3rd International DZL Symposium

“Lung Regeneration and Beyond – BREATH meets REBIRTH”

Abstract Book incl. Program

May 08–10, 2014
Hannover, Germany

rebirth Cluster of Excellence

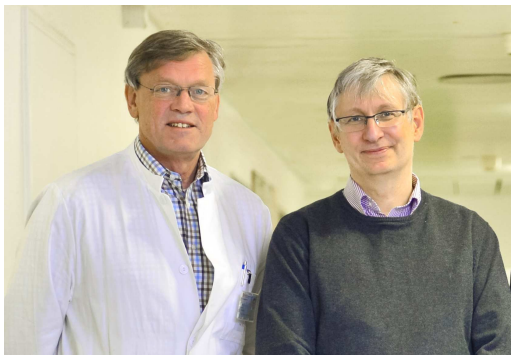
BREATH HANNOVER

DZL Deutsches Zentrum für Lungenforschung

Preface

Dear Guests,

It is our pleasure to welcome you to our symposium! We are very much looking forward to an exciting series of lectures and discussions dealing with the organ lung, its regeneration – and beyond.



The two large scientific networks at Hannover Medical School, BREATH and REBIRTH, are a cornerstone of MHH's external funding. While they could theoretically exist independently, we are very proud of the fact that they actually cover several topics which overlap and involve a number of staff and scientists who work for and in both networks.

During our joint symposium we are going to share a substantial amount of time in joint sessions, concentrating on overarching topics like transplantation, artificial organs and tissue engineering, regenerative therapy and stem cell research.

We are going to hear from exceptionally renowned speakers from around the world as well as from our own young scientists. The balanced mix of young and senior, national and international, basic scientists and clinicians, is the special appeal of this symposium.

While in the case of most others organs, there is a replacement or surrogate device which will, at least for a reasonable amount of time, secure the survival of a patient, this is not the case with the lung. And while other organs have a good amount of self-repair potential, the lung in most cases of serious injury is only capable of pathological repair, resulting in scarred tissue no longer able to fulfill its function.

Therefore, in lung research the hope for the future lies in regenerative science. As in many other cases, we can learn from findings in related fields, like research in the areas of the heart, liver and blood, as we will hear during this symposium.

Two related research initiatives are joining our symposium as cooperating partners: CARPuD (Cellular Approaches for Rare Pulmonary Diseases) and NIFE (Lower Saxony Centre for Biomedical Engineering, Implant Research and Development).

The goal of the partners of the CARPuD network is the development of innovative therapies for the treatment of rare lung diseases like alpha-1-antitrypsin deficiency, cystic fibrosis or surfactant deficiencies, for which, in many cases, lung transplantation remains the only available therapeutic option up to now.

The focus of NIFE lies in the area of implant research. The topics include the development of biocompatible materials for implants, infection biology, imaging, laser medicine and regenerative therapy. NIFE's research spans several organ systems and deals with the whole innovation chain.

Scientists from both cooperating partners will present their work during our symposium, and you are more than welcome to also join their lectures. All together we believe to have brought together the

highest quality of presentations in the area of lung research and regenerative medicine for this symposium.

We would also like to invite you to enjoy the beauty of Hannover's Herrenhausen Palace and Baroque Garden, as well as the New Town Hall in the city center of Hannover where we will hold our symposium dinner on Friday evening.

We wish you all a stimulating meeting,

Tobias Welte and Axel Haverich

Professor Dr. Tobias Welte

Director of BREATH
Deputy Chairman of the German Center for Lung Research

Professor Dr. Dr. Axel Haverich

Coordinator of the REBIRTH Cluster of Excellence
Member of the BREATH Management Board

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Committees & Reviewers

Symposium Presidents

Axel Haverich, Coordinator of the REBIRTH Cluster of Excellence, Member of the BREATH Management Board

Tobias Welte, Director of BREATH, Deputy Chairman of the German Center for Lung Research

BREATH Management Board:

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Axel Haverich, Coordinator of the REBIRTH Cluster of Excellence, Medical Director, Department of Cardiothoracic, Transplantation and Vascular Surgery (HTTG), Hannover Medical School

Ulrich Martin, Research Director, Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO)

German Center for Lung Research (DZL)

German Center for Lung Research e.V. (DZL e.V.)
Chairman and Speaker of the German Center for Lung Research: Werner Seeger
DZL Scientific Officer: Megan Grether

Airway Research Center North (ARCN)
Director: Klaus F. Rabe
DZL Manager ARCN: Jörn Bullwinkel

Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH)
Director: Tobias Welte
DZL Manager BREATH: Annegret Zurawski

Comprehensive Pneumology Center Munich (CPC-M)
Director: Oliver Eickelberg
DZL Manager CPC-M: Antje Brand

Translational Research Center Heidelberg (TLRC-H)
Director: Marcus Mall
DZL Manager TLRC-H: Birgit Teucher

Universities of Giessen and Marburg Lung Center (UGMLC)
Director: Werner Seeger
DZL Manager UGMLC: Sylvia Weissmann

Abstract Reviewers & Program Committee

We would like to thank the following persons for identifying and attracting speakers to our symposiums, and/or for their time and efforts to review abstracts.

Bauersachs, J., REBIRTH
Cantz, T., REBIRTH
Eickelberg, O., CPC-M
Fehrenbach, H., ARCN
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Gruh, I., REBIRTH
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Thomas, M., TLRC-H
Tümmler, B., BREATH
Vogelmeier, C., UGMLC
Von Mutis, E., CPC-M
Wacker, F., BREATH
Warnecke, G., BREATH
Welte, T., BREATH
Zurawski, A., BREATH

Program

	Joint Session DZL/REBIRTHDZL
	DZL Session
	REBIRTH Session
	Poster Session

Thursday, May 8th

	Auditorium	Seminar Room 1	Seminar Room 3	Seminar Room 4
01:00 pm	**Reception**			
01:30 pm	**Welcome:** C. Baum (1:30 pm)			
	Current Status of … (Chair: A. Haverich, M. Gladwin)			
	…Lung Diseases and Regeneration T. Welte (1.40 pm)			
	…Lung Transplantation D. v. Raemdonck (2:00 pm)			
	…Tissue Engineering "Embryonic stem cells for cardiac repair - from basic science to clinical translation" P. Menasché (2:30 pm)			
03:00 pm	**Coffee Break**			
03:30 pm	**Current Status of …** (Chair: T. Welte, M. Ochs)	**Heart 1 - Myocardium:** (Chair: I. Gruh, R. Zweigerdt) R. Zweigerdt (3:30 pm) I. Gruh (3:55 pm) J. Heineke (4:20 pm) D. Hilfiker-Kleiner (4:40 pm)	**Blood-based therapies:** (Chair: A. Schambach, J. Skokowa) A. Schambach (3:30 pm) T. Moritz (3:55 pm) J. Skokowa (4:20 pm) M. Eder (4:40 pm)	**Liver:** (Chair: T. Cantz, M. Ott) Y. Nahmias (3:30 pm) A. Vogel (4:00 pm) A. D. Sharma (4:20 pm) R. Gutierrez (4:40 pm) O. Papp (4:50 pm)
	…Heart and Lung Research "Towards systems medicine in airways disease" I. M. Adcock (3:30 pm)			
	…Heart and Lung Tolerance Studies in Large Animals J. C. Madsen (3:55 pm)			
	…Lung Imaging I: "Amplifying the signals for compensatory lung growth" C. C. W. Hsia (4:20 pm)			
	…Lung Imaging II: "On the potential for lung growth in the adult human" J. P. Butler (4:45- 5:10 pm)			
05:00 pm	**Dinner**			
06:00 pm	**Poster Session I** (odd poster numbers; ballroom, seminar room 2 & foyer)			
07:30 pm	**Speakers Reception** (informal conclusion at palace bar)			

Joint Session (Auditorium, 1:30 – 3:30 pm)

Baum, Christopher
Hannover Medical School, Germany
Welcome

Welte, Tobias
Hannover Medical School, Germany
Current Status of Lung Diseases and Regeneration

van Raemdonck, Dirk
University Hospital Leuven, Belgium
Current Status of Lung Transplantation

Menasché, Philippe
Hôpital Européen Georges Pompidou, France
Current Status of Tissue Engineering: "Embryonic stem cells for cardiac repair - from basic science to clinical translation"

DZL Session (Auditorium, 3:30 – 5:10 pm)

Adcock, Ian M.
Imperial College London, UK
Current Status of Heart and Lung Research: "Towards systems medicine in airways disease"

Madsen, Joren C.
Massachusetts General Hospital, USA
Current Status of Heart and Lung Tolerance Studies in Large Animals

Hsia, Connie C. W.
UT Southwestern Medical Center, USA
Current status of Lung Imaging I: "Amplifying the signals for compensatory lung growth"

Butler, James P.
Harvard Medical School, USA
Current Status of Lung Imaging II: "On the potential for lung growth in the adult human"

REBIRTH: Heart 1 – Myocardium (Seminar Room 1, 3:30 – 5:00 pm)

Zweigerdt, Robert
Hannover Medical School, Germany
Suspension culture and cardiomyogenic differentiation of human pluripotent stem cells in stirred bioreactors

Gruh, Ina
Hannover Medical School, Germany
From pluripotent stem cells to myocardial tissue engineering

Heineke, Jörg
Hannover Medical School, Germany
Paracrine regulation of cardiac hypertrophy and failure

Hilfiker-Kleiner, Denise
Hannover Medical School, Germany
Novel role of STAT3 for substrate metabolism in the heart

REBIRTH: Blood-based therapies (Seminar Room 3, 3:30 – 5:00 pm)

Schambach, Axel
Hannover Medical School, Germany
Retroviral vectors: Fascinating tools for genetic engineering of hematopoietic cells

Moritz, Thomas
Hannover Medical School, Germany
Towards a curative genetic treatment of Pulmonary Alveolar Proteinosis (PAP)

Skokowa, Julia
Hannover Medical School, Germany
New therapeutic strategies for stimulation of granulopoiesis

Eder, Matthias
Hannover Medical School, Germany
miRNAs in hematopoiesis

REBIRTH: Liver (Seminar Room 4, 3:30 – 5:00 pm)

Nahmias, Yaakov
School of Engineering, Faculty of Science, The Hebrew University, Israel
Challenges and opportunities in liver tissue engineering

Vogel, Arndt
Hannover Medical School, Germany
Mechanisms of liver regeneration

Sharma, Amar Deep
Hannover Medical School, Germany
microRNAs in stem cell based liver regeneration

Gutierrez, Rodrigo
Hannover Medical School, Germany
Patient-derived adult liver stem cells

Papp, Oliver
Hannover Medical School, Germany
Stem cells in biliary regeneration

Friday morning, May 9th

	Auditorium	Seminar Room 1	Seminar Room 3	Seminar Room 4	Seminar Room 5
09:00 am	**REBIRTH in Translation 1** (Chair: I. Gruh, K. C. Wollert) **Mining the human bone marrow cell secretome for new therapeutically active proteins** K. C. Wollert (9:00 am) **From heart to lung and back - engineering the path** S. Korossis (9:30 am) **Translation of research results into products from the perspective of a SME** M. Harder (10:00 am)	**Disease Area Asthma & Allergies** (Chair: H. Fehrenbach, G. Hansen) P. S. Hiemstra (9:00 am) T. Roeder (9:35 am) S. Webering (10:00 am) A. Lorenz (10:15 am)	**Disease Area Diffuse Parenchymal Lung Disease** (Chair: O. Eickelberg, A. Guenther) A. Giangreco (9:00 am) M. Königshoff (9:35 am) S. Bellusci (10:00 am) L. Knudsen (10:15 am)	**Disease Area Cystic Fibrosis** (Chair: M. Mall, B. Tümmler) J. M. Beekmann (9:00 am) L. Wiehlmann (9:45 am) R. Agrawal (10:00 am) S. Gräber (10:15 am)	**Disease Area Endstage Lung Disease** (Chair: R. Hatz, A. Haverich) S. Jockenhövel (9:00 am) U. Martin (9:35 am) A. Zakrzewicz (10:00 am) K. Jansson (10:15 am)
10:30 am	**Coffee Break**				
11:00 am	**Panel Discussion: What the industry wants from science - and vice versa** (Chair: T. Welte, Moderator: M. Grether) A. Biedermann M. Braddock P. Nicklin W. Seibold C. Stein R. Herzog M. Stein-Gerlach A. Haverich N. Krug W. Seeger	**Heart 2 - Valves & Vessels** (Chair: S. Cebotari, A. Hilfiker) A. Hilfiker (11:00 am) W. Wolkers (11:30 am) F. Bengel (12:00 am)	**Blood & Immunology** (Chair: A. Krueger, R. Stripecke) R. Stripecke (11:00 am) C. Figueiredo (11:25 am) A. Krueger (11:50 am) C. Guzmán (12:10 am)	**CARPuD** (Chair: T. Cantz, G. Hansen) B. J. Scholte (11:00 am) U. Martin (11:30 am) R. Eggenschwiler (11:55 am) N. Lachmann (12:10 am) C. Happle (12:20 am)	**NIFE Pooling Resources - Creating Synergies** (Chair: M. Elff, M. Stiesch) M. Elff (11:00 am) M. Stiesch (11:25 am) A. Kirschning (11:50 am) H. Meyer (12:10 am)
12:30 am	**Lunch Break**				

REBIRTH in Translation 1 (Auditorium, 9:00 – 10:30 am)

Wollert, Kai C.
Hannover Medical School, Germany
Mining the human bone marrow cell secretome for new therapeutically active proteins

Korossis, Sotirios
Hannover Medical School, Germany
From heart to lung and back: engineering the path

Harder, Michael
corlife GbR, Germany
Translation of research results into products from the perspective of an SME

DZL: Asthma & Allergies (Seminar Room 1, 9:00 – 10:30 am)

Hiemstra, Pieter S.
Leiden University, The Netherlands
Airway epithelial repair and differentiation and the effect of cigarette smoke exposure

Roeder, Thomas
Christian-Albrechts-Universität Kiel, Germany
Deregulation of ORMDL3 expression induces stress responses and modulates repair pathways

Webering, Sina
Research Center Borstel, Germany
RORγt-specific RNAi decreases allergic airway inflammation and airway hyperresponsiveness in a mouse model of neutrophilic asthma

Lorenz, Annika
Hannover Medical School, Germany
Investigating the role of BAFF in different mouse models of allergic asthma

DZL: Diffuse Parenchymal Lung Disease (Seminar Room 3, 9:00 – 10:30 am)

Giangreco, Adam
University College London, UK
How intrinsic cell signaling regulates airway regeneration

Königshoff, Melanie
Comprehensive Pneumology Center, Großhadern, Germany
Developmental signal pathways in pulmonary fibrosis

Bellusci, Saverio
Justus-Liebig University Giessen, Germany
Interplay of FGF and Wnt signaling in regulating mesenchymal progenitor cell lineage formation during lung development and repair after injury

Knudsen, Lars
Hannover Medical School, Germany
Alveolar derecruitment and collapse induration as crucial mechanisms in lung injury and fibrosis

DZL: Cystic Fibrosis (Seminar Room 4, 9:00 – 10:30 am)

Beekman, Jeffrey M.
University Medical Center Utrecht, The Netherlands
Stem cell derived organoids to test new therapies in CF

Wiehlmann, Lutz
Hannover Medical School, Germany
Population biology of chronic airway infections with pseudomonas aeruginosa with CF and COPD

Agrawal, Raman
University of Heidelberg, Germany
Silencing of miR-148b ameliorates cystic fibrosis-like lung diseases in βENaC-overexpressing mice

Gräber, Simon
University of Heidelberg, Germany
ICM is sensitive to detect potentiation of CFTR-mediated Cl- secretion in patients with cystic fibrosis and the G551D mutation treated with ivacaftor

DZL: Endstage Lung Disease (Seminar Room 5, 9:00 – 10:30 am)

Jockenhövel, Stefan
Helmholtz Institute of RWTH Aachen University & Hospital, Germany
Respiratory Tissue Engineering – Concepts & Biomaterials

Martin, Ulrich
Hannover Medical School, Germany
iPS derivatives for treatment of respiratory diseases

Zakrzewicz, Anna
Justus-Liebig-University Giessen, Germany
Interleukin 18 in the pathogenesis of experimental bronchiolitis obliterans syndrome (BOS)

Jansson, Katharina
Hannover Medical School, German, y
Treatment with donor specific alloantigen before or on the day of lung transplantation in a large animal model

DZL: Panel Discussion (Auditorium, 11:00 – 12:30 am)

Welte, Tobias
Hannover Medical School, Germany

Grether, Megan
DZL e.V.

Biedermann, Alexander
AstraZeneca, Germany

Braddock, Martin
AstraZeneca, UK

Nicklin, Paul
Boehringer Ingelheim Pharma GmbH & Co. KG, Germany

Seibold, Wolfgang
Boehringer Ingelheim Pharma GmbH & Co. KG, Germany

Herzog, Ruth
German Cancer Research Center (DKFZ) , Germany

Stein, Christian
Ascenion, Germany

Stein-Gerlach, Matthias
Max-Planck-Innovation, Germany

Haverich, Axel
Hannover Medical School, Germany

Krug, Norbert
Fraunhofer ITEM, Germany

Seeger, Werner
Justus-Liebig-University Giessen, Germany

REBIRTH: Heart 2 – Valves & Vessels (Seminar Room 1, 11:00 – 12:30 am)

Hilfiker, Andres
Hannover Medical School, Germany
Decellularized heart valve matrices for tissue engineering

Wolkers, Willem
Leibniz University of Hannover, Germany
Preservation of heart valve scaffolds in a dry state

Bengel, Frank
Hannover Medical School, Germany
Molecular in vivo imaging of the cardiovascular system

REBIRTH: Blood & Immunology (Seminar Room 3, 11:00 – 12:30 am)

Stripecke, Renata
Hannover Medical School, Germany
Adaptive human immune regeneration in mice: models for preclinical testing of immune therapies

Figueiredo, Constanca
Hannover Medical School, Germany
In vitro production of HLA universal platelets

Krueger, Andreas
Hannover Medical School, Germany
Micro(RNA)-management of lymphocyte development

Guzmán, Carlos
Helmholtz Centre for Infection Research, Germany
Use of adjuvants with well-defined molecular targets to tailor innate and adaptive immune responses

REBIRTH: CARPuD (Seminar Room 4, 11:00 – 12:30 am)

Scholte, Bob J.
Erasmus MC Cell Biology, The Netherlands
Animal and cellular models of chronic lung disease, prospects of gene and cellular therapy

Martin, Ulrich
Hannover Medical School, Germany
iPS cell-derived transplants for cystic fibrosis and surfactant deficiencies

Eggenschwiler, Reto
Hannover Medical School, Germany
iPS cell-derived transplants for α-1-antitrypsin deficiency

Lachmann, Nico
Hannover Medical School, Germany
Intratracheal transplantation of macrophages as novel pulmonary cell therapy

Happle, Christine
Hannover Medical School, Germany
Intratracheal transplantation of macrophages for pulmonary diseases

REBIRTH: NIFE: Pooling Resources - Creating Synergies (Seminar Room 5, 11:00 –12:30 am)

Elff, Manfred
Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Germany
NIFE - A translational research center

Stiesch, Meike
Hannover Medical School, Germany
Biofabrication for NIFE

Kirschning, Andreas
Leibniz University of Hannover, Germany
Preparation and functionalization of biomedical materials – applications for extracorporeal lung devices

Meyer, Heiko
Laser Zentrum Hannover, Germany
Novel laser based imaging techniques and applications

Friday afternoon, May 9th

	Auditorium	Seminar Room 1	Seminar Room 3	Seminar Room 4	Seminar Room 5
01:30 pm	**Poster Session II** (even poster numbers; ballroom, seminar room 2 & foyer)				
03:00 pm	**Coffee Break**				
03:30 pm	**REBIRTH in Translation 2** (Chair: M. Graf, U. Martin)	**Disease Area Chronic Obstructive Lung Disease** (Chair: J. Hohlfeld, K. Rabe) W. E. Fibbe (3:30 pm) A. Pichl (4:10 pm) E. Frenzel (4:30 pm) S. Seehase (4:45 pm)	**Disease Area Pulmonary Hypertension** (Chair: D. Jonigk, W. Seeger) M. Gladwin (3:30 pm) G. Warnecke (4:10 pm) M. Gierhardt (4:40 pm)	**Disease Area Lung Cancer** (Chair: U. Klingmüller, M. Thomas) R. Rosell (3:30 pm) S. Depner (4:15 pm) O. Ammerpohl (4:30 pm) A. Tufman (4:45 pm)	**Disease Area Acute Lung Injury** (Chair: J. Lohmeyer, U. A. Maus) D. Weiss (3:30 pm) R. Morty (4:10 pm) J. Quantius (4:40 pm)
	Stem cell applications in a pharma company M. Graf (3:30 pm)				
	Innovative health technologies: legal and ethical aspects of translating research into practice N. Hoppe (4:00 pm)				
	From innovation to reimbursement: how are new treatments introduced into the German health care system M. Perleth (4:30 - 5:00 pm)				
07:00 pm	**Congress Dinner and Party "Der Gartensaal" New Town Hall** (pre-registration required)				

REBIRTH in Translation 2 (Auditorium, 3:30 – 5:00 pm)

Graf, Martin
Hoffmann-La Roche AG, Switzerland
Stem cell applications in a pharma company

Hoppe, Nils
Leibniz University of Hannover, Germany
Innovative health technologies: legal and ethical aspects of translating research into practice

Perleth, Matthias
Federal Joint Committee (Gemeinsamer Bundesausschuss - G-BA), Germany
From innovation to reimbursement: how are new treatments introduced into the German health care system

DZL: Chronic Obstructive Lung Disease (Seminar Room 1, 3:30 – 5:00 pm)

Fibbe, Willem E.
Leiden University, The Netherlands
Control of inflammation by mesenchymal stromal cells

Pichl, Alexandra
Justus-Liebig-University Giessen, Germany
The soluble guanylate cyclase in smoke-induced lung emphysema

Frenzel, Eileen
Hannover Medical School, Germany
Acute phase protein α1-antitrypsin - a novel regulator of angiopoietin-like protein 4 transcription and secretion

Seehase, Sophie
Research Center Borstel, Germany
Alveolar epithelial cells type II show a high sensitivity to cigarette smoke extract

DZL: Pulmonary Hypertension (Seminar Room 3, 3:30 – 5:00 pm)

Gladwin, Mark
University of Pittsburgh, USA
Development of a rat model of metabolic syndrome related Group II PH and therapy with nitrite and metformin

Warnecke, Gregor
Hannover Medical School, Germany
Lung transplantation for severe pulmonary hypertension – Awake ECMO for postoperative left ventricular remodelling

Gierhardt, Mareike
Justus-Liebig-University Giessen, Germany
P66shc deficient mice developed decreased right heart hypertrophy via a Cyclophilin D dependent mechanism in hypoxia-induced pulmonary hypertension

DZL: Lung Cancer (Seminar Room 4, 3:30 – 5:00 pm)

Rosell, Rafael
Autonomous University of Barcelona, Spain
Molecular mechanisms within bronchial carcinoma

Depner, Sofia
German Cancer Research Center (DKFZ), Germany
Role of BAMBI in the regulation of EMT processes in human lung cancer cell lines

Ammerpohl, Ole
University Clinic Schleswig-Holstein, Germany
Aberrant DNA methylation patterns in lung cancer

Tufman, Amanda
Ludwig-Maximilian-University Munich, Germany
Individualising radiochemotherapy in NSCLC

DZL: Acute Lung Injury (Seminar Room 5, 3:30 – 5:00 pm)

Weiss, Daniel
University of Vermont, USA
Stem cell therapy in the case of acute lung injury

Morty, Rory
Justus-Liebig-University Giessen, Germany
TGF-ß directs trafficking of the epithelial sodium channel ENaC which has implications for ion and fluid transport in acute lung injury

Quantius, Jennifer
University of Giessen Lung Center, Germany
Influenza virus impairs fibroblast growth factor receptor 2b dependent epithelial regeneration from a distal airway epithelial progenitor pool

Saturday, May 10th Program & List of Speakers

	Auditorium
	Beyond Transplantation (Chair: K. Rabe, T. Welte)
09:00	**Endogenous Regenerative Therapy:** W. Seeger
09:30	**A Patient's an Politicians View on Transplantation:** G. Günther
10:00	**Generation of anterior foregut derivatives from human pluripotent stem cells:** H.-W. Snoeck
10:30	**Coffee Break**
	Beyond Transplantation (Chair: U. Martin)
11:00	**(Pluripotent) Stem cell therapy related to lung diseases:** A. Wong
11:30	**Pathways regulating lung stem cells and regeneration:** E. Morrisey
12:00	**Bio Implants:** A. Haverich
12:25	**Wrap-Up:** T. Welte, W. Seeger, A. Haverich
12:30	**Dinner**

Joint Session

Seeger, Werner
Justus-Liebig-University Giessen, Germany
Endogenous regenerative therapy

Günther, Gerhard
Member of the State Parliament of Thüringen
A patient's and politician's view on transplantation

Snoeck, Hans-Willem
Columbia University, New York, USA
Generation of anterior foregut derivatives from human pluripotent stem cells

Wong, Amy
The Hospital for Sick Children, Toronto, Canada
Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein

Morrisey, Edward
University of Pennsylvania, USA
Pathways regulating lung stem cells and regeneration

Haverich, Axel
Hannover Medical School, Germany
Bio Implants

Welte, Tobias
Hannover Medical School, Germany
Wrap-Up

General Information

Conference Office

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E-Mail: Symposium2014@mh-hannover.de

Symposium Venue

We are very much looking forward to welcoming you in Hannover's newly restored historical Herrenhausen Palace, surrounded by its beautiful baroque gardens.

Schloss Herrenhausen
Herrenhäuser Straße 5
30419 Hannover, Germany

www.schloss-herrenhausen.de

Access to the Baroque Gardens

The Herrenhausen Gardens are an internationally famous ensemble of garden arts and culture that rank among the most important historical gardens in Europe.

Aside from the symposium, we invite you to visit the beautiful Herrenhausen Gardens as well as the associated museum.

Tickets are available free of charge at the reception desk during the following coffee breaks:

Thursday, May 8th	03:00 - 03:30 pm
Friday, May 9th	10:30 - 11:00 am
Friday, May 9th	03:00 - 03:30 pm

WiFi Internet Access at the Symposium Venue

WiFi Internet Access at the Herrenhausen Palace is available free of charge during the whole symposium. For login information please ask at the organizers' desk.

Poster Session

The scientists will present their posters in joint DZL/REBIRTH sessions in the ballroom, seminar room 2 and in the foyer.

Poster Session I (odd poster numbers)	Thursday, May 8th	18:00-19:30 pm
Poster Session II (even poster numbers)	Friday, May 9th	13:30-15:00 pm

Posters #1 – 20:	foyer (next to the auditorium)
Posters #21 – 40:	seminar room 2 (ground floor)
Posters #41 – 116:	ballroom

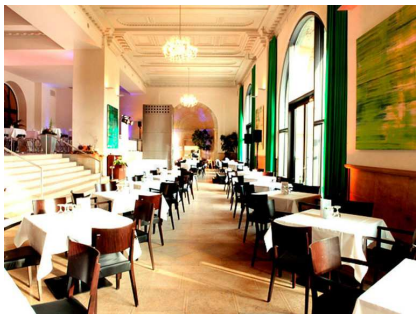
Evening Event "Der Gartensaal"

The congress dinner and party will take place at Hannover's New Town Hall on Friday, May 9th in the "Gartensaal" restaurant, starting at 19:00.

Registration prior to the symposium was required. A limited number of tickets are, however, still available at the organizers' desk for 25 € (contribution towards expenses).

