) was detectable. Accordingly, protein levels of these growth factors were significantly increased in MK lysates. Remarkably, in vitro differentiated MKs showed a significantly higher content in growth factors in comparison to conventional PLT lysates ($p<0.01$). Also, typical PLT miRNAs were detectable in in vitro differentiated MKs. An upregulation of miRNA-16 (3-fold), miRNA-21 (10-fold) and miRNA-126 (5.5-fold) was measured in MK lysates in comparison to standard PLT lysates. These findings suggest that the in vitro generation of MKs represents an efficient strategy to widely exploit their regenerative potential in innovative cell therapies.

Abstract No. P099

Generation of a NKX2.1/P63 knockin human induced pluripotent stem cell reporter line for monitoring the generation of respiratory cells

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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 or submucosal gland (SMG) stem cells will be required. A requirement of this strategy is the development of an efficient and robust protocol for the generation of the desired lung stem 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. This 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. P100

The New York Blood Center - RheinCell Therapeutics Allogeneic iPSC Haplobank

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We develop cGMP grade Cell Therapeutic OFF-THE-SHELF Products for a wide range of indications in Regenerative Medicine at publicly affordable costs. RheinCell Therapeutics (RCT) is reprogramming human cord blood units collected for clinical use; all produced under licences by the PEI or the FDA. In particular, ethnically diverse fully HLA homozygous CB units representing the more common haplotypes are being reprogrammed with non intergrating episomal plasmids harboring the transgenes OCT4, SOX 2, KLF4, L-MYC, which were introduced by nucleofection, cultured under feeder free and animal free conditions. From four reprogrammings thus far eleven chromosomally intact iPSC clones with no mutations detected were generated sterily; characterized by pluripotency markers, embryoid body formation, vector clearance, DNA fingerprinting. The potential for self-renewal and tri-lineage differentiation of these HLAh clones are being assessed by the hPSC Scorecard panel. RheinCell Therapeutics considers these HLAh iPSC clones as OFF-THE-SHELF INTERMEDIATES kept frozen under liquid Nitrogen for later development of final products for any clinical settings, for example for patients with Age Related Macular Degeneration. Since RheinCell Therapeutics can operate under full scale clean room conditions to provide such an allogeneic fully HLA homozygous pluripotent Master Cell Bank for fully HLA matched recipients, the present priority involves the clarifications of a dynamic donor consent form, created on and for an international legal basis. In parallel the elaboration of Regulatory Requirements for ATMPs within Europe and North America will have to be determined before such products from RheinCell Therapeutics shall be able to enter the practice of Medicine on a larger scale.

Abstract No. P101

Encapsulated human embryonic stem cells partially differentiated in vitro, express insulin mRNA and C-peptide when transplanted into diabetic mice.

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Background: Differentiated human embryonic stem cells (hESCs) are a potential source of surrogate β-cells for treatment of type 1 diabetes. Microencapsulation of the differentiated cells is a means of immunoprotection from host once transplanted. We aimed to determine if microencapsulation prior to differentiation would produce a superior outcome after encapsulated cells were transplanted.

Methods: Endeavour 1 hESCs cultured on feeder fibroblasts were encapsulated in 500µm alginate microcapsules (2% w/v) for differentiation to endocrine pancreatic lineage[1]. This process was verified using RT-qPCR at days 0,3,6,9. NOD/SCID mice were made diabetic with streptozotocin and implanted intraperitoneally with encapsulated hESCs. Body weight and blood glucose concentrations were measured up to 83 days with insulin administered if required (0.5U/day, weight loss >10%). After animals were euthanized, capsules were collected for viability, mRNA expression and glucose stimulation. Plasma was assayed for human C-peptide.

Results: Prior to transplantation, lineage commitment of hESCs was confirmed through expression of specific markers for definitive endoderm (*SOX17, FOXA2*), primitive foregut (*HNF1B, HNF4A*) and pancreatic endoderm (*PDX1*). At 83 days post-implantation the cells retained differentiation markers expression, without losing viability, and expressed mature β-cell mRNAs including *INS*. Circulating human C-peptide at days 30 and 83 were 3.1±0.5 and 4.1±0.1pmol/L, respectively, and undetectable in diabetic control mice. Implanted mice gained weight without requiring exogenous insulin while the controls lost weight (≥10%) despite receiving regular insulin. Implantation of encapsulated hESCs did not affect blood glucose levels but all readings, including in control diabetic mice, were >33.3mmol/L.

Conclusion: Our preliminary data confirm that encapsulated hESCs undergo appropriate lineage commitment *in vitro* and then, after transplantation into diabetic mice, continue to differentiate to express insulin and secrete C-peptide. Our findings present a streamlined and scalable method for differentiation of hESCs for the treatment of type 1 diabetes.

1. Kroon E, et al. Nat biotech 26.4(2008):443-452.

Abstract No. P102

Layer-by-Layer technology as a supportive tool for pluripotent stem cell technologies

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Objectives: Induced pluripotent stem cells (iPSCs) have the ability to differentiate in any specialized somatic cell type, which makes them an attractive target for a wide variety of scientific as well as of clinical approaches. The exploitation of these prospects requires an efficient manipulation of gene expression patterns to initiate directed differentiation. However, their growth in compact colonies renders them difficult to access through viral vectors or biophysical particles. Methods: In this study we have compared the accessibility of iPSC colonies to Layer-by-Layer (LbL) coated polymer microcarriers as a valuable microplatform with the one obtained for virus particles. Virus particles and LbL microcarriers were applied in the reverse approach, before plating of the iPSCs, and in the direct approach after one or three days of iPSC cultivation.

Results: Both, the reverse as well as the direct application approach to one and three day old iPSC colonies were associated with an efficient up-take rate. Especially smaller colonies showed a high interaction rate with Lbl particles, while colonies with a diameter above 200 µm were only positive for LbL interaction at the rim region.

Conclusion: The modular design of LbL carriers enables their use as a promising drug delivery system for multiple biomedical applications and to maximize targeted differentiation.

Abstract No. P103

Signaling by BMP-, Wnt/beta-catenin-, and ATRA is necessary for anterior-posterior patterning of hESC-derived definitive endoderm

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The differentiation of hESCs into beta cells requires initially the formation of definitive endoderm followed by anterior-posterior patterning into the primitive gut tube domains foregut (SOX2+) and hindgut (CDX2+). Then specific domains are patterned, which give rise to organ primordia. Activities of Wnt, FGFs and BMPs in the posterior and all-trans retinoic acid (ATRA), TGF-β-ligands, Wnt- and BMP-inhibitors in the anterior half of the endoderm are responsible for anterior-posterior patterning. However, it is unclear how these interactions can be translated into a differentiation protocol for hESCs. Two hESCs lines were differentiated into DE-cells and purified by MACS. Next, the effects of Wnt/beta-catenin-, TGF-β-, ATRA-, and FGF2-signaling were tested by combinations of ligands and inhibitors. Differentiated cells were analyzed by RT-qPCR and immunofluorescence upon lineage selection towards foregut or hindgut identity. Treatment of DE-cells with CHIR-99021, BMP4, ATRA and bFGF for 48h resulted in a CDX2+ hindgut population expressing HOXC5, HOXC6, and HOXB8. If Wnt- and BMP4-signaling were suppressed SOX2/FOXA2-positive cells could be detected. Activin A treatment did not yield in SOX2-positive cells. ATRA could posteriorize the foregut population in a concentration-dependent manner towards the foregut/midgut boundary. These cells expressed HNF6, HNF1B, and FOXA2 typical for posterior foregut cells. Gene expression of MNX1, SHH, HOXC5, and HOXA3 was detected, whereas the anterior markers TBX1 and HEX1 were suppressed. Duodenal PDX1-positive precursor cells were detected as first signs of pancreatic progeny, which then upon further differentiation, yielded in multipotent NKX6.1-positive progenitors. The identity of the anterior-posterior axis of the endoderm is controlled by BMP-, Wnt/beta-catenin-, and ATRA-signaling. BMP- and Wnt/beta-catenin-inhibition were sufficient to induce SOX2-positive cells, whereas activation of Wnt/beta-catenin-signaling promotes hindgut development. TGF-β activation has no instructive role. ATRA is able to posteriorize and dorsalize foregut cells in a concentration-dependent manner and suppresses anterior foregut in exchange of PDX1-positive pancreatic cells.

Abstract No. P104

Significance of microRNAs for the differentiation of human embryonic stem cells into definitive endoderm and mesoderm

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Pluripotent stem cells hold great promises for a potential cell replacement therapy of type 1 diabetes. One important class of post-transcriptional regulators influencing gene expression are microRNAs (miRNAs). Thus, the miRNA transcriptomes of purified definitive endoderm (DE), mesoderm and undifferentiated embryonic stem cells (ESCs) were comparatively analyzed. Then, selected miRNA candidates were functionally investigated during differentiation to identify early cell fate regulators. Human ESCs (HUES8, HES3) were differentiated into DE (CHIR-99021/ActivinA) and mesoderm (CHIR-99021/BMP4). FACS-based cell sorting of differentiated populations was performed via CXCR4, EpCAM and NCAM. Specific marker gene expression was analyzed by RT-qPCR and miRNA transcriptomes were collected by qPCR-based arrays. Functional evaluation of selected miRNAs was performed upon transient transfection with mimics or inhibitors during differentiation and applying luciferase-based reporter assays. DE-marker genes (FOXA2, SOX17) were significantly induced in FACS-sorted CXCR4-positive DE-cells, while mesodermal marker genes (VEGFR2, PDGFRa, CD34) were highly expressed in purified EpCAM-negative/NCAM-positive mesodermal cells. Analysis of the miRNA-arrays identified 19 DE-specific (e.g. miR-371, miR-489, miR-1263) and 28 mesoderm-specific miRNAs (e.g. miR-10a, miR-196b, miR-483). Validation of selected candidates verified the miR-371-373 cluster, miR-1263 and miR-489 as highly enriched in DE-cells. Functional analysis demonstrated that upon transfection of miR-1263 mimic CXCR4-positive DE-cells arise earlier in a significantly increased quantity. Furthermore, KLF4 was experimentally validated as target of miR-1263. Out of the mesodermal enriched miRNAs miR-199a, miR-214-3p and miR-483-3p were used for functional tests. Transfection of miR-483 mimic during mesoderm differentiation yielded in higher amounts of PDGFRa-positive cells, whereas miR-483-3p inhibition reduced their quantity. This study identified miRNAs exhibiting particular functions for the early endodermal and mesodermal lineage. MiR-1263 facilitates endodermal differentiation potentially by regulating the pluripotency transcriptional network. In addition, miR-483 was identified as important regulator for the mesodermal PDGFRa-positive subpopulation, which is considered as progenitor population for cardiac, smooth muscle and mesenchymal lineages.