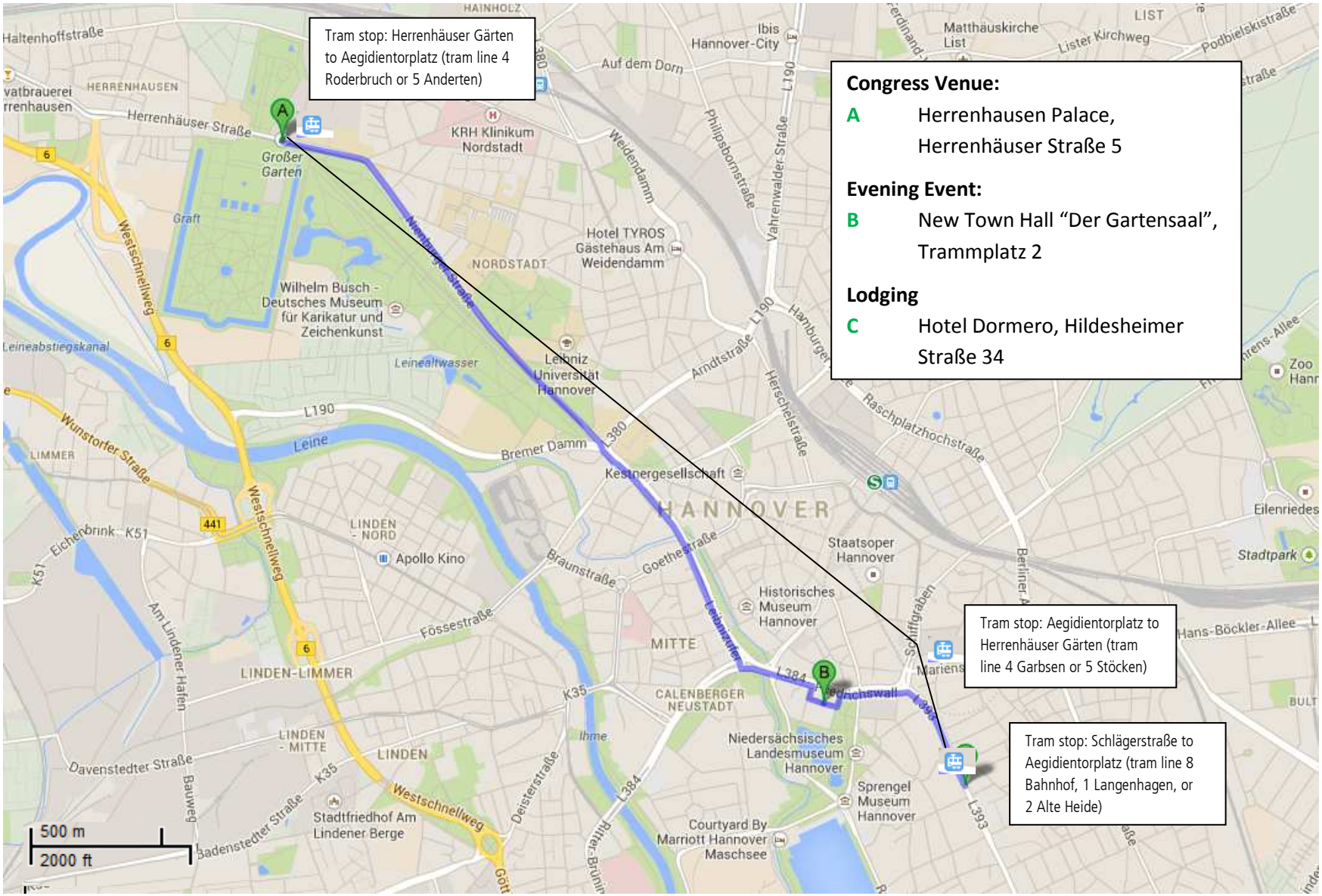
Address:
Restaurant "Der Gartensaal"
Trammplatz 2
30159 Hannover
Tel.: +49 511 168-45874

The New Town Hall is within walking distance of the tram stop "Aegidientorplatz".



Certification (ÄKN)

The State Medical Chamber of Lower Saxony granted 16 credits for the conference. Please come to the registration desk to receive your certification.



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Abstracts of oral presentations

Joint Session (Auditorium, 1.30 – 3:00 pm)

Current Status of Lung Diseases and Lung Regeneration

Tobias Welte[1, *]

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Lung diseases besides cardiovascular and malignant diseases are among the three major disease groups worldwide. In perspective they will advance to the top within the next 10 years.
Lung diseases can be divided into proliferative – lung cancer, interstitial lung disease, pulmonary hypertension - or degenerative diseases - COPD and in particular pulmonary emphysema.
During the last decade it has become possible to stop the progression of some of this diseases, however, causal therapies that lead to cure of the underlying disease have not been developed. Only in patients with cystic fibrosis the baseline defect could be corrected by treatment with new pharmacological compounds, so cure can be achieved for patients with one special mutation of the baseline defect. For the other progressive pulmonary diseases, lung transplantation is the only therapeutic option for patients progressing to respiratory insufficiency. In Germany, nearly half a million patients with end-stage lung disease are estimated, which is a huge number compared to nearly 400 lung transplants per year actually performed in the country .

Two routes could help to improve this imbalance: on one hand the development (technologically, humanization of artificial membranes) of extracorporeal systems, which are today usable only for a limited time, into a chronic organ replacement therapy. On the other hand stem cell-based therapy could create functional cell and tissue structures to correct genetic defects and replace destroyed lung tissue. For both options, there are promising initial data, but also more open questions than answers.

Current Status of Lung Transplantation

Dirk Van Raemdonck[1, 2, *], *Geert M Verleden*[3, 4]

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*Presenting author

Lung transplantation (LTx) has come of age [1]. Since the introduction of successful LTx nearly three decades ago, this treatment has now become an effective and safe therapy for selected patients suffering from a variety of end-stage pulmonary diseases offering a prolonged and improved quality of life. Survival in recent years is expected to be around 90%, 80%, 70%, and 50% at 1, 3, 5, and 10 years after the procedure. Three major obstacles that remain limiting its clinical usefulness and success as standard therapy for more patients are 1) donor organ shortage, 2) primary graft dysfunction, and 3) chronic allograft dysfunction.

1) Alternative sources to expand the limited donor pool [2] such as the use of lungs from donors after circulatory arrest [3, 4] and ex-vivo lung perfusion for assessment of questionable and reconditioning of

unacceptable pulmonary grafts [5], are emerging strategies. The use of ventilatory and, more recently, circulatory support with extracorporeal membrane oxygenation (ECMO) serve as a "bridge" to LTx in the management of patients with rapidly advancing (cardio)respiratory failure until a suitable donor becomes available.

2) Severe primary graft dysfunction (PGD3) in the first 72 hours after LTx remains a feared complication in about 30% of recipients. Newer preservation strategies such as normothermic lung preservation is hoped to significantly reduce the incidence of PGD with less early morbidity [5, 6]. The use of ECMO post-LTx has emerged as a prophylactic strategy in recipients with significant pulmonary arterial hypertension and serves as a rescue therapy for patients confronted with PGD3 with increasing success.

3) Chronic lung allograft dysfunction (CLAD) is the major limiting factor for long-term survival. Several phenotypes have been identified recently [7]. Macrolide treatment is effective in patients with the neutrophilic phenotype of chronic allograft dysfunction while retransplantation is the only effective therapy for patients with obstructive bronchiolitis (BOS) and restrictive allograft (RAS) syndromes.

Further research is needed to find new ways to create bioartificial organs and to induce selective immune tolerance to accept the allograft in the absence of toxic immunosuppressants [8]. These two steps would allow LTx to become an effective and safe therapy for many more patients suffering from end-stage pulmonary disease.

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Embryonic stem cells for cardiac repair - from basic science to clinical translation

Philippe Menasché[1,*]

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*Presenting author

The rationale for the use of embryonic stem cells (ESC) in patients with heart failure primarily stems from the assumption that regeneration of scarred myocardium likely requires the supply of cells endowed with a true cardiomyogenic differentiation potential, regardless of whether they act by generating a new myocardial tissue by themselves or by harnessing endogenous repair pathways. This approach is made possible by the intrinsic pluripotentiality of ESC which allows to drive their fate *in vitro* towards a cardiac lineage and, so far, our approach has been to generate early SSEA-1-positive cardiac progenitors (rather than fully mature cardiomyocytes) with the assumption that the transplanted progenitors would use local cues to instruct them to differentiate into cardiomyocytes and vascular cells. The experimental results have so far been promising as these cells have been shown to differentiate into cardiomyocytes and to improve heart function, including in a clinically relevant scenario of allogeneic transplantation in nonhuman primates. Asides from ethical issues, the clinical translation of this ESC-based program has entailed a stepwise approach including the following steps : (1) the expansion of a clone of pluripotent hESC to generate a master cell bank under Good Manufacturing Practice conditions (GMP); (2) a growth factor-induced cardiac specification; (3) the purification of committed cells by immunomagnetic sorting to yield a SSEA-1-positive cell population strongly expressing the early cardiac transcription factor *Isl-1*; (4) the incorporation of these cells into a fibrin scaffold and the assessment of the functional benefits of this cell construct; (5) a safety assessment focused on the loss of teratoma-forming cells by *in vitro* (transcriptomics) and *in vivo* (cell injections in immunodeficient mice) measurements; (6) an extensive cytogenetic and viral testing; and (7) the characterization of the final cell product and its release criteria. Put together, these data have led to an approval for a first-in-man clinical trial of transplantation of these SSEA-1^+ progenitors in patients with severely impaired cardiac function.

DZL Plenary Session: Current Status of … (Auditorium, 3:30 – 5:15 pm)

Current status of heart & lung disease: Towards systems medicine in airways disease

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*Presenting author

Airways diseases affect over 500m people worldwide and have a huge cost to public health and productivity across all age groups. It has become increasingly clear that asthma and COPD for example are not single diseases but syndromes and that better treatments and/or cures will necessitate closer understanding of the differences in these patient subtypes. Initial clustering of asthma has been performed using clinical variables but it is clear that clustering according to patterns of different 'omic analysis will be necessary to discern molecular phenotypes and thereby discover pathways that drive disease in individual patients. Data will be presented from the U-BIOPRED consortia on severe asthma on how topological analysis may be used to integrate complex datasets and derive new phenotypic clusters of patients with severe asthma. In addition, concepts from this approach may highlight new areas of research into other chronic airways diseases such as COPD and into other areas such as heart disease.

Current Status of Heart and Lung Tolerance Studies in Large Animals

Joren C. Madsen[1,*]

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*Presenting author

Tolerance of kidney allografts has been achieved in non-human primates (NHPs) and in humans using a combination of non-myeloablative conditioning and donor bone marrow transplantation (DMBT) that results in transient donor chimerism. However, mixed-chimerism protocols that achieve long-term tolerance of kidney allografts in NHPs have consistently failed to induce tolerance in recipients of heart or lung allografts. It is well known that some organs, such as kidney and liver, are tolerance-prone while others, such as heart and lung, are tolerance-resistant. It has been hypothesized that only protocols that result in durable donor chimerism would be able to achieve tolerance in resistant heart and lung allografts. However, our recent results demonstrate, for the first time, that tolerance of heart and lung allografts can be achieved in NHPs via transient mixed-chimerism by enhancing the contributions of host regulatory mechanisms. Indeed, our results suggest that it is the ability of a mixed-chimerism protocol to augment or expand regulatory T cells (Tregs), rather than its durability, that determines its ability to induce tolerance of resistant organs. Thus, our current studies seek to combine mixed chimerism with novel strategies designed to amplify the contributions of Tregs in order develop clinical tolerance protocols that can be rapidly translated to human recipients of heart and lung allografts.

Amplifying Compensatory Lung Growth

Connie C. W. Hsia[1,*]

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*Presenting author

Pneumonectomy (PNX), a robust model that mimics the consequences of destructive lung disease, is often employed to study the adaptive mechanisms of the remaining functioning lung units. Following PNX, the remaining alveoli and capillaries experience increased mechanical stress resulting from lobar expansion and the redirection of pulmonary perfusion. Mechanical stress is thought to increase tissue permeability and ion flux, and transduce multiple adaptive pathways leading to the accelerated gain in alveolar tissue and capillary volumes as well as surface areas, thereby augmenting the gas exchange capacity of the remaining lung units. Because the lung must fit within its thoracic container, both developmental and post-PNX lung growth are inherently dependent on the physical interactions between the thorax and the lung parenchyma. In the highly stratified large mammalian lung, post-PNX lung growth is characterized by initial cellular proliferation and/or hypertrophy and progressive scaffold remodeling, leading to delayed functional compensation that span many months. The prolonged time course of compensation is essential for minimizing structural distortion and maximizing functional gain. This presentation will summarize the use of imaging techniques to study the fundamental signals, mechanisms, and limits of compensatory lung growth as well as the various interventional approaches that have been employed to amplify the endogenous signals for alveolar re-growth.

On the potential for lung growth in the adult human

James P. Butler[1,2*]

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[2]Department of Environmental Health, Harvard School of Public Health, Boston, MA. USA
*Presenting author

Conventional wisdom holds that lung regeneration post-pneumonectomy does not occur in adult humans. We have evidence in a post pneumonectomy patient that contradicts this, based on (1) serial measurements of lung and tissue volumes from computerized tomographic (CT) images, and (2) measurements of regional lung microstructure (acinar radial-axial length scale **R** and alveolar depth **h**) using hyperpolarized ^{3}He magnetic resonance imaging (MRI). The patient was studied over 15y following right pneumonectomy at age 35y.

Spirometry and CT: Post-pneumonectomy, the patient's forced expired volume in 1s (FEV1) fell from 2.92 to 1.03L (99.7% to 35% predicted); Forced vital capacity (FVC) fell from 3.69 to 1.75L (104% to 45% predicted). Over 15y, there was a progressive increase in FEV_1 (to 60% predicted) and FVC (to 73% predicted). Compliance (0.135-0.150 L/cmH_2O) and resistance (3.5-6.0 cmH_2O/(L/s)) for the remaining lung at 1y and 14y were normal. Serial CT scans showed a rapid rise in gas and total lung volumes, and a near-doubling of tissue volume.

MRI: 15y post-pneumonectomy, acinar dimensions (**R**=330±20 μm (mean±SD)), were normal (**R**=322±21 μm, mean±intra-individual SD). Importantly, **R** was strikingly uniform—varying by ~5% over the entire remaining lung. With increased volume, the normal value of **R** implies a 64% increase in alveolar number. Alveolar depth (**h**) was 70±30 μm, significantly less than and more heterogeneous than normal (138±22 μm).

Absent lung growth, the lung's shape stability predicts an increase in acinar dimensions in the herniating lung. The observations of (1): the uniformity and near-normal values of acinar dimensions in the herniating region, and (2): the increase in lung and tissue volumes are inconsistent with simple hyperexpansion, and support the hypothesis that true lung growth can occur in an adult human. The patient's vigorous exercise program invites hypotheses on the importance of lung stretch in promoting lung growth.

Support: NIH HL094567, HL096471, HL070037, HL054885, HL52586

REBIRTH: Heart 1 – Myocardium (Seminar Room 1, 3:30-5:00 pm)

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

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

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[2]Biomedical Research in Endstage and Obstructive Lung Disease (BREATH), Member of the German Center for Lung Research (DZL), Hannover

*Presenting author

Human pluripotent stem cells (hPSC) present an attractive source to generate large amounts of lineage specific progenies for innovative cell therapies, tissue engineering, in vitro disease modelling and drug screening assays. The therapeutic and industrial applications of hPSCs will require large cell quantities generated in defined conditions. We have recently established single cell inoculated suspension cultures of hPSCs (Zweigerdt et al., Nature Prot. 2011) which form aggregates in stirred tank reactors (Olmer et al., Tissue Eng. 2012) in the defined medium mTeSRTM (STEMCELL Technologies). This work enabled the translation of conventional, adherence-dependent "2D" culture of hPSC to "3D" suspension culture. Since stirred tank bioreactors allow straightforward up scaling and comprehensive monitoring of process parameters these systems are widely used for the mass culture of conventional mammalian cell lines. Application of stirred reactors to hPSC culture, however, is in its infancy. Aiming at low

medium consumption but integration of all probes relevant for process monitoring (pO2, pH, biomass) a parallel "mini bioreactor system" consisting of individually controlled vessels (DASGIP / Eppendorf) was utilized. After establishing stirring-controlled aggregate formation up to 2x108 hiPSCs were generated per run in 100 ml culture scale applying batch-feeding. Yet, only linear cell growth was achieved suggesting suboptimal process conditions. Here we will present how perfusion-feeding results in substantially improved process characteristics and hPS cell yields. Expanded cells were directly used for the efficient differentiation into cardiomyocytes in stirred tank reactors in a chemically defined medium resulting >80% cardiomyocyte purity without additional lineage enrichment. Technical modifications of the bioreactor system will be highlighted including: impeller design, online biomass sensor integration, establishing a cell retention system and utilization of disposable bioreactor vessel (BioBLU® 0.3) combined with the DASbox® culture control system.

From pluripotent stem cells to myocardial tissue engineering

Ina Gruh[1,*]

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*Presenting author

Myocardial tissue engineering (MTE) strategies may provide new therapeutic options for the treatment of cardiovascular disease. MTE involves the generation and cultivation of appropriate cell sources, the design of adequate extracellular matrices (ECMs), as well as the development of bioreactor and transplantation strategies. Pluripotent stem cells (PSCs), i.e. embryonic stem cells (ESCs) and induced PSCs (iPSCs) hold the capability of differentiating into cells of all three germ layers, including cardiomyocytes (CMs). Recent advance in reprogramming technology including generation of transgene-free iPSCs raised expectations on the clinical application of iPSCs in the foreseeable future.

On our way from pluripotent stem cells to myocardial tissue engineering, we developed an agarose microwell approach to reproducibly aggregate and differentiate human and murine ESCs and iPSCs. A defined small-molecule based protocol was used for human PSC differentiation, resulting in high cardiomyogenic efficiency. Antibiotic selection of transgenic cell lines enabled us to efficiently purify CMs (>99%) from ESCs, which were used as non-dissociated „cardiac bodies" for the generation of bioartificial cardiac tissue (BCT).

The functional assembly of strong BCTs in vitro requires chemical and mechanical stimulation. We identified four different parameters critically affecting the formation of BCTs from murine and human PSC-derived CMs: i) use of purified CMs in cardiac body aggregates, ii) the addition of fibroblasts, iii) supplementation with ascorbic acid and iv) application of growing stretch. We were able to generate BCTs from human ESCs and iPSC displaying so far unparalleled forces (4.4 mN/mm2). Other stimulation strategies including optogenetic control of BCT contractility are currently under investigation.

Together with progress concerning patient-specific iPSC generation and their clinical applicability, our findings could facilitate the generation of autologous bioartificial vascularized cardiac tissue from human iPSCs for future tissue replacement therapy of damaged and diseased myocardium.

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Novel role of STAT3 for substrate metabolism in the heart

Britta Stapel[1], Michael Kohlhaas[2], Melanie Ricke-Hoch[1], Arash Haghikia[1], Sergej Erschow[1], Philipp Fischer[1], Juhani Knuuti[3], Johanna Silvola[3], Anna Roivainen[3], Matti Jauhiainen[4], J. Bankstahl[5], M. Scherr[6], F. Bengel[5], Christoph Maack[2] and Denise Hilfiker-Kleiner[1,]*

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*Presenting author

Enhanced neurohumoral activation has been suggested to drive heart failure in ischemic and dilated cardiomyopathy. Expression and activation of the transcription factor STAT3 is reduced in failing human hearts. Mice with a cardiomyocyte-restricted deletion of STAT3 (*aMHC-Cre$^{tg/+;}$STAT3$^{flox/flox}$*, CKO) display normal cardiac function up to 4 months of age but develop heart failure later on. We explored the relevance of STAT3 for adaptive mechanisms in response to neurohumoral stress in the heart.

Three months old male CKO and wildtype mice (*STAT3$^{flox/flox}$*, WT) were treated with angiotensin II (AngII), with the a-adrenergic agonist phenylephrine (PE), with the b-adrenergic agonist isoproterenol (Iso:) or with NaCl via osmotic minipumps for 2 weeks.

Cardiac function, hypertrophy and survival were similar in WT and CKO in response to AngII and PE. Iso caused increased inflammation, myocyte necrosis, reduced LV function and poor survival in CKO compared to WT that could be rescued by the b-blockers metoprolol or nebivolol. Attenuation of oxidative stress by the MnSOD mimetic TEMPOL or inhibition of the mitochondrial permeability transition pore by cyclosporine A provided no rescue.

Iso-treatment decreased fatty acid uptake and content to a similar degree in CKO and WT hearts. Cardiac glucose uptake was comparable in both genotypes but total pyruvate and PDH activity were lower in Iso-treated CKO compared to WT mice. This was associated with a lower energy status (ADP/ATP ratio) albeit respiratory chain activity in isolated mitochondria was normal. Pharmacological increase in glycolysis by Perhexiline (PER) or Etomoxir (ETO) or treatment with Ethyl pyruvate partially rescued LV function and survival of Iso-treated CKO mice. Further molecular analysis showed substantially decreased expression of ErbB4, which is at least in part responsible for the decrease in glycolysis in Iso-treated CKO mice.

In conclusion, cardiac STAT3 is necessary for cardiac protection against chronic b-adrenergic stress; this is independent of its classical role in anti-oxidative defense and disposable for protection against AngII and PE treatment. b-adrenergic stimulation rapidly depletes the heart from fatty acid as an energy

substrate and a novel circuit involving STAT3 and ErbB4 signaling seems to be necessary to maintain glycolysis as the major energy source under this condition. This finding may explain the adverse effects of catecholamine treatment in patients with chronic heart failure.

REBIRTH: Blood-based Therapies (Seminar Room 3, 3:30-5:00 pm)

Retroviral vectors: Fascinating tools for genetic engineering of hematopoietic and pluripotent cells

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*Presenting author

Retroviral/lentiviral vectors have demonstrated a promising track record for genetic modifications in experimental systems and more importantly in clinical gene therapy studies, e.g. for the treatment of severe combined immunodeficiency. However, unfortunately also side effects related to the integrating nature of retroviral vectors were observed. This has been attributed to the integration preference of the underlying retrovirus family member as well as the load of regulatory elements, e.g. promoter/enhancer sequences.

Within this talk, we will exemplify how safer integrating vectors with clinical perspectives can be designed and how one can also take advantage of retroviral intermediates to efficiently mediate transient gene expression. Furthermore, we will give an overview about the fascinating options of retroviral tools for the generation and genetic modification of induced pluripotent stem cells (iPS).

In summary, rational changes in vector design can help and contribute to improve the risk-benefit assessment of retroviral vectors and to develop useful tools for applications in gene therapy and regenerative medicine.

Towards a Curative Genetic Treatment Approach for Pulmonary Alveolar Proteinosis (PAP)

Thomas Moritz[1,*], *Christine Happle*[2], *Nico Lachmann*[1], and *Gesine Hansen*[2]

[1]Reprogramming and Gene Therapy Group, REBIRTH Cluster of Excellence, Institute of Experimental Haematology, Hannover
[2]Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover
*Presenting author

We here introduce the concept of intratracheal transplantation of macrophage progenitors (pulmonary cell transplantation; PCT) as a novel, cause-directed, and well-tolerated therapy for hereditary pulmonary alveolar proteinosis (herPAP). HerPAP constitutes a rare lung disease caused by mutations in the granulocyte/macrophage-colony-stimulating factor (GM-CSF) receptor genes (CSF2RA or CSF2RB), resulting in disturbed alveolar macrophage (AM) differentiation, massive alveolar proteinosis, and life-

threatening respiratory insufficiency. So far, treatment is symptomatic only including repetitive whole lung lavage in general anesthesia.

We provide evidence from two murine disease models, that PCT of murine or human macrophage progenitor cells yields efficient pulmonar engraftment associated with significant improvement of clinical and functional parameters for more than nine months. On the way to a gene therapy approach for herPAP employing PST we already have generated SIN-lentiviral constructs expressing the human CSF2RA-cDNA (Lv.EFS.CSF2RA.EGFP). Conferring these vectors to CD34+ cells of a CSF2RA-deficient patient rescued hGM-CSF dependent colony formation as well as granulocytes and monocyte differentiation. Furthermore, as gene-corrected MPs derived from patient-specific iPSC appear as a particularly safe and readily available autologous cell source for PST, we also have investigated this approach demonstrating stable CSF2RA-expression and reconstituted of GM-CSF dependent functions.

New therapeutic strategies for stimulation of granulopoiesis

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*Presenting author

G-CSF receptor (CSF3R) activation upon ligand binding induces myeloid cell proliferation, survival, and differentiation. Acquired somatic mutations within the CSF3R gene and/or defects in the CSF3R downstream signaling pathways abrogate myeloid differentiation and might lead to either leukemic transformation or congenital neutropenia (CN). We assumed that G-CSFR downstream signaling is severely defective in CN, leading to "maturation arrest" of granulopoiesis. Identification of the abnormal signal transduction cascade activated by treatment with G-CSF of CN patients may lead to the identification of new therapeutic options for stimulation of granulopoietic differentiation. Indeed, in the last years we were able to identify new mechanisms of G-CSF action in CN patients and in healthy individuals. We found completely disturbed transcriptional regulation of granulopoiesis in CN bone marrow due to a lack of the myeloid-specific transcription factors LEF-1 and C/EBPa, diminished phosphorylation and activation of the adaptor protein HCLS1, severely reduced levels of the anit-protease SLPI and hyperactivation of the STAT5 protein. We also found that G-CSF induces granulocytic differentiation of CN myeloid cells by activation of emergency granulopoiesis via Nampt/SIRT1/C/EBPß pathway. Nampt is an enzyme nicotinamide phosphorybosiltransferase important for NAD+ generation from nicotinamide. Subsequent investigations in healthy individuals, CN patients and patients with cyclic neutropenia (CyN) revealed granulopoietic functions of high-dose nicotinamide treatment. We also found that treatment of CN myeloid progenitors with bortezomib led to inhibition of the proteosomal degradation of LEF-1 protein induced by binding of hyperphosphorylated STAT5 protein and NARF U3 ubiquitin ligase. I will discuss these findings in my talk.

Micro RNAs (miRNAs) in hematopoiesis

Matthias Eder[1,*], *Joanna Jagielska*[1], *Chun-Wei Lee*[1], and *Michaela Scherr*[1]

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*Presenting author

miRNAs are non-coding single-stranded RNAs involved in post-transcriptional regulation of gene expression. They are differentially expressed in a cell-type specific manner and regulate the cellular proteome in a complex and specific manner. We hypothesized that miRNAs may be used to identify potential therapeutic targets if they are differentially expressed and have disease- and/or context specific functions. Based on this hypothesis we identified repression of miR-17~92 in a subgroup of acute lymphoblastic leukemia (ALL) and demonstrated its pro-apoptotic function. Quantitative proteome-wide expression analysis identified BCL2 as a target of the miR-17 and miR-18a family. Finally, pharmacologic BCL2-inhibition proved very effective in a murine ALL-primograft xenotransplantation model (Scherr 2014). In myeloid differentiation, we previously found increasing miR-125b-expression in differentiating 32D cells. Interestingly, over-expression of miR-125b completely blocks differentiation in a dose-dependent manner (Surdziel 2011). We therefore analyzed miR-125b-effects on mature granulocytes in a search for potential targets to modulate granulocytic function and/or generation. We generated miR-125b chimeric mice by lentiviral gene transfer into lineage-depleted bone marrow cells and transplantation into sub-lethally irradiated syngeneic mice. 8-10 weeks after transplant engraftment of transgenic cells and miR-125b-expression reached up to 84% and 206-fold, respectively. Using a thioglycolate induced peritonitis model we analyzed granulocytic migration and survival of bone marrow and peritoneal fluid derived granuloyctes. Interestingly, miR-125 transgenic granulocytes from the bone marrow but not form peritoneal fluid show about 2-fold higher migration rates as compared to controls. Similarly, miR-125b transgenic granulocytes from bone marrow but not from peritoneal fluid survive better in short term in vitro cultures in the presence of PMA, TNFa , and LPS. These data demonstrate some function of miR-125b and its regulated targets on granulocytic function and survival. We will expand these studies to finally identify the targets mediating these effects which may be suitable for pharmacological intervention in the future.

REBIRTH: Liver (Seminar Room 4, 3:30-5:00 pm)

Development of predictive tools for liver toxicology: micro-well bioreactors and pluripotent stem cells

Danny Bavli[1], *Sebastian Prill*[2], *Yishai Avior*[1], *Magnus Jaeger*[2], and *Yaakov Nahmias*[1,*]

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*Presenting author

The liver is the main organ responsible for the modification, clearance, and transformational toxicity of most drugs and toxins. Primary hepatocytes, the parenchymal cells of the liver, are the main cell type responsible for drug metabolism. Regretfully, primary human hepatocytes are scarce and rapidly lose metabolic function in culture. Attempts to differentiate hepatocytes from pluripotent stem cells similarly

result in minimal metabolic activity. In this talk we will describe our recent efforts create predictive models of liver toxicity using microfluidics, integrated sensors, and three-dimensional cultures. We report on the fabrication of micro-well bioreactor capable of maintaining hepatocyte organoids for over 28 days in vitro. Cell viability is continuously monitored using on-chip frequency-based luminescence-quenching (FBLQ) nano-scale oxygen probes, and integrated off-chip glucose and lactate sensors. The approach is sensitive enough to identify sub-threshold effects of toxicity across multiple toxicological end points. We will also present a rapid differentiation of hepatocytes from human embryonic stem cells using a protocol mimicking important aspects in post-partum development. Finally, end-point assays demonstrate utility of stem cells derived hepatocytes for toxicological screening.

Mechanismen of liver regeneration

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The liver has the remarkable ability to regenerate itself. In order to better understand molecular mechanisms of liver regeneration during chronic injury, we primarily use a mouse model of hereditary tyrosinemia type 1 (HT1). HT1 is an autosomal-recessive disease caused by a genetic inactivation of the enzyme fumarylacetoacetate hydrolase (FAH).

In this project, we were interested in the role of p21 and the mTOR pathway for liver regeneration. p21 is one of the main effectors of p53 that induces cell cycle arrest and senescence in response to triggers such as DNA damage. In order to determine the role of p21, Fah/p21-/- mice were generated. We provide evidence that abundantly expressed p21 completely prevents proliferation of hepatocytes during severe liver injury. Interestingly however, we also found that moderately expressed p21 supports proliferation of hepatocytes with moderate liver injury. Mechanistically, we observed a striking correlation between p21 expression, mTOR activation and hepatocyte proliferation. mTOR activation was suppressed by Sestrin-2 in Fah/p21-/- mice with moderate liver injury, in which liver regeneration was impaired. To determine the role of mTOR, the rapamycin analogue RAD001 was used. RAD001 significantly suppressed proliferation of hepatocytes during liver injury. The ability of everolimus to inhibit proliferation of hepatocytes during chronic liver injury did not depend on p21, but required intact p53 signaling. Mechanistically, we show that RAD001 not only inhibits global protein synthesis, but also regulates the expression of specific cell cycle related proteins in Fah deficient mice taken off NTBC and in WT mice following partial hepatectomy.

In conclusion, we provide evidence that the degree of liver injury and the strength of p21 activation determine its effects on hepatocyte proliferation and hepatocarcinogenesis. Moreover, our data uncover a molecular link in the complex mTOR, Nrf2 and p53/p21-signaling network

MicroRNAs in stem cell based liver regeneration

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MicroRNAs have been shown to be implicated in liver development; however, their precise role in hepatocyte formation remains to be understood in detail. Furthermore, the ability of microRNAs to improve hepatocyte differentiation from embryonic stem cells (ESCs) remains to be determined. To address the aforementioned questions, we compared microRNA profiles of ESCs-derived hepatic cells with fetal liver and primary adult hepatocytes. By comprehensive microRNA screening, we discovered miR-199a-5p, which is a negative regulator of hepatocyte differentiation. Loss of miR-199a-5p improved hepatic differentiation in vitro. In addition, miR-199a-5p inhibition in ESCs-derived hepatic cells led to better engraftment and liver repopulation upon transplantation in a mouse model of liver disease. Hence, our recent findings provide evidence that microRNAs modulation is a promising approach to generate functional hepatic cells.

Human adult liver stem cells derived from patient samples

Rodrigo Gutierrez Jauregui[1,*], *Lara Higging-Woods*[1], *Nora Fekete-Drimusz*[1], *Urda Rüdrich*[2], *Michael P. Manns*[1], *Florian WR Vondran*[1], and *Michael Bock*[1]

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Background and Aims: Basic and translational research on primary human liver cells is currently hindered by the scarcity of appropriate cell material due to short supply, lack of cell proliferation and their rapid dedifferentiation in vitro. The optimal source, for instance for pharmacological testing and cell therapies, would be human liver stem cells propagated in vitro and subsequently induced towards a mature phenotype.

Methods: Primary hepatocyte cell suspensions obtained from patient resectates are received from our surgical collaboration partner in MHH. We established a specific tissue culture protocol for the enrichment of adult liver stem cells within these samples, finally leading to stably proliferating stem cell populations. Adult liver stem cell cultures are then characterized and compared to liver cell lines and fresh primary human hepatocytes by immunofluorescence microscopy and RT-qPCR gene expression analyses employing an extensive panel of cellular markers.

Results: High nucleus-to-cytoplasm ratio and incredible phenotypic sensitivity to the slightest modifications of culture conditions already indicate stemness of the cell populations. Most significant cell characteristics are high levels of markers for progenitor cells (notably EpCAM and PK M2). In addition, stable signatures of both, the hepatocytic and cholangiocytic phenotype are detectable by immunoflourescence and RT-qPCR (Albumin, TDO, TWF1 and AE2, amongst others).

Conclusions: A method was developed to enrich adult liver stem cell populations from patient liver samples. Upon characterisation and comprehensive evaluation of the cells differentiation potential, a

plethora of possible uses, ranging from basic research to clinical applications are within reach in the not-so-far future.

References:

Christ B, Pelz S. Implication of hepatic stem cells in functional liver repopulation. Cytometry A., 2013, 83, 1, 90-102

Stem cells in biliary regeneration

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Biliary regeneration is still an underinvestigated issue in liver regeneration upon acute and chronic liver damage. Noteworthy, misregulation of bile duct homeostasis is one of the major causes of chronic liver graft failure, known as ischemic-type biliary lesions (ITBL), after liver transplantation. Multiple other critical conditions can lead to secondary sclerosing cholangitis (SSC) exhibiting severe bile duct destruction.

Little is known about the endogenous regeneration of bile duct cells from common hepatic progenitor cells. We expect to gain new insights into endogenous mechanisms and expression pattern of hepatobiliary genes, especially SOX9, KRT19 and KRT7, and microRNAs during differentiation of bipotent progenitor cells into biliary epithelial cells by establishing a biliary differentiation protocol for human embryonic and induced pluripotent stem cells (ESCs and iPSCs). For lineage tracing approaches we are using a lentiviral GFP reporter construct under control of a KRT19 promoter. We also aim to establish stable reporter knock-in ESCs for further biliary lineage tracing experiments to depict fate determining steps during differentiation/regeneration in vitro and in vivo. Thereby we tend to investigate the molecular regulation of biliary regeneration in different models of bile duct damage, especially in Mdr2 knock-out mice, resembling the pathology of progressive familiar intrahepatic cholestasis type 3 (PFIC-3), caused by mutations in phospholipidfloppase gene ABCB4.

In summary, diseases of the intrahepatic biliary tree are a heterogeneous group of congenital and acquired liver disorders commonly known as cholangiopathies. Understanding the role of biliary epithelial cells in liver regeneration and the mechanisms of their activation as well as their relationship towards liver progenitor cells will be of strong interest for providing authentic disease models and developing novel ESC- and iPSC-based therapeutic approaches.

REBIRTH in Translation 1 (Auditorium, 9:00- 10:30 am)

Mining the human bone marrow cell secretome for new therapeutically active proteins

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Intracoronary infusion of autologous bone marrow cells (BMCs) has been proposed as a therapeutic strategy to enhance tissue perfusion, reduce scar formation, and improve heart function after myocardial infarction (MI). Clinical trials have shown that BMC therapy can lead to a modest improvement of heart function in patients with acute MI. Trial results have varied, however, which may be related to the current lack of standardization of cell isolation protocols. The best current evidence indicates that adult BMCs do not provide a significant source of new cardiac myocytes. Instead, transcriptome and proteome analyses have shown that bone marrow-derived progenitor cell populations release a broad repertoire of well-known cytokines, chemokines, and growth factors that may promote tissue protection and repair in a paracrine manner. We hypothesized that characterization of the human BMC secretome provides an opportunity to identify specific paracrine factors and develop them as protein therapeutics that could be applied non-invasively and that could be more easily standardized than an autologous cell product. We performed a bioinformatic secretome analysis in bone marrow cells from patients with acute MI to identify novel secreted proteins with therapeutic potential. Using functional screens we discovered a secreted protein encoded by an open reading frame on chromosome 19 that promotes cardiac myocyte survival and angiogenesis. We named this protein myeloid-derived growth factor (MYDGF). We show in bone marrow chimeric mice that bone marrow-derived cells produce MYDGF endogenously to protect and repair the heart after myocardial infarction. While MYDGF-deficient mice develop larger infarct scars and more severe contractile dysfunction, treatment with recombinant MYDGF reduces scar size and contractile dysfunction after myocardial infarction. This study is the first to assign a biological function to MYDGF and may serve as a prototypical example for the development of protein-based therapies for ischemic tissue repair.

From Heart to the Lung and Back: Engineering the Path

Sotirios Korossis[1,2,*], *Lucrezia Morticelli*[1,2], *Andres Hilfiker*[1], *Igor Tudorache*[1], *Sergei Cebotari*[1], and *Axel Haverich*[1,2]

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The cardiopulmonary system, comprising the heart and the lungs, represents one of the major focuses of the research conducted in HTTG at Hannover Medical School. The research strategy has adopted a holistic approach in the confrontation of cardiopulmonary disease, with the work focusing on the development and assessment of a number of surgical solutions for the heart valves, myocardium and lungs. Most importantly, the work has adopted a truly multidisciplinary perspective, positioned at the

interface of biology, medicine and engineering, with a view to pulling basic biomedical research from the bench to the bedside though multilateral implant development and testing. Along these lines, biomechanical and haemodynamic function testing platforms have been developed for assessing the effectiveness of decellularised heart valves, cardiac patches and bioartificial lungs in replacing/repairing organ and tissue function. These testing platforms have also been employed to study native tissues in order to provide important design specifications for the development of implants with improved durability and function. Moreover, near-physiological in vitro model systems, able to simulate normal and pathological conditions, have been developed both for generating living replacement tissues and for investigating the onset and progress of a number of cardiovascular diseases. In addition, virtual testing platforms, employing state-of-the-art computational models have been developed with a view to forecasting the postoperative performance of cardiac and pulmonary implants in a pre-operative setting using the patient's specific anatomy and haemodynamics, effectively facilitating implant selection in non-urgent elective surgery. The aim of this talk is to give an overview of the work in the field conducted at Hannover Medical School.

Translation of research results into products from the perspective of a SME

Michael Harder[1,*]

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This paper intentionally does not deal with questions about starting a business, but presents two aspects to the foreground that are often underestimated.

(i) Products based on regenerative medicine have the potential to give therapeutic answers to medical needs. From the regulatory point the term "regenerative medicine" assembles very different classes of products: tissue preparations, medical devices, pharmaceuticals, Advanced Therapy Medicinal Products (ATMP) and combination thereof. It is very important to work out in which class the product is regulated to determine the appropriate route for preclinical and clinical development. The different classes are characterized by different sets of rules and quality requirements and they are supervised by very different competent authorities.

(ii) Regenerative medicine products often suffer from "coupled processes", short shelf life and often individualized application. "Coupled processes " are based on a sequence of process steps without the possibility for controlled interruption. This places extremely high demands on the manufacturing organization and logistics. For use in the routine, coupled processes are difficult to implement or increase costs to unaffordable heights. It is therefore very important to stabilize primary and intermediate products and the product itself in order to compensate production interruptions and to allow affordable supply chains. Decoupled processes are also safer and therefore much easier approved by the competent authorities.

DZL: Asthma & Allergies (Seminar Room 1, 9:00-10:30 am)

Airway epithelial repair and differentiation

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The airway epithelium is the first barrier to inhaled substances such as allergens, micro-organisms and pollutants, and this may help explain its proposed central role in asthma and respiratory allergies. Traditionally, the airway epithelium has been viewed as a passive physical barrier that mediates mucociliairy clearance, but it is now clear that the airway epithelium is actively involved in regulating inflammation, immunity and airway remodelling. In addition, the composition of the airway epithelium is markedly affected by inflammation, as illustrated by the capacity of the Th2 cytokine IL-13 to increase formation of mucus-secreting goblet cells. In asthma, the airway epithelium shows marked differences, including inflammation, epithelial injury, and a decreased barrier activity resulting in increased sensitivity to inhaled substances. In addition, genome-wide association studies have identified several genes that are associated with asthma and are expressed in the airway epithelium. These genes include genes involved in epithelial barrier formation. Recent studies have provided a more detailed insight into the mechanisms involved in airway epithelial repair and differentiation. Using a model in which human primary airway epithelial cells are cultured at the air-liquid interface, and subsequently exposed to whole cigarette smoke, diesel exhaust or Th2 cytokines, we are studying modulation of repair and differentiation of these cells. Results will be presented on the effect of these exposures on production of antimicrobial peptides and cytokines, endoplasmic reticulum stress, and epithelial repair and differentiation.

Deregulation of ORMDL3 expression induces stress responses and modulates repair pathways

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The asthma-susceptibility gene ORMDL3 is an ER transmembrane protein previously associated with sphingolipid metabolism, the unfolded protein response, Ca2+ homeostasis, T-cell activation, and antiviral responses. However, the functional relevance of ORMDL3 in asthma pathogenesis remains elusive. Using the fruit fly Drosophila melanogaster as a model, we mimicked the situation found in patients at risk for asthma by increasing the expression of ormdl, the sole Drosophila homolog of ORMDL3, in the airway epithelium. Although ormdl overexpression did not overtly affect epithelial integrity, it increased the susceptibility to airborne stressors, such as cigarette smoke and hypoxia.

When confronted by daily doses of cigarette smoke, flies overexpressing ormdl in the airway epithelia had a significantly shortened lifespan compared to matched controls. Moreover, these animals exhibited a much stronger behavioral response to hypoxia, and signaling systems such as the unfolded protein response and the TOR/PI3K pathway lost their ability to react to this stressor. In addition, overexpression of ormdl in the airways drastically reduced the output of signaling pathways associated with repair mechanisms, including EGFR and Notch signaling. These molecular changes were accompanied by changes in the lipid profile that resembled the situation observed in asthmatic airways. On the basis of these findings, we conclude that ORMDL proteins increase the stress status of the airway epithelium, which increases susceptibility to stress factors and increases the probability of developing asthma.

RORγt-specific RNAi decreases allergic airway inflammation and airway hyperresponsiveness in a mouse model of neutrophilic asthma

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Introduction: Recent studies suggest T helper 17 (Th17) cells as important players in the progression of asthma towards a severe phenotype. Characterized by the production of pro-inflammatory cytokines like TNF-α, IL-1ß, IL-22 and IL-17A, Th17 cells appear to act as general promoters of chronic inflammatory responses. These effector functions are regulated by the transcription factor Retinoic acid-related Orphan Receptor gamma (RORγ) t, which is essential for their differentiation. Thus, RORγt represents an ideal target not only to investigate the actual contribution of Th17 cells in the formation of severe asthma, but also as a promising novel target for a therapeutic intervention.

Therefore, the aim of this study is to diminish RORγt expression by using siRNA and to characterize its effects on Th17 cell activity in-vitro and on neutrophilic asthma in-vivo.

Methods: We generated OVA-specific Th17 cells in-vitro, which were transfected with siRNA candidates targeting RORγt. Afterwards, the in-vivo relevance of siRNA-mediated downregulation of RORγt was characterized in a mouse model of neutrophilic asthma.

Results: We could show that siRNA-transfected Th17 cells revealed not only a reduced expression of RORγt in-vitro but also of proinflammatory cytokines like IL-17A and IL17F. Intra-tracheal application of the RORγt-specific siRNA, which was most active in the in-vitro setting, inhibited the development of airway hyperresponsiveness (AHR) to methacholine and decreased bronchoalveolar lavage IL-17A, TNF-α and KC levels. Consequently, application of the RORγt-specific siRNA significantly reduced the number of neutrophils and of lymphocytes.

Conclusion: These results indicate that targeting RORγt could be a new approach for the treatment of neutrophilic asthma

Investigating the role of BAFF in different mouse models of allergic asthma

Anika Lorenz[1,3,*], *Mandy Busse*[1], *Kathleen Dalüge*[1], *Ann-Kathrin Behrendt*[2], *Gesine Hansen*[1,3,*] and *Almut Meyer-Bahlburg*[1,3,**]

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Introduction: The cytokine B cell activating factor of the TNF family (BAFF) is crucial for the homeostatic development, differentiation and proliferation of B cells in the periphery. It is well known that elevated BAFF levels are associated with autoimmune diseases but its role in allergic diseases including asthma is barely understood.

Aims and objectives: To better comprehend the role of BAFF in allergic asthma we started analyzing BAFF in murine asthma models.

Methods: Wildtype (WT) and B cell deficient (µMT) mice were immunized with ovalbumin (OVA) or house dust mite (HDM). Bronchoalveolar lavage fluid (BALF) was measured morphometrically, airway hyperreactivity (AHR) by invasive lung function and BAFF via ELISA.

Results: Asthmatic WT mice showing lung eosinophilia and severe AHR have significantly elevated BAFF serum levels compared to controls. Additionally, in OVA-induced respiratory tolerant mice, BAFF levels are lower than in allergic mice. Kinetic studies demonstrate that BAFF levels increase, the more often allergen is administered intranasally suggesting local BAFF production in the asthmatic lung. Furthermore, BAFF determination in BALF showed increased levels in allergic compared to control mice. To test, whether BAFF is related to elevated IgE levels during asthma, BAFF production in µMT mice was analyzed. Allergen treated µMT mice develop a similar allergic phenotype compared to WT mice and show increased BAFF levels in serum and BALF even in IgE absence.

Conclusions: In asthma models, allergic mice show elevated systemic and local BAFF levels, which increase with the frequency of allergen uptake via the lung and are independent of IgE presence. Thus, BAFF inhibition, recently permitted for treatment of systemic lupus erythematodes, might represent a new therapeutic target in allergic asthma.

DZL: Diffuse Parenchymal Lung Disease (Seminar Room 3, 9:00-10:30 am)

How Intrinsic Cell Signaling regulates Airway Regeneration

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Chronic respiratory diseases including pulmonary fibrosis, chronic obstructive pulmonary disease (COPD) and lung cancer represent the second most common cause of death among Europeans. Despite this significant disease burden and numerous therapeutic interventions, mortality rates for these conditions have remained largely unchanged for many years. These data suggest that a new understanding of the causes of chronic respiratory disease is needed to improve patient survival.

We and others have previously shown that airway epithelial progenitor cell behaviour influences chronic respiratory disease aetiology. Here, we describe the relevance of key progenitor cell signalling pathways in controlling airway regeneration and their relevance to chronic respiratory disease initiation. Our results suggest that conducting airway progenitor cells are key regulators of chronic and diffuse parenchymal lung diseases including fibrosis. Our data also suggest that restoring progenitor cell function in early stage chronic lung disease may play an important therapeutic role in limiting disease progression, morbidity and mortality.

Developmental signal pathways in pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is the most common and aggressive form of idiopathic interstitial pneumonia (Raghu et al., 2011). The etiology of IPF is unknown and treatment options are still limited (Baroke et al., 2013; du Bois, 2010). Pathological hallmarks included alveolar epithelial injury and hyperplasia, aberrant wound healing, formation of fibroblast foci, as well as excessive matrix deposition resulting in disrupted lung architecture and respiratory insufficiency (Selman et al., 2001; Wolters et al., 2013). Disturbed growth factor signaling within the epithelial-mesenchymal unit has been shown to contribute to the pathobiology of IPF. Among them, the transforming growth factor-β1 (TGF-β1) has been identified as a key pro-fibrotic mediator (Fernandez and Eickelberg, 2012). In addition, reactivation of developmental pathways, in particular Wnt signaling, has been demonstrated in both experimental and human lung fibrosis (Chilosi et al., 2003; Königshoff et al., 2008; Selman et al., 2008).

In epithelial cells, WNT/β-catenin reactivation was reported to be a prosurvival factor in alveolar epithelial type II (ATII) cells after bleomycin injury, and to drive ATII-to-ATI cell differentiation in vitro (Flozak et al. 2010; Tanjore et al. 2013). Similarly, active WNT/β-catenin signaling was recently demonstrated to attenuate experimental emphysema, indicating that this pathway drives alveolar epithelial cell repair (Kneidinger et al. 2013). In pulmonary fibrosis, however, alveolar epithelial cell

repair and/or differentiation are impaired because alveolar epithelial cells appear hyperplastic and hypertrophic, indicating that the WNT/β-catenin–driven attempt to repair and regenerate is insufficient (Königshoff et al. 2010).

The following questions will be addressed and discussed:

a) What are the cell-specific mechanism by which WNT/β-catenin signaling exerts its effect on cellular function?

b) How does crosstalk of developmentally active signal pathways (such as TGF-β and WNT) influence fibrogenesis?

Interplay of FGF and Wnt signaling in regulating mesenchymal progenitor cell lineages formation during lung development and repair after injury

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Over the years our research group has focused on the formation of the different mesenchymal cell lineages during lung development and their respective function during repair/disease progression. We previously showed that during development, Fgf10-positive cells located in the distal lung mesenchyme during the early pseudoglandular stage are progenitors for airway SMCs . In addition, We have reported that FGF signaling in the mesenchyme impairs the entry of the mesenchymal progenitors into the SMC lineage both in vitro and in vivo. Furthermore, we have shown that inactivation of beta-catenin in the mesenchyme leads to the loss of amplification of the mesenchymal progenitor cells due the loss of FGF9/FGFR2c signaling. We have also found that in the adult mice Wnt activation was triggered in airway SMC following naphthalene injury. Such increase in Wnt signaling is linked to increased mesenchymal cell proliferation and Fgf10 expression in these cells. Fgf10 in turn is instrumental for the repair of damaged bronchial epithelium. Moreover, we have reported that inhibition of beta-catenin signaling in the mesenchyme triggered by the silencing of miR142 leads to arrested proliferation and premature differentiation of the smooth muscle cells. Lineage tracing using our recently generated Fgf10CreERT2 knock in mice showed that Fgf10-positive cells in the embryonic lungs labeled at embryonic day (E) 11.5 are progenitors for smooth muscle cells (SMCs), resident mesenchymal stromal (stem) cells and lipofibroblasts. Lipofibroblasts (LIFs) found in the late fetal and postnatal lung parenchyma are juxtaposed to alveolar type II stem cells and have been proposed to contribute to the maintenance of their stemness. Although LIFs have been studied in postnatal lungs, their exact cellular origin and mechanism of differentiation are unknown. Our recent results demonstrate an essential role for Fgf10 signaling in directing the differentiation of Fgf10-positive cells towards the LIF lineage during late lung development.

Alveolar derecruitment and collapse induration as crucial mechanisms in lung injury and fibrosis

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Idiopathic pulmonary fibrosis (IPF) and bleomycin-induced pulmonary fibrosis are associated with surfactant system dysfunction, alveolar collapse, and collapse induration (irreversible closure). These events play critical but undefined roles in the loss of lung function and disease progression. To quantify how surfactant inactivation leads to lung injury and fibrosis we employed design-based stereology and invasive pulmonary function tests 1, 3, 7, and 14 days (D) following intratracheal bleomycin-instillation in rats. Active surfactant subtypes declined significantly by D1, leading to progressive alveolar closure (derecruitment) and an associated decrease in organ-scale compliance. Alveolar epithelial damage was more pronounced in closed alveoli compared to ventilated alveoli. At the ultrastructural level, we observed collapse induration in the bleomycin treated rats on D7 and D14 as indicated by collapsed alveoli overgrown by a hyperplastic alveolar epithelium. This pathophysiology was also observed for the first time in human IPF lung explants. Prior to the onset of collapse induration (D7), the lungs were easily recruited, and lung elastance could be kept low after recruitment by application of positive end-expiratory pressure (PEEP). By contrast, at later time points the recruitable fraction of the lung was reduced by collapse induration, causing elastance to be elevated at high levels of PEEP. We conclude that surfactant inactivation leading to alveolar collapse and subsequent collapse induration is the primary pathway for the loss of alveoli in this animal model and is the dominant factor in the degradation of lung function. Our ultrastructural observations suggest that collapse induration is also important in human IPF.

DZL: Cystic Fibrosis (Seminar Room 4, 9:00-10:30 am)

Stem Cell Derived Organoids to test New Therapies in CF

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Cystic fibrosis (CF) is a life shortening genetic disease caused by mutations of the CFTR gene. We have recently developed a functional CFTR assay in intestinal adult stem cell cultures termed organoid cultures. These cultures allow large-scale expansion of primary tissue of individual patients, and provide a patient-specific platform for testing of residual CFTR function and the efficacy of CFTR-targeting drugs. We isolate intestinal crypts from rectal biopsies after intestinal current measurements used for CF diagnostics. These crypts form spheres with a central lumen, and grow into self-organizing epithelial structures consisting of a single epithelial polarized cell layer, which recapitulates the in vivo tissue architecture. CFTR is expressed at the apical membrane facing the lumen of an organoid. Activation of CFTR by forskolin induced rapid fluid secretion into the lumen of the organoids, causing CFTR-dependent swelling of organoids. Swelling was restored in CF organoids by CFTR-targeting drugs. We found CFTR-genotype dependent swelling and response to drugs quantitatively associated with published data concerning clinical disease features and response to drugs in vivo. In collaboration, we also used these cultures to indicate that synergistic combinations of drugs can robustly restore mutant CFTR function, and that the CFTR gene can be corrected in adult stem cells using CRISPR-CAS9 gene editing approach.

In summary, we have developed CFTR function assays in patient-specific organoids. The dynamic range of this assay allows us to distinguish between severe CF, mild CF, and healthy controls, and allow us to measure CFTR-genotype specific responses to CFTR-targeting drugs that match quantitative with published in vivo therapeutic responses. In addition, this model is highly suited to identify new therapeutic approaches for CF and to study patient-variability in therapy response.

Population biology of Pseudomonas aeruginosa in chronic CF and COPD airway infections

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To analyse the population structure of Pseudomonas aeruginosa, more than 1400 independent isolates from diverse environmental and clinical habitats and geographic origins were investigated by SNP-typing of core genome and markers of the accessory genome. More than 50% of all isolates belonged

to less than 25 dominant clones widespread in disease and environmental habitats. Moreover, most clones group in only a few clonal complexes. These complexes seem to be phylogenetically ancient and related to specific sets of genomic islands, e.g. exoU islands. This implies that recombinations between strains of different complexes are rare events.

While most clones were found in the environment, only a subgroup of these strains has been found to date in humans. Moreover, beside some extremely frequent generalists, most strains were related to a specific mode of infection or habitat. For example, the clones dominating in cystic fibrosis (CF) are also dominant in chronic obstructive pulmonary disease (COPD), acute lung infections and urinary tract infections.

To investigate the microevolution of P. aeruginosa in the human lung, serial CF airway isolates of the globally most frequent clones C and PA14 were collected over 20 years since the onset of colonization. The intraclonal evolution in CF lungs was resolved by genome sequencing of first, intermediate and late isolates and subsequent multimarker SNP genotyping of the whole strain panel.

While the PA14 clone diversified into three branches in the patient's lungs and acquired 15 nucleotide substitutions and a large deletion during the observation period, the clone C genome remained invariant during the first years in CF lungs; however, 15 years later 947 transitions and 12 transversions were detected in a mutL mutant strain. Late persistors in CF lung habitats were compromised in growth and cytotoxicity, but their mutation frequency was normal even in mutL mutant clades.

Silencing of miR-148b ameliorates cystic fibrosis-like lung diseases in βENaC-overexpressing mice

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MicroRNAs are involved in diverse biological and pathological processes. Here, we studied the potential role of miRNAs in the in vivo pathogenesis of cystic fibrosis (CF)-like lung disease in βENaC-overexpressing (βENaC-Tg) mice. We performed miRNA array analysis in lung tissue of βENaC-Tg and wild-type mice. Differentially expressed miRNAs were validated by qRT-PCR and their target genes were identified by bioinformatics analysis and luciferase reporter assays. Tissue specific localization was performed by in situ hybridization using locked nucleic acid-modified DNA probe. Direct functional studies were performed by knockdown of miRNA expression in the lungs of βENaC-Tg mice using antagomirs. The effects of knockdown were studied by lung histology, analysis of inflammatory cells in bronchoalveolar lavage and pulmonary function testing using flexiVent system. Genetic association studies in CF patients were performed by analyzing miR148b-Sat1 allele frequency. We demonstrate that miR-148b is upregulated in the lungs of βENaC-Tg mice and predominantly localized in conducting

airway and alveolar epithelial cells. Luciferase reporter assay in Hela cells suggests Mig-6 (mitogen inducible gene-6), a protein previously shown in normal lung development, as a potential target of miR-148b. Antagomir-mediated knockdown of miR-148b in the lung of βENaC-Tg mice reduced emphysema formation, goblet cell metaplasia and neutrophillic inflammation. Further, we observed upregulation of miR-148b in human cystic fibrosis and COPD lung tissue, as well as its localization in airway and alveolar epithelial cells. Finally, our genetic association studies establish that distribution of miR148b-Sat1 allele, closely linked to the MIR148b genomic locus in human, is associated with disease manifestation among F508del-CFTR homozygous sibling pairs. Collectively, these results indicate that deregulation of miR-148b may play an important role in the pathogenesis of CF and COPD and may serve as a novel therapeutic target.

ICM is sensitive to detect potentiation of CFTR-mediated Cl- secretion in patients with cystic fibrosis and the G551D mutation treated with ivacaftor

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Background: Sensitive outcome measures of CFTR function may facilitate the implementation of mutation-specific therapy with CFTR modulators in patients with cystic fibrosis with non-G551D mutations. Intestinal current measurement (ICM) is a sensitive assay for functional assessment of mutant CFTR in rectal biopsies and was recently shown to detect potentiator effects of 1-EBIO ex vivo (Roth E. et al., PLOS ONE 2011). The aim of this study was to determine, if ICM is sensitive to detect potentiation of CFTR-mediated Cl^- secretion in rectal epithelia from CF patients with a G551D mutation treated with ivacaftor. Methods: Rectal biopsies were obtained from 8 patients carrying a G551D-CFTR mutation before and at least four weeks after the start of ivacaftor therapy. Rectal tissues were mounted in micro-Ussing chambers and CFTR-mediated Cl^- secretion was determined from Cl- secretory responses induced by cAMP (IBMX/forskolin)- and Ca^{2+} (charbachol)-mediated stimulation.

Results: Before ivacaftor therapy, ICM detected variable residual CFTR-mediated Cl- secretion in rectal tissues from CF patients with a G551D mutation. In the presence of ivacaftor therapy, CFTR-mediated Cl^- secretory responses were increased in all 8 patients. Conclusion: We conclude that ICM is sensitive to detect in vivo potentiation of mutant CFTR function by treatment with ivacaftor. Our results indicate that ICM may be a useful bioassay to determine therapeutic responses at the level of the basic CF defect of ivacaftor and potentially other clinical CFTR modulators in CF patients with non-G551D mutations.

DZL: Endstage Lung Disease (Seminar Room 5, 9:00-10:30 am)

iPS cell-derivatives for treatment of respiratory diseases

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*Presenting author

The availability of disease-specific human induced pluripotent stem cells (hiPSCs) with their almost unlimited potential for proliferation and differentiation now offers novel opportunities in personalized medicine. In case of hereditary lung diseases such as cystic fibrosis (CF) and pulmonary hypertension, human iPSCs will be the basis not only for advanced in vitro systems for disease modelling, drug screening and compound evaluation, but also for *ex vivo* gene therapy and cell-based therapeutic concepts.

hiPSC generation has now become a routine approach and scalable production of large amounts of iPSCs is possible. Novel genome engineering technologies not only allow for efficient introduction of required transgenes but also footprint-less correction of disease-specific mutations for generation of isogeneic WT control cells. Although not as advanced as for other organs such as the heart, remarkable advance is currently observed with regard to the development of robust differentiation protocols that will allow production of iPSC-derived respiratory epithelia.