Abstract No. P105

Evaluation of the definitive endoderm differentiation bias between individual hESC lines by standardized methods

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Individual human embryonic stem cell (hESC) lines often demonstrate a differentiation bias towards a specific germ layer, which may hamper the efficiency of hESC-based biomedical applications. A better understanding of the molecular mechanisms causing this phenomenon is therefore needed. However, an accurate quantification of differentiation bias is challenging, as culture conditions also influence differentiation outcome. Here, we compared standardized methods to quantify definitive endoderm (DE) differentiation potential of four hESC lines. All lines carried a balanced chromosomal content and were cultured in identical conditions on laminin-521™ in Nutristem™ medium. First, we used our in-house optimized embryoid body (EB) formation protocol to generate equal-sized EBs, followed by 21-days of spontaneous differentiation in APEL medium. Gene expression analysis did not show a lineage bias between differently sized EBs within the same line, but we detected consistent differences between individual hESC lines. Next, we performed a 3-day DE induction with a defined seeding density. DE samples from hESC line VUB14 showed a statistically significant reduction in expression levels of SOX17, FOXA2 and GATA4 in comparison to the other hESC lines. Also, immunocytochemistry analysis showed that the VUB14-derived DE population had the lowest percentage of SOX17-positive cells and the highest number of POU5F1-positive cells. These results were in concordance with the EB spontaneous differentiation experiment. Additionally, we performed a 12-day EB differentiation and evaluated gene expression levels using the TaqMan® hPSC Scorecard™ Panel. These results showed that VUB14 in general had the lowest tri-lineage differentiation potential amongst the evaluated lines. Our data shows that standardized EB and DE differentiation followed by gene expression analysis can serve as a reliable tool for evaluating DE differentiation bias. Our next step will be evaluating a potential reason for VUB14's resistance to differentiation and establishing whether this hindrance is maintained during further differentiation towards hepatocytes.

Abstract No. P106

Large scale chemically defined cardiac differentiation of human pluripotent stem cells (hPSC) in dynamic suspension cultures

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Novel regenerative therapies and drug discovery assays require large amounts of human cardiomyocytes. We have recently shown that cardiomyocytes can be generated from human pluripotent stem cells (hPSC) cultivated and differentiated as free floating aggregates in controlled stirred tank bioreactors (Kropp et al., Stem Cells Transl Med, in press; Kempf et al., Nature Prot, 2015; Kempf et al., Stem Cell Reports 2014). However, currently applied differentiation media have limitations such as high costs and the presence of bovine serum albumin. Therefore, a more cost-efficient, chemically defined and xenofree protocol has to be developed that is more suitable for large scale suspension cultures. Stirred tank bioreactors facilitate process up-scaling and enable the control of key parameters such as pH, dissolved oxygen and defined feeding strategies. In this study, hPSC aggregates were generated in 150 ml scale stirred tank bioreactors. After 48h of cultivation 20 ml aliquots were transferred into Erlenmeyer flasks and cultured on orbital shakers. This strategy allows screening of several media and chemical compounds including the WNT pathway modulators CHIR99021 and IWP-2. Applying the cell lines HES3 NKX2-5$^{eGFP/w}$ (Elliott et al., Nat Methods, 2011) and hHSC_Iso4_ADCF_SeV-iPS2 (unpublished) for differentiation in the chemically defined medium CDM3 (Burridge et al., Nat Methods, 2014), beating aggregates were obtained consisting of >70% of alpha myosin heavy chain positive cells on day 10 as shown by flow cytometry. With the optimized, fully chemically defined and xenofree differentiation protocol more than 10 Mio cardiomyocytes were generated in 20 ml Erlenmeyer flasks. These results were reproducibly obtained with both cell lines underscoring robustness of the protocol. Current work aims at process up-scaling to 1 L bioreactor scale, targeting at producing >500 Mio cardiomyocytes in a single run.

Kempf H, Kropp C, Olmer R, Martin U, Zweigerdt R. Cardiac differentiation of human pluripotent stem cells in scalable suspension culture. Nature Protocols 2015;10:1345-1361

Burridge PW, Matsa E, Shukla P, Lin ZC, Churko JM, Ebert AD, et al. Chemically defined generation of human cardiomyocytes. Nat Methods 2014;11(8):855-60

Abstract No. P107

An animal component-free, chemically defined media formulation for cryopreservation ensuring high cell recovery and viability

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Human pluripotent stem cells (PSC) play an important role in disease modelling and drug discovery. Moreover, they hold great promise for future clinical applications, especially due to their potential usage in cell therapy. Therefore, optimal storage and cryopreservation of stem cells and progenies is of major importance. Our new animal component-free, chemically defined media formulation has been specifically designed for use with xeno- and serum- free culture systems as often used in stem cell cultures. The chemically defined nature of the formulation will allow a rapid translation into a clinical grade medium designed following the recommendations of USP <1043 > on ancillary materials. Human induced pluripotent stem cells (iPSC) frozen in this cryopreservation formulation showed a reliable and reproducible viability post thaw and fast recovery after replating. Furthermore, typical stem cell morphology and high expression of various pluripotency markers could be observed shortly after thawing. Likewise, iPSC-derived dopaminergic neurons and cardiomyocytes were successfully cryopreserved, the latter showing a contractile phenotype only 24-48 hours after thawing. Additionally other stem cell types like hematopoietic stem cells (HSC) or mesenchymal stem cells (MSC) not only showed high recovery but also a high functionality after thawing. HSCs stored in our cryopreservation medium showed normal formation of all colony forming units (CFUs), including CFU-GEMMs, indicating full preservation of HSC differentiation capacity. MSCs cryopreserved in the formulation showed typical marker expression and the ability to inhibit the proliferation of T-cells in an immunosuppression assay immediately after thawing, i.e. without prior cultivation. In summary, our media formulation ensures high viability, fast recovery and functionality of PSCs, PSC-derived cells, MSCs and HSCs after cryopreservation and storage.

Abstract No. P108

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 cell processing platform CliniMACS Prodigy® we previously developed a cultivation and expansion workflow for iPS cells. Now we have translated the differentiation to mesencephalic dopaminergic (mesDA) progenitor cells to the device. Adapting this protocol from an embryonic body based to a fully adherent differentiation paradigm enabled straight forward upscaling of a lab protocol to a medium-scale production process within the closed system. 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 300 million mesDA progenitor cells, before cells were passaged for final differentiation until d16. Here, an in-process control showed a typical mesDA phenotype, analysed by flow cytometry and immunocytochemistry. The final differentiation step typically led to minimal cell expansion. Taken together, we have developed a method for adherent, closed-system cultivation of PSCs and differentiation to dopaminergic progenitor cells. Extrapolating the cell numbers retrieved would correspond to 150-250 patient doses assuming 5 million cells per cryopreseved unit. However, the process holds further potential for upscaling. In a next step, we aim at implementing a magnetic separation procedure to enable purification of the final cell product.

Abstract No. P109

Glycoproteomic analysis of human pluripotent stem cells and stem cell-derived cardiomyocytes

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Human pluripotent stem cells, both induced (hiPSCs) and embryonic (hESCs), are promising tools for future regenerative medicine approaches, for instance for the production of cardiomyocytes to be used as tissue transplants. Importantly, for application in patients, the generated cells have to be highly pure and well characterized by using specific markers, typically protein or glycan structures on the cell surface. To date, however, only few cell surface markers for cardiomyocytes are known. The aim of our study is to overcome this scarcity of markers by characterizing the glycoproteome of hiPSCs and hESCs, cardiomyocyte progenitors and cardiomyocytes derived thereof. We applied labeling of sialic acid residues on the glycoproteins using the PAL (periodate oxidation and aniline-catalyzed oxime ligation; Zeng *et al.*, 2009) technique in combination with SILAC (stable isotope labeling by amino acids in cell culture; Ong *et al.*, 2002) to achieve a quantitative comparison of the different time points of cardiomyogenic differentiation. Pilot experiments led to the identification and quantification of 316 proteins in a comparison of hiPSCs harvested at d0 and d15 of differentiation after PAL labeling. According to gene ontology (GO) analysis, plasma membrane or extracellular proteins were strongly enriched by PAL, comprising ~75% in the PAL-labeled sample versus ~20% in a whole cell proteome analysis. Out of the 316 proteins identified by PAL, 105 and 62 proteins were up- or down-regulated, respectively, comparing d15 with d0. Of note, known surface markers of cardiomyocytes (SLC8A1 (Shattock *et al.*, 2015) and VCAM1 (Uosaki *et al.*, 2011)) were found to be up-regulated in hiPSC-CMs, while markers of pluripotent stem cells (CDH1, GLUT1 and THY1 (Boheler *et al.*, 2014)) were in the fraction of down-regulated proteins upon differentiation. Thus, with the combination of the PAL and the SILAC technique, we could show effective labeling, identification and quantification of glycosylated proteins.

Abstract No. P110

Repair of Focal Cerebral Ischemia-Reperfusion Injury by Human Trophoblast Progenitor Cells in Rats

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Objective: Stroke is the third most common cause of death after coronary heart disease and all cancers all over the world. Trophoblast stem cells develop from polar trophoectoderm and give rise to the cells generate the placenta. Pre-implantation and implantation microenvironment resemble with post-ischemic hypoxic neural environment. The purpose of this study is to try to repair the brain damage after focal ischemia in rats via human trophoblast progenitor cells (hTPCs).

Methods: hTPCs which isolated form abortion material were characterized by flow cytometry and immunoflourescent staining. In vivo cerebral ischemia model was generated in rats upon middle cerebral artery occlusion and after 24 hours, hTPCs were injected through tail vein. For histopathological measurements %2 TTC (2,3,5-Triphenyltetrazolium) staining performed to visualize the infarction zone in ischemic brain tissue. In addition, to determine the myelin loss in the central nervous system Luxol Fast Blue staining was done.

Results: hTPCs were positive for trophoblast stem cell markers CDX2 and EOMES in 92.5% and 92.7%, respectively. Also they identified to be positive for these markers via immunohistochemisry. Through %2 TTC staining infarct volumes were demonstrated in all groups. This area was smaller in the hTPC injected group. Visible myelin loss recovery was observed in hTPC injected group with LBF staining. Additionally, differentiation of hTPCs to neuron-like cells and characterization of them upon immunofluorescent was performed.

Conclusion: Cerebral ischemia is defined as reduction of cerebral blood flow below the critical threshold value in all or a specific region of the brain which may cause damage. Treatment of neurological diseases is very difficult because of the limited neurogenesis in the central nervous system, presence of active inhibitors that delay recovery and prevention of healing by glial scar tissue. In this study we tested the hypothesis that hTPCs could be therapeutically involved in repair of stroke.