This presentation will provide an overview on recent developments in iPSC generation, controlled iPSC culture, novel genome engineering approaches and targeted differentiation into relevant cell lineages. Finally, opportunities for *ex vivo* gene repair and personalized cell therapy based on disease corrected respiratory iPSC derivatives as well as current risks and limitations are discussed.

Interleukin 18 in the pathogenesis of experimental bronchiolitis obliterans syndrome (BOS)

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Introduction: Bronchiolitis obliterans syndrome (BOS), characterized by bronchiolitis obliterans, vascular remodeling and general fibrosis is a major cause of mortality after lung transplantation. Elevated levels of IFNγ-dependent chemokines are predictive factors for the development of BOS. IFNγ expression can be induced by IL18, a pro-inflammatory cytokine, secreted mainly by macrophages upon inflammasome activation. IL18 can be involved in the remodeling of airways and vessels, fibrosis and impairment of endothelial progenitor cell function. However, its potential contribution to BOS has not been yet assessed.

Material and Methods: The Fischer 344 to Lewis rat strain combination was used for orthotopic left lung transplantation. Isogenic transplantations were performed in Lewis rats. Recipients were treated with ciclosporine for 10 days and 28 days after transplantation, LPS was instilled into airways. The

mRNA and protein expression of IL18 was measured on days 28, 29 and 33 after transplantation by quantitative RT-PCR and western blot, respectively.

Results: Left lung isografts and allografts as well as control right lungs expressed stable mRNA levels of pro-IL18, whereas pro-IL18 protein was elevated in left lung allografts on days 28 and 29. Interestingly, mature form of IL18 was detected predominantly in left allografts on day 29 and was absent in right control lungs independent on the day investigated. In agreement with this observation, mRNA expression of inflammasome components like caspase1 and ASC was elevated in left allografts on day 29.

Conclusions: IL18 might play important role in the development of BOS. Control of inflammasome activation and IL18 secretion might represent a novel therapeutic strategy to prevent lung graft destruction.

Treatment with donor specific alloantigen 28 days before or on the day of lung transplantation – a comparison in a large animal model

Katharina Jansson[1,3,*], *Wiebke Sommer*[1,3], *Murat Avsar*[1], *Jawad Salman*[1], *Ann-Kathrin Knöfel*[1,3], *Jeanette Hahn*[1,3], *Marion Hewicker-Trautwein*[2], *Danny Jonigk*[1,3], *Christine Falk*[1], *Axel Haverich*[1,3] and *Gregor Warnecke*[1,3]

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Purpose: Administration of donor-specific alloantigen during transplantation has been shown to induce T-cell regulation and long term transplant tolerance in our large animal model before. In rodents, it was also possible to induce allograft acceptance if the donor-antigen was administered 28 days in advance. Here, we wished to translate this protocol into our lung transplantation model in minipigs and compare it to our already established protocols.

Methods: Lung transplantation from MHC-mismatched donors was performed in 41 minipigs. 23 of those animals received donor-splenocytes perioperatively (group1), whereas in 18 animals the splenocytes were administered 28 days before transplantation (group2). All animals were treated with Tacrolimus and Steroids 28 days following transplantation. Concomittant with donor-antigen the animals received either non-myeloablative irradiation or depleting anti-CD4 and/or -CD8 antibodies. Both groups include 4 (group1) respectively 6 (group2) animals which underwent no immunomodulation at all.

Results: In our minipig model, it was not possible to induce reliable allograft acceptance if the minipigs were treated with donor-antigen 28 days before transplantation. From the total of group2, only 16,7% achieved long term allograft survival (>178d), compared to group1 with 26,1% after all. After censoring animals that died due to other causes related to this experiment than rejection (like bleeding from thrombocytopenia) there still remained 73,3% animals with rejection in group2 but only 46,4% in group1 before postoperative day 178 (p=0.01). Median survival in the day -28 animals was 64 days, whereas in the perioperatively treated animals it was 239 days. Time course and histology suggest

sensitization and consecutive hyperacute rejection in animals pretreated with donor-splenocytes 28 days before lung transplantation even though anti-CD4 and/or –CD8 antibodies were co-administered.

Conclusion: Administration of donor-splenocytes 28 days before transplantation appears to rather promote sensitization, but at the time of transplant promotes tolerance in this large animal lung transplantation model.

REBIRTH: Heart 2 – Valves & Vessels (Seminar Room 1, 11:00-12:30 am)

Decellularized Heart Valve Matrices for Tissue Engineering

Andres Hilfiker[1,*]

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*Presenting author

Untreated heat valve disease is a life threatening illness leading to heart failure and subsequent death. 280,000 heart valve replacement therapies are conducted every year worldwide. Basically, two types of prostheses are commercially available, the mechanical heart valves and the bioprosthetic heart valves, which are mainly cryopreserved homografts or glutaraldehyde fixed valves of porcine or bovine origin. Unfortunately, over 60% of the patients develop severe complications within ten years, mostly based on lifelong medication for anticoagulation in the case of the thrombogenicity of mechanical valves and loss of function though calcification in the case of fixed bioprostetic valves.

Decellularized allogeneic heart valves offer a better solution as demonstrated by experimental settings in the sheep model and in first clinical applications. Such grafts do not need anticoagulation and severe immunogenic problems leading to calcification could not be found. This holds true for both, pulmonary heart valves as well as for aortic heart valve replacements. As shown in the animal model, recipients own cells repopulate the cell-free unfixed matrix. This invasion allows the remodeling and adaptation to the recipients required environment.

As in the clinical situation human decellularized allografts are required a strong limitation is based on its availability. Focusing on xenogeneic alternatives immunologic handicaps i.e. super acute, acute and chronic rejection reactions have to be addressed and solved. However, promising developments will increase the chance of having decellularized xenogeneic heart valves matrices available in required quantities and sizes in close future.

Preservation of heart valve scaffolds in a dry state

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Storage of biological material in a dry state has clear advantages compared to frozen storage. Long-term storage becomes available without the use of liquid nitrogen and bulky, energetically expensive freezers. We have investigated the effects of drying on decellularized heart valve tissues using sucrose as protectant. Diffusion kinetics of sucrose in the scaffolds was studied using attenuated total reflection infrared spectroscopy. These studies implicated that a 4 h incubation step at 37 degrees C is sufficient to homogeneously load the scaffolds with sucrose. After loading with sucrose, the scaffolds were either freeze- or vacuum-dried, and the structure was evaluated by histology staining. Freeze-drying in the absence of sucrose caused an overall disintegrated appearance of the histological architecture. Sucrose (5% w/v) protects during drying, but freeze-dried scaffolds were found to have a more porous structure, likely due to ice crystal formation. Rapid freezing, which reduces ice crystal size, reduced the pore size. No pores were observed when samples were incubated in high concentrations of sucrose (80% w/v) and the overall architecture closely resembled that of fresh tissue. Vacuum drying lacks a freezing step and has the advantage that drying times are much shorter. Vacuum-dried tissue displayed an intact histological architecture similar to freeze-dried tissue. Taken together, both freeze-drying and vacuum-drying hold promise for preservation of decellularized heart valve scaffolds in a dry state and could possibly replace vitrification or cryopreservation approaches in the near future.

Molecular In Vivo Imaging of the Cardiovascular System

Frank Bengel[1,*]

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Imaging is expected to play an essential role when regenerative cardiovascular therapies are developed from small and large animal models towards clinical application. On the one hand, robust surrogate endpoints for clinical trials are needed. Established clinical techniques targeting myocardial perfusion, function and viability will serve for this purpose initially. On the other hand, a series of novel molecular markers for noninvasive assessment of mechanisms involved in tissue regeneration (e.g. inflammation, angiogenesis or scar formation) are under development, and therapeutic agents such as cells and proteins can be labeled directly for tracking of their in vivo fate. These novel techniques will provide insight into the mechanistic underpinnings of emerging therapies. When integrated into the development of novel therapies, molecular imaging holds the potential to be jointly developed towards a future clinical practice where specific molecular therapy is guided by results from specific molecular imaging. The advent of hybrid imaging systems, which allow for fusion of standard clinical magnetic resonance and CT techniques with highly specific molecular radionuclide probe signals, is expected to further boost the success of image-guided therapies to support regenerative therapy.

REBIRTH: Blood & Immunology (Seminar Room 3, 11:00-12:30 am)

Dendritic cell-mediated immune humanization of mice: preclinical models of stem cell transplantation

Renata Stripecke[1,*]

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*Presenting author

Predictive mouse models of human hematopoietic stem cell transplantation(HSCT) to pinpoint experimentally the spatio-temporal events during human adaptive immune reconstitution in vivo are still lacking. In particular, HSCT modeling in immune-deficient is currently limited due to incomplete maturation of T and B lymphocytes. We are exploring novel clinically translatable approaches, by which novel types of adoptively transferred engineered dendritic cells can effectively accelerate regeneration of the immune system after adult or cord blood HSCT. Nod-Rag-/-Il2gc-/- mice transplanted with stem cells and adoptively transferred with donor derived lentivirus-induced self-differentiated dendritic cells expressing the pp65 antigen (SmyleDC/pp65) developed lymph node-like structures (LN-LS), showed expansion of effector T helper and T cytotoxic cells and production human antibodies reactive against pp65. The clinical translation of SmyleDC/pp65 for HSCT and its practical uses for humanized animal experimental models to address potency of vaccines, gene therapy and immune therapeutic approaches will be discussed. In particular, differences between modelling peripheral blood and cord blood transplantation will be discussed in view of potency models.

In vitro production of HLA universal platelets

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Refractoriness to platelet (PLT) transfusion caused by alloimmunization against HLA class I antigens constitutes a significant clinical problem. Thus, it would be desirable to have PLT units devoid of HLA antigens. Previously, we showed that the generation of HLA class I-silenced (HLA-universal) PLTs from CD34+ cells using an shRNA targeting β2-microglobulin transcripts is feasible. Furthermore, we assessed the functionality of HLA-silenced PLTs and their ability to escape HLA antibody-mediated cytotoxicity in vitro and in vivo. For the large-scale production of this blood component, we have differentiated PLTs from induced pluripotent stem cells (iPSCs). Platelet activation in response to ADP and thrombin were assessed in vitro. The immune-evasion capability of HLA-universal megakaryocytes (MKs) and PLTs was tested in lymphocytotoxicity assays using anti-HLA antibodies. To assess the functionality of HLA-universal PLTs in vivo, 1x106 HLA-silenced MKs were infused into NOD/SCID/IL-2Rγc-/- mice with or without anti-HLA antibodies. PLT generation was evaluated by flow cytometry using anti-CD42a and CD61 antibodies. HLA-universal PLTs demonstrated to be functionally similar to blood-derived PLTs. Lymphocytotoxicity assays showed that HLA-silencing efficiently protects MKs against HLA antibody-mediated complement-dependent cytotoxicity. 80-90% of HLA-expressing MKs, but only 3% of HLA-silenced MKs were lysed. In vivo, both HLA-expressing and HLA-silenced MKs showed human PLT production (up to 0.5% within the PLT population) when anti-HLA antibodies were

absent. However, in presence of anti-HLA antibodies HLA-expressing MKs were rapidly cleared from the circulation of mice, while HLA-silenced MKs escaped HLA antibody-mediated cytotoxicity and human PLT production was detectable up to 11 days. Our data show that HLA-silenced PLTs are functional and efficiently protected against HLA antibody-mediated cytotoxicity. In addition, we have shown the feasibility to use iPSCs for the large-scale production of MKs and PLTs. Hence, provision of HLA-universal PLT units may become an important component in the management of patients with PLT transfusion refractoriness.

Micro(RNA)-Management of Lymphocyte Development

Natalia Zietara[1], *Marcin Lyszkiewicz*[1], *Malte Regelin*[1], *Jonas Blume*[1], *Jens Pommerencke*[1], and *Andreas Krueger*[1,*]

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*Presenting author

Lymphopoiesis, and T cell development in particular, can be separated into distinct phases comprising differentiation, proliferation and selection events. These phases are tightly regulated by both cell-extrinsic and cell-intrinsic factors. It has been proposed that miRNAs contribute to the lineage decisions during lymphopoiesis. However, individual miRNAs regulating intrathymic T cell development remain to be identified. In order to assess the role of miRNAs in thymopoiesis we have generated and analyzed mice carrying targeted deletions of various candidate miRNAs. We identified two miRNA families critical for distinct stages of T cell development. We found that early T cell development and responsiveness of immature thymocytes to IL-7 depend on expression of the miR-17~92 cluster. Furthermore, we identified members of the miR-181 family, especially miR-181a/b-1, as critical regulators of agonist selection in the thymus. In consequence, miR-181a/b-1–/– mice had a profound defect in generation of invariant NKT cells and displayed reduced numbers of intrathymic Treg cells.

Thus, we have demonstrated that various miRNAs are key players in the control of distinct stages of intrathymic T cell development.

Use of adjuvants with well-defined molecular targets to tailor innate and adaptive immune responses

Thomas Ebensen[1], *Peggy Riese*[1], *Christine Rueckert*[1], *Kai Schulze*[1], *Sebastian Weissmann*[1], and *Carlos A. Guzmán*[1,*]

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Vaccination is the most powerful tool to fight infectious diseases. While traditional vaccines contain either live or inactivated pathogens, modern vaccines consist of defined subunits. Since the immunogenicity of subunit vaccines is often reduced, adjuvants should be included into the formulation. At present, only a few adjuvants are available for human use, These adjuvants induce poor mucosal immunity and exhibit a limited capacity to tailor T cell responses. Our adjuvant development program led to the discovery of several well-defined synthetic immune modulators, which are also active when

administered by the mucosal route as well as in poor responders. One promising compound class comprises the cyclic-di-nucleotides (CDNs), which are prokaryotic signaling molecules with strong immune modulatory effects on dendritic cells and macrophages by activation of type I interferons and TNF production. Co-administration of CDNs with purified antigens induces strong humoral and cellular responses with a balanced Th1/Th2/Th17 phenotype, and cytotoxic cells. Influenza A virus vaccines adjuvanted with CDNs confer protection against virus challenge in different preclinical models. CDNs can also stimulate the immune system of aging subjects. Another candidate adjuvant is a PEGylated derivative of CD1 agonist α-galactosylceramide (αGC). In contrast to the CDNs, αGC elicits its immune stimulatory activities by the activation of NKT cells. This leads to a specific suppression of Th17 induction, and stimulation of strong Th2 responses. These unique features of αGC allow the fine tuning of Th17 immune responses, which can be harmful in some settings. It was also demonstrated that αGC affects the maturation status and biological activity of different NK cell subsets, and that this can be exploited to promote efficient viral clearance in therapeutic settings. Taken together, this new generation of fully synthetic adjuvants with well-defined molecular targets represents a powerful tool for the rational design of vaccines or immune therapies.

REBIRTH: CARPuD (Seminar Room 4, 11:00-12:30 am)

Animal and cellular models of chronic lung disease, prospects of gene and cellular therapy.

Bob J Scholte[1,*]

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*Presenting author

Different forms of chronic lung disease (COPD, Asthma, IPF, Cystic fibrosis), are characterized by similar pathological processes. Chronic inflammation associated with mucous hyperplasia, airway obstruction, tissue injury and fibrotic repair result in irreversible loss of function. Effective preventive or curative therapies are not available despite intensive research.

We study cystic fibrosis (CF) lung disease in both animal and cellular models. This frequent congenital disorder caused by mutations in the CFTR anion transporter is characterized by severe progressive lung disease. Distal airway obstruction and bronchiectasis is evident in most infants with CF. Chronic infection with opportunistic bacterial pathogens is the main cause of morbidity and reduced lifespan.

To elucidate the molecular mechanisms involved in tissue injury and repair, we have challenged mice carrying the most common CFTR mutation (F508del) with lung injury and inflammation. The results show that the lungs of mutant mice show enhanced responses, resulting in increased mucous hyperplasia and fibrosis. In organotypic cell culture of airway cells our data confirm that CFTR deficiency increases output of growth factors and cytokines.

We use these experimental platforms to study experimental therapeutics. In addition to small molecule therapy, gene therapy can be considered. However, classical non-viral and viral gene transfer to intact airway epithelium has not been successful in the clinic, and a breakthrough in this field remains elusive so far.

In the advent of novel induced pluripotent stem-cell (iPS) based technology combined with novel gene editing tools we can now consider an alternative approach. Genetically corrected patient derived stem cells can theoretically be used to improve the function of deficient organs. This could be achieved by transplanting suitable progenitor cells to the organ in situ, or by generating artificial organs in culture. We will outline the considerable practical challenges that lie ahead.

iPS cell-derived transplants for Cystic Fibrosis and Surfactant Deficiencies

Ulrich Martin [1,*]

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*Presenting author

Human induced pluripotent stem cells (iPSCs) offer promising new perspectives for the treatment of lung diseases, e.g. cystic fibrosis (CF) or surfactant deficiencies, by cellular or tissue replacement therapy, disease modeling and drug screening. On that account, we are aiming at an efficient protocol for the differentiation of PSCs into functional lung epithelial (progenitor) cells and more mature respiratory derivatives.

Murine iPSCs were generated from CCSP-promoter reporter mice. In addition, human PSC NKX2.1-eGFP knock in reporter cell lines were established through homologous recombination. In murine PSC lines we demonstrate that the glucocorticoid dexamethasone plus cAMP-elevating agents (DCI) strongly induces differentiation into Clara-like cells with expression of the Clara cell marker CCSP. While KGF synergistically supports the inducing effect of DCI on alveolar markers with increased expression of surfactant protein (SP)-C and SP-B, an inhibitory effect on CCSP expression was shown. Furthermore, the use of iPSCs from transgenic mice with CCSP promoter-dependent lacZ expression enabled detection of derivatives with Clara cell typical features. Human PSC reporter lines were utilized to monitor and optimize differentiation into NKX2.1-eGFP lung progenitors. Furthermore, we were able to detect hPSC-derived CFTR+ cells with a cholangiocyte-like phenotype, which may be useful to screen for novel drugs targeting CF disease.

The in vitro generation of murine and human PSCs into respiratory derivatives will enable the evaluation of innovative cellular therapies in animal models of lung diseases and provides the basis for innovative therapeutic options for the treatment of respiratory diseases like CF.

Genetic correction of A1AT deficiency iPSC using CRISPR/Cas9

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Alpha 1-antitrypsin (A1AT) inhibits a wide variety of proteases in the serum by covalent binding. A congenital point mutation (E342K) causes A1AT polymerization, resulting in its retention in the endoplasmic reticulum of hepatocytes. Homozygous individuals (ZZ) have only 10% of normal A1AT serum concentration, which is not sufficient to inhibit neutrophil elastase, leading to breakdown of

elastin in the lung and causing emphysema. Moreover, accumulation of misfolded protein in hepatocytes can cause liver cirrhosis and hepatocarcinoma.

Here, we use CRISPR/Cas9 based genomic precision engineering in induced pluripotent stem cells (iPSC) of severe A1AT deficiency patients. We evaluated the gene targeting potency of different A1AT-specific gRNAs with Cas9_D10A nickase in an in vitro reporter assay. We used the two most potent opposite strand gRNAs to introduce a double nick with 5' overhang and were able to reach up to 1% gene targeting efficiency. Finally, we compared the gene targeting accuracy of Cas9 nuclease and Cas9_D10A nickase in patient-specific A1AT deficiency iPSC by co-transfection of a piggyBac-flanked puromycinΔtk-selectable donor. Analysis of different clones by multiplex PCR showed that most of the nuclease treated ones had off-target integrations, while all of the nickase targeted clones showed correct monoallelic integration of the donor. Moreover, when we used the double nick strategy, we found biallelic donor integration in 40% of all clones. Selected biallelic targeted clones were chosen for transfection with piggyBac transposase, followed by gancyclovir counterselection.

Taken together, we demonstrate that CRISPR/Cas9 is a highly efficient and accurate tool for precision genome engineering of patient specific human iPSC.

iPS cell-derived macrophages as in vitro model of Pulmonary Alveolar Proteinosis

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

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

REBIRTH: NIFE: Pooling Resources - Creating Synergies (Seminar Room 5, 11:00-12:30 am)

NIFE - A Translational Research Center

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The Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE) in Hannover takes the region and German state Lower Saxony into the international focus and spotlight as one of the leading facilities of biomedical research and development. NIFE has recently been founded by the Hannover Medical School (MHH), the University of Veterinary Medicine Hannover, Foundation (TiHo), and the Leibniz University of Hannover (LUH) in cooperation with the Laser Zentrum Hannover e.V. (LZH). The focus is on implant research and development.

NIFE is an answer to meet the requirements and challenges in modern biomedical technology and makes a consequent use of the expertise of the involved scientists. It concentrates the expertise of engineers, natural scientists and health professionals in one joint research center at one location under "one roof". Depending on their outstanding expertise in biomedical technology, the interdisciplinary teams are focused to work together on the improvement of existing and the development of new and innovative implants.

The major target is to prevent the early loss of implants and to improve implant function. The research program of NIFE aims on the reduction of implant infection, the enhancement of the biocompatibility of the implants and the minimisation of pathological reactions of the tissue. NIFE has build up the whole value chain for the translation of the research results into clinical practice.

Due to the expertise of the regional scientific partners in the field of regenerative and reconstructive medicine the center focus on cardiovascular, orthopaedic, auditory, neuronal and dental implants.

Through the foundation of NIFE, Hannover emphasizes its outstanding status in biomedical engineering and becomes even more attractive for the settlement of national and international life science companies.

Preparation and functionalization of biomedical materials – applications for extracorporeal lung devices

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*Presenting author

One major problem with medical devices is the fact that surrounding tissue is not able to recognize the artificial surface as tissue like material. In case of blood oxygenators, this can induce blood coagulation which leads to blocking of the surface and inhibition of gas exchange. For metal implants such as pacemakers, incomplete tissue integration frequently promotes infection and additionally leads to implant migration.

The report will describe our ongoing research programme to modify and humanize the surface of biomedical materials using adhesion factors as cell biocompatible entities. One approach is to covalently attach RGD-peptides to the implant surface and thereby promoting cellular adhesion and allowing the integration of the device in the tissue. The RGD-modified blood oxygenator can be seeded with lung endothelia cells which stealth the surface from the blood stream.

We combine different technologies of which open-air plasma initially serves to increase the wetability of the material allowing the introduction of different reactive chemical functionalities. Finally, adhesion factors such as RGD-peptides are attached by what is called "Click" chemistry, a technique that can be used to create linkages in a bioorthogonal manner.

The report will provide a detailed insight into the techniques and scopes and limitations employed here.

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Novel Laser based imaging techniques and applications

Heiko Meyer [1,*], *Marko Heidrich* [1], *Raoul-Amadeus Lorbeer* [1], *Manuela Kellner* [2], *Andreas Winkel* [2], *Matthias Ochs* [2], *Alexander Heisterkamp* [3], *Meike Stiesch* [2], *Mark Philipp Kuehnel* [2], and *Tammo Ripken*[1]

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Laser based microscopic imaging has evolved vastly within the past decades. However the demand of the biological and biomedical community for imaging techniques in the mesoscale with microscopic resolution has grown rapidly during the past ten years. A variety of such techniques has been developed since then, as e.g. Optical Projection Tomography (OPT), Selective Plane Illumination Microscopy (SPIM) and Ultramicroscopy as well as Scanning Laser Optical Tomography (SLOT), filling the gap between microscopic and macroscopic imaging techniques. This talk aims towards the comparison of microscopic and mesoscale imaging exemplarily on some biological model organisms as e.g. Locusta migratoria and Danio rerio and biological tissues. More specifically, the direct comparison of data acquired with multiphoton microscopy, Ultramicroscopy, µCT and SLOT on lung tissue is shown. The future goal is the underlying comprehension of the acquired data in comparison between the different imaging methods and modalities and their transfer into clinical imaging.

REBIRTH in Translation 2 (Auditorium, 3:30-5:00 pm)

Stem Cell Applications in a Pharma Company

Martin Graf[1,*]

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*Presenting author

At Hoffmann-La Roche we started to work with human stem cells 5 years ago. Our goal is to develop more relevant human in vitro assays for screening and testing of drug candidates. We implemented differentiation protocols to several cell lineages such as neurons, endothelial cells, adipocytes, cardiomyocytes, hepatocytes and podocytes. We currently do not intend to use stem cells as therapeutics.

One main driver to start with stem cell research was the invention of reprogramming by Shinya Yamanaka in 2006. At Roche we have set up a collection of induced pluripotent stem cells (iPSCs) from a variety of patients with diverse diseases. But we are also using more and more genome editing to model monogenic diseases. I will present several case studies to highlight how we are using stem cells in high throughput screening (HTS) and in disease modelling.

We have established a number of collaborations that enables us to move forward in this fast developing field. At the end of 2012 we started a huge IMI project (Innovative Medicines Initiative) the StemBANCC project. Together with 33 partners we are in the process to generate iPSCs from 500 patients in the area of peripheral nervous system disorders, central nervous system disorders and diabetes and to study these diseases in vitro.

From innovation to reimbursement: how are new treatments introduced into the German health care system

Matthias Perleth[1,*]

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*Presenting author

The German benefit catalogue can be considered to be comprehensive, including prevention and screening, immunisation, diagnostic procedures, treatment of disease and transportation. Treatment includes all necessary and state-of-the-art ambulatory medical care, dental care, drugs, non-physician care, medical aids, hospital care, home nursing care, palliative care and rehabilitation. Only few services are explicitly excluded (e.g. drugs for common cold, life-style drugs).

Decision-making for funding of innovative diagnostic and therapeutic methods in Germany's statutory health insurance (SHI) follows a dichotomy: in ambulatory (outpatient) care, only methods with proven benefit after assessment by the Federal Joint Committee (G-BA) are reimbursed while in inpatient care, all methods may be provided unless they are excluded due to proven harm or lack of benefit. A large part of the assessments are commissioned to the Institute for Quality and Efficiency in Health Care (IQWiG).

In January 2012, as part of a new innovation-friendly federal initiative (to support small and medium companies), a new section 137e was added to the Social Code Book V (SGB V), allowing for the application of manufacturers of an innovative medical device (as part of a diagnostic or therapeutic method) to the G-BA to initiate a pivotal clinical trial. Manufacturers have to provide data that show the potential of the innovation to generate a patient-relevant benefit. It is expected that the first trial will be initiated during the next year. Thus, the G-BA has not only the power to decide on the benefit of innovations by analysing existing data, but by generating lacking evidence to fill in existing gaps.

DZL: Chronic Obstructive Lung Disease (Seminar Room 1, 3:30-5:00 pm)

Control of Inflammation by Mesenchymal Stromal Cells

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*Presenting author

In addition to hematopoietic stem cells, the bone marrow also contains mesenchymal stromal cells (MSCs). These cells were first recognized more than 40 years ago by Fredenstein et al. who described a population of adherent cells from the bone marrow which were non-phagocytic, exhibited a fibroblast like appearance and could differentiate in vitro into bone, cartilage, adipose tissue, tendon and muscle. Human MSCs were first identified in post-natal bone marrow and later in a variety of other tissues, including periosteum, muscle connective tissue, umbilical cord blood, adipose tissue and fetal tissues, amniotic fluid and placenta. One of the hallmarks of MSCs is their multipotency, defined as the ability to differentiate into several mesenchymal lineages, including bone, adipose tissue and cartilage.

Several experimental studies have indicated that MSCs are endowed with potent immune modulatory properties directed at cells involved in immune responses. Due to their immunomodulatory and engraftment-promoting properties, MSCs have been tested in the clinical setting both to facilitate hematopoietic engraftment and to treat steroid resistant acute graft-versus-host disease. MSCs are also applied in other immune-mediated disorders, including solid organ transplantation and auto-immune disorders, such as Crohn's disease and multiple sclerosis.

More recently, experimental findings in clinical trials have focused on the ability of MSCs to home to injured tissues and to produce paracrine factors with anti-inflammatory properties. The mechanisms through which MSCs exert their therapeutic effect rely on the capacity to home to sites of injury, the ability to suppress excessive immune responses and the ability to secrete soluble factors capable of stimulating the survival and recovery of injured cells. The collective data suggest that MSCs may play a crucial role in balancing inflammation by recruiting monocytes and macrophages to adopt an anti-inflammatory phenotype resulting in suppressing of T-cell proliferation and promotion of regulatory T cells. During the presentation, the orchestrating role of MSCs in controlling inflammation and immune responses and in maintaining tissue homeostasis will be discussed.

A possible role of serotonin for the development of tobacco smoke-induced lung emphysema and pulmonary hypertension

Alexandra Pichl[1,*], *Michael Seimetz*[1], *Athanasios Fysikopoulos*[1], *Mariola Bednorz*[1,*], *Nirmal Parajuli*[1], *Daniela Haag*[1], *Rudolf Reiter*[2], *Jan Grimminger*[1], *Werner Seeger*[1], *Ralph Schermuly*[1], *Friedrich Grimminger*[1], *Hossein A. Ghofrani*[1], and *Norbert Weissmann*[1]

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*Presenting author

Chronic obstructive pulmonary disease (COPD) is a major cause of death and disability worldwide. An estimated portion of 30-70% of COPD patients also suffer from pulmonary hypertension (PH). Studies indicate that activation of serotonin-mediated pathways contribute to development of PH. Moreover, vascular alterations have been suggested to contribute to emphysema development.