Abstract No. P111/T03

BSA-free differentiation of hPSCs into cardiomyocytes

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Cardiovascular diseases cause a high number of deaths in the world and around 17 million people are affected every year. The heart has a very low regeneration capacity and donor organs for transplantation are scarce. Therefore regeneration of lost myocardium with stem cell-derived cardiomyocytes is an attractive strategy for regenerative medicine. hPSCs can be differentiated into cardiomyocytes but the molecular mechanism behind that differentiation are poorly understood. It is likely that differentiating cells secrete paracrine factors into the supernatant that function as important regulatory determinants. A recent and very effective protocol leading to high proportions of cardiomyocytes from hPSCs depends on modulation of Wnt signaling at early stages of differentiation. The differentiation medium according to Lian et al. [1] contains 2.5 mg/ml BSA, originating from the applied B27 supplement. Considering that secreted paracrine factors are found at concentrations in the range of ng/ml, the enormous excess of BSA in cell culture supernatants hampers analytics to identify little amounts of secreted proteins. Therefore the development of a BSA-free cardiomyocyte differentiation protocol is pivotal for the subsequent mass spectrometry-based analysis of secreted proteins. According to Roth et al., we generated a custom made B27 and omitted the BSA. hESCs and hiPSCs could be successfully differentiated under BSA-free conditions into cardiomyocytes and our results are in line with a recent publication describing the suitability of BSA-free conditions for cardiomyogenic differentiation [2]. For secretome analysis, seven time points of the differentiation process were analyzed. In total, more than 5,000 proteins could be identified and there relative levels at the different time points were quantitatively assessed by label-free quantification. Beyond the identified proteins there are many factors involved in signaling pathways that are related to cardiomyocyte development. We further identified several cytokines in the supernatants of differentiating cardiomyocytes with not yet know function on cardiomyogenesis.

1. *Lian X, Zhang J, Azarin SM, Zhu K, Hazeltine LB, Bao X, Hsiao C, Kamp TJ, Palecek SP, (2012), Nature Protocols, 8(1):162-75*

2. *Lian X, Bao X, Zilberter M, Westman M, Fisahn A, Hsiao C, Hazeltine LB, Dunn KK, Kamp TJ, Palecek SP, (2015), Nature Methods, 12, 595–596*

Abstract No. P112

Downregulation of endogenous WNT antagonists as a potential marker for resistance of human pluripotent stem cells to dissociation-induced apoptosis

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Quality control of human pluripotent stem cells (hPSCs) is a crucial issue for the manufacture of hPSC-based therapeutic products, because characteristics of hPSCs would be altered by culture conditions. Although hPSCs undergoes massive apoptosis triggered by Rho activation and Rac inactivation after complete dissociation into single cells, long-term culture could make hPSCs resistant to the dissociation-induced apoptosis. Theoretically, dissociation-resistant hPSCs can be more prone to generate tumor, compared with normal hPSCs. Thus contamination with dissociation-resistant hPSCs needs to be avoided in the quality control of hPSCs used as starting materials of therapeutic products. However, no biological marker is currently available for the detection of the dissociation-resistant hPSCs. To identify such biomarkers, we compared the gene expression profiles of dissociation-resistant human embryonic stem cells (hESCs) and their parental cell lines. Microarray analyses and quantitative RT-PCRs showed that eight genes were differentially expressed more than 2-fold in dissociation-resistant hESCs. Among them, we focused on endogenous WNT antagonists, which were downregulated in dissociation-resistant hESCs, since non-canonical WNT signal is known to regulate the activities of Rho and Rac. Treatment with recombinant WNT antagonists increased and decreased annexin V-positive apoptotic cells and the number of colonies, respectively, after dissociation and passaging of dissociation-resistant hESCs. In contrast, treatment with WNT5A that regulates non-canonical WNT signal decreased apoptotic cells and increased the number of colonies after passaging of parental hESCs. These results suggest that non-canonical WNT signal is involved in the acquisition of the resistance to dissociation-induced apoptosis and that downregulation of endogenous WNT antagonists is a potential marker to detect dissociation-resistant hPSCs.

Abstract No. P113

Development of a Feeder-Free PSC Culture System Enabling Translational & Clinical Research

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Pluripotent stem cell (PSC) culture using the xeno-free Essential 8™ Medium/truncated recombinant human Vitronectin system has been shown to support normal PSC properties and provide a large pool of cells for disease modeling and drug development. As research moves from translational to clinical research, general regulatory guidance from the US Food and Drug Administration (FDA) indicates that, cGMP manufactured, or clinical grade reagents should be used whenever available as ancillary reagents to minimize downstream risk to patients. Thus, we sought to identify regulatory compliant, animal-origin-free alternatives for growth factors contained within the Essential 8™ Medium and incorporate cGMP manufacturing processes for the recombinantly expressed, truncated human Vitronectin, producing a qualified ancillary system for PSC expansion. Here we present data to support a seamless transition from the xeno-free Essential 8™ Medium system to the Cell Therapy Systems (CTS™) animal-origin free system. Compatibility is shown with existing cGMP-manufactured passaging reagents: Versene Solution for clumped cell passaging and CTS™ TrypLE™ Select combined with RevitaCell™ Supplement for single cell passaging. Upon expansion, PSCs are shown to maintain normal PSC properties, including morphology, pluripotency, karyotype, and trilineage differentiation potential. Together this system provides a consistent, feeder-free PSC culture medium for translational and clinical research.

Abstract No. P114

hiPSC derived endothelial cell types from scalable agitated erlenmeyer flasks for biofunctionalization and tissue engineering

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Applications like full endothelialisation of gas exchange membranes in extracorporal membrane oxygenation (ECMO) devices for improved hematocompatibility, cell therapy of pulmonary hypertrophy or tissue engineering require large numbers of (patient-specific) endothelial cells (ECs). The isolation of ECs from peripheral blood or explanted vessels is well established however especially cells from older individuals show a limited proliferation capacity. Patient specific ECs from pluripotent stem cells (hiPSCs) might be an alternative suitable cell source. The opportunity to generate large amounts of undifferentiated hiPSC in defined media under scalable conditions allows for the generation of cell numbers in dimensions which are suitable for envisioned applications. By differentiation of these well monitored cell populations a virtually unlimited number of (autologous) ECs may become available for disease modelling, tissue engineering approaches and biofunctionalization of ECMO devices. The growth factors BMP4 and VEGFA as well as modulation of the WNT pathway were utilized for the differentiation of the scalable suspension cultures in agitated Erlenmeyer flasks to endothelial cell types. Resulting cell populations were analyzed by flow cytometry and quantitative RT PCR (qRT-PCR) for expression of endothelial cell markers. Differentiation approaches resulted in up to 75% of CD31 positive cells on day 7 of differentiation. Expression of endothelial cell markers like, VEGFR2 (FLK1), von Willebrand Factor (vWF) and VE-cadherin (CD144) could be shown by qRT-PCR. Sorting of CD31pos populations and further detailed phenotypic analysis is ongoing. With the established protocol we were able to generate endothelial cell types from scalable cultures. In the future the resulting patient- (and lung disease-) specific iPSC-derived ECs will represent a novel cell source for disease modelling or biofunctionalization of gas exchange membranes as well as for vascularization of tissue engineered constructs. In addition, TALEN-based gene correction in iPSCs might enable novel concepts of ex vivo gene therapy for respiratory diseases.

Abstract No. P115

Structure induced neuronal differentiation of human adipose derived 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 nanostructures 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. Polydimethylsiloxane (PDMS) micro-structured surfaces were used in this study 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 linages, provide a novel tool for damaged neuronal tissue reconstitution, or for production of patient-specific neural cells.

Abstract No. P116/PS4

Induced human hepatocyte-like cells formation by transcription factor-mediated direct reprogramming

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Hepatocyte transplantation is limited by the shortage of donor livers. Therefore, we aimed to generate a source for donor organ independent cells for the treatment of liver diseases. Previously, we reported the attenuation of liver fibrosis in mice via generation of iHeps from myofibroblasts by in vivo reprogramming. Here, we demonstrate that ectopic expression of the transcription factors (TFs) FOXA3, GATA4, HNF1A and HNF4A from a polycistronic lentiviral vector converts human neonatal fibroblasts into cells with hepatocyte phenotype in vitro. Human iHeps showed epithelial morphology, evidence of glycogen storage, LDL uptake and expression of marker genes similar to hepatocytes. In addition, human iHeps showed no chromosomal alterations when compared to human fibroblasts. Global transcriptional profiling comparing control transduced fibroblasts and primary human hepatocytes indicated a profound phenotype change in human iHeps similar to the human hepatocytes. Furthermore, human iHeps secreted albumin, generated urea, and showed efficient cytochrome activity. Thus, our findings suggest that ectopic expression of the 4TFs also converts human neonatal fibroblasts into human iHep.

Abstract No. P117

In vitro culture of full thickness human skin punches for wound healing assays employing sweat gland stroma-derived nestin+ stem cells

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Chronic skin wounds affecting the epidermal and dermal compartment of the body's outermost barrier are considered a major medical challenge in ageing societies. In consequence of conditions such as diabetes mellitus, chronic venous insufficiency and ischemia, cutaneous wound healing is often dysfunctional, giving rise to chronic ulcers. In the attempt to support and possibly normalize the incomplete healing process of chronic wounds, (stem) cell-based wound care has been proposed as a promising therapeutic option. In this study, we employed a serum-free airlift culture system of wounded human full thickness skin punches to evaluate wound closure and vessel formation under the influence of adult nestin+ stem cells derived from the mesenchyme of sweat glands (sweat gland stroma-derived stem cells; SGSCs). SGSCs were obtained via explant cultures of eccrine and apocrine sweat glands isolated from tissue resected during axillary plastic surgery. Excess skin derived from plastic surgeries concerning the scalp, breast or abdominal region was further utilized to generate 4 mm skin punches. In order to model a full thickness skin defect, these punches were subsequently wounded with a standardized incomplete inner 2 mm punch by means of an ArFl-excimer laser (193nm) prior to topical application of SGSCs. Within one week of *in vitro* culture, SGSCs mediated complete wound closure and restoration of a multi-layered epidermis (localized expression of CK14 and CK10). Notably, the re-epithelialization of inner wounds was more pronounced in skin punches treated with SGSCs compared to commercially available dermal fibroblasts of the eye lid. We thus suggest that nestin+ stem cells derived from the mesenchyme of sweat glands may exert additional benefits on skin wound healing in humans.