The aim of the study was to clarify the role of serotonin and the serotonin inhibitor Terguride on the development of tobacco smoke-induced emphysema and PH in a mouse model.

WT mice (C57BL6/J) were exposed to cigarette smoke for 6 hours/day, 5 days/week for 8 months. Mice were split into different experimental groups (Placebo smoke-exposed, Placebo non-exposed and Terguride smoke-exposed). Terguride-treated animals received the drug twice per day by gavage. Gene and protein expression analysis were performed by quantitative real-time PCR and western blotting. Development of PH and emphysema were determined by measurement of lung compliance, in vivo hemodynamics, right ventricular heart mass alterations and as well by alveolar and vascular morphometric analyses.

The mRNA as well as protein analyses revealed a significant upregulation of 5-HT2A and 5-HT2B receptors in tobacco smoke-exposed mice. Similar alterations were found in lungs from human COPD patients compared to healthy donors. Non-treated smoke-exposed mice developed pulmonary hypertension and emphysema upon smoke exposure. In contrast, smoke-exposed Terguride-treated mice were prevented from PH and vascular remodeling. In addition, the smoke-induced increase in lung compliance as well as structural measures for emphysema development remained on a normal level in Terguride-treated smoke-exposed mice.

We concluded that Terguride has a protective effect on the development of tobacco smoke-induced pulmonary hypertension and emphysema development in mice.

Acute phase protein α1-Antitrypsin - a novel regulator of angiopoietin-like protein 4 transcription and secretion

Eileen Frenzel[1,*], *Sabine Wrenger*[1,3], *Stephan Immenschuh*[1], *Rembert Koszulla*[2,4], *Tobias Welte*[1,3] and *Sabina Janciauskiene*[1,3]

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*Presenting author

The angiopoietin-like protein 4 (angptl4, also known as peroxisome proliferator-activated receptor (PPAR) gamma induced angiopoietin-related protein) is a multifunctional protein associated with acute phase response. The mechanisms accounting for the increase in angptl4 expression are largely unknown. This study is the first to show that human α1-antitrypsin (A1AT) up-regulates expression and release of angplt4 in human blood adherent mononuclear cells and in primary human lung microvascular endothelial cells in a concentration- and time-dependent manner. Mononuclear cells treated for 1 h with A1AT (from 0.1 to 4 mg/ml) increased mRNA of angptl4 from 2 to 174-fold, respectively, relative to controls. In endothelial cells the maximal effect on angptl4 expression was achieved at 8 h with 2 mg/ml of A1AT (11-fold induction versus controls). In ten emphysema patients receiving A1AT therapy (Prolastin) plasma angptl4 levels were higher relative to patients without therapy [ng/ml, mean (95% confidence interval) 127.1 (99.5-154.6) versus 76.8 (54.8-98.8),

respectively, p=0.045] and correlated with A1AT levels. The effect of A1AT on angptl4 expression was significantly diminished in cells pre-treated with a specific inhibitor of ERK1/2 activation (UO126), irreversible and selective PPARγ antagonist (GW9662), or genistein, a ligand for PPARγ. GW9662 did not alter the ability of A1AT to induce ERK1/2 phosphorylation, suggesting that PPARγ is a critical mediator in the A1AT-driven angptl4 expression. In contrast, the forced accumulation of hypoxia inducible factor 1-α, an up-regulator of angptl4 expression, enhanced the effect of A1AT. Thus, acute phase protein A1AT is a physiological regulator of angptl4, another acute phase protein.

Alveolar epithelial cells type II show a high sensitivity to cigarette smoke extract

Sophie Seehase[1,3,*], *Bettina Baron-Luehr*[1], *Christian Kugler*[2], *Ekkehard Vollmer*[1], and *Torsten Goldmann*[1,3]

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*Presenting author

Alveolar epithelial cells type II (AECII) play an important role in the normal pulmonary function as well as in the host defense and immune response. The human tumor cell line A549 is the most popular model of AECII.

In the presented study, the effects of cigarette smoke extract (CSE) on primary AECII and A549 as well as the epithelial adenocarcinoma cell line H1975 were investigated.

Tumor-free lung tissue from patients who underwent lobectomy due to cancer at the LungenClinic Großhansdorf was used to isolate AECII. Briefly, after crushing the lung tissues, AECII were separated by negative selection via a CD45. Subsequently, cells were seeded on collagen-coated 96-well plates at low density (4 x 10^4 cells/ well). A549 cells were seeded at 2 x 105 cells/ well, H1975 at 2 x 10^4 cells/well. All three cell types were maintained in DMEM-F12 supplemented with 10% FCS overnight. CSE was obtained using commercially available cigarettes (West light) by drawing smoke of one cigarette slowly through a water pump into a tube containing 10 mL of ddH2O (=10% CSE). Stimulation was performed under serum-free conditions. Cells were stimulated with increasing concentrations of CSE (0.1 - 5%) for 1 h. Cells were cultured for further 4 h. Cell viability was measured via MTT assay.

Acute CSE exposure with 0.5% CSE induced a significant cytotoxic effect in AECII (IC50: 0.21% CSE), which was not reversible by dexamethasone (4 mg/mL) or roflumilast (15 µM). In H1975, a concentration of 5% CSE caused a significant reduction of cell viability. By contrast, no cytotoxic effect was detectable in A549 cells.

Primary AECII are a model to investigate cigarette smoke induced inflammatory effects, better suited than the widely used tumor cell line A549. Moreover, CSE-induced AECII damage is not reversible by anti-inflammatory treatment.

DZL: Pulmonary Hypertension (Seminar Room 3, 3:30-5:00 pm)

Development of a rat model of metabolic syndrome related Group II PH and therapy with nitrite and metformin

Mark Gladwin[1,]*

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*Presenting author

Nitrate and nitrite have traditionally been considered dietary toxins that increase the risk of stomach cancer. Recent scientific discoveries suggest that nitrate and nitrite are in fact natural signaling pathways in the human body, via a NO synthase independent reductive pathway from nitrate-to-nitrite-to-NO. Nitrite is now appreciated as a biological reservoir of nitric oxide (NO), present in plasma, red cells and organ systems, that is reduced to NO during physiological and pathological hypoxia. Current studies by multiple research groups indicate that nitrite forms via reduction of dietary nitrate to nitrite by commensal mouth bacteria, in addition to NOS-dependent nitrite formation from NO oxidation. Nitrite then contributes to critical physiological functions such as blood pressure control, hypoxic vasodilation, mitochondrial respiration and the cellular resilience to ischemic stress. Pre-clinical and clinical studies suggest that inhaled and oral nitrite may be able to prevent and reverse established pulmonary arterial hypertension and phase II proof of concept trials are currently in progress in the US and Europe. We review recent data suggesting that the nitrate-nitrite-NO pathway limits key features of the metabolic syndrome and ameliorates Group II pulmonary hypertension with preserved ejection fraction. It is proposed that the nitrate – nitrite – NO pathway represents a fundamentally conserved pathway for physiological and pathological hypoxic NO-signaling in biology.

Lung Transplantation for severe pulmonary hypertension – Awake ECMO for postoperative left ventricular remodeling

Igor Tudorache[1], Wiebke Sommer[1], Christian Kühn[1], Olaf Wiesner[1], Johannes Hadem[1], Thomas Fühner[1], Fabio Ius[1], Murat Avsar[1], Nicolaus Schwerk[1], Jens Gottlieb[1], Tobias Welte[1], Christoph Bara[1], Axel Haverich[1], Marius M. Hoeper[1] and Gregor Warnecke[1,]*

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Background: Bilateral lung transplantation (BLTx) is an established treatment for end-stage pulmonary hypertension (PH). Ventilator weaning failure and death are more common as in BLTx for other indications. We hypothesized that left ventricular (LV) dysfunction is the main cause of early postoperative morbidity/mortality and investigated a weaning strategy using awake veno-arterial extracorporeal membrane oxygenation (v/a ECMO).

Methods: In 23 BLTx for severe PH ECMO used during BLTx was continued for a minimum of five days. Echocardiography, left atrial (LA) and Swan-Ganz catheters were used for monitoring. Early extubation after transplantation was attempted under continued ECMO.

Results: Preoperatively, all patients had severely reduced cardiac index (mean 2.1 l/min/m^2). On post-operative day (POD) two, reduction of ECMO flow resulted in an increase in LA and decrease of

systemic blood pressure. On the day of ECMO explantation (median POD8), LV diameter had increased; LA and blood pressure remained stable. Survival rates at three and 12 months were 100% and 96%, respectively, comparing favourably with historical controls. *Conclusion:* In patients with BLTx for severe PH the LV may be unable to handle normalized LV preload during the early postoperative period. LV function normalizes within days and this period can be effectively bridged with awake v/a ECMO.

P66shc deficient mice develope decreased right heart hypertrophy via a Cyclophilin D dependent mechanism in hypoxia-induced pulmonary hypertension

Mareike Gierhardt[1,*], *Natascha Sommer*[1], *Rolf Schreckenberg*[2], *Klaus-Dieter Schlueter*[2], *Ardeschir H Ghofrani*[1], *Ralph T Schermuly*[1], *Rainer Schulz*[2], and *Norbert Weissmann*[1]

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In acute and chronic hypoxia the response of the pulmonary vasculature is suggested to be regulated via mitochondrial reactive oxygen species (ROS). In response to cellular stress the mitochondrial regulator protein p66shc enhances the ROS-production probably via the pro-apoptotic protein cyclophilin D (CypD). We hypothesized in p66shc-deficient mice lower hypoxic pulmonary vasoconstriction (HPV) and pulmonary hypertension (PH) related to lower hypoxia-induced ROS-production.

HPV was determined in isolated lungs of p66shc and CypD deficient mice, as well as in mice lacking both proteins, and compared to lungs of wild type (WT) mice. The thromboxane mimetic U46619 and potassium chloride (KCl) were used as hypoxia-independent vasoconstrictive stimuli. PH was quantified after exposure of mice to 10% oxygen for 4 weeks by in vivo hemodynamics, and morphometric analysis.

Mice deficient of p66shc, CypD or both proteins exhibited lower responses to acute hypoxia, U46619 and KCl compared to WT mice. In chronic hypoxia-induced pH only p66shc deficient mice exhibited lower right ventricular pressure, right ventricular hypertrophy and hematocrit compared to WT mice. In mice lacking CypD or both proteins, no significant changes of these parameters in chronic hypoxia were detected. There was no change in lung remodeling between all groups.

We conclude that the mitochondrial ROS producing protein p66shc regulates right heart hypertrophy and right ventricular pressure during chronic hypoxia, probably via a CypD dependent mechanism.

DZL: Lung Cancer (Seminar Room 4, 3:30-5:00 pm)

Molecular mechanisms within bronchial carcinoma

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*Presenting author

Non-small-cell lung cancer (NSCLC) is commonly diagnosed at the metastatic stage, with median survival of one year. The identification of driver mutations in the epidermal growth factor receptor (EGFR) in a subset of lung adenocarcinomas as the primary oncogenic event led to a model of targeted therapies and to the genetic profiling of NSCLC. Currently, the use of EGFR tyrosine kinase inhibitors (TKIs) confers remission in 60% of patients, but responses are still short-lived. The pre-existing EGFR T790M mutation could be a subclonal driver responsible for these transient responses. In addition, AXL overexpression and reduced MED12 function are hallmarks of resistance to TKIs in EGFR-mutant NSCLCs. Crosstalk between signalling pathways is another mechanism of resistance; therefore, the identification of the molecular components involved could lead to the development of combination therapies co-targeting these components in lieu of EGFR TKI monotherapy.

Adaptive resistance can occur almost immediately after starting targeted therapy through a rapid rewiring of cancer cell signalling. By losing ERK negative feedback on RTK expression (EGF, FGF),[91] cancer cells are exposed to the stimuli of several ligands, and the ensuing activation of several RTKs reprograms all the canonical signalling pathways. The overexpression of several RTKs (EGF, AXL, HER3, FGF, NRG) was observed in breast cancer cell lines treated with a MEK inhibitor[92] and in BRAFV600E melanoma cell lines treated with BRAF inhibitors.[93] This rebound effect of overexpression of several RTKs, including ERBB3, also occurs in lung cancers driven by KRAS or EGFR mutations when treated with MEK, PI3K or dual PI3K/mTOR inhibitors.[94]

Molecular analysis of tumours from serial rebiopsies starting hours after initiating treatment with EGFR TKIs could identify which signalling components (eg, RTKs) are selectively upregulated during treatment. This would permit efficient co-targeting of these adaptive changes, precluding frank resistance in the tumour through synthetic lethality.

Role of BAMBI in the regulation of EMT processes in human lung cancer cell lines

Sofia Depner[1,6,*], *Sebastian Marwitz*[2,5], *Dmytro Dvornikov*[1], *Ruth Merkle*[1], *Philippe Lucarelli*[1], *Ole Ammerpohl*[3,5], *Reiner Siebert*[3,5], *Martin Reck*[4,5], *Peter Zabel*[2,5], *Ekkehard Vollmer*[2], *Torsten Goldmann*[2,5] and *Ursula Klingmüller*[1,6]

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Lung cancer, with its predominant form non-small cell lung cancer (NSCLC), is the leading cause of cancer related-deaths world-wide. One of the hallmarks of lung cancer is the high rate of mutations affecting different signaling pathways including member of the TGFbeta (transforming growth factor beta) signaling cascade. TGFbeta is known to have a dual role in carcinogenesis as it functions as a tumor suppressor by inhibiting cell proliferation and can act as a tumor promoter by inducing epithelial-to-mesinchymal transition (EMT). In lung cancer it has been observed that high TGFbeta levels correlate with poor prognosis and can be used as an independent risk factor for pulmonary metastasis.

The analysis of patient derived lung cancer tissues and tumor-free control samples by immunohistochemistry, transcriptome and array-based epigenetic methylome studies provided evidence for the down regulation of the TGFbeta pseudo-receptor BAMBI expression in tumor tissues. To examine the role of BAMBI in modulating TGFbeta signaling, we restored its expression in lung cancer cell lines using an inducible retroviral vector system. We showed that reconstitution of BAMBI expression resulted in reduced TGFbeta induced SMAD phosphorylation and a marked decrease in the expression of EMT markers at the mRNA and protein level. Furthermore, a reduction of cell motility was observed in a 2D migration assay and 3D collagen invasion assay.

Thus, the absence of BAMBI expression in NSCLC cell lines elevates responsiveness towards TGFbeta signaling and suggests a possible mechanism contributing to progression of lung cancer.

Aberrant DNA methylation patterns in lung cancer

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Lung cancer is the most common cause of cancer related death in males and the third common cause in females in Germany. Identification of molecular mechanisms by which environmental factors contribute to the development of lung cancer might improve not only lung cancer prevention but also prognosis and therapy of this cancer entity. DNA methylation, an enzymatically catalyzed and reversible covalent modification of the DNA, belongs to the family of epigenetic modifications allowing the cell to adapt its genetic activity according to the environmental conditions. In the German Center for Lung Diseases (DZL) we investigate alterations in the DNA methylation pattern in lung cancer tissue samples and corresponding normal controls using an array-based BeadChip approach.

First, to investigate the effect of tissue preprocessing on array based DNA methylation analyses, we compared the impact of fixation on the outcome of BeadChip analysis. Lung cancer tissue and corresponding tumor free lung tissue samples were collected, separated into defined pieces and subsequently fixed with either formalin or the non-crosslinking HOPE-technique prior to paraffin embedding. Cryo-preserved material acted as control. We showed that using the HOPE-technique instead of formalin largely prevents the introduction of formalin-fixation related artifacts.

Subsequently, we investigated the DNA methylation pattern of 90 lung tumor samples of selected entities (e.g. squamous cell carcinoma, adenocarcinoma, and large cell carcinoma) and compared the

methylation status between entities and the corresponding normal controls. Altogether, we identified >620 loci aberrantly methylated in lung cancer as compared to controls (FDR<$1.62x10^{-24}$, τ/τ_{max}> 0.415). This list will be compared with a list of loci found differentially methylated in blood between lung cancer cases and controls, to identify any overlaps.

Individualisation of radiochemotherapy (RTCT) for locally advanced non-small cell lung cancer (NSCLC)

Amanda Tufman[1,7,*], *Astrid Borgmeier*[1,7], *Claus Belka*[1,7], *Kurt Ulm*[2], *Fei Tian*[1,7], *Michael Flentje*[3], *Philipp Schnabel*[4,8], *Torsten Goldmann*[5,6], and *Rudolf Maria Huber*[1,7]

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*Presenting author

Background:

Individualised therapy has not yet found its place in the treatment of stage III NSCLC. Although RTCT can be curative, many tumours progress despite multimodal treatment.

The CTRT 99/97 Bronchial Carcinoma Therapy (BROCAT) study investigated radiotherapy (RT) vs. RTCT after induction chemotherapy. The German Intergroup Lung Trial (GILT) investigated consolidation CT following simultaneous RTCT. Here we present clinical and translational predictors of outcome in these two large randomised trials.

Methods:

We analysed histology subgroups within BROCAT, comparing adenocarcinoma, squamous cell and large cell tumours. We also examined site of first progression (PR), comparing local, central nervous system (CNS), and systemic PR. Within GILT we collected tumour biopsies and established a cooperation within the DZL to analyse potentially prognostic and predictive molecular markers.

Results:

BROCAT (n=214) found longer progression free survival (PFS) with RTCT vs RT after CT, and a trend to longer overall survival (OS).

Site of first PR differed between the study arms ($p < 0.047$), with more CNS and distant metastases after RT (CNS 21%, distant 36%) vs. RTCT (CNS 7%, distant 24%), and more local and thoracic PRs after RTCT (55% vs. 34%).

Histology in BROCAT: 59 adenocarcinoma, 171 squamous cell, 28 large cell, 10 mixed and 35 NSCLC not otherwise specified (NOS). There was a trend to longer OS in all histologies. Squamous cell carcinoma had longer PFS after RTCT, and large cell tumours showed a trend to shorter PFS after RTCT.

The GILT trial (n=279) found no significant OS benefit for consolidation CT after RTCT; however, some subgroups stood out. 27 samples from the GILT trial are being analysed for a panel of molecular markers.

Conclusion:

Further clinical and translational efforts are needed to increase our ability to tailor treatment to the patient and disease in stage III NSCLC.

DZL: Acute Lung Injury (Seminar Room 5, 3:30-5:00 pm)

Stem Cell Therapy in the case of Acute Lung Injury

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*Presenting author

MSC-based cell therapies are increasingly being investigated for use in pulmonary diseases and critical illnesses. As such a steadily increasing number of pre-clinical studies demonstrates efficacy of MSC administration in ameliorating disease-specific endpoints in a wide range of lung injury models. This includes mouse, sheep, and explanted human lung models of acute lung injury (ALI) resulting from endotoxin or bacterial-induced injuries. The mechanisms underlying acute ling injury and the acute respiratory distress syndrome (ARDS) in part involve a dysregulated intense acute inflammatory response to the underlying injury, exactly the type of injury for which MSC administration may have best efficacy. Pre-clinical models, postulated mechanisms of MSC actions, and initial clinical experiences of MSCs in patients with ALI/ARDS will be examined.

Influenza virus impairs fibroblast growth factor receptor 2b dependent epithelial regeneration from a distal airway epithelial progenitor pool

Jennifer Quantius[1,*], *Carole Schmoldt*[1], *Katrin Hoegner*[1], *Elie El Agha* [1], *Werner Seeger* [1,2], *Juergen Lohmeyer* [1,2], *Saverio Bellusci* [1,2], and *Susanne Herold* [1,2]

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IV (influenza virus) pneumonia is associated with apoptotic damage of the alveolar epithelial barrier and therefore efficient alveolar repair is crucial for recovery. Lineage tracing studies suggest that the adult lung contains epithelial progenitor cells which proliferate after injury. Fibroblast growth factor 10 (FGF10) plays a major role in lung development and is also known to have reparative, anti-apoptotic potential after injury.

We therefore investigated if FGF10 would support alveolar epithelial repair processes after IV-induced pneumonia.

Following IV infection or naphthalene treatment, epithelial progenitor cells (EpProg), defined as EpCamhighCD49fhighCD104+ Sca-1int showed increased resistance to apoptosis and revealed high proliferation rates. This response was likely mediated by upregulation of the FGF10 receptor FGFR2b on EpProg post IV infection or naphthalene treatment. However, EpProg were found to be primary targets of IV infection, which resulted in reduced FGFR2b upregulation and renewal capacity in the infected

compared to the non-infected fraction of EpProg, likely due to virus-induced blockade of the wnt signaling pathway mediating FGFR2b upregulation. Notably, the extent of EpProg infection correlated with the pathogenicity of different IV strains, suggesting that the severity of viral pneumonia might be associated with impairment of FGF10/FGFR2b-mediated epithelial cell renewal. Intratracheal application of recombinant or overexpression of FGF10 increased the reparative response of EpProg, whereas dominant negative FGFR2b overexpression resulted in reduced proliferation rates, sustained alveolar leakage and poor outcome.

We provide evidence that IV-induced blockade of the FGF10/FGFR2b axis may result in reduced epithelial cell renewal capacity and poor outcome and that induction of an FGFR2b-dependent pathway may represent a therapeutic approach to overcome IV-induced impairment of epithelial renewal and to drive tissue repair after injury.

Plenary Session: ...and Beyond (Auditorium, 9-10:30 am, 11-12:30 am)

Generation of anterior foregut derivative of from human pluripotent stem cells

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The generation of anterior foregut endoderm (AFE) derivatives from human pluripotent stem cells (hPSCs) would have major translational impact, as, among others, thymus and respiratory system are derived from this embryonic structure. The ability to generate functional thymic epithelial cells from pluripotent stem cells would have applications in modeling human immune responses in mice, in tissue transplantation, and in modulating autoimmune and infectious disease. Generating lung and airway epithelial cells from human pluripotent stem cells (hPSCs) has applications in regenerative medicine for lung diseases, drug screening and disease modeling, and provides a model to study human lung development. We have developed a strategy to differentiate hPSCs into AFE, and used this as a platform for studies aimed at achieving differentiation of these cells into lung and thymus. We established, based on developmental paradigms, a highly efficient method for directed differentiation of hPSCs into lung and airway epithelial cells. Long-term differentiation yielded cultures where >90% of the cells were committed to a lung or airway epithelial fate and contained goblet, Clara, ciliated, type I and, after addition of maturation media containing dexamethasone, predominantly (> 50%) type II alveolar epithelial cells. Inhibiting or removing agonists to signaling pathways critical for early lung development in the mouse, retinoic acid, Wnt and BMP, modeled defects observed in corresponding genetic mouse knockouts, thus validating this approach. Importantly, the type II alveolar epithelial cells generated were capable of surfactant protein-B uptake and release, providing evidence of specific function. Furthermore, we developed a flow cytometric approach to isolate type II cells from the cultures based on their function. Finally, we also developed a three-dimensional culture system for anterior foregut endoderm that allowed esophagus, thymus as well as lung and airway differentiation.

Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein

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*Presenting author

The limited availability of differentiated patient-specific CF lung epithelium remains a major roadblock for the potential development of therapeutic drugs to treat CF. Generated from skin fibroblasts, patient cells can be reprogrammed with 4 transcription factors (OCT4, SOX2, KLF4 and C-MYC) to become embryonic stem cell-like called induced pluripotent stem (iPS) cells. Tissue-specific cells generated from iPS cells hold great promise for patient-specific disease modeling, drug discovery and personalized medicine. We developed an in vitro differentiation protocol for generating functional CFTR-expressing

airway epithelia from human pluripotent stem cells. We identified key growth factors that mimic lung endoderm developmental pathways in vivo followed by air-liquid interface culture to direct the differentiation of pluripotent stem cells into mature large airway epithelia. The result was maturation of patches of tight junction-coupled differentiated airway epithelial cells. These cells showed up-regulated expression of several characteristic proximal lung markers, including, importantly, apical localization of the CFTR protein for proper chloride transport. CFTR transport functions were active in the differentiated cells as demonstrated by responsiveness to cAMP agonists and a CFTR potentiator in a modified iodide efflux assay for CFTR activity. The ability to generate a renewable source of patient-specific airway cells offer great hope for personalized medicines in drug discovery, tissue engineering and cell transplantation to treat many lung diseases including CF.

Pathways regulating lung stem cells and regeneration

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The respiratory system is a highly complex structure and is one of the few tissues in constant exposure to the external environment. The trachea, lungs, and cardiopulmonary vasculature that comprise the respiratory system have been the focus of extensive investigations in recent years. These studies have provided important new information about the mechanisms driving embryonic and postnatal development, as well as the differentiation of pluripotent human stem cells (hESCs and iPSCs) into lung cell types. However, there is still much to learn about the ability of the adult respiratory system to repair itself after damage and to replace cells lost in response to injury and disease. We have focused on defining the cell lineages responsible for generating and regenerating the respiratory system and the molecular pathways that regulate these processes. In particular, we have shown that development of cardiovascular and pulmonary systems is orchestrated by a common progenitor called the cardiopulmonary progenitor or CPP. We have also demonstrated an important role for epigenetic modifiers including HDACs in regenerating lung epithelium after injury. These and other topics will be discussed.

Bio-Implants

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In our research cluster on regenerative medicine, REBIRTH, we follow four routes towards regenerative therapies. Endogenous regeneration, cell and gene therapies, tissue engineering and bio-hybrid devices. While the first two therapeutic approaches will be applied within the surroundings of internal medicine, the latter two entities may represent future surgical devices, "Bio-Implants".

In lung research, tracheal substitutes were the first tissue-engineered constructs to be applied clinically. Previous implants from decellularized tissues, cryopreserved allografts, as well as aortic substitutes did not gain widespread acceptance due to limited long-term success. Macchiarini et al. then inaugurated the in vivo fabrication of tracheal substitutes, consisting of allogeneic cartiledge and stem cell based cellular re-composition of tissue, mainly the respiratory epithelium. His first attempts were highly recognized by the international scientific community, but no other groups have so far been able to get a similar approach to clinical success. It will require even more experimental work to successfully reconstruct functional pulmonary tissue for use in patients.

Bio-hybrid devices, by contrast, may represent important intermediate steps to replace lung function in end-stage organ failure. Current approaches towards bio-functionalisation of ECMO surfaces using endothelial cells may be an important step in this direction, also heading towards long-term application in patients and, potentially, implantability.

All these technologies have to compare with the current gold standard of permanent lung replacement, allogeneic transplantation. Availability and patient selection in lung transplantation will have to be titrated against the options of efficacy and long-term duration of alternative technologies in the future.

Poster Abstracts

German Center for Lung Research (DZL)

Disease Area: Asthma & Allergies (Poster 1-5)

P1: Species comparison of interleukin-13 induced airway hyperreactivity in precision-cut lung slices

Olga Danov[1,3,*], *Sharon Melissa Jimenez Delgado*[1,3], *Helena Drake*[1,3], *Susann Schindler*[1], *Olaf Pfennig*[2], *Christine Förster*[2], *Armin Braun*[1,3] and *Katherina Sewald*[1,3]

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Interleukin-13 is a key cytokine of asthma and elevated in asthmatics resulting in airway hyperresponsiveness (AHR). AHR is a hallmark of allergic asthma defined as exaggerated bronchoconstriction in response to contractile stimuli. Research for the development of new drugs is mainly based on appropriate in vivo and in vitro models. There is a need for translational models with improved predictivity for human. For comparison of different species we used precision-cut lung slices (PCLS) and assessed IL-13 induced hyperreactivity in PCLS of mice, rats, and humans.

PCLS were prepared from Balb/c mice, Brown Norway rats, and humans. IL-13 receptor was stained in the airways of mouse, rat and human PCLS by immunohistochemistry. Airways of all species were pre-incubated with 100 ng/mL IL-13. Subsequently, bronchoconstriction was induced by addition of methacholine (MCh) and visualized by videomicroscopy.

IL-13 receptor was present in epithelial cells and smooth muscle cells in PCLS of all species. Methacholine-induced bronchoconstriction in mouse exhibited an EC50 of 80 nM and decreased by pre-incubation with IL-13 to 50 nM, in rat from 220 nM to 170 nM and human from 180 nM to 47 nM MCh. In general, pre-incubation of PCLS in the presence of IL-13 resulted in all species in stronger bronchoconstriction at maximum methacholine concentration. Maximal constriction of initial airway area resulted in mouse in Cmax 61 % by control and decreased in IL-13 pre-incubated tissue to 80 %, in rat by 49 % to 69 % and human by 85 % to 94 % compared to untreated tissue.

This study shows that IL-13 receptor is similar distributed in epithelial cells and smooth muscle cells of all three species. IL-13 induced airway hyperreactivity in all tested species with different methacholine sensitivity. In future studies, PCLS will be used for pre-clinical studies to valuate the antagonist efficacy.