Abstract No. P118

Differentiation of Human Pluripotent Stem Cells into CFTR-expressing (Lung) Epithelial Cells

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Pluripotent stem cells (PSCs), like embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), offer promising new options for the treatment of lung diseases by cellular/tissue replacement therapies, disease modelling and drug screening. These regenerative approaches crucially rely on the efficient differentiation of PSCs into lung epithelial cells which is the aim of our present study. We made use of the human (h)ESC reporter cell line hES3 NKX2.1-eGFP (kindly provided by the lab of A. Elefanty) expressing eGFP under the endogenous promoter of NK2 homeobox 1 transcription factor (NKX2.1). Furthermore, we took advantage of a lab-internal generated double transgenic cell line based on the hES3 NKX2.1-eGFP cells additionally expressing dTomato controlled by the endogenous CFTR promotor. Using the commercially available STEMdiff™ Definitive Endoderm (DE) Kit (TeSR™-E8™ Optimized) by Stemcell Technologies resulted in the robust and efficient generation of a highly enriched DE population of > 93 % CXCR4^{pos}/c-Kit^{pos} cells and CXCR4^{pos}/EpCAM^{pos} cells demonstrated by flow cytometric analysis. DE generation was additionally verified by co-expression of the transcription factors FOXA2 and SOX17 based on immunofluorescence staining. Further differentiation resulted in a distinct FOXA2^{pos}/SOX2^{pos} anterior foregut endoderm (AFE) population of about 80 %, which finally gave rise to up to 55 % NKX2.1-eGFP^{pos} cells. Subsequent maturation of purified NKX2.1-eGFP^{pos} cells demonstrated the formation of CFTR-dTomato^{pos}/NKX2.1-eGFP^{pos} co-expressing cells around day 40 of differentiation, whose detailed phenotypic analysis is currently ongoing. In summary, we were able to generate almost pure DE followed by the induction of a distinct AFE population resulting in a decent percentage of NKX2.1-eGFP^{pos} cells. Further maturation demonstrated the generation of CFTR-dTomato^{pos}/NKX2.1-eGFP^{pos} co-expressing cells, most likely associated with the lung lineage. Future work will focus on additional optimization of the differentiation strategy and phenotypic and functional analysis of the cells.

Abstract No. P119

Identification of microRNA-125b-5p as a novel regulator of acute liver failure

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Acute liver failure (ALF), due to viral hepatitis or drug-induced hepatotoxicity, is a clinically devastating multi-organ syndrome after sudden and severe hepatocellular dysfunction. Till date, orthotopic liver transplantation is the only curative and definitive treatment option for advanced ALF. However, the number of available donor livers is limited and the mortality rate due to long waiting list is alarming. Recent reports have provided evidence that microRNAs (miRNAs) are sensitive and informative biomarker of ALF. Therefore, we aimed to develop an effective therapy of ALF based on miRNA modulation. Through functional miRNA mimic library screening, our study identified miR-125b-5p as key regulators of ALF. Overexpression of miR-125b-5p in primary hepatocytes dramatically inhibited cell death in acetaminophen or FAS-induced ALF whereas inhibition of miR-125b-5p substantially increased hepatocyte sensitivity to cell toxins. More importantly, delivery of miR-125b-5p either based on adeno-associated virus or in vivo mimic robustly ameliorated hepatotoxicity and prolonged mice survival in mouse models of ALF. Taken together, our findings suggest that miR-125b-5p mimic inhibits ALF and may serve as one of the potential therapeutic approaches for ALF treatment.

P120 – P124: Stem cells in regenerative therapies: mesenchymal stem/stroma cells

P120 Effects Of Placental-Derived Mesenchymal Stem Cells On Proliferation and Apoptosis Mechanisms in Chronic Kidney Disease
Busra Cetinkaya

P121 Opposite early SOX9 response of human iPS cells compared to MSCs during in vitro chondrogenesis
Solvig Diederichs

P122 Phase II Clinical Trial Of Using Adipose Derived Stem Cell In Spinal Cord Injury Treatment
Nguyen Dinh Hoa

P123 Human Adipose Stromal Cells resist the detrimental effects of hyperglycaemic modified extracellular matrix in contrast to human retinal pericytes
Karen Bieback

P124 TGF-beta is a strong selection pressure for MSC cultures revealed by barcode transduced umbilical cord pieces
Anton Selich

Abstract No. P120

Effects Of Placental-Derived Mesenchymal Stem Cells On Proliferation and Apoptosis Mechanisms in Chronic Kidney Disease

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OBJECTIVES: The feature of chronic kidney failure (CKF) is loss of kidney functions due to erosion of healthy tissue and fibrosis. Recent studies showed that mesenchymal stem cells (MSCs) differentiated to tubular epitelial cells thus renal function and structures renewed. MSCs can engraft to kidney thus they can prevent renal injury. Therefore, we aimed to investigate whether placental-derived mesenchymal stem cells can repair fibrosis that occurs as a result of chronic kidney failure and they are effective in mechanisms of proliferation and apoptosis.

METHODS: We constituted rat model of CKF by applying Aristolochic acid (AA) in this study. $6x10^5$ mesenchymal stem cells that were isolated from amnion membrane transplanted into tail vein of rats. At the end of 30 day and 60 day recovery period, we examined expressions of PCNA, Ki67, p57 and Parp-1 by western blotting. Immunoreactivity of PCNA, Ki67 was detected by immunohistochemistry. Besides, apoptosis was detected by TUNEL. Serum creatinine and urea was measured. RESULTS: We observed that expression of PCNA and Ki67 proliferation markers increased in stem cell groups compared to CKF group but expression of PARP-1 apoptosis marker decreased and it was supported with TUNEL. Also p57 that is cell cycle inhibitory protein increased in CKF group when compared to control, stem cell groups and sham groups. Serum creatinine and urea levels in CKF group increased when compared control, stem cell groups and sham groups.

CONCLUSION: When AA applied to rats, fibrosis increased importantly. While proliferation decreased, apoptosis increased in CKF groups. After injection of MSCs to rats, proliferation increased and apoptosis decreased. Functionally, serum creatinine and urea levels decreased in stem cell groups compared to CKF groups. Therefore, placental-derived mesenchymal stem cells can ameliorate renal failure.

This study was supported by Research Foundation of Akdeniz University, Turkey (project number: TYL-2014-131).

Abstract No. P121

Opposite early SOX9 response of human iPS cells compared to MSCs during in vitro chondrogenesis

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Induced pluripotent stem cells (iPSC cells) are highly attractive for cartilage regeneration and promise to surpass mesenchymal stromal cells (MSCs) that differentiate along the undesired endochondral pathway. In order to illuminate mechanistic differences during in vitro chondrogenesis of iPS cells versus MSCs, we investigated the early regulation of the chondrogenic master transcription factor SOX9, whose upregulation is considered a success indicator for cartilage formation. Upon generation of mesenchymal progenitor (iMPCs) from human iPS cells, SOX9 was induced and reached highly variable protein levels. In contrast, MSCs showed reproducibly high SOX9 levels. In iMPCs, but not MSCs, high levels of the SOX9-antagonizing hsa-miR-145 correlated with low SOX9 protein quantity. Chondrogenic stimulation with TGF-beta induced Smad protein phosphorylation in iMPCs as efficiently as in MSCs, thus indicating that iMPCs were able to adequately respond to chondrogenic stimulation. Strikingly, SOX9 protein levels in iMPCs dropped significantly lower from day 0 to day 4 of chondrogenic stimulation, while MSCs showed upregulated SOX9 protein at this time. Along with an altered condensation behaviour compared to MSCs and despite early SOX9 downregulation, iMPC pellets developed collagen type II positive chondrocytes. Chondrogenesis was better in the iPS cell line with higher SOX9 protein und lower miR-145 levels prior to chondrogenic stimulation and was further improved by co-treatment with TGF-beta+BMP-4. This appeared to shorten the duration of SOX9 protein decline but came at the expense of undesired hypertrophy. In conclusion, there is a negative SOX9 response to TGF-beta stimulation in iMPCs which is opposite to MSCs and may explain the inadequate chondrogenesis of iPS cells. This surprising opposite regulation clearly demonstrates that the role of SOX9 for chondrogenic differentiation is far from understood. Overexpression of SOX9, thus, appears as a promising approach to overcome this limitation of iMPC chondrogenesis.

Abstract No. P122

Phase II Clinical Trial Of Using Adipose Derived Stem Cell In Spinal Cord Injury Treatment

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BACKGROUND AIMS: To observe the clinical effect and safety adipose derived-stem cells (ADSCs) in treating spinal cord injury (SCI) by subarachnoid space, intrathecal, intravenous injection.

METHODS: From March 2012 to March 2015, a total of 48 completed SCI patients were enrolled in a clinical trial and were divided equally in treatment and control group. 32 patients in the treatment group received 4 injections. Spinal stable and spinal decompression surgery and first injection with dosage was 1.5 – 4 x 10^6 cells, second and third ADSCs transplantation took place after 30^{th} days, and 45^{th} days respectively. The 30 million cells/ 8 ml of Sodium Chloride 0.9% was delivered into intrathecal injection. Fourth ADSCs injection took place after 75^{th} days. 100 million cells/ 10 ml of Sodium Chloride 0.9% was dissolved in 200 ml Sodium Chloride 0.9% and intravenous injected in 1 hour. MRI, American Spinal Injury Association scoring system, somatosensory evoked potential, Barthel activities of daily index and SF-36 were used to evaluate neural function and ability to perform activities of daily living.

RESULTS: Analysis of subsequent treatment results indicated significant improvements in sensory, motor and autonomic nerve function as assessed. In treatment group, 6 months after transplantation, eight patients 51.61% improved from AIS A to AIS B, 6.45% improved from AIS A to AIS C, 12 months after transplantation, and two patients (10%) improved from AIS C to AIS D. While only one patient (6.25%) improved from AIS A to AIS B in control group. The most common adverse event, 6.25% reported back pain, 3.2% recorded headache and disappeared within 24–48 h without treatment, 6.5% reported fever (not recorded meningitis) and 3.2% reported uneasiness.

CONCLUSIONS: ADSCs therapy by subarachnoid space, intrathecal, intravenous injection is safe and can improve neurologic function and quality of life in most patients with complete SCI.

Abstract No. P123

Human Adipose Stromal Cells resist the detrimental effects of hyperglycaemic modified extracellular matrix in contrast to human retinal pericytes

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Diabetic retinopathy, the leading cause of blindness in the Western world, is characterized by an early loss of pericytes accompanied with thickening of the basement membrane and changes of the extracellular matrix (ECM) of connected endothelial cells. Adipose stromal cells (ASC) largely overlap with pericytes regarding the immune phenotype and endothelial stabilization capacity. This plus their known immunomodulatory and proregenerative features renders them attractive to prevent progression of diabetic retinopathy. It is, however, unclear whether ASC and pericytes behave differently in conditions mimicking the diabetic milieu. Human ASC and human retinal pericytes (HRP, primary and Bmi-1 immortalized) were seeded on ECM produced by human umbilical vein endothelial cells (HUVEC) under varying glucose conditions: normal glucose (NG, 5.6 mmol/L), high glucose (HG, 28 mmol/L) or intermitted phases of hyperglycemia (every 3h during daytime). Adhesion and cell confluency on the ECM were monitored using kinetic live cell imaging. Apoptotic events were assessed using a fluorescent caspase 3 substrate. In contrast to HRP, ASC resisted the detrimental effects of constant hyperglycemic modified ECM of endothelial cells (ECMNG ASC and HRP =100%, ECMHG ASC= in average 110% and ECMHG HRP 85%, $p \leq 0.05$). Only intermitted phases of hyperglycemia affected the adhesion and growth of ASC (95% versus ECMNG 12-16hours after seeding). The higher sensitivity of HRP to hyperglycemic-modified ECM was apparent by the occurrence of apoptotic events, rarely seen in ASC. Our data document that ASC, in contrast to HRP, resist the detrimental effects of constant hyperglycemic modified matrix of endothelial cells. Thus they may serve as corrective against hyperglycemia-induced pericyte death or dysfunction reducing microvascular complications. Our data, however, also suggest that good glycemic control of patients may be required. Whether the immunomodulatory and secretory properties of ASC contribute further to prevent/treat diabetic complications is a matter of current studies.

Abstract No. P124

TGF-beta is a strong selection pressure for MSC cultures revealed by barcode transduced umbilical cord pieces

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MSC are applied in the treatment of a variety of diseases, but the reproducibility of results in clinical application remains poor. Cultivated umbilical cord pieces (UCP) give rise to mesenchymal stromal cell explant monolayer (MSC-EM) for months and are supposed to contain a MSC niche. Previously, we could show the efficient transduction of whole UCP with stably integrating lentiviral vectors coding for one of three fluorescent proteins and a highly variable barcode. Transduction of MSC progenitors within UCP is a unique opportunity to follow clones from the stem cell niche along the inevitable expansion phase in culture. Several groups proposed an influence of the medium on the functional characteristics of MSC and we hypothesized clonal selection to contribute in part to the observed results. We used our whole UCP transduction method to mark MSC progenitors with 2 barcode vectors and used the 3rd vector to transduce resulting MSC-EMs (serial transduction). The serial transduction allowed us to distinguish between culture-acquired differences in MSC functionality and those inherent to different progenitor populations. We cultured a single MSC-EM separately in either basal medium (alpha-MEM, 10% human AB-Serum) or in the presence of epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) or transforming growth factor beta (TGF-beta). We analyzed the cultures by flow cytometry, deep sequencing and differentiation assays (osteoblasts, adipocytes and chondrocytes). Cultures with EGF or bFGF proliferated faster than cells in basal medium during passage 2-4. Remarkably, the same MSC-EM did not proliferate in TGF-beta containing medium during 2-3 passages. We observed a loss of a complete sub-population, which was still present in the other conditions. Moreover, we have first hints towards growth factor induced variability in MSC-EM differentiation capabilities of selected clones.

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Abstract No. P125

'CureMotorNeuron': Novel iPSC approach to advance ALS research

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ALS is a neurodegenerative disorder that affects both lower motor neurons in brainstem and spinal cord, and the upper motor neurons in the motor cortex. Loss of motor neurons with both familial and sporadic ALS is relentlessly progressive, and most patients die within three to five years after symptom onset. The high unmet medical need is compounded by clinical heterogeneity, lack of robustly predictive in vitro/in vivo disease models and limited understanding of the molecular mechanisms of disease pathogenesis. 'Cure MotorNeuron', our strategic partnership with the Harvard Stem Cell Institute, uses motor neurons derived from a panel of well characterized human induced pluripotent stem cell lines both from familial and sporadic ALS patients as basic models of disease. We have adapted and standardized motor neuron differentiation and phenotypic screening in 384-well format and are progressing towards further disease modeling and systematic screening for new mechanisms, targets and compounds that have therapeutic value for this life-threatening disease.

Abstract No. P126/T20

A new model to study neurotoxicity of drug metabolites based on chemical conversion to Neurons on a chip in tandem with liver-on-a-chip

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Microscale engineering technology can mimic organ microenvironments through integration of multi-cellular and -functional devices and thereby offers a unique niche to study physiology and pathophysiology of human tissues. Recently, several models have been developed to investigate neuroregeneration and neurodegeneration towards personalized medicine, in which patient-specific iPSCs are exploited. However, this process including generation of patient-specific iPSCs and differentiation to neurons are very complicated and time-consuming. In an effort to establish a reliable and reproducible chemical reprogramming protocol, we used small molecules derived from high throughput screenings based on their ability to induce pluripotency-associated transcription factors involved in reprogramming and transdifferentiation. Surprisingly, we found one compound is sufficient to convert fibroblasts to Tuj1+/MAP2+ neuron-like cells with comparable efficiency to virus-mediated reprogramming (5%). Optimization by combination with other small molecules and growth factors led to discover a chemical cocktail able to directly generate neurons from patient fibroblasts with more than 80% efficiency. More intriguingly, Tuj1+ neuron-like cells can be observed within days and MAP2+/NeuN+/Tau+ within one week. In cooperation with ChipShop (Jena, Germany), we applied this cocktail to a microfluidic chip-based system and reproducibly achieved conversion of fibroblasts to Tuj1+/Chat+ neuron-like cells within days. Finally, we developed a microfluid-based model in a tandem neuron-on-a-chip, liver-on-a-chip design to study patient-specific neurotoxicity of drug metabolites, which can be potentially applied for personalized disease model and neurotoxicity study of drugs and drug metabolites via high throughput screening.

This work was supported by BMBF Grant FKZ 0315398B and FKZ 031A303E.

Cheng, X. et al. Identification of 2-[4-[(4-Methoxyphenyl)methoxy]-phenyl]acetonitrile and Derivatives as Potent Oct3/4 Inducers. J Med Chem 2015, 58, 4976-83

Cheng, X. et al. Chemically reprogramming human fibroblast into neuron-like cells by single small molecule. Submitted

Abstract No. P127

Recapitulation of virus infection-related disorders during early embryonic development through induced pluripotent stem cells

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Objectives: The process of cell reprogramming enabled the generation of human induced pluripotent stem cells (iPSCs) as a promising approach for both, basic research and regenerative medicine. They will also revolutionize the field of virology through their ability to differentiate into specialized human somatic cells such as neurons or hepatocytes. Additionally, they are a valuable in vitro model for virus-associated pathological outcomes during the otherwise inaccessible early phase of human development.

Methods: For two human iPS cell lines the course of infection with viruses associated with congenital disorders (rubella virus and cytomegalovirus) and with miscarriage events (measles virus and coxsackievirus B3) were characterized. The viral replication rate was assessed in comparison to the viral impact on iPSC colony morphology, metabolic activity and general survival rate as well as on their pluripotent properties.

Results: While human cytomegalovirus appears to be able to infect pluripotent stem cells only after initiation of differentiation, coxsackievirus B3 establishes a lytic infectious cycle, especially on bigger iPSC colonies (diameter of more than 200 µm). The most striking effect was observed after infection with measles virus, which resulted in loss of densely packed iPSC colony structure due to syncytium formation. Only for rubella virus a persistent infection could be established.

Conclusion: The use of these selected and distinct viruses shows the great potential of iPSCs for in vitro modelling of virus infections during the vulnerable period of early human development.

Abstract No. P128

Optimization of stem cell culture conditions for maintenance and differentiation into peripheral neurons

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Peripheral chronic pain is a debilitating condition and current treatment is often insufficient. Investigation of human peripheral neurons is limited due to low accessibility of biopsies for research. Recent progress in stem cell research offers the possibility to assess human peripheral neurons. Using a small molecule approach, stem cells can be differentiated into peripheral sensory neurons. This constitutes a valuable tool for detailed investigation of the human sensory neurons physiology and disease modeling. Standard protocols for cultivation and differentiation of the reprogrammed cells are dependent on animal-derived compounds that are present in the coating and culture media. Such compounds may limit reproducibility of the experiments due to stock-to-stock variations and make stem cell research expensive. Therefore, a growing interest in new xeno-free coating and media has emerged. Here, we differentiated neurons from human embryonic stem cells (ESCs) using three different media (mTeSR1, TeSR-E8 and iPS-Brew XF) and two coating strategies (matrigel hESC-Qualified Matrix and Vitronectin XF). Our Results indicate that all tested conditions are suitable for culturing ESCs, which are then applicable for neuronal differentiation. Differences in cell survival and differentiation efficiency are observed. Using immunostainings, calcium imaging and patch clamp electrophysiology, we show that cells have neuronal morphology and express ion channels including voltage-gated sodium channels and TRPs, which are highly expressed in the human peripheral sensory neurons. A detailed electrophysiological study showed that stem cell-derived neurons are functional and reveal no differences in key neuroelectrophysiological characteristics between cells from varying expansion conditions. This shows that the applied culture conditions are suitable for optimization of a method to generate sensory neurons appropriate for physiological studies of nociception, disease modelling and drug testing.

Abstract No. P129

Human Induced Pluripotent Stem Cells with KIT D816V Mutation for Modeling Leukemia

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Induced pluripotent stem cells (iPS cells) represent particularly appealing tools for modeling of human diseases *in vitro*. Myeloproliferative neoplasms (MPN) are chronic malignant diseases of the myeloid lineage and are associated with mutations in key signaling pathways. A mutation in the stem cell factor (SCF) receptor tyrosine kinase KIT (KIT D816V) leads to a constitutive active form of the receptor and resistance against the tyrosine kinase inhibitor Imatinib (Gleevec/Glivec). Thus, KIT D816V patients in the terminal phase of disease are essentially left without effective therapy, rendering this a fatal disease. Here we generate iPS cells from KIT D816V patients in order to better understand the mechanistic underlying this pathology and to develop a model for screening of novel drugs on a patient-specific background. We additionally introduced the KIT D816V mutation in human ES cells by CRISPR/Cas9n technology to generate isogenic pairs of ES cells with and without the KIT D816V mutation. The presence of the mutation was confirmed by allele specific PCR and Sanger sequencing. Patient derived iPS cells are differentiated into hematopoietic stem cells (HSC) that show CD31, CD43 and CD45 expression. Stimulation of Wnt pathway is used to obtain HSC with definitive differentiation potential to mimic the patient's situation. KIT D816V HSCs obtained from patient derived iPS cells and ES cells are being analyzed for KIT downstream signaling and screened for novel inhibitors. In summary, the patient specific KIT D816V iPS cells and KIT D816V HSC derived thereof, overcome the limitation of cell numbers from primary patient samples and are used for the development of novel therapeutic strategies. Finally, the patient-specific KIT D816V iPS cell clones can be used as a valuable *in vitro* model for further investigations of this pathology and might give new insights into the disease pathophysiology.