P2: Deregulation of ORMDL3 expression induces stress responses and modulates repair pathways

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The asthma-susceptibility gene ORMDL3 is an ER transmembrane protein previously associated with sphingolipid metabolism, the unfolded protein response, Ca2+ homeostasis, T-cell activation, and antiviral responses. However, the functional relevance of ORMDL3 in asthma pathogenesis remains elusive. Using the fruit fly Drosophila melanogaster as a model, we mimicked the situation found in patients at risk for asthma by increasing the expression of ormdl, the sole Drosophila homolog of ORMDL3, in the airway epithelium. Although ormdl overexpression did not overtly affect epithelial integrity, it increased the susceptibility to airborne stressors, such as cigarette smoke and hypoxia. When confronted by daily doses of cigarette smoke, flies overexpressing ormdl in the airway epithelia had a significantly shortened lifespan compared to matched controls. Moreover, these animals exhibited a much stronger behavioral response to hypoxia, and signaling systems such as the unfolded protein response and the TOR/PI3K pathway lost their ability to react to this stressor. In addition, overexpression of ormdl in the airways drastically reduced the output of signaling pathways associated with repair mechanisms, including EGFR and Notch signaling. These molecular changes were accompanied by changes in the lipid profile that resembled the situation observed in asthmatic airways. On the basis of these findings, we conclude that ORMDL proteins increase the stress status of the airway epithelium, which increases susceptibility to stress factors and increases the probability of developing asthma.

P3: Characterization of lipophilic house dust mite-allergens with regard to the allergic phenotype

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The common causes for respiratory allergies and allergic asthma are house dust mites (HDM). Not all HDM-allergic patients are detected by use of commercially available aqueous HDM-extract. Especially with regard to lipophilic allergens a lot of information is still lacking which is due to their low

concentration in the respective extracts or because they are hidden in a complex matrix. However, there is evidence from other allergen sources that lipophilic allergens are associated with severe clinical reactions. For studying these allergens in more detail as probable cause for HDM-asthma and for improving diagnostic tests, lipophilic allergens need to be identified, isolated and characterized in order to determine their allergenic risk.

In the case of house dust mite some hydrophobic allergens have already been identified (e. g. Der p 5, 7, 13, and 14). For studying interactions of these allergens with the epithelial cells of the respiratory tract a high amount of purified and well characterized single allergens is needed. Therefore, we started with the expression of recombinant allergens (e. g. Der p 5, 7, and 13) in *E. coli* and *Pichia pastoris*.

In parallel, we use sera from patients with different clinical phenotypes for further component-resolved investigations with the recombinant HDM-allergens Der p 1, 2, 4, 5, 7, 10, 11, 14, 15, 18, 21, and 23, applying immunoblot analysis. Up to now 43 sera of which the majority showed an IgE-reactivity against the major allergens Der p 1 and 2, were tested; one patient reacted with Der p 7, five with Der p 5 and one with Der p 21.

Future work will focus on the significance of selected hydrophobic HDM-allergens which are associated with allergic asthma. We will address the interaction of the single allergens with epithelial cells, probable receptor activation and cell uptake.

P4: Investigating the role of BAFF in different mouse models of allergic asthma

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Introduction: The cytokine B cell activating factor of the TNF family (BAFF) is crucial for the homeostatic development, differentiation and proliferation of B cells in the periphery. It is well known that elevated BAFF levels are associated with autoimmune diseases but its role in allergic diseases including asthma is barely understood.

Aims and objectives: To better comprehend the role of BAFF in allergic asthma we started analyzing BAFF in murine asthma models.

Methods: Wildtype (WT) and B cell deficient (µMT) mice were immunized with ovalbumin (OVA) or house dust mite (HDM). Bronchoalveolar lavage fluid (BALF) was measured morphometrically, airway hyperreactivity (AHR) by invasive lung function and BAFF via ELISA.

Results: Asthmatic WT mice showing lung eosinophilia and severe AHR have significantly elevated BAFF serum levels compared to controls. Additionally, in OVA-induced respiratory tolerant mice, BAFF levels are lower than in allergic mice. Kinetic studies demonstrate that BAFF levels increase, the more often allergen is administered intranasally suggesting local BAFF production in the asthmatic lung. Furthermore, BAFF determination in BALF showed increased levels in allergic compared to control mice. To test, whether BAFF is related to elevated IgE levels during asthma, BAFF production in µMT mice

was analyzed. Allergen treated µMT mice develop a similar allergic phenotype compared to WT mice and show increased BAFF levels in serum and BALF even in IgE absence.

Conclusions: In asthma models, allergic mice show elevated systemic and local BAFF levels, which increase with the frequency of allergen uptake via the lung and are independent of IgE presence. Thus, BAFF inhibition, recently permitted for treatment of systemic lupus erythematodes, might represent a new therapeutic target in allergic asthma.

P5: RORγt-specific RNAi decreases allergic airway inflammation and airway hyperresponsiveness in a mouse model of neutrophilic asthma

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Introduction: Recent studies suggest T helper 17 (Th17) cells as important players in the progression of asthma towards a severe phenotype. Characterized by the production of pro-inflammatory cytokines like TNF-α, IL-1ß, IL-22 and IL-17A, Th17 cells appear to act as general promoters of chronic inflammatory responses. These effector functions are regulated by the transcription factor Retinoic acid-related Orphan Receptor gamma (RORγ) t, which is essential for their differentiation. Thus, RORγt represents an ideal target not only to investigate the actual contribution of Th17 cells in the formation of severe asthma, but also as a promising novel target for a therapeutic intervention.

Therefore, the aim of this study is to diminish RORγt expression by using siRNA and to characterize its effects on Th17 cell activity in-vitro and on neutrophilic asthma in-vivo.
Methods: We generated OVA-specific Th17 cells in-vitro, which were transfected with siRNA candidates targeting RORγt. Afterwards, the in-vivo relevance of siRNA-mediated downregulation of RORγt was characterized in a mouse model of neutrophilic asthma.

Results: We could show that siRNA-transfected Th17 cells revealed not only a reduced expression of RORγt in-vitro but also of proinflammatory cytokines like IL-17A and IL17F. Intra-tracheal application of the RORγt-specific siRNA, which was most active in the in-vitro setting, inhibited the development of airway hyperresponsiveness (AHR) to methacholine and decreased bronchoalveolar lavage IL-17A, TNF-α and KC levels. Consequently, application of the RORγt-specific siRNA significantly reduced the number of neutrophils and of lymphocytes.

Conclusion: These results indicate that targeting RORγt could be a new approach for the treatment of neutrophilic asthma.

Disease Area: COPD (Poster 6- 14)

P6: Acute phase protein α1-Antitrypsin - a novel regulator of angiopoietin-like protein 4 transcription and secretion

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The angiopoietin-like protein 4 (angptl4, also known as peroxisome proliferator-activated receptor (PPAR) gamma induced angiopoietin-related protein) is a multifunctional protein associated with acute phase response. The mechanisms accounting for the increase in angptl4 expression are largely unknown. This study is the first to show that human α1-antitrypsin (A1AT) up-regulates expression and release of angplt4 in human blood adherent mononuclear cells and in primary human lung microvascular endothelial cells in a concentration- and time-dependent manner. Mononuclear cells treated for 1 h with A1AT (from 0.1 to 4 mg/ml) increased mRNA of angptl4 from 2 to 174-fold, respectively, relative to controls. In endothelial cells the maximal effect on angptl4 expression was achieved at 8 h with 2 mg/ml of A1AT (11-fold induction versus controls). In ten emphysema patients receiving A1AT therapy (Prolastin) plasma angptl4 levels were higher relative to patients without therapy [ng/ml, mean (95% confidence interval) 127.1 (99.5-154.6) versus 76.8 (54.8-98.8), respectively, p=0.045] and correlated with A1AT levels. The effect of A1AT on angptl4 expression was significantly diminished in cells pre-treated with a specific inhibitor of ERK1/2 activation (UO126), irreversible and selective PPARγ antagonist (GW9662), or genistein, a ligand for PPARγ. GW9662 did not alter the ability of A1AT to induce ERK1/2 phosphorylation, suggesting that PPARγ is a critical mediator in the A1AT-driven angptl4 expression. In contrast, the forced accumulation of hypoxia inducible factor 1-α, an up-regulator of angptl4 expression, enhanced the effect of A1AT. Thus, acute phase protein A1AT is a physiological regulator of angptl4, another acute phase protein.

P7: Peptide of alpha1-antitrypsin: potential novel therapy

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Background: We confirmed that alpha1-antitrypsin (A1AT) exhibits immunomodulatory activities that are unrelated to inhibition of neutrophil elastase. A1AT does not suppress but rather modulates production of pro- and anti-inflammatory substances, dependent on the cell/tissue response magnitude (output) to pro-inflammatory stimuli. A1AT down-regulates a hyper-immunity or hyper-inflammation

without impairing the normal immune or inflammatory response necessary to defend against infection or injury.

Methods: Human blood neutrophil and monocyte isolation by gradient centrifugation, explanted lung tissue culture, A1AT peptide synthesis, gene expression (RT-PCR), western blots, ELISA, cell adhesion, phagocytosis and viability assays, spectrophotometry, flow cytometry.

Results: We have selected synthetic C-terminal peptide of A1AT, which significantly modulates endotoxin-induced pro-inflammatory cytokine and chemokine release and expression (like IL-8, MCP-1, IL-1β, IL-6 and TNFα), increases neutrophil phagocytic activity and shows no toxic effects on human cell and lung tissue models ex vivo.

Conclusion: We propose that A1AT-based short peptides that mimic immunomodulatory properties of our own endogenous A1AT are excellent candidates for the drug development.

Research Support: Hannnover Medical School, German Center for Lung Research (DZL)

References:
Janciauskiene S and Welte T. 2013 Cardiovascular & Hematological Disorders-Drug Targets

P7a: The role of surfactant protein D in fibrotic lung remodelling

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Objective: Chronic obstructive pulmonary disease (COPD) is a strong risk factor for cardiovascular disease, since even moderate airflow reduction increases the danger of ischemic heart disease, stroke, arrhythmias, heart failure, and sudden cardiac death. COPD is associated with enhanced inflammatory response in the airways and the lung tissue accompanied by a complex remodelling process with fibrosis as one possible outcome. Surfactant protein D (SP-D), a collectin with immunmodulatory function, has been implicated in the pathogenesis of COPD and is seen as a potential biomarker for idiopathic interstitial fibrosis, although its role in this disease remains elusive. In this study we aimed to test the hypothesis that SP-D plays an important role in the fibrotic remodelling by characterizing a mouse model deficient in SP-D.

Methods: SP-D knockout mice were analyzed at different time points (after 3 and 6 months, 1 and 1.7 years) by design-based stereology and lung function was assessed. We further determined the expression of fibrotic marker genes including microRNAs in lung tissue applying quantitative real-time polymerase chain reaction.

Results: Compared to wilde-type littermates, SP-D-deficient mice exhibited thicker alveolar septae, predominantly in the interstitium, and this increase was strengthened over time. We further observed a significant elevation of pro-fibrotic microRNAs (miR-21 and miR-155) in lung tissue of SP-D-deficient mice as well as a deregulation of collagen, transforming growth factor β1, and vimentin.

Conclusion: These findings indicate that the deficiency in SP-D favours a pro-fibrotic remodelling in the lung altering the morphology of the alveolar interstitium as well as the expression of fibrosis-related genes.

P8: Can health-economic modeling be adjusted to assess the cost-effectiveness of prognostic testing in COPD management?

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Background:

Genetic mutations like ADRB2 polymorphisms are suspected to influence treatment outcomes in COPD [1]. Clinical application of pharmacogenetic testing may improve treatment and cost outcomes in COPD. This paper analyzes whether effectiveness and cost-effectiveness of genetic testing for mutations and stratified treatment can be assessed by testing and adapting an existing COPD Markov model.

Methods:

The German COPD model is a comprehensive Markov model (disease stages I-IV according to GOLD, post-surgery, post-LTx, death) and considers mild, moderate and severe exacerbations [2]. It was developed to conduct cost-utility analyses. The model was extensively cross-validated in an international COPD modeling workshop led by Erasmus University Rotterdam. For evaluating stratified approaches, the model needs to be adjusted to compare COPD patients who either receive or do not receive a genetic test before pharmacological treatment.

Results:

Structure and input parameters of the economic CODP model were successfully cross-validated comparing output with several international COPD models. The existing model comprises the entire course of disease, thus it can be adjusted to analyze treatment strategies, and extended for stratified approaches. The model showed good applicability for evaluating cost and effects of different and complex COPD interventions. If novel and experimental markers, e.g. ADRB2 polymorphisms, report sufficient differential impact on treatment while having good sensitivity and specificity, stratified treatment approaches can be meaningfully compared to usual care treatment without genetic testing using this model. Furthermore, information on the prevalence of the mutation in the population as well as on test costs and uncertainty is needed for relevant population-based modeling.
Conclusion:

A German COPD model has been found valid in testing. The model enables adjustments to derive cost-effectiveness estimates of stratified treatment according to prognostic markers in the management of COPD. A consistent reporting of test effects and test characteristics is necessary for reliable cost-effectiveness estimations.

References:

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Menn P, Leidl R, Holle R. A lifetime Markov model for the economic evaluation of chronic obstructive pulmonary disease. Pharmacoeconomics. 2012 Sep 1;30(9):825-40.

P9: The primary care routine data registry BeoNet: health services and health economic research in COPD and ELD

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Objectives: Reliable primary care data are needed for health services and health economic research in lung disease like COPD and ELD. Within the German Center for Lung Research, the BeoNet- ("Beobachtungspraxen-Netzwerk") Registry will gather full primary care routine data from electronic patient records for real-time monitoring as well as for longitudinal trans-sectoral cohort studies and combine these data with patient reported outcomes.

Methods: A real-time, standardized collection of primary care routine data will be established. Basic claims data from electronic patient records of participating general practitioners, pneumologists and pediatricians as well as data on different aspects of care like on diagnosis, treatments and procedures, medication, disease management, treatment frequencies and accounting are accumulated, transferred via standardized interfaces and compiled for analysis.

Data on other healthcare utilization, health-related quality of life and further disease-specific parameters will be gathered by additional questionnaires and linked with the electronic health records.

Results: Important milestones during the pilot phase of the BeoNet-Registry have been achieved so far: (1) project approvals by the ethics committees of Hannover and Munich as well as for the data security concept were obtained; (2) cooperation with software developers was initiated to ensure data extraction and transport; (3) specific questionnaires for additional data were selected, tested and adopted.

The ongoing recruitment of the network of surgeries and the construction of the data test-network has been initiated. Until now 51 general physicians agreed to participate, 91 are interested. Physicians engaged in specific health care research projects will recruit target-patients. Over 26.000 patient IDs and the coresponding data entries were transferred as a test dataset from the electronic patient records to the database. First queries are in progress.

Conclusions: For the improvement of pulmonary healthcare strategies the BeoNet-Registry provides a quality-proved data base for standardized assessments of disease-specific costs, quality of life and for objective outcome evaluation.

P10: Alveolar epithelial cells type II show a high sensitivity to cigarette smoke extract

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Alveolar epithelial cells type II (AECII) play an important role in the normal pulmonary function as well as in the host defense and immune response. The human tumor cell line A549 is the most popular model of AECII.

In the presented study, the effects of cigarette smoke extract (CSE) on primary AECII and A549 as well as the epithelial adenocarcinoma cell line H1975 were investigated.

Tumor-free lung tissue from patients who underwent lobectomy due to cancer at the LungenClinic Großhansdorf was used to isolate AECII. Briefly, after crushing the lung tissues, AECII were separated by negative selection via a CD45. Subsequently, cells were seeded on collagen-coated 96-well plates at low density (4 x 10^4 cells/ well). A549 cells were seeded at 2 x 105 cells/ well, H1975 at 2 x 10^4 cells/well. All three cell types were maintained in DMEM-F12 supplemented with 10% FCS overnight. CSE was obtained using commercially available cigarettes (West light) by drawing smoke of one cigarette slowly through a water pump into a tube containing 10 mL of ddH2O (=10% CSE). Stimulation was performed under serum-free conditions. Cells were stimulated with increasing concentrations of CSE (0.1 - 5%) for 1 h. Cells were cultured for further 4 h. Cell viability was measured via MTT assay.

Acute CSE exposure with 0.5% CSE induced a significant cytotoxic effect in AECII (IC50: 0.21% CSE), which was not reversible by dexamethasone (4 mg/mL) or roflumilast (15 µM). In H1975, a concentration of 5% CSE caused a significant reduction of cell viability. By contrast, no cytotoxic effect was detectable in A549 cells.

Primary AECII are a model to investigate cigarette smoke induced inflammatory effects, better suited than the widely used tumor cell line A549. Moreover, CSE-induced AECII damage is not reversible by anti-inflammatory treatment.

P11: Development of emphysema in bENaC-overexpressed mice is accelerated by acute cigarette smoking exposure

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Chronic obstructive pulmonary disease (COPD) is characterized by chronic bronchitis, small airway remodeling and emphysema. As a hallmark of COPD, mucus accumulation may result in airway hypersensitivity and small airway obstruction. Accelerated b-Epithelial Na+ channel (bENaC) in the

lower airway epithelial membrane of mice leads to airway surface liquid depletion and mucocilia clearance dysfunction, which causes mucus accumulation and further, COPD like disease(Mall, M.A. A.M.J.Respir, 2008). However, it remains unclear how increased mucus production contributes to emphysema and COPD development.

10 day old bENaC mice and litter mate controls were exposed to filtered air or cigarette smoke, for 50min twice daily, 4 days at a particle concentration of 500mg/m3. On the 5th day lung function and cytospin from Bronco Alveolar Lavage (BAL) fluid was performed. H&E and PAS staining of lung sections were performed to analyze morphological changes.

We observed spontaneous emphysema development in the lung of bENaC mice. However, this emphysema development was exacerbated after acute cigarette smoke exposure, as demonstrated by higher compliance values (0.014+/-0.002 mL/cmH2O vs 0.006+/-0.0006 mL/cmH2O, $P<0.01$) and increased airspace enlargement compared to WT animals. Interestingly, emphysema development was associated with increased macrophage and neutrophil cell number in BAL fluids.

Our data demonstrate that the spontaneous development of emphysema accompanied with the accumulation of mucus in the airways of bENaC transgenic mice results in an impairment of lung function, and a severer inflammatory response following cigarette smoke exposure that may contribute to an accelerated COPD pathogenesis.

P12: In vitro ciliary development in an epithelial stem equivalent cell line M3E3/C3 derived from the fetal Syrian hamster lung

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Failure in airway regeneration after injury may result in profound consequences like a disease with bronchiolitis obliterans. Re-building of ciliated cells is a good marker for resumed airway activity. We decided to investigate whether M3E3C3,a fetal hamster stem equivalent cell line, is capable of in vitro ciliogenesis. Immunoreactivity and qRT-PCR have revealed that this cell line expresses a stem cell marker Sox2 and another marker, Clara cell secretory protein (CCSP) specific for the stem cells in the bronchiolar region. An embryonic stem cell factor Oct4 was negative.

The cells 3D-cultured at the air-liquid interface (ALI) exhibited pseudostratification together with apical cytoplasmic ezrin and growth of long cilia when combined with mesenchymal cells (MC) embedded in an underlying collagen (I) gel, inside which the medium flowed vertically and reciprocally. A transcription factor Foxj1, whose expression critically regulating ciliary development was measured by qRT-PCR, became active early in the 3D culture at ALI and rapidly diminished a few days before the start of the ciliogenesis. The ciliated cells were also detectable by immunoreaction to β-tubulin IV antibody or optical recognition of ciliary beating. They can grow when the ALI conditions are met even without the co-presence of MC, but the morphology of the cilia is often dwarfed, as observed by electron microscopy. However, on a Matrigel film even under ALI conditions alone the cilia appear to

develop normally. Fibrillar collagen (I) and Matrigel, instead of the living MC could not support pseudostratification in the epithelium, although beating cilia were induced despite their size or form.

The results show that the airway stem equivalent cell line can be coaxed in vitro into forming a pseudostratified epithelial architecture and cilia in the adequate microenvironment. This stem equivalent cell line may thus constitute a useful experimental system for exploring the mechanisms of airway epithelium regeneration.

P13: Immunoaging augments sensitivity to cigarette smoke-induced COPD

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The pathogenesis of COPD is related to an abnormal inflammatory response of the lungs to cigarette smoke, toxic gases and particles, which leads to emphysema, chronic bronchitis and subsequent decline in lung function. Age-related changes of immune system functions have been described, but are complex phenomena incompletely understood. We hypothesize that in a chronic CS-induced mouse model the pathogenesis of COPD is characterized by an elevated immune response in aged mice.

2 and 12 months old C57BL/6 mice were exposed to CS concentration of 500 mg/m3 TPM for 3 months. BAL fluid was sampled to perform differential cell counts and inflammatory cell recruitment in lung tissue was measured by FACS. Lung function and emphysema development were also determined.

Only in aged mice an increase in lung compliance was noticeable after 3 months of CS exposure compared to control and young animals. Emphysema development in CS-exposed aged mice compared to younger animals substantiated these findings. A significantly greater volume of iBALT structures in aged mice after CS exposure was shown by quantification of lung tissue inflammation. Staining for MMP12 in lung tissue indicated significantly higher macrophage accumulation and activation in CS-exposed aged mice, which was in accordance with increased MMP12 expression and an elevated MMP12/TIMP1 ratio. Differential cell counts of BAL cytospins revealed significantly higher lymphocytes only in CS-exposed aged animals. Interestingly, an increase in Th17 cells in the lung could only be shown in CS-exposed aged mice compared to control animals.

These results strongly suggest that lung inflammation after CS exposure is augmented in aged mice, which might be related to an age-induced change in gene expression profiles. This suggests a role for age-related inflammatory changes in the pathogenesis of COPD.

P14: Evaluation of small molecule FRET reporter for the diagnosis and monitoring of proteolytic activity in chronic obstructive lung disease model

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Proteases such as neutrophil elastase (NE) and matrix metalloprotease 12 (MMP-12) are key factors in inflammatory processes and contribute the gradual destruction of extracellular lung matrix in chronic inflammation. We now hypothesize that activity levels of inflammation-relevant proteases may be useful indicators for the onset and progression of obstructive lung diseases such as cystic fibrosis or COPD. With the recently published small molecule FRET protease reporters NEmo and LaRee, for detection of NE and MMP-12 activity, respectively, it is possible to monitor protease activity at the single cell level.

The goal of this study is to apply protease activity measurements to sputum specimens from patients with chronic obstructive lung diseases. We investigated the impact of sample generation and experimental conditions on the performance and outcome of diagnostic protease monitoring in sputum samples in general. We tested the new approach on a double blinded sample set from the Fraunhofer ITEM Hannover (BREATH). In this study healthy subjects were exposed to irritating conditions or control air in an incubation room. Sputum was produced and processed by standard operation procedure. Through comparison of by-hand analysis, we succeeded in receiving satisfactory data sets via automated cell analysis of hundreds of cells per sample using novel macros. This assay format is now expanded to employ additional small molecule protease probes for cathepsin B and S.

Our novel approach of the assessment of protease activity at the single cell level applied to multiple protease types may result in a new tool for diagnosis and monitoring. The detection of patient specific protease activity patterns may improve the differentiated diagnosis and therapeutic strategies.

Disease Area: Cystic Fibrosis (Poster 15- 23)

P15: Functional analysis of regulatory variants that determine the outcome of the monogenic disease cystic fibrosis

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The clinical outcome of patients with Cystic Fibrosis is highly variable, even among patients who harbour the same CFTR mutation genotype, indicating that environmental and non-CFTR genetic factors such as modifying genes shape the course of the disease. Two contrasting alleles (C or T) of SNP rs7910656 on intron 2 of the FAS gene have been associated with mild and severe CF outcome.

To gain mechanistic insight into how the SNPs of FAS alter the course and the outcome of the disease, we performed in- silico analysis using ENCODE data which showed that rs7910656 is on a nucleosome free region and occupied with RNA POLII and H3k36me3. We have also identified a novel secondary promoter start site which is +4.5kb from rs7910656. Analysis into differential transcription factor occupancy of rs7910656 and controls rs2147420, rs1571019 showed that 29 TFs, mostly of immune regulating family occupied rs7910656 while 6 and 7TFs are on rs2147420and rs1571019 respectively. We also have found that the C-allele of rs7910656 is bound by 6 TFs (NFKB, STAT4, HIF1A, MAFA, NURR1 and TEAD) which are not observed for the T-allele. More so, the C-allele makes multimeric complexes with NFKB (p65, p50, C-Rel), binds with three motifs to STAT4 and binds with one motif to HIF1A, while T-allele does not bind with NFKB and HIF1A but binds with two motifs to STAT4.

We have designed probes for the TFs and have developed a nuclear extraction protocol that preserves the transcription factors in their native states (phosporylated and acetylated) and have carried out an Electrophoretic Mobility Shift Assay with biotinylated probes of p65 subunit of NFKB,C and T-allele of rs7910656. We found the robust binding of p65 subunit and have shown that C-allele binds more robustly than T-allele, which is consistent with our in-silico predictions.

P16: Silencing of miR-148b ameliorates cystic fibrosis-like lung diseases in βENaC-overexpressing mice

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MicroRNAs are involved in diverse biological and pathological processes. Here, we studied the potential role of miRNAs in the in vivo pathogenesis of cystic fibrosis (CF)-like lung disease in βENaC-overexpressing (βENaC-Tg) mice. We performed miRNA array analysis in lung tissue of βENaC-Tg and wild-type mice. Differentially expressed miRNAs were validated by qRT-PCR and their target genes were identified by bioinformatics analysis and luciferase reporter assays. Tissue specific localization was performed by in situ hybridization using locked nucleic acid-modified DNA probe. Direct functional studies were performed by knockdown of miRNA expression in the lungs of βENaC-Tg mice using antagomirs. The effects of knockdown were studied by lung histology, analysis of inflammatory cells in bronchoalveolar lavage and pulmonary function testing using flexiVent system. Genetic association studies in CF patients were performed by analyzing miR148b-Sat1 allele frequency. We demonstrate that miR-148b is upregulated in the lungs of βENaC-Tg mice and predominantly localized in conducting airway and alveolar epithelial cells. Luciferase reporter assay in Hela cells suggests Mig-6 (mitogen inducible gene-6), a protein previously shown in normal lung development, as a potential target of miR-148b. Antagomir-mediated knockdown of miR-148b in the lung of βENaC-Tg mice reduced emphysema formation, goblet cell metaplasia and neutrophillic inflammation. Further, we observed upregulation of miR-148b in human cystic fibrosis and COPD lung tissue, as well as its localization in airway and alveolar epithelial cells. Finally, our genetic association studies establish that distribution of miR148b-Sat1 allele, closely linked to the MIR148b genomic locus in human, is associated with disease manifestation among F508del-CFTR homozygous sibling pairs. Collectively, these results indicate that deregulation of miR-148b may play an important role in the pathogenesis of CF and COPD and may serve as a novel therapeutic target.

P17: ICM is sensitive to detect potentiation of CFTR-mediated Cl- secretion in patients with cystic fibrosis and the G551D mutation treated with ivacaftor

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Background: Sensitive outcome measures of CFTR function may facilitate the implementation of mutation-specific therapy with CFTR modulators in patients with cystic fibrosis with non-G551D mutations. Intestinal current measurement (ICM) is a sensitive assay for functional assessment of mutant CFTR in rectal biopsies and was recently shown to detect potentiator effects of 1-EBIO ex vivo (Roth E. et al., PLOS ONE 2011). The aim of this study was to determine, if ICM is sensitive to detect potentiation of CFTR-mediated Cl^- secretion in rectal epithelia from CF patients with a G551D mutation treated with ivacaftor. Methods: Rectal biopsies were obtained from 8 patients carrying a G551D-CFTR mutation before and at least four weeks after the start of ivacaftor therapy. Rectal tissues were

mounted in micro-Ussing chambers and CFTR-mediated Cl^- secretion was determined from Cl- secretory responses induced by cAMP (IBMX/forskolin)- and Ca^{2+} (charbachol)-mediated stimulation. Results: Before ivacaftor therapy, ICM detected variable residual CFTR-mediated Cl- secretion in rectal tissues from CF patients with a G551D mutation. In the presence of ivacaftor therapy, CFTR-mediated Cl^- secretory responses were increased in all 8 patients. Conclusion: We conclude that ICM is sensitive to detect in vivo potentiation of mutant CFTR function by treatment with ivacaftor. Our results indicate that ICM may be a useful bioassay to determine therapeutic responses at the level of the basic CF defect of ivacaftor and potentially other clinical CFTR modulators in CF patients with non-G551D mutations.

P18: Rescue of function in a cystic fibrosis mouse model by transfer of hematopoetic stem cells

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Cystic fibrosis (CF) is the most common hereditary disease in the Caucasian population. The disease causing gene CFTR was identified in 1989. So far, more than 1,800 mutations have been identified in the *CFTR* gene [1], which codes for a cyclic adenosine monophosphate–regulated chloride channel. The malfunction of this channel leads to a progressive loss of function in the CF lung, which is the decisive factor for morbidity and mortality of most CF patients. Beside mucus accumulation in the airways, also an impaired phagocytosis in alveolar macrophages seems to play an important role in the pathogenesis of chronic infection and inflammation in the CF lung [2]. The Gram-negative, ubiquitous opportunistic pathogen *Pseudomonas aeruginosa* is the key organism in causing CF lung disease.

CF mouse models are an extremely suitable tool to investigate *Pseudomonas* lung pathogenicity. In our study presented here, we tested whether the increased genetic susceptibility of CF mice to airway infection with *P. aeruginosa* can be reduced to levels of wild-type mice by transfusion of hematopoietic stem and precursor cells (HPSPCs) of *Cftr* wildtype mice. Therefore, we subjected recipient *B6.Cftr*$^{TgH(neoim)Hgu}$ mice to a lethal dose of irradiation and injected them with hematopoietic progenitor cells from B6 wildtype donor mice. After a six weeks phase of reconstitution mice were infected intratracheally with a disease causing dose of the cytopathic *P. aeruginosa* strain PAO1. The course of infection was monitored over 144 h measuring murine lung function, body weight, rectal temperature and survival of the mice. *B6.Cftr*$^{TgH(neoim)Hgu}$ mice which received isogenic HPSPCs served as controls.

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P19: The effector protein ExoY secreted by Pseudomonas aeruginosa augments the inflammatory reaction in the respiratory tract of mice

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Infection with the gram-negative opportunistic pathogen *P. aeruginosa* causes serious pulmonary, urogenital and systemic inflammation in immunodeficient patients. Most prominently, *P. aeruginosa* is the key organism responsible for pneumonia in cystic fibrosis patients, defining the course of the disease as well as its prognosis. *P. aeruginosa* produces a wide variety of effector proteins and injects them into host cells via the type III secretion system [1]. One of the effectors, injected by this needle-like structure is ExoY, which can be found in 90% of clinical isolates from *P. aeruginosa*. Despite of the highly frequent ExoY occurrence, its function is still unknown. While previous publications described ExoY to be apathogenic, our recent in vivo studies demonstrated a distinct role of ExoY as a pathogenic factor of *P. aeruginosa*.

In our murine infection model we infected B6 mice intratracheally with 1 x 10^{6-8} colony forming units (cfu) of two *P. aeruginosa* strains, the first expressing a functional ExoY, the second the catalytically inactive ExoY mutant K81M [2]. Mice were sacrificed 0-48 h after infection and bacterial infection was characterized by analyzing the migration of neutrophils into the lung and inflammatory cytokines in the respiratory tract.

Infection doses of 10^7 cfu/mouse lead to ExoY-dependent, severe pathological changes in lung tissue and to increased mortality. Even more distinct effects were seen at concentrations of 10^8 cfu/mouse. Within 4 to 8 hours severe signs of infection and lung inflammation were observed. Lethality occured 24-48 hours after infection. Inflammatory lung reaction was characterized by interstitial edema, hemorrhagic infiltration and necrotic/ apoptotic areas in the tissue. Secretion of proinflammatory factors such as IL-6, IL-1, MCP-1 and KC was significantly increased in the ExoY infected groups.

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P20: Population biology of Pseudomonas aeruginosa in chronic CF and COPD airway infections

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To analyse the population structure of Pseudomonas aeruginosa, more than 1400 independent isolates from diverse environmental and clinical habitats and geographic origins were investigated by SNP-typing of core genome and markers of the accessory genome. More than 50% of all isolates belonged to less than 25 dominant clones widespread in disease and environmental habitats. Moreover, most clones group in only a few clonal complexes. These complexes seem to be phylogenetically ancient and related to specific sets of genomic islands, e.g. exoU islands. This implies that recombinations between strains of different complexes are rare events.

While most clones were found in the environment, only a subgroup of these strains has been found to date in humans. Moreover, beside some extremely frequent generalists, most strains were related to a specific mode of infection or habitat. For example, the clones dominating in cystic fibrosis (CF) are also dominant in chronic obstructive pulmonary disease (COPD), acute lung infections and urinary tract infections.

To investigate the microevolution of P. aeruginosa in the human lung, serial CF airway isolates of the globally most frequent clones C and PA14 were collected over 20 years since the onset of colonization. The intraclonal evolution in CF lungs was resolved by genome sequencing of first, intermediate and late isolates and subsequent multimarker SNP genotyping of the whole strain panel.

While the PA14 clone diversified into three branches in the patient's lungs and acquired 15 nucleotide substitutions and a large deletion during the observation period, the clone C genome remained invariant during the first years in CF lungs; however, 15 years later 947 transitions and 12 transversions were detected in a mutL mutant strain. Late persistors in CF lung habitats were compromised in growth and cytotoxicity, but their mutation frequency was normal even in mutL mutant clades.

P21: Strategies to identify clinically relevant interaction partners of cystic fibrosis modifying genes: analysis of SCNN1B

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The course of the monogenic disease cystic fibrosis is influenced by non-genetic factors and by CF modifier genes. During the last years, several CF modifying genes were identified and the clinically relevant variants were mapped by the base in the patient population of the European CF Twin and Sibling study. The identified genetic variants largely are located within the non-coding sequence of the human genome, thus implying that they do not change the amino acid sequence of the corresponding modifier gene but instead reflect an essential regulatory mechanism. During the last years, we have used complementary technologies to describe the molecular mechanism that governs the expression of the cystic fibrosis modifier gene SCNN1B. To identify the causative variants, 3 microsatellites and 45 SNPs were genotyped on 101 families with a total of 171 F508del-CFTR homozygous CF patients. Resequencing of two 8000 bp fragments for which discordant and concordant sibling pairs carry contrasting genetic information has revealed six possible causative SNPs. Bioinformatic predictions and subsequent testing of the predicted interaction partners as candidate genes have so far confirmed one transcription factor as a CF modifying gene. Using both alleles of the six identified SCNN1B SNPs in a comparative electrophoretic mobility shift assay and subsequent protein sequencing of the excised bands has revealed previously unknown novel DNA-protein interactions that are likely to mediate SCNN1B expression. Continuous support by the Fritz-Thyssen-Stiftung is gratefully acknowledged.

P22: Imaging cilial motion by endoscopic optical coherence microscopy

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Endoscopic imaging gives minimally invasive access to the airways and can provide important diagnostic information. We have shown that optical coherence microscopy (OCM), the combination of confocal microscopy and optical coherence tomography is able to image airway tissue with subcellular resolution. Due to its high imaging speed and lack of tissue damage OCM is a promising technique for clinical imaging if it can be integrated into endoscopic imaging devices.

Here, we present endoscopic OCM imaging of airway tissue using a rigid GRIN lens based endoscopes. Beating motion of cilia and particle transport was visualized with up to 50 Hz temporal resolution.

Presently,the probe can be used for small animal imaging. Work is under progress to convert the rigid endoscopy into flexible probe which can be used for human imaging

P23: Downregulation of Hemeoxygenase-1 and Altered Cellular Homeostasis in Cystic Fibrosis

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Hemeoxygenase-1 (HO-1), an inducible heat shock protein, is upregulated in response to multiple cellular insults via oxidative stress, lipopolysaccharides (LPS), and hypoxia.

In this study, we investigated the role of toll-like receptor 4 (TLR4), hypoxia-inducible factor-1α (HIF-1α) and iron on HO-1 expression in cystic fibrosis (CF). Immunohistochemical analysis of TLR4, HO-1, ferritin and HIF-1α were performed on lung sections of CFTR-/- and wildtype mice. CFBE41o- and 16HBE14o- cell lines were employed for in vitro analysis via immunoblotting, immunofluorescence, real-time PCR, luciferase reporter gene analysis and iron quantification.

We observed a reduced TLR4, HIF-1α, HO-1, and ferritin in CFBE41o- cell line and CF mice. Knockdown studies using TLR4-siRNA in 16HBE14o- revealed significant decrease of HO-1, confirming the role of TLR4 in HO-1 downregulation. Inhibition of HO-1 using tin protoporphyrin in 16HBE14o- cells resulted in increased iron levels suggesting a probable role of HO-1 in iron accumulation. Additionally, sequestration of excess iron using iron chelators resulted in increased HRE response in CFBE41o- and 16HBE14o- implicating a role of iron in HIF-1α stabilization and HO-1.

To conclude, our results demonstrate that downregulation of HO-1 expression in CF is resulted due to reduced TLR4 expression and increased intracellular iron and decreased HIF-1α.

Disease Area: DPLD (Poster 24- 40)

P24: Aberrant expression and activity of histone deacetylases (HDAC) in lungs of patients with sporadic idiopathic pulmonary fibrosis (IPF)

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Introduction: Histone deacetylases (HDACs) are enzymes that remove acetyl groups from an ε-N-acetyl lysine amino acid on histones, resulting in epigenetic repression of gene transcription. HDACs can also catalyze deacetylation of many non-histone proteins, such as the tumor suppressor p53, resulting in inhibition of its pro-apoptotic activity. HDACs thus pivotally control gene expression and cellular signaling. Due to their anti-apoptotic activity, HDACs are upregulated in many cancers. Here, we describe for the first time a biochemical characterization of Class-I/-II/-III-HDACs in lungs from patients with sporadic IPF (n=16) and organ donors (n=26). Methods: Lung tissue was analyzed by RT-PCR, immunoblotting and immunohistochemistry (IHC). Results: Compared to donors, protein-levels of Class-I- (HDAC1,2,3 and 8) and Class-II-HDACs (HDAC4,5,7,9,10), and of the Class-III-HDAC Sirtuin-1 were significantly elevated in IPF lungs. By means of IHC, strong nuclear induction of HDACs 1-3 and Sirtuin-1 was observed in myofibroblasts of fibroblast foci (FF) and in abnormal bronchiolar basal cells at sites of aberrant re-epithelialization in IPF lungs, but not in donors. Similarly, induced cytoplasmic expression of Class-II-HDACs: 4,5,7,9,10 and of the Class-III-HDAC Sirtuin-2 could be encountered in FF and basal cells in IPF. Importantly, type-II alveolar epithelial cells (AECII) of IPF-lungs did not reveal notable expression of Class-I/-II/-III-HDACs, possibly due to severe ER stress in this cell type. But IPF-AECII indicated induced cytoplasmic expression of HDAC6 – a Class-IIb-HDAC involved in aggresome formation. Conclusions: We suggest that fibroblast proliferation, fibroblast-to-myofibroblast differentiation and the apoptosis-resistant phenotype of fibroblasts and myofibroblasts in IPF may be mediated due to enhanced expression and action of Class-I/-II/-III-HDACs. Similarly, aberrant overexpression of HDACs in basal cells of IPF lungs may cause the exaggerated, proliferative character of this cell type in IPF and thus govern the process of bronchiolization in this disease. We conclude that HDACs may be novel molecular targets for IPF therapy.

P25: Inhibition of profibrotic signaling in fibroblasts from patients with idiopathic pulmonary fibrosis by histone deacetylase-inhibitors or pirfenidone

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a fatal disease characterized by distorted pulmonary structure and the excessive deposition of extracellular matrix (ECM) proteins, such as collagen. Myofibroblasts are the primary collagen-producing cells in IPF lungs, and their accumulation within pathologic lesions called fibroblast foci (FF) is a hallmark of IPF. To explore new drugs for IPF, we investigated the therapeutic potential of histone deacetylase inhibitors (HDACi), because we have discovered a significant overexpression of Class-I/-II/-III HDAC enzymes in IPF fibroblasts/myofibroblasts. Methods: Primary IPF fibroblasts were incubated for 30h with the HDACi panobinostat (LBH589, 85nmol) or valproic acid (VPA, 1.5mM), or with the IPF drug pirfenidone (2.7mM). Results: Treatment of primary IPF fibroblasts with the pan-HDACi panobinostat resulted in significantly reduced expression of genes associated with fibrogenesis (ACTA2, COL1A1, COL3A1, FN), cell survival (BIRC5=survivin), proliferation (CCND1), as well as in suppression of HDAC7, and was paralleled by induction of severe ER stress (ATF6, CHOP) and apoptosis (p21, PUMA, cleaved caspase-3). Blockade of Class-I-HDACs by VPA was also associated with reduced expression of BIRC5, but profibrotic gene expression was not greatly altered. Finally, the direct comparison panobinostat - versus pirfenidone therapy showed also for pirfenidone treated IPF fibroblasts a significant downregulation of COL1A1, COL3A1, and FN, but not of CCND1. Furthermore, the profibrotic genes CNN1 and P4HTM were exclusively reduced by pirfenidone -, but not by panobinostat treatment. Importantly, pirfenidone treatment lead also to a significant downregulation of the cancer-associated gene BIRC5, but was not associated with induction of ER stress and pro-apoptotic signaling. Finally, pirfenidone did not greatly affect expression of HDAC proteins. Conclusions: We conclude that generation and apoptosis resistance of IPF fibroblasts/myofibroblasts are mediated due to enhanced activity of HDAC proteins, and that panobinostat can present a novel therapeutic option (in addition to pirfenidone) for progressive fibrotic lung diseases.

P26: Regulation of autophagy in pulmonary fibrosis

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Importance of lysosome related pathways under conditions of idiopathic pulmonary fibrosis is not extensively studied till date. Autophagy has been known from several years as a self-eating catabolic pathway, activated mainly to degrade the cell's own unused organelles, macromolecules and long-lived proteins via the lysosomal system. Here, we aimed to study the regulation of autophagy in idiopathic pulmonary fibrosis as well as in murine model of bleomycin induced lung fibrosis.

Markers of different forms of autophagy were analyzed in the lungs of IPF patients, healthy donors and vehicle or bleomycin treated mice. Levels of p62, LC3BI or LC3BII or their ratios did not vary between IPF and healthy donor lungs. Immunohistochemistry on lung sections revealed an intense staining for autophagy proteins in AECII as well as in fibroblasts of IPF lungs as compared to donor lungs. An increase in the levels of transcription factor EB (TFEB), and its nuclear localization was observed in AECII of IPF lungs. Chaperone-mediated autophagy (CMA) levels decreased in IPF lungs, as denoted by the levels of lysosome-associated membrane protein 2a (LAMP2a). On the contrary, bleomycin injured mouse lungs showed an increase in the autophagy markers, LC3BII, p62, Atg7 and Atg12. Levels of TFEB protein was also increased in bleomycin treated mice lungs. An overall increase in CMA was observed.

We conclude that different autophagy pathways are differentially regulated in IPF and in bleomycin injured mouse lungs.

P27: Interplay of FGF and Wnt signaling in regulating mesenchymal progenitor cell lineages formation during lung development and repair after injury

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Over the years our research group has focused on the formation of the different mesenchymal cell lineages during lung development and their respective function during repair/disease progression. We previously showed that during development, Fgf10-positive cells located in the distal lung mesenchyme during the early pseudoglandular stage are progenitors for airway SMCs . In addition, We have reported that FGF signaling in the mesenchyme impairs the entry of the mesenchymal progenitors into the SMC lineage both in vitro and in vivo. Furthermore, we have shown that inactivation of beta-catenin in the mesenchyme leads to the loss of amplification of the mesenchymal progenitor cells due the loss of FGF9/FGFR2c signaling. We have also found that in the adult mice Wnt activation was triggered in airway SMC following naphthalene injury. Such increase in Wnt signaling is linked to increased

mesenchymal cell proliferation and Fgf10 expression in these cells. Fgf10 in turn is instrumental for the repair of damaged bronchial epithelium. Moreover, we have reported that inhibition of beta-catenin signaling in the mesenchyme triggered by the silencing of miR142 leads to arrested proliferation and premature differentiation of the smooth muscle cells. Lineage tracing using our recently generated Fgf10CreERT2 knock in mice showed that Fgf10-positive cells in the embryonic lungs labeled at embryonic day (E) 11.5 are progenitors for smooth muscle cells (SMCs), resident mesenchymal stromal (stem) cells and lipofibroblasts. Lipofibroblasts (LIFs) found in the late fetal and postnatal lung parenchyma are juxtaposed to alveolar type II stem cells and have been proposed to contribute to the maintenance of their stemness. Although LIFs have been studied in postnatal lungs, their exact cellular origin and mechanism of differentiation are unknown. Our recent results demonstrate an essential role for Fgf10 signaling in directing the differentiation of Fgf10-positive cells towards the LIF lineage during late lung development.

P28: Regulation of macroautophagy in amiodarone induced pulmonary fibrosis

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Amiodarone (AD) is a highly efficient antiarrhythmic drug, however, it may cause interstitial pneumonia as well as lung fibrosis. Apoptosis of alveolar epithelial type II cells (AECII) has been suggested to play an important role in this disease, but the precise molecular mechanisms are unclear. Here, we aimed to establish a murine model of AD induced lung fibrosis and assess the role of autophagy. Intratracheal administration of AD induced extensive lung fibrosis, accumulation of surfactant phospholipids and surfactant proteins (SP) in mice. Induction of autophagy and apoptosis were encountered in AECII of AD treated mice over time. AD treated MLE12 and primary AECII showed increased proSP-C and LC3B positive vacuolar structures and underwent apoptosis in dependency of LC3B. In vitro, AD induced autophagosome-lysosome fusion and increased the autophagy flux. In vivo, LC3B was localized at the limiting membrane of lamellar bodies, which were closely connected to the autophagosomal structures in the AECII. Our data suggest that AD causes activation of macroautophagy, intracellular surfactant accumulation in the AECII and extensive AECII apoptosis, resulting in lung fibrosis.

P29: Endogenous Fgfr2b ligands are dispensable for fibrosis formation and resolution in bleomycin-injured mice

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Increased fibroblast growth factor 10 (Fgf10) expression in vivo and administration of exogenous FGF7 recombinant protein enhance lung repair due to bleomycin injury by sending survival signals to lung epithelial cells via tyrosine kinase fibroblast growth factor receptor 2b (Fgfr2b). Given the therapeutic effects of these ligands during bleomycin injury, we hypothesized that activation of the Fgfr2b endogenous pathway is critical for lung repair. Furthermore, as new studies for the treatment of Idiopathic Pulmonary Fibrosis (IPF) have begun to target tyrosine kinase receptors, we aimed to 1) assess the levels of FGF10 and FGF7 signaling in IPF lungs, and 2) assess the recruitment of the endogenous Fgfr2b pathway after bleomycin lung injury in mice. Though FGF7 and FGF10 transcripts were increased in IPF patient lungs, receptors as well as downstream targets were significantly decreased. In contrast, wild type mice undergoing spontaneous repair after bleomycin injury, expressed Fgf10 and downstream targets from 14 days post injury, indicating potential recruitment of this pathway during repair. Surprisingly, however, mice deficient in endogenous Fgfr2b signaling did not develop significantly more fibrosis than wild type animals. However, the dysregulated signaling observed in end-stage IPF lungs and the recruitment of Fgf10 during bleomycin injury of wild type mice, may indicate the potential therapeutic use of exogenous FGF10 to promote fibrosis resolution in IPF patients.

P30: Mitochondrial autophagy in the development of amiodarone induced pulmonary fibrosis

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Amiodarone (AD) is an anti-arrhythmic drug with very well-known vasodilatory properties. Severe pulmonary toxicity has been reported in patients receiving AD even at low doses, with the most common histological finding being chronic interstitial pneumonia. The precise mechanism underlying AD-induced pulmonary toxicity remains unknown. Dysfunctional mitochondria and enhanced oxidative stress via the production of reactive oxygen species (ROS) have been reported in AD-induced pulmonary toxicity. Recent unpublished studies from our group revealed AECII (Alveolar epithelial cells type II) specific autophagy alongside with AECII apoptosis and lysosomal stress in the lungs of AD treated mice and mouse lung epithelial (MLE) 12 cells. This convinced us to hypothesize that AD alters mitochondrial autophagy (mitophagy) and causes subsequent cell death, as an outcome of increased oxidative stress. Apart from the well-known autophagy markers like LC3BII, p62, ATG7, ATG12-5, important mitophagy markers like Pink and NIX/BNIP3L was significantly increased in AD treated mice lungs. In addition, PUMA, Bax and Cytochrome C, which are involved in mitochondrial dysregulation, were also elevated in response to AD treatment in mice lungs and in AECII. Hemeoxygenase-1 (HO-1), a pivotal antioxidant protein was increased in AD treated mice lungs, AECII and MLE12 cells. Since HO-1 forms a major link between oxidative stress and autophagy, we hypothesized that AD induced autophagy might be HO-1 dependent. In contrast, both in vitro knockdown (via siRNA) and chemical inhibition of HO-1 increased the levels of LC3BII. Similarly, LCB knockdown further increased the AD induced HO-1 protein levels in MLE12 cells. This indicates that AD induces HO-1 independent autophagy and HO-1 seems to be protective in function. We conclude that amiodarone induces mitochondrial autophagy, which might accelerate the apoptosis of AECII and thereby lung fibrosis in response to amiodarone treatment.

P31: Integrative molecular and anatomical characterization of idiopathic pulmonary fibrosis

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Rationale: Idiopathic pulmonary fibrosis (IPF) is a chronic, debilitating disease which is characterized by excessive collagen deposition and destruction of lung architecture leading to respiratory insufficiency. Lung transplantation still remains the only definite therapeutic option, since the etiology of IPF is poorly understood and no adequate medical treatment is currently available. In IPF there appears to be a correlation between the spatial localization of fibrotic changes and disease progression. The present study characterizes histopathological changes seen in IPF lungs with molecular changes and anatomical/three-dimensional distribution.

Methods: Freshly explanted IPF lungs from patients undergoing lung transplantation were inflated and scanned by computer tomography to identify and sample representative areas of fibrotic reorganization. The internal structure of the samples was analyzed in three-dimensional datasets by using scanning laser optical tomography (SLOT). Subsequently, areas of interest were laser-microdissected and a set of fibrosis-associated genes and corresponding endogenous controls were analyzed by real-time PCR and immunohistochemistry. All results were correlated with the individual clinical findings.

Results: The three-dimensional position of fibroblastic foci was visualized properly by using SLOT. An integrative model of IPF could be generated by correlating the 3D change patterns with the molecular microenvironment.

Conclusions: Correlation of the techniques outlined above (CT, SLOT, microsropy, 3D reconstruction, laser-microdissection, RT-PCR and immunohistochemistry) with clinical changes enables us to characterize IPF on a macroscopic, microscopic and molecular level for the first time.

P32: Development of a new mouse model to assess the therapeutic efficacy of GRP78 for prevention and resolution of ER stress in vivo

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It is known, that ER-stress plays a role in several, very different diseases like artherosclerosis, dilated cardiomyopathy or neurodegenerative diseases. Maladaptive ER-stress was also indicated to play a prominent role in IPF and seems to represent a key trigger in the pathogenesis of this disease.

As 78kDa glucose-regulated protein/immunoglobulin-binding protein (Grp78/Bip) is the key chaperone of the ER, and as it has been shown to counterbalance ER stress-induced apoptosis induced by etoposide or proteasome inhibition in many cell lines in vitro, we plan to assess the therapeutic efficacy of GRP78/Bip for preventing and resolving ER stress in vivo.

For this purpose we were generating transgenic mice with conditional overexpression of full-length GRP78 in alveolar epithelial type II cells (AECII). In detail, the cDNA encoding mmGrp781-654 was cloned into the pBI-L_Tet vector. After oocyte injection through a cooperation with EMBL (Dr. P. Moreira, EMBL Mouse Biology Unit; Campus A. Buzzati-Traverso, Monterotondo, Italy), breeding of heterozygous founders was performed, followed by crossbreeding with homozygous transactivator SP-C rtTA mice.

The resulting double-transgenic mice with inducible conditional overexpression of Grp78 in AECII will be subjected to different models of lung fibrosis (Pepstatin A, Amiodarone and Bleomycin) with overexpression of GRP78 shut on or shut off. For induction of GRP78 overexpression double-transgenic mice will be fed with doxycycline enriched food (Tet-On). Age-matched animals with the transgene being shut off (Tet-Off) will serve as controls.

We suggest, that GRP78 overexpressing mice will be protected from ER stress and subsequent development of lung fibrosis and that this mouse model will provide insight into the mechanism by which Grp78 overexpression protects from ER stress and lung fibrosis induced by DNA damage and oxidative stress (bleomycin-model) or induced by accumulated proSP-B precursors (Amiodarone- and Pepstatin A-model).

P33: Biological role of the proapoptotic transcription factor C/EBP homologous protein (CHOP) in Idiopathic Pulmonary Fibrosis (IPF)

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We have recently identified severe and pro-apoptotic Endoplasmic Reticulum (ER) stress as major pathomechanism for alveolar epithelial cell (AEC) injury in Idiopathic Pulmonary Fibrosis (IPF). In line with this, the pro-apoptotic transcription factor CHOP, which functions as a crucial mediator of ER stress-induced apoptosis, was strongly induced in AECII from patients with IPF. We therefore aimed to fully disclose the transcriptional regulation and biological role of epithelial CHOP expression in AECII-like cells in vitro, and in pulmonary fibrosis in vivo. We performed promotor analysis of the human CHOP gene in silico, followed by reporter gene assays for several proximal CHOP promoter fragments in A549 and MLE12 cells in the presence or absence of ER stress-inducing agents. We performed transient overexpression of FLAG- and c-myc-tagged CHOP constructs in alveolar epithelial cell lines A549 (human) and MLE12 (murine), followed by the assessment of CHOP expression and post-translational modification and regulation of apoptosis. The in silico-analysis of the 2.7-kb 5´-flanking region of the human CHOP gene revealed approximately 15 different putative transcription factor binding sites in addition to the already known ER stress-response elements (ERSE) and Amino-Acid-response elements (AARE). Surprisingly, next to the common transcription factor binding sites (ERSE and AARE), the CHOP promoter has another transcription factor binding site(s) which seems to play a role in CHOP expression. Additionally, CHOP could be successfully overexpressed in MLE12 and A549 cells, with a maximum of protein expression 24-48 hours after transfection. Parallel to the expression of CHOP, caspase-3 activation and hence induction of apoptosis could be encountered in these cell lines. Moreover, overexpression studies suggest the presence of unknown post-translational modifications in CHOP proteins. Based on the data generated herein, we expect to be able to develop novel, AECII specific, antiapoptotic treatment strategies for treatment of IPF.

P34: Deficient Autophagy in Hermansky-Pudlak Syndrome associated lung fibrosis

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Hermansky-Pudlak syndrome (HPS) is a lysosome realated disorder. Patients with HPS types -1, -2 & -4 develop pulmonary fibrosis called Hermansky-Pudlak syndrome associated Interstitial Pneumonia (HPSIP). HPSIP lungs show enlarged alveolar type II cells (AECII) with giant lamellar bodies. We previously reported lung fibrosis in a HPSIP mouse model (HPS1/2), accompanied with surfactant accumulation and apoptosis of AECII due to severe lysosomal stress and ER stress. Data from human HPS1 patient corroborated with the HPS1/2 mice data. Here, we aim to analyze autophagy, an important lysosomal degradation pathway, under HPSIP conditions.

Immunohistochemistry was performed and on serial paraffin lung sections from HPS1, HPS2, HPS1/2 and WT control mice and on lung sections from human HPS1 patients and healthy donors for autophagy related proteins LC3B, p62 and TFEB and for AECII marker, pro SP-C. Immunogold labelling for LC3B was performed on mice lungs fixed in paraformaldehyde and gluteraldehyde.

Immunohistochemistry revealed that the AECII of HPS1/2 mice and human HPS1 did not stain for LC3B, while a convincing signal was observed within macrophages of the same sections and within AECII & macrophages of WT mice and healthy donors. Electron microscopy results confirm the qualitative observation of less labeling of LC3B on the limiting membrane of lamellar bodies in HPS mice compared to WT mice. Immunohistochemistry showed decreased staining for p62 and TFEB within AECII of HPS1/2 compared to WT mice.

Our results point towards defective autophagy within AECII under HPSIP conditions both in mice and men. An in depth analysis of this pathway is underway to further understand the role of defective autophagy in the development of HPSIP.

P35: Role of Bcl-xL in hepatocyte growth factor-elicited epithelial protection in idiopathic pulmonary fibrosis

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Hepatocyte growth factor (HGF) is a cytokine with pleiotropic functions during wound healing and repair. Its anti-fibrotic effects were shown in animal models of lung fibrosis and linked to improved cellular survival and proliferation and reduced myofibroblast accumulation. HGF-elicited, pro-survival pathways have yet not been investigated in detail in lung epithelial cells. Based on literature, our study is focused on Bcl-xL, pro-survival protein involved in mitochondrial control of apoptosis.

Analysis of IPF lung homogenates revealed significantly increased expression of Bcl-xL when compared to donor lungs. In human IPF, much less in donor lungs, Bcl-xL protein is highly expressed in hyperplastic alveolar epithelial type II cells, basal cells and bronchial epithelial cells. Furthermore, Bcl-xL expression co-localized with specific HGF receptor c-Met. In vitro data shows decreased expression of Bcl-xL in murine epithelial MLE12/15 cells in response to oxidative stress-induced apoptosis. Under these conditions, HGF treatment resulted in increased survival of cells that correlated with increased Bcl-xL expression. The same effect of HGF is seen after treatment of cells with the potent ER-stress inducer thapsigargin. Anti-apoptotic effect of HGF was abolished after pre-incubation with c-Met inhibitor. Knock-down of Bcl-xL protein made epithelial cells much more sensitive to injury caused by oxidative stress, as well as ER stress, however did not affect HGF pro survival activity.

In conclusion, our data shows that HGF has a strong pro-survival effect on alveolar epithelial cells. Its interdependency with Bcl-xL protein needs to be further investigated, however Bcl-xL seems to be an important factor in epithelial response to injury.

P36: Alveolar derecruitment and collapse induration as crucial mechanisms in lung injury and fibrosis

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Idiopathic pulmonary fibrosis (IPF) and bleomycin-induced pulmonary fibrosis are associated with surfactant system dysfunction, alveolar collapse, and collapse induration (irreversible closure). These events play critical but undefined roles in the loss of lung function and disease progression. To quantify how surfactant inactivation leads to lung injury and fibrosis we employed design-based stereology and invasive pulmonary function tests 1, 3, 7, and 14 days (D) following intratracheal bleomycin-instillation in rats. Active surfactant subtypes declined significantly by D1, leading to progressive alveolar closure (derecruitment) and an associated decrease in organ-scale compliance. Alveolar epithelial damage was more pronounced in closed alveoli compared to ventilated alveoli. At the ultrastructural level, we observed collapse induration in the bleomycin treated rats on D7 and D14 as indicated by collapsed alveoli overgrown by a hyperplastic alveolar epithelium. This pathophysiology was also observed for the first time in human IPF lung explants. Prior to the onset of collapse induration (D7), the lungs were easily recruited, and lung elastance could be kept low after recruitment by application of positive end-expiratory pressure (PEEP). By contrast, at later time points the recruitable fraction of the lung was reduced by collapse induration, causing elastance to be elevated at high levels of PEEP. We conclude that surfactant inactivation leading to alveolar collapse and subsequent collapse induration is the primary pathway for the loss of alveoli in this animal model and is the dominant factor in the degradation of lung function. Our ultrastructural observations suggest that collapse induration is also important in human IPF.