Abstract No. P130

The role of Adiponectin signalling in an iPSC-based model of nonalcoholic fatty liver disease

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Metabolism in hepatocytes is highly susceptible to nutritional cues. In the presence of abundant calories derived from fat and carbohydrates, hepatocytes store fatty acids as triacylglycerides in lipid droplets (LDs). This effect is increased by the action of insulin and results in the development of nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of the metabolic syndrome. While early stages of NAFLD are benign and reversible, many patients develop fibrosis (NASH), cirrhosis and even hepatocellular carcinoma (HCC). Adiponectin has been associated with many positive aspects on metabolism which result in increased insulin sensitivity as well as reduced gluconeogenesis and LDs in the liver. Adiponectin plasma levels are reduced in obesity, type 2 diabetes and insulin resistance in contrast to healthy individuals. Hepatocytes express Adiponectin receptors 1 and 2 (AdipoR 1+2) which are involved in regulating glucose and lipid metabolism, inflammation and oxidative stress. The metabolism-associated Adiponectin signalling activates the AMP-activated protein kinase (AMPK) and Peroxisome proliferator-activated receptor α (PPARα) pathways which both increase fatty acid oxidation. In addition, AMPK is also involved in reducing gluconeogenesis. We have established an *in vitro* model for NAFLD based on the differentiation of induced pluripotent stem cells (iPSCs) into hepatocyte like cells (HLCs). Treatment of HLCs with oleic acid (OA) induces the formation of LDs in parallel with an increase of the LD-coating protein PLIN2. In addition, we observed regulated expression of metabolism related genes. The influence of an individual's genetic background on LD incorporation and metabolism changes are assessed by using iPSCs derived from NAFLD patients and lean controls. Within this model, we investigate the beneficial effects of Adiponectin on HLC metabolism using a small molecule analogue called AdipoRon. Metabolic adaptations are monitored by gene expression analysis, quantification of LDs, measurement of reactive oxygen species (ROS) and analysis of mitochondrial integrity.

Abstract No. P131

Investigation of the energy metabolism of iPSC-derived basal ganglia neurons and glia from patients with Leigh syndrome

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Leigh Syndrome (LS) is a severe neurodegenerative and mitochondrial fatal childhood disorder. It is a genetically and biochemically heterogeneous disease that leads to impaired oxidative phosphorylation. The SURF1 mutations are known to be the most frequent cause of LS with defects in cytochrome c oxidase (COX). COX, as part of complex IV, is the terminal enzyme of electron transport chain (ETC) and couples electron transport from cytochrome c to molecular oxygen to form water by transporting protons from the matrix into the cytosol (Zhu, 1998). In this project, we are focusing on two patients harbouring exclusively p.Val177Gly and p.Gly257Arg mutations on SURF1 gene which are recessively inherited. Both of these mutations are missense mutations that lead to loss of function of Surf1 protein which is assumed to be involved in early step of complex IV assembly whereas its function remains unknown (Kovářová N, 2012). The underlying reasons why basal ganglia region is the most affected area still remain unknown. There is no effective therapy available and in vivo models to study the disease are lacking. The aim of the project is to develop a novel (iPSC)-based neuronal model of LS and to investigate the molecular mechanisms underlying the disease specific neuronal cell death and additionally glial dysfunction that could potentially allow identification of novel therapeutic strategies. After generation of clinically feasible iPSCs; the transcription activator-like effector nuclease (TALEN) system is employed to correct and introduce the particular p.Val177Gly mutation. Generated and edited iPSCs will be differentiated into midbrain dopaminergic neurons (mDANs) and astrocytes. Eventually, mitochondrial and metabolic mechanisms of differentiated cell types will be investigated and based on the findings, phenotype-based assays will be developed.

Abstract No. P132

The Generation of 3D Cerebral Organoids for Mimicking Neurodegenerative Aspects of Psychiatric Diseases

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Background and Aim of the Study: Three dimensional (3D) organotypic cultures offer promising tools for research on the causal connection of psychiatric diseases to neurobiological dysfunction. 3D organoids provide a powerful tool to study cells within 3D microenvironment. Degeneration of neurons can be reflected by the disturbed distribution, maturation, functionality or viability. Genomic studies indicate that single nucleotide polymorphisms (SNPs) are related to the development of Alzheimer's disease. Patient-specific induced-pluripotent stem cells (iPS cells) provide a powerful tool for the analysis of SNPs. Patient-derived iPS cells have the patient-specific genetic background and allow the analysis of diseases within disease-specific in vitro models. Aim of the study was to establish 3D cerebral organoid cultures suitable for the in vitro analysis of disease-specific Alzheimer-associated SNPs.

Materials and Methods: We generated iPS cells from patients-derived B lymphoblastoid cell lines. Pluripotency of patient-specific iPS cells was verified by alkaline phosphatase staining and immunofluorescence anaylsis. Neural stem cell lines were used for the generation of neurospheres suitable for the generation of 3D cerebral organoids. The differentiation of cortical neurons focused on neurons, synapses, and astrocytes. Organoids were cut into sections for the immunofluorescence analysis suitable to detect aggregation and patterning of cells. Staining of neural proteins revealed layer-specific localization of neural marker proteins.

Results and Conclusion: The analysis of mRNA and protein expression revealed the induction of crucial neurodevelopmental markers. The induction of neural stem cells was characterized by SOX2 and PAX6. Neural induction towards cortical neurons and glia was monitored by a set of neurodevelopmental marker genes including NESTIN, GFAP and NGN3. Mature markers suitable to detect cortical layer patterning included revealed a layer-like expression pattern for several mature makers including TBR1. In conclusion, organotypic 3D disease models provide a powerful tool for the analysis of neurodegenerative disorders such as Alzheimer's disease.

Abstract No. P133

Tyrosine hydroxylase deficient iPSCs as a model for neurometabolic diseases

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Tyrosine hydroxylase (TH; OMIM 605407) is the rate-limiting enzyme for the generation of dopamine and catalyses the hydroxylation of L-tyrosine to L-dopa. Deficiency of the enzyme leads to autosomal recessive Dopa-responsive dystonia which has a prevalence of ~1:1.000.000 births in Europe leading to severe central deficiency of dopamine, serotonin, epinephrine and norepinephrine with progressive symptoms including psychometric retardation, muscular hypotonia, extrapyramidal movement disorders, epilepsy and oculogyric crisis starting in early childhood. Currently, L-dopa in combination with carbodopa is the therapy of choice. Less severe phenotypes respond well to the treatment but more severe phenotypes frequently show hypersensitivity to L-dopa and only moderate to no benefit at al. As patient material for this type of rare disease is not easily available to study underlying pathomechanisms or screen for better therapeutics human induced pluripotent stem cells (hiPSCs) generated from patient cells and differentiated derivatives can serve as model systems to overcome these issues. Here, we report the generation of hiPSCs from a patient suffering from tyrosine hydroxylase deficiency. Fibroblasts of the patient were reprogrammed to TH-deficient iPSCs using episomal plasmids and further characterized (karyotype, marker gene expression, transgene silencing, differentiation potential). TH-deficient iPSCs have been differentiated to neurons and characterized biochemically. As expected, TH activity assays revealed a reduced formation of L-dopa from tyrosine in the affected cells. Currently, the TH-deficient cells are corrected using CRISPR/CAS 9 technology, serving as control for an RNA-Seq approach to identify misregulated RNA species in TH-deficient neurons.

Abstract No. P134

Calcium, calpains and decreased autophagy conspire to drive aggregation of ataxin-3 in neurons from Machado-Joseph disease

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Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 is a devastating neurological disorder driven by a polyglutamine repeat expansion in the C-terminus of ataxin-3 and represents the most frequent form of inherited spinocerebellar ataxias worldwide. Recently, we showed protein aggregation in iPSC-derived MJD-neurons could be initiated via excitatory stimulation resulting in Ca2+ entry and subsequent calpain-mediated cleavage of ataxin-3. However, the role of intracellular Ca2+ stores in ataxin-3 aggregation remained undefined. Therefore, we explored whether and to what extent intracellular Ca2+ stores contribute to the generation of ataxin-3 aggregates. Inhibiting Ca2+-induced calcium release (CICR) from endoplasmic reticulum (ER) by ryanodine profoundly diminished ataxin-3-positive SDS-insoluble aggregates induced by glutamate, pointing to an amplifying and important role for calpain-mediated ataxin-3 aggregation. Direct Ca2+ release from ER induced by ATP via metabotropic P2Y receptors (P2YR) or inhibition of sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) also yielded SDS-insoluble aggregates, underlining the relevance of intracellular Ca2+ release for aggregation. Expression of P2YR already in immature neural cells enabled ATP-induced ataxin-3 aggregation even in one week-old neurons. Moreover, ATP-stimulation led to microscopically visible neuronal intranuclear inclusions (NIIs) positive for ataxin-3 and ubiquitin, a neuropathological hallmark of MJD. However, NII induction by intracellular Ca2+-release showed high variability between independent experiments. We reasoned fluctuations in cellular proteostasis might underlie this variability. Indeed, careful calibration of media conditions towards inhibition of autophagic flux resulted in reliable NII formation selectively in MJD neurons. Consistently, pharmacological inhibition of autophagy in mature neurons yielded ataxin-3-positive SDS-insoluble aggregates whereas autophagy induction by rapamycin prevented aggregation. Furthermore, glutamate stimulation lead to cleavage of the autophagy core proteins BECN1 and ATG5 most likely by calpains. The data presented here indicates an intertwining of calpain activation by dysregulated Ca2+ homeostasis and decreased autophagy where both processes conspire to drive aggregation of ataxin-3 in MJD.

Abstract No. P135

Human induced pluripotent stem cell derived peripheral neurons to predict and model neurotoxicity in high throughput and high content assays

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A major challenge in characterizing the potential neurotoxic risk of chemical agents or drugs is the paucity of available and relevant hazard data sets. Currently, thousands of chemicals are being evaluated for drug discovery purposes, however, only a relatively small number of these have been adequately characterized for adverse effects such as being causative agents for neurological damage. Many studies have indicated that in vitro neurite outgrowth assays can be used to identify harmful chemical effects on the developing nervous system and/or peripheral neurons and this approach has been widely adopted by the pharmaceutical industry for drug discovery and safety pharmacology. Due to the advances in stem cell technologies, we have developed a semi-automated 384-well High Throughput Assay using highly characterised hiPSC derived peripheral neurons (Peri.4U, Axiogenesis). The assay combines a non-lytic cell viability assessment (RealTime-Glo™, Promega) with a flexible High Content Screening (HCS) assay (Perkin Elmer, Opera) that detects neuron-specific betaIII-Tubulin, activated Caspase-3 and nuclei staining. The assays offer Z' >0.5 for “total neurite outgrowth intensity”, “mean Caspase-3 intensity” and “nuclei number”. To validate the assay we tested a commercial available collection of toxic reagents (approximately 150 compounds) at 10 µM. Hits were profiled in 11-point dose-response studies. Using CSIRO Neurite Analysis (Perkin Elmer, Columbus) we were able to characterize neurite outgrowth in detail, including maximum neurite length, number of extremities, roots, segments and nodes. Due to the hypothesis that differences in damage among drugs reflect differences in their mechanisms of action we analysed neurite outgrowth in detail, including maximum neurite length, number of extremities, roots, segments and nodes. These data will be used to perform statistical association tests to identify and to describe phenotypic changes which are correlated with the mechanism of action of known and unknown compounds.

Abstract No. P136

Human iPSC-derived macrophages reveal impaired IFNg-signaling in "Mendelian Susceptibility to Mycobacterial Disease" due to IFNgR1 deficiency

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Mendelian Susceptibility to Mycobacterial Disease (MSMD) is a rare primary immunodeficiency characterized by recurrent severe infections caused by otherwise only weakly virulent mycobacteria. MSMD is associated with mutations in different genes, all leading to an impaired activation of macrophages (Mac) by T-cells and a defective innate immunity. In particular, mutations in the interferon-gamma (IFNγ)-receptor-1 or -2 (IFNγR1/2) genes result in a life-threatening phenotype with most patients dying in early childhood. To gain new insights into MSMD pathophysiology, we generated patient specific iPSCs from $CD34^+$ peripheral blood cells of one patient suffering from compound heterozygous IFNγR1 deficiency that had previously received bone marrow transplantation from a family member donor harboring a heterozygous mutation. In total, 36 *bona-fide* iPSC clones have been established, reflecting the blood chimerism of approx. 50%. Established iPSCs were characterized by morphology, SSEA4/Tra1-60 expression, endogenous reactivation of OCT3/4, SOX2, and NANOG, and three germ layer differentiation capacity. Interestingly, 90% of compound heterozygous iPSCs had chromosomal abnormalities by fluorescence-R banding, whereas normal karyotype was observed for heterozygous clones. When subjecting compound-heterozygous (iMSMD-cohet) or heterozygous (iMSMD-het) iPSC to macrophage (Mac) differentiation, no abnormalities were observed. Both Mac populations showed typical morphology in brightfield and cytospin and stained positive for CD14/CD11b/CD45/CD86/CD163. While IFNγ-independent characteristics such as phagocytosis in iMSMD-het-Mac and iMSMD-cohet-Mac resembled wildtype (WT)-iPSC-Mac, iMSMD-cohet-Mac revealed impairment of IFNγ-signaling by lack of phosphorylated STAT1 and up-regulation of HLA-DR upon stimulation. Moreover, induction of IFNγ-downstream targets IRF1, SOCS-3 and IDO in iMSMD-cohet-Mac was impaired compared to WT- and iMSMD-het-Mac. In summary, we demonstrate the simultaneous generation of heterozygous and compound heterozygous iPSC lines from one patient suffering from IFNγR1 deficiency. Impaired IFNγ signaling upon hematopoietic differentiation towards macrophages make these lines highly suited to investigate novel treatment options for MSMD or gain new insights of IFNγ-signaling on macrophages.

Abstract No. P137

Live subtype identification during cardiac differentiation using genetically encoded voltage sensors

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During cardiac differentiation of pluripotent stem cells all different cardiomyocyte subtype lineages can be found. Despite great improvements in directed cardiac differentiation protocols the process of cardiomyocyte subtype emergence during in-vitro cardiac differentiation is poorly understood. However, in the light of emerging applications of stem cell-derived cardiomyocyte screening platforms and the increasing importance of stem cell-based disease modelling (e.g. in studying congenital heart disease), the understanding of this process is crucial. We have developed a system to express genetically encoded FRET-based voltage sensors in human induced pluripotent stem cell-derived cardiomyocytes and cardiac progenitors under the control of cardiomyocyte subtype-specific promoter elements. This enables recording of action potentials exclusively in the cardiomyocyte lineage of interest and sequential measurements of single cells. These features do not only allow live visualization of cardiomyocyte subtype emergence during in-vitro differentiation, but also functional analysis of their electrophysiological properties over-time.

Abstract No. P138

Characterization of an iPS cell-based disease model for phosphomannomutase 2 congenital disorder of glycosylation (PMM2-CDG)

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PMM2-CDG, which was previously known as congenital disorder of glycosylation-Ia (CDG-Ia) is an inherited disease caused by mutations in the gene encoding phosphomannomutase 2 (*PMM2*). PMM2-CDG affects multiple organ systems and is generally associated with severe psychomotor and mental retardation. The enzyme PMM2 catalyzes the conversion of mannose-6-phosphate to mannose-1-phosphate, which is the precursor for the synthesis of the activated donor sugar GDP-mannose (GDP-Man). All types of glycosylation, N- and O-glycosylation as well as C-mannosylation depend on GDP-Man. C-mannosylation has been found on type I cytokine receptors and proteins with thrombospondin type 1 repeats. However, the observed disease phenotypes of PMM2-CDG were so far only being associated with aberrant N-glycosylation although the molecular reasons for the pathophysiology are poorly understood. PMM2-CDG manifests during embryonic development and thus we established an induced pluripotent stem cell-based model for PMM2-CDG by reprogramming of PMM2-CDG patient-derived fibroblasts into iPSCs (PMM2-iPSCs). These PMM2-iPSCs showed significantly lowered levels of high-mannose-type N-glycans, which were further reduced upon stable knock-out of PMM2 in PMM2-iPSCs[1]. In order to study whether C-mannosylation is also affected by PMM2-CDG, we aimed to express target structures for C-mannosylation in control and diseased iPSCs and to compare their levels of C-mannosylation. Tagged soluble fragments of the target proteins for C-mannosylation, thrombospondin-1 and UNC-5 were expressed in iPSCs and could be enriched by affinity chromatography from cell culture supernatants. Analysis of C-mannosylation by mass spectrometry is currently being performed.

[1] Thiesler et al. (2016) Mol Cell Proteomics 1435-52

Abstract No. P139

Disease modelling, safety screening and drug development using IPSCs: automated patch clamp, extracellular field potential and impedance platforms

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Electrophysiological characteristics and activity footprint of induced pluripotent stem cell (iPSC) derived cardiomyocytes and neurons, recently became a priority in disease modeling research, drug development and safety screening. We describe the development and optimization of cell-based assays that are sensitive and provide reproducible results for safety pharmacology. We present evaluation data from automated patch-clamp and MEA systems performed on iPSC cardiomyocytes, giving information on ionic currents, action potentials and activity patterns. Changes in the impedance signal indicate effects on cell contractility and shape whereas the field potential parameters provide information about the electrophysiological activity of the beating network of cells. In accordance with the Comprehensive In Vitro Proarrhythmia Assay (CiPA) guidelines, standard reference compounds were tested on iPSCMs. Example traces of action potential recordings, voltage-clamp measurements and also contractility and EFP/MEA recordings before and after compound applications were compared. Additionally, we demonstrate the application for the MEA systems for usage of iPSC derived neurons in drug development and characterization of in vitro disease models – Parkinson´s disease, ALS, epilepsy, fragile X, and autism – with the ultimate goal of identifying treatments. The high throughput, ultra-high resolution (millisecond events with microvolt amplitudes), high electrode count (allows population network activity measurements) and accuracy these systems provide will significantly accelerate progress toward such treatments. Multiwell optogenetic stimulation further excels MEA-based disease modeling and drug discovery. In summary, we show complementary electrophysiology platforms, which provide unmatched information on a compound's safety profile, drug discovery and development of phenotypic disease-in-a-dish cellular models. Reduced cell usage, increased throughput and integration into robotic environments improve cost efficiency, precision and are speeding up the whole HTS process of drug development and safety screening.

Abstract No. P140

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

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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 that closely recapitulate the properties of the clinically most affected organs. To address these unmet needs we focus on the generation of induced pluripotent stem cell (iPSC) lines from CF-patients homozygous for F508del mutation with mild and severe phenotype and with known intragenic recombination. CF-iPSCs were generated via reprogramming of CD34+ cells isolated from small volumes of non-mobilized peripheral blood. The resulting CF-iPSCs were analysed regarding their karyotype, pluripotency status and potential to differentiate. Moreover, different transgenic iPSC and embryonic stem cell (ESC) lines were generated overexpressing a halide sensitive yellow fluorescent protein (YFP) monitoring CFTR-function, in combination with the overexpression of an artificial CFTR or an endogenous CFTR-tomato-fluorescence-reporter. Several CF-iPSC lines were established and characterized in detail. The generated YFP-reporter cell lines showed stable transgene expression also during in vitro differentiation and the general functionality of the YFP-reporter could be confirmed. Functional measurements to test the CFTR dependent halide sensitivity of our transgenic cell lines are currently ongoing and show promising results. Differentiation of YFP-expressing CFTR-tomato-reporter iPSCs towards cholangiocytes revealed YFP+/tomato+ cells, displaying CFTR-channel specific response after Forskolin application. Hence, the stable integration of the halide reporter into CF-iPSCs in combination with integration of the CFTR-tomato-reporter should enable disease modelling of F508del-based CF with regard to the individual genetic context and the implementation of high-throughput screening for novel correctors and potentiators of CFTR-trafficking mutations.

Abstract No. P141

Generation of large numbers of floor plate derived, midbrain- specified DA neurons from human PSCs for scaled applications

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Stem cell derived midbrain dopaminergic (DA) neurons provide an excellent cell source for disease modeling and drug screening for Parkinson's disease. To expedite this effort, we have developed culture system which can make authentic midbrain DA neurons starting from human pluripotent stem cells. Our system was designed to simplify and standardize the whole differentiation process while compressing timelines and adding increased flexibility in to this complex differentiation workflow. The process has 3 distinct steps: (1) specification of hPSC to midbrain floor plate (mFP) cells, (2) expansion and cryopreservation of derived mFP cells, and (3) maturation to DA neurons. Our system has the unique feature of enabling the generation of expanded banks at the midpoint, with mFP cells expanded at least 200 fold (2 passages) prior their cryopreservation (optional step). However, we were challenged to further increase the size of the cell bank to facilitate certain applications requiring large numbers of cells and standardization, such as high through put drug screening. To achieve this goal we developed an alternative workflow enabling stable growth of mFP cells up to passage 10 (p10). The cryopreserved mFPp10 were able to be directly recovered on assay format and after 7 days in maturation, phenotype marker of TH was examined using high throughput imaging system. The standardized work flow resulted in consistent differentiation of our reference PSC line over time. However we have noticed significant variation in maturation efficiency can occur across PSC lines and looked for ways to reduce this variability. To improve the differentiation efficiency, we tested a number of conditions and ultimately incorporated an extended differentiation procedure for low performing lines. As a result, we could get comparable differentiation efficiency from this line. Thus, we could use our system successfully across multiple cell lines.

Abstract No. P142

Establishing a co-culture system to study the interaction of neurons and peripheral immune cells in neurodegeneration

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Neuronal loss and neuroinflammation are common features of neurodegenerative diseases like Alzheimer`s Disease (AD), Parkinson´s Disease (PD), or multiple sclerosis (MS). Besides neurotoxicity induced by immune cells of the central nervous system (CNS), infiltrating peripheral immune cells, especially T lymphocytes, contribute to the neurodegeneration in MS, but also in AD and PD. To study the role of T lymphocytes on neuronal cell death in PD in a human in vitro system, we established a novel autologous co-culture system. Dopaminergic (DA) neurons, the degenerating neuronal phenotype in PD, were generated from human induced pluripotent stem cells (hiPSC) derived from PD patients and healthy controls. They were co-cultured with ex vivo activated autologous T lymphocytes isolated from peripheral blood of PD patients or controls. Various co-culture settings, including the media composition, cultivation time as well as the ratio of T lymphocytes and neurons were investigated and optimized. Interestingly, when applying PD vs. control cells in the novel co-culture setup, increased levels of neurotoxicity could be detected in PD neurons compared to control neurons. Thus, PD patients are more susceptible to neurotoxicity either due to more vulnerable DA neurons or due to an altered T lymphocyte population with an increased pro-inflammatory signature. In conclusion, this novel co-culture setup offers a unique possibility to study neuronal-immune interaction in neurodegenerative diseases in an authentic human system. It can be used to define the role of various immune cells (T cells, B cells, macrophages) in neurodegenerative diseases like PD, AD or MS and thus provide important knowledge for the development of new personalized therapeutic approaches.

Abstract No. P143

Functional analysis of Nexilin in hiPSC-derived cardiomyocytes

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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 human. Additionally, zebrafish and mice lacking Nexilin in heart develop DCM. The underlying pathomechanisms are still unkown. The main aim of this study is to generate hiPSCs lacking Nexilin as well as hiPSCs carrying DCM Nexilin mutations, and functionally characterize derived cardiomyocytes in order to get a better understanding of the molecular mechanisms leading to DCM in human. 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 and protein level using the markers Oct4, Klf4 and Nanog to show pluripotency of hiPSCs. After differentiation, characterization of differentiated cardiomyocytes is performed using heart specific expression markers such as MYL2, Troponin T, Desmin, GATA4 and HCN4 at RNA and protein level. The next step will be the generation of Nexilin knockout and DCM mutations iPS cells using the CRISPR/Cas system. Knocking out Nexilin in iPSCs is in progress. Introducing DCM mutations will follow. After that resulted cell lines will be differentiated into cardiomyocytes and the same aforementioned markers will be used for detailed molecular characterization of iPSC-derived cardiomyocytes from different lines and compared to controls. Additionally, phenotypic characterization, sarcomere integrity and expression levels of cardiac transcription factors such as Smad proteins and Tbx5 will be analysed to narrow down the function of Nexilin.

Hassel, D. et al., 2009. Nexilin mutations destabilize cardiac Z-disks and lead to dilated cardiomyopathy.

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Abstract No. P144

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

Anja Trillhaase[1,], Undine Haferkamp[1], Beatrice Strohbücker[1], Ali Torkamani[2], Jeanette Erdmann[1], and Zouhair Aherrahrou[1]*

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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 analyse the effect of 9p21 locus on calcification using iPS-derived smooth muscle cells (SMC). Induced-pluripotent stem cells (iPSCs) with a deletion of 60 Kb region within the 9p21 locus was successfully generated and validated. A protocol for differentiating these iPSCs into SMCs was established in our laboratory and studies on calcification in-vitro using iPSC-derived SMCs from WT, KO and Heterozygous iPSC lines for 9p21 are on going. The iPS-derived SMC express as expected SMC markers both at RNA and protein level. For the calcification assay calcifying media containing Pi, CaCl2 or ß-glycerophosphate will be added to induce calcification in iPS-derived SMCs. Calcification deposits in the 3 lines of iPS-derived SMCs will be confirmed using calcification specific staining such as Alizarin-red-S and calcein, and quantified using a calcium assay kit. Results from the 3 genotypes WT, Het and KO will be compared. The assay may be used in future as read out to screen for calcification inhibitors.

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Schunkert, H. et al., 2011. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease.

Abstract No. P145/T19

Stiff matrix induces switch to pure beta-cardiac myosin heavy chain expression in human embryonic stem cell-derived cardiomyocytes

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Cardiomyocytes generated from human pluripotent stem cells (hPSCs) represent a powerful tool for in vitro modelling of cardiac diseases such as hypertrophic and dilated cardiomyopathies. Yet, knowledge about the expression and functional impact of different sarcomeric protein isoforms like myosin heavy chain (MyHC) in hPSCs is poorly understood. After differentiation, human embryonic cardiomyocytes (hESC-CMs) typically express high levels of the fast atrial myosin heavy chain (α-MyHC). In human ventricular cardiomyocytes, however, the slow β-MyHC predominates. Here we aimed to generate hESC-CMs with exclusive β-MyHC protein expression in individual cardiomyocytes and to characterize the contractile properties of single cardiomyocytes with different MyHC- isoform composition. MyHC- isoform composition and contractile properties of single cardiomyocytes were evaluated in prolonged in vitro culture in cardiac bodies (soft matrix) versus cardiomyocytes plated onto laminin coated glass coverslips (rigid matrix). Using a specific antibody against ventricular β-MyHC and a newly generated anti-atrial α-MyHC-specific antibody we found that individual cardiomyocytes grown in cardiac bodies for a maximum of 110 days mostly contained a mixture of α- and β-MyHC. Only a minority of about 10% of cardiomyocytes expressed β-MyHC exclusively. However, cardiomyocytes plated on laminin-coated coverslips shifted MyHC-expression towards 66% and 87% of all cardiomyocytes expressing exclusively β-MyHC after 35 and 75 days, respectively. This isoform switch was accompanied by morphological changes towards more elongated cardiomyocytes with highly organized sarcomeres. Surprisingly, twitch kinetics and calcium transients were found unaffected by the MyHC-isoform in the sarcomeres while cardiomyocytes grown on laminin-coated coverslips in general displayed faster twitch kinetics and calcium transients. In conclusion, stiff substrate induces pure β-MyHC-protein expression in hESC-CMs, with several contractile parameters close to ventricular cardiomyocytes, thus providing a well-defined in vitro system for modelling of cardiomyopathies and drug screening approaches.

Abstract No. P146

A robust platform for generation and high throughput functional analysis of human induced pluripotent stem cell-derived cardiomyocytes

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Induced pluripotent stem cells (iPSCs) and their differentiated progeny provide useful tools for safety testing and promise to provide more predictive results from assays that translate to pre-clinical and clinical outcomes with greater fidelity than current heterologous expression systems or non-human cardiomyocytes. Moreover, disease-specific iPSCs can be derived directly from patients with known disease phenotypes or can be mimicked by introducing known mutations into the iPSC genome by CRISPR/Cas9 or TALEN systems. The ability to derive human cardiomyocytes from these iPSCs supports the study of cardiac diseases in vitro, and facilitates the discovery of drugs that could compensate for cardiac abnormalities. However, these drug screening efforts would be improved by more reliable, reproducible and scalable cardiomyocyte differentiation from human iPSCs. Here we describe a workflow that facilitates high-throughput functional screening with hiPSC-derived cardiomyocytes. This workflow relies on a robust system to efficiently generate cardiomyocytes from multiple hiPSC lines, yielding cardiomyocyte purities of over 50%, which can be further increased using a metabolic enrichment step. We show that these iPSC-derived cardiomyocytes can be replated into 384-well plates to support calcium flux and membrane potential assays (Fluo-4 & FluoVolt™ dyes). We demonstrate that function of the iPSC-derived cardiomyocytes can be measured accurately as shown by the expected pharmacology and physiology of a variety of known and selective ion channel activity modulators. In summary, we provide tools that can be used in HTS platforms to reliably produce and analyze human cardiomyocytes at a scale sufficient to support functional cardiomyocyte screening assays. These assays can be used for lead identification screens or lead optimization profiling that support drug discovery efforts for cardiac disease as patient- or disease-specific cellular contexts may be generated using patient-derived or CRISPR/TALEN edited iPSCs. Also they can be directly implemented in safety studies to identify compounds that may have cardiac liabilities.

Abstract No. P147

Establishment of a human stem cell-based model of alcohol use disorders

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Alcohol use disorders (AUDs) can lead to serious health problems and represent an increasing health-care burden, as there are no efficient treatments available. Previous GWAS studies identified a haplotype block containing RASGRF2 rs26907 to be associated with alcohol consumption (Stacey et al. 2012). Experiments conducted in RASGRF2 mice models showed that dopaminergic (DA) neurons exhibit aberrant dopamine release (Easton et al. 2014; Newton 2004; Stacey et al. 2012). This effect may also partly be due to the modulation of neuronal calcium homeostasis, given that RASGRF2 is a calcium/calmodulin factor that can influence calcium influx at synaptic terminals (Schwechter, Rosenmund, and Tolias 2013). We aim to build on these findings in order to develop a human model system of AUDs using induced pluripotent stem cells (iPSCs) and reprogramming technologies. Using this model, we will determine the role of RASGRF2 in the physiology of human DA neurons and the specific response to acute and prolonged ethanol exposure. This may allow on the one hand to bridge the gap between the findings obtained in mice and the genetic and imaging data in humans and on the other hand to identify potential agents capable of counteracting the disease phenotypes in human DA neurons. We will overexpress RASGRF2 in DA neurons using a lentiviral-based system in order to investigate the contribution of RASGRF2 and alcohol exposure on DA neurons. The RASGRF2 knockout (KO) DA neurons will be generated using the CRISPR\Cas9 system. We will then investigate the presence of disease-associated phenotypes in RASGRF2 DA neurons using calcium imaging, high content screening based imaging of neural branching and measurement of dopamine under short-term and prolonged alcohol exposure.

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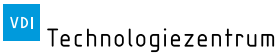

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