P37: Wnt-Heparan Sulfate Proteoglycan interaction in fibrotic lung disease

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a lethal lung disease of yet unknown etiology. Recently, (re)activation of developmental pathways, such as Wnt/β-catenin in the alveolar epithelium has been linked to disease development. Regulation of Wnt/β-catenin activation has been shown to be dependent on Heparan Sulfate Proteoglycans (HSPGs). The role of Wnt-HSPG interaction in IPF, however, remains to be elusive.

Methods: Murine and human lung epithelial cells were treated with Heparin, Desulfated (DS) Heparins or Heparinase II and Chondroitinase ABC, respectively. A Heparin binding assay was used to assess binding of Wnt3a to Heparin and DS Heparins. Immunofluorescence staining of ex vivo lung tissue for Wnt proteins was conducted. In addition, lung tissue and bronchoalveolar lavage fluid (BALF) from bleomycin-treated mice were screened for Wnt ligand expression.

Results: WNT3a is localized to bronchial and alveolar epithelial cells with increased expression in fibrotic regions. Canonical WNT3a and WNT10b levels are elevated in bronchoalveolar lavage fluid (BALF) of mice with bleomycin-induced lung fibrosis. Immunofluorescence staining of ex vivo lung tissue slices revealed upregulated WNT3a expression in bleomycin-treated mouse lungs compared to controls. Heparin treatment of murine lung epithelial cells resulted in inhibition of TOP/FOP-flash reporter, indicating decreased canonical WNT signaling. In contrast, Heparinase II and Chondroitinase ABC treatment resulted in upregulated β-catenin-dependent gene transcription. Interestingly, 6-O-DS Heparin treatment of A549 cells did not decrease WNT signaling, while binding of WNT3a to 6-O-DS Heparin was not altered, thereby suggesting less inhibition of WNT signaling upon binding to 6-O-DS Heparin.

Conclusion: Taken together, our findings revealed that HS sulfation and degradation modulate WNT/β-catenin signal activity. Altered sulfation states during extracellular matrix (ECM) remodeling in IPF pathology might contribute to (re)activation of developmental pathways like WNT/β-catenin.

P38: Novel alveolar epithelial cell differentiation markers in lung injury and repair

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Objective: The alveolar epithelium, consisting of mainly alveolar epithelial type 1 (AT1) and type 2 (AT2) cells, represents a major site of tissue destruction in idiopathic pulmonary fibrosis. Several studies indicate that adult AT2 cells are able to self-renew and exert progenitor function for AT1 cells upon alveolar injury in vivo. However, cell differentiation pathways enabling this plasticity are poorly understood. Here, we used the primary culture of murine AT2 cells as model system to identify novel proteins and pathways involved in epithelial transdifferentiation.

Methods/Results: Expression profiles of primary transdifferentiating AT2 cells were analyzed applying 2D gel electrophoresis and mass spectrometry. Beside others, we found enolase 1 (ENO1) to be upregulated, whereas carbonyl reductase 2 (CBR2) was decreased in transdifferentiating AT2 cells, as further confirmed by quantitative RT-PCR analysis and immunoblotting. This was accompanied by reduction in AT2 cell derived pro surfactant protein C (proSPC) expression and increased AT1 cell T1a expression, as well as an activation of the Wnt/β-catenin pathway. We applied a lung tissue culture model of murine precision cut lung slices ex vivo to further analyze transdifferentiation in the 3D natural spatial lung environment. We observed Wnt/β-catenin signal activation and alveolar epithelial cell transdifferentiation upon lung tissue cultures ex vivo. Interestingly, the inhibition of Wnt/β-catenin signaling in cultured AT2 resulted in decreased expression of ENO1 and T1a and stabilization of CBR2. In an in vivo model of lung fibrosis, which exhibits activated Wnt/β-catenin signaling, decreased

expression of CBR2 and proSPC correlated in AT2 cells, whereas ENO1 along with T1a expression was increased.

Conclusion: Proteomic analysis revealed novel proteins differentially expressed in differentiating AT2 cells. Interestingly, newly identified proteins were regulated by β-catenin in vitro and in experimental fibrosis in vivo, suggesting a role in epithelial repair processes upon lung injury.

P39: In vivo effects of TGF-β1 in lung surfactant regulation, lung meachanics and structure

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Transforming growth factor beta 1 (TGF-β1) is a signalling protein with a wide range of biological activities. TGF-β1 is thought to have a pivotal role in fibrogenesis, where TGF-β1 induces myofibroblast migration and increases extracellular matrix synthesis, including collagen. Moreover, it has been described that TGF-β1 is a negative modulator of the regulation of surfactant associated proteins A (SP-A), B (SP-B) and C (SP-C) in vitro. Pulmonary surfactant is a lipid-protein complex that lowers surface tension at the respiratory air-liquid interface, stabilizing the lungs against physical forces tending to collapse alveoli. SP-B and SP-C deficiency has been found in patients suffering from lung diseases and related to potential mechanical stress of the lung epithelium. We have characterized lung surfactant protein composition 1 and 2 weeks after adenoviral mediated gene transfer of active TGF-β1 into lungs. Gene expression of surfactant proteins is down-regulated pointing at deficient transcriptional regulation that might include a deficient activity of TTF-1, during TGF-β1 overexpression. Deficiency on SP-B and SP-C at early stages correlates with high surface tension under dynamic cycling of isolated surfactant in Captive Bubble Surfactometry (CBS). In addition, high surface tension correlates with decreased quasistatic lung compliance and increased collapsibility of distal airspaces. Stereological data demonstrate a correlation between septal wall thickness and quasistatic compliance 2 weeks after gen-transfer. At the ultrastructural level thickening of septal walls, could be attributed to an increase in interstitial cells, formation of dense alveolar oedema and increase in profiles of epithelial type II (AEII) cells. The latter could be also attributed to Epithelial to Mesenchymal Transition (EMT), indicated by down-regulation of epithelial markers associated to up-regulation of mesenchymal molecular markers. We can conclude that in vivo TGF-β1 is a strong negative regulator of surfactant metabolism, originating a mechanical stress that may contribute to EMT at following stages.

P40: Effect of stretch and hyperoxia on the stress response of the newborn mouse lung

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Introduction

Prolonged mechanical ventilation of preterm infants with oxygen-rich gas (MV-O2) as a lifesaving treatment often leads to chronic lung disease, also known as bronchopulmonary dysplasia (BPD). The disease is characterized by extracellular remodelling and inflammatory changes leading to impaired alveolar and vascular development. We aimed at investigating the effect of MV-O2 on the protein stress response of the immature lung focussing on cellular protein quality control pathways such as ER-stress, autophagy and proteasome function using a mouse-model of mechanical ventilation and neonatal pulmonary myofibroblasts (MFBs) which are treated with stretch, TGF-β, or hyperoxia in vitro.

Materials and Methods:

5-7 days old C57BL/6 mice were ventilated at 180 breaths/min with/without oxygen (FiO2=0,4 or FiO2=0,21) for 2 or 8 hours; the controls spontaneously breathed room air or O2 for 2 or 8 hours. At the end of each experiment, lungs were harvested in liquid nitrogen; homogenized lysate was used for protein- and RNA analysis. For in vitro analysis, MFBs were isolated from lungs of 5-7 day old mice and subjected to stretch or hyperoxia experiments with/without TGF-β treatment for 24 hours.

Results

Preliminary results demonstrate a 1.5 fold increase in ER-stress (Binding immunoglobulin protein) and a significant decrease in cell-proliferation (Proliferating Cell Nuclear Antigen) in the ventilated lungs when compared to unventilated control littermates. Other stress response systems were not found to be significantly regulated in contrast to findings from adult lung tissue.

Conclusion

Our preliminary results indicate that the cellular protein quality control system in the neonatal lung shows a specific response to stress induced by mechanical stretch and hyperoxia. Ongoing experiments using different markers and functional assays will characterize this response in more detail.

Disease Area: Pulmonary Hypertension (Poster 41- 43)

P41: P66shc deficient mice develope decreased right heart hypertrophy via a Cyclophilin D dependent mechanism in hypoxia-induced pulmonary hypertension

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In acute and chronic hypoxia the response of the pulmonary vasculature is suggested to be regulated via mitochondrial reactive oxygen species (ROS). In response to cellular stress the mitochondrial regulator protein p66shc enhances the ROS-production probably via the pro-apoptotic protein cyclophilin D (CypD). We hypothesized in p66shc-deficient mice lower hypoxic pulmonary vasoconstriction (HPV) and pulmonary hypertension (PH) related to lower hypoxia-induced ROS-production.

HPV was determined in isolated lungs of p66shc and CypD deficient mice, as well as in mice lacking both proteins, and compared to lungs of wild type (WT) mice. The thromboxane mimetic U46619 and potassium chloride (KCl) were used as hypoxia-independent vasoconstrictive stimuli. PH was quantified after exposure of mice to 10% oxygen for 4 weeks by in vivo hemodynamics, and morphometric analysis.

Mice deficient of p66shc, CypD or both proteins exhibited lower responses to acute hypoxia, U46619 and KCl compared to WT mice. In chronic hypoxia-induced pH only p66shc deficient mice exhibited lower right ventricular pressure, right ventricular hypertrophy and hematocrit compared to WT mice. In mice lacking CypD or both proteins, no significant changes of these parameters in chronic hypoxia were detected. There was no change in lung remodeling between all groups.

We conclude that the mitochondrial ROS producing protein p66shc regulates right heart hypertrophy and right ventricular pressure during chronic hypoxia, probably via a CypD dependent mechanism.

P42: A possible role of serotonin for the development of tobacco smoke-induced lung emphysema and pulmonary hypertension

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Chronic obstructive pulmonary disease (COPD) is a major cause of death and disability worldwide. An estimated portion of 30-70% of COPD patients also suffer from pulmonary hypertension (PH). Studies indicate that activation of serotonin-mediated pathways contribute to development of PH. Moreover, vascular alterations have been suggested to contribute to emphysema development.

The aim of the study was to clarify the role of serotonin and the serotonin inhibitor Terguride on the development of tobacco smoke-induced emphysema and PH in a mouse model.

WT mice (C57BL6/J) were exposed to cigarette smoke for 6 hours/day, 5 days/week for 8 months. Mice were split into different experimental groups (Placebo smoke-exposed, Placebo non-exposed and Terguride smoke-exposed). Terguride-treated animals received the drug twice per day by gavage. Gene and protein expression analysis were performed by quantitative real-time PCR and western blotting. Development of PH and emphysema were determined by measurement of lung compliance, in vivo hemodynamics, right ventricular heart mass alterations and as well by alveolar and vascular morphometric analyses.

The mRNA as well as protein analyses revealed a significant upregulation of 5-HT2A and 5-HT2B receptors in tobacco smoke-exposed mice. Similar alterations were found in lungs from human COPD patients compared to healthy donors. Non-treated smoke-exposed mice developed pulmonary hypertension and emphysema upon smoke exposure. In contrast, smoke-exposed Terguride-treated mice were prevented from PH and vascular remodeling. In addition, the smoke-induced increase in lung compliance as well as structural measures for emphysema development remained on a normal level in Terguride-treated smoke-exposed mice.

We concluded that Terguride has a protective effect on the development of tobacco smoke-induced pulmonary hypertension and emphysema development in mice.

P43: Arachidonic acid/cytochrome p450-derived mediators decrease hypoxic pulmonary vasoconstriction in isolated, ventilated and perfused mouse lungs

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Introduction:

Hypoxic pulmonary vasoconstriction (HPV) is an essential physiological mechanism that adapts perfusion to ventilation to optimize gas exchange by redistributing the blood flow to well-ventilated areas, thereby improving alveolar oxygenation. Disturbances in HPV can lead to life-threatening hypoxemia. Intrinsic and modulatory pathways of HPV are not fully elucidated. Arachidonic acid-derived mediators are known to be potent vasoregulators in different organs in health and disease. In the lung, little is known about the role and physiological function of arachidonic acid/cytochrome p450-derived mediators. Our study focuses on the cytochrome p450 oxygenase pathway, during which 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs) are synthezised. The aim of this study was to investigate the effect of four EETs (5,6-, 8,9-, 11,12- and 14,15-EET) and 20-HETE on HPV.

Methods:

Experiments were performed in isolated, ventilated and perfused lungs of wild-type mice. The strength of HPV in response to a change from normoxic to hypoxic ventilation (21 % O2 and 1% O2, 10 minutes) was quantified. The effect of EET- and 20-HETE-applications on acute HPV and normoxic vascular tone was compared to control experiments with solvent applications.

Results:

Application of EETs did not affect normoxic pulmonary vascular resistance. However, hypoxia-induced vasoconstriction was significantly reduced in response to application of 5,6-, 8,9-, 11,12- and 14,15-EET via the perfusate. Application of 20-HETE induced vasoconstriction during normoxia and inhibited subsequent hypoxic pulmonary vasoconstriction.

Conclusion:

EETs as well as 20-HETEs influence hypoxic pulmonary vasoconstriction (HPV) via different mechanisms. Future studies with stereoisomers of EETs as well as with inhibitors for EET synthezising enzymes will give further insight into EET-mediated pulmonary vasoregulation.

Disease Area: Endstage Lung Disease (Poster 44- 55)

P44: Treatment with donor specific alloantigen 28 days before or on the day of lung transplantation – a comparison in a large animal model

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Purpose: Administration of donor-specific alloantigen during transplantation has been shown to induce T-cell regulation and long term transplant tolerance in our large animal model before. In rodents, it was also possible to induce allograft acceptance if the donor-antigen was administered 28 days in advance. Here, we wished to translate this protocol into our lung transplantation model in minipigs and compare it to our already established protocols.

Methods: Lung transplantation from MHC-mismatched donors was performed in 41 minipigs. 23 of those animals received donor-splenocytes perioperatively (group1), whereas in 18 animals the splenocytes were administered 28 days before transplantation (group2). All animals were treated with Tacrolimus and Steroids 28 days following transplantation. Concomittant with donor-antigen the animals received either non-myeloablative irradiation or depleting anti-CD4 and/or -CD8 antibodies. Both groups include 4 (group1) respectively 6 (group2) animals which underwent no immunomodulation at all.

Results: In our minipig model, it was not possible to induce reliable allograft acceptance if the minipigs were treated with donor-antigen 28 days before transplantation. From the total of group2, only 16,7% achieved long term allograft survival (>178d), compared to group1 with 26,1% after all. After censoring animals that died due to other causes related to this experiment than rejection (like bleeding from thrombocytopenia) there still remained 73,3% animals with rejection in group2 but only 46,4% in group1 before postoperative day 178 (p=0.01). Median survival in the day -28 animals was 64 days, whereas in the perioperatively treated animals it was 239 days. Time course and histology suggest sensitization and consecutive hyperacute rejection in animals pretreated with donor-splenocytes 28 days before lung transplantation even though anti-CD4 and/or –CD8 antibodies were co-administered.

Conclusion: Administration of donor-splenocytes 28 days before transplantation appears to rather promote sensitization, but at the time of transplant promotes tolerance in this large animal lung transplantation model.

P45: Towards the development of a bioartificial lung - Endothelialisation of TiO2 coated oxygenator membranes

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Introduction:

Currently, the use of extra corporeal membrane oxygenation (ECMO) devices, which is indicated for patients awaiting lung transplantation, is limited to a few weeks only, due to thrombus formation and deposition of blood components within the device. Therefore, the basic idea is to improve the haemocompatibility by endothelialisation of the poly-4-methyl-1-pentene gas exchange membranes (PMP), which necessitates the development of coating techniques for the mediation of endothelial cell adhesion to the hydrophobic polymers. The pulsed vacuum cathodic arc plasma deposition (PVCAPD) technique has been shown to enable the coating of thermosensitive polymers. Hence the eligibility of Titaniumoxides (TiO2) deposited on PMP using PVCAPD as an effective coating technique for enabling the endothelialization was assessed.

Methods:

PMP film samples were coated with TiO2 via PVCAPD and analyzed using SEM and EDX. Umbilical cord blood derived endothelial cells (hCBECs) were seeded on such samples and incubated for 24 h. Established monolayers were investigated for expression of activation-relevant marker genes and subjected to a leucocyte adhesion assay. Flow resistance and self-healing capacity were assessed in a laminar flow chamber applying 30 dyne/cm2 for 24h.

Results:

SEM and EDX analysis confirmed the homogeneous deposition of nanoscalic TiO2 particles. hCBECs exclusively adhered to areas of PMP film coated with TiO2. Gene expression analysis revealed that endothelial cells seeded on TiO2 coated surface retained the non-activated, anti-thrombogenic state, additionally confirmed by a leucocyte adhesion assay. Furthermore, the established monolayer was resistant to high physiologic shear rate of 30 dyne/cm2, for 24 hours. Besides planar PMP, the coating technique was successfully applied to 3D hollow fibres where ECs established confluent monolayers.

Conclusion:

This study demonstrated that TiO2 coating via PVCAPD is a promising technique for coating thermo-sensitive PMP gas exchange membranes, enabling the generation of a non-activated and flow-resistant EC monolayer.

P46: Do B-cells contribute to experimental Bronchiolitis obliterans syndrome?

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Objectives: Recently, we established a clinically relevant experimental model for human bronchiolitis obliterans syndrome (BOS), which involves orthotopic transplantation of rat lung allografts followed by intratracheal application of lipopolysaccharide (LPS) (Atanasova et al. 2013, J Heart Lung Transplant 32: 1131). Chronic lung damage does neither develop in allografts treated with vehicle instead of LPS nor in pulmonary isografts. Alloreactive and autoreactive antibodies have been detected in BOS patients but their pathogenic role is disputed. Here, we investigate B cell infiltration into experimental lung allografts as well as deposition of immunoglobulins and C4d.

Material and methods: Orthotopic left lung transplantation was performed in the Fischer 344 to Lewis strain combination followed by application of ciclosporine (5 mg/kg) for 10 days. Lewis rats served as isograft recipients. Four weeks after transplantation, LPS (0.5 mg/kg body weight) was instilled into the trachea. Lungs were harvested before (day 28) and after LPS application (days 29, 33, and 40) for immunohistochemistry.

Results: Perivascular and peribronchiolar areas of lung allografts were more strongly infiltrated by B cells in comparison to right native lungs and isografts. Interestingly, an influx of B cells into the alveolar region was induced in response to LPS-application only in allografts. Immunoglobulin-positive cells were markedly increased in the alveolar space of lung allografts compared to isografts at days 33 and 40. C4d deposits were mainly found in the wall of small blood vessels as well as on the respiratory epithelium of lung allografts but not of isografts.

Conclusion: These results suggest that B cells play a role in the development of BOS by producing antibodies against donor tissue. As described before for renal allograft rejection, deposition of C4d could be a prognostic factor for lung allograft survival.

Founding: DFG No. GR 1094/6-1

P47: Interleukin 18 in the pathogenesis of experimental bronchiolitis obliterans syndrome (BOS)

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Introduction: Bronchiolitis obliterans syndrome (BOS), characterized by bronchiolitis obliterans, vascular remodeling and general fibrosis is a major cause of mortality after lung transplantation. Elevated levels of IFNγ-dependent chemokines are predictive factors for the development of BOS. IFNγ expression can be induced by IL18, a pro-inflammatory cytokine, secreted mainly by macrophages upon inflammasome activation. IL18 can be involved in the remodeling of airways and vessels, fibrosis and impairment of endothelial progenitor cell function. However, its potential contribution to BOS has not been yet assessed.

Material and Methods: The Fischer 344 to Lewis rat strain combination was used for orthotopic left lung transplantation. Isogenic transplantations were performed in Lewis rats. Recipients were treated with ciclosporine for 10 days and 28 days after transplantation, LPS was instilled into airways. The mRNA and protein expression of IL18 was measured on days 28, 29 and 33 after transplantation by quantitative RT-PCR and western blot, respectively.

Results: Left lung isografts and allografts as well as control right lungs expressed stable mRNA levels of pro-IL18, whereas pro-IL18 protein was elevated in left lung allografts on days 28 and 29. Interestingly, mature form of IL18 was detected predominantly in left allografts on day 29 and was absent in right control lungs independent on the day investigated. In agreement with this observation, mRNA expression of inflammasome components like caspase1 and ASC was elevated in left allografts on day 29.

Conclusions: IL18 might play important role in the development of BOS. Control of inflammasome activation and IL18 secretion might represent a novel therapeutic strategy to prevent lung graft destruction.

P48: Simulation of physiologic conditions in diseased lung grafts for drug exposition using the Organ Care System - a new model

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The only curative treatment option for patients with end-stage lung disease is lung transplantation, since no curative pharmacological drug for diseases is known so far. In order to analyze the effect of new drugs on diseased tissue and vasculature, we developed an ex vivo lung perfusion (EVLP) setup, in

which explanted diseased lungs from lung transplant recipients cab be treated with new drugs whilst undergoing normothermic perfusion and ventilation.

Here, we present data from our first three lungs, that were put on EVLP in order to proof the feasibility of the experimental setup.

Methods

For EVLP, the Organ Care System (OCS) a transportable EVLP unit with integrated ventilator was used. During transplant procedure, the diseased lungs of three end-stage IPF patients were explanted, leaving a sufficient cuff of the pulmonary artery as well as the main bronchus. Reperfusion was performed with ~1.0-1.2l/min and ventilation was started aiming for a tidal volume of 250-300ml, in respect to patient's body height and lung size.

Results

The lungs were kept in the OCS for 17, 24 and 26 hours in order to test the longest possible conservation period in the perfusion unit.

After cessation of EVLP, the lungs were preserved with formaline flush through the pulmonary artery. Histological examination showed characteristic histological features of the known underlying disease. More importantly, there was no visible difference between OCS-treated organs and untreated organs regarding tissue vitality. All OCS-treated grafts were free of histopathological correlates indicating necrosis, lysis or apoptosis.

Conclusion

For that reason, OCS treatment reliably protects lung tissue and keeps it in a vital state. This observation was independent from the duration of OCS treatment. This new tool can now be used for testing drugs for end stage lung diseases with the opportunity of finding histopathological effects on lung tissue and/or vasculature.

P49: Generation of a NKX2.1 knockin human induced pluripotent stem cell reporter line for monitoring the generation of respiratory cells

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One promising therapeutic option to cure hereditary pulmonary diseases like cystic fibrosis might be a cell replacement therapy comprising the generation of patient specific autologous induced pluripotent stem cells (iPSCs), followed by the correction of the genetic mutation, differentiation into the needed airway cell type and replacement of the endogenous cells. For long term restoration, most likely airway progenitor or stem cells like basal cells or submucosal gland duct stem cells will be required. A prerequisite is the development of an efficient and robust protocol for the generation of the desired airway stem cells from human iPSCs (hiPSCs). The transcription factor NK2 homeobox1 (NKX2.1) is expressed in lung epithelial progenitor cells which can give rise to airway stem cells. Thus, NKX2.1 represents a suitable marker for optimizing differentiation protocols. The aim of the present study was

the generation of a hiPSC reporter line targeting the NKX2.1 locus. Therefore, two hiPSC lines, established in our lab, were screened for efficient differentiation into definitive endoderm and NKX2.1 expressing cells. Based on the results, the hHSC_F1285_T-iPS2 line was then used for the transfection with the NKX2.1 targeting vector (kind gift of Andrew G. Elefanty), which consists of two homology arms for homologous recombination flanking an eGFP coding sequence and a floxed antibiotic selection cassette. One correctly targeted clone out of 191 neomycin resistant clones was identified by PCR analysis. Southern blot analysis using an eGFP probe verified that the vector had integrated correctly into one of the two NKX2.1 alleles without any further integration sites. Differentiation of the identified clone with our established protocol resulted in eGFP expressing cells first occurring on day 12 of differentiation. The established hiPSC NKX2.1 reporter line represents an optimal tool for the improvement of protocols for the pulmonary differentiation of hiPSCs.

P50: Changes in local alpha-1-antitrypsin expression during the pathogenesis of experimental bronchiolitis obliterans syndrome

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Alpha-1-antitrypsin (AAT) is the prototypical member of the serin protease inhibitor (SERPIN) family. It is mainly produced by the liver, but can also be produced by monocytes, macrophages and alveolar epithelial cells. AAT is released in large amounts during the acute phase of inflammation and limits tissue damage due to its antiprotease activity. Anti-inflammatory effects independent of antiprotease function are currently investigated. AAT might also play a role during Bronchiolitis obliterans syndrome (BOS), the major cause of death of patients after lung transplantation, which limits survival rates to 53% after 5 years. Ischemia/reperfusion injury, acute rejection and respiratory infections are main risk factors for the development of BOS.

Our group has recently developed a model for human BOS in rats. This model consists of allogeneic left lung transplantation from Fischer 344 (F344) to Lewis (LEW) rats, a short course of immunosuppression followed by intratracheal instillation of lipopolysaccharide (LPS) 28 days after transplantation. Control allograft recipients were treated with vehicle. Isogeneic transplantation was performed in the LEW rat. We analyzed AAT mRNA expression by real-time RT-PCR in lung tissues of transplanted rats, as well as AAT protein expression by immunohistochemical staining on paraffin sections.

Isogeneic transplantation provoked a graft-specific decrease in AAT mRNA-expression, which was transiently reverted upon LPS application. In allografts, however, the LPS-mediated increase in AAT expression was not observed. LPS-induced differences were also reflected in the intensity of the immunohistochemical staining of alveolar walls.

Our results suggest that AAT supplementation early after lung transplantation might prevent chronic allograft rejection in the long run.

P51: Modeling Cystic Fibrosis in vitro: A new possible platform for patient customised drug screening and ex-vivo gene therapy?

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Cystic fibrosis (CF) is the most common lethal monogenic recessive disease in the caucasian population. Over 1900 mutations are known in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, but the most common mutation is the F508del resulting in misfolding of the CFTR protein.

Until today, classical gene therapy trials have not been successful in CF patients, and currently approved CFTR modulators are only effective in a minority of CF patients. In contrast, stem cell-based approaches offer new perspectives for diseases like CF. Besides their almost unlimited proliferation and differentiation potential, human induced pluripotent stem cells (hiPSCs) can easily undergo genetic modification. Thus, patient-derived hiPSCs represent a suitable cell source for autologous ex-vivo gene therapy approaches to cure CF. In addition to cell replacement therapies, these cells might serve as a basis for patient-specific drug and toxicology screenings of CFTR modulators in vitro.

Our strategy comprises the establishment of patient-specific hiPSCs, the targeted ex-vivo correction of individual CFTR mutations and the differentiation of the iPSCs into CFTR expressing cells. Generation of hiPSCs from somatic cells of CF patients was already successful and genetic correction of these mutated iPSCs was performed by two different gene targeting strategies based on homologous recombination via Zinc-Finger- or TAL-effector nucleases. For proof of concept, we are aiming at the differentiation of non-disease specific human embryonic stem cells (ECS, as control) and of uncorrected and corrected CF hiPSCs into CFTR expressing cells. During differentiation of the hESC line, increasing levels of CFTR mRNA and of mature CFTR protein were detectable. CFTR mRNA expression was verified in differentiating non-corrected and gene-corrected CF hiPSCs as well. Current work is focused on the generation of CFTR reporter cell lines, facilitating monitoring of CFTR expression, optimisation of the differentiation protocol and characterisation of the CFTR positive cells.

P52: Miniaturization of the Organ Care System® into rat lungs for the establishment of ex-vivo therapy

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Objectives

The Organ Care System® (OCS) is a well-established system used in human lung transplantation, allowing for warm perfusion and ventilation of the donor lungs. Besides lung retrieval, this system therefore offers an innovative opportunity for clinical ex-vivo therapy of diseased lungs for different indications, e. g. tumor therapy. While the patient is on extracorporeal membrane oxygenation, the otherwise inoperable lungs can be treated in the OCS, followed by autotransplantation. For the development of ex-vivo therapy, a miniaturized OCS for small animals model was established.

Methods

Wistar rats were euthanized (per group n=5), the left lung was connected to the miniaturized OCS, while the right lung was stored on ice. The OCS lungs were ventilated and perfused at body temperature under continuous monitoring (e. g. pressure, blood gas analysis). Four different perfusion solutions were analyzed (Steen solution® ± blood, OCS solution® ± blood). Thereafter the lungs were processed histologically and examined pathologically (e. g. HE staining).

Results

The miniaturized OCS worked technically faultless, in particular the perfusion and ventilation went well. For all perfusion solutions stable pH, pO2, pCO2, oncotic pressure and systemic pressure could be observed. The base excess has to be stabilized by application of sodium hydrogen carbonate using both perfusion solution combined with blood. Furthermore, the lactate increased in these two combined perfusion solutions until the end of the experiment up to 7,0mmol/l, while the two others indicated lactate levels up to 1,0mmol/l. Pathological work up revealed no significant morphological changes, except for focal atelectasis. There were no delimitable differences in between the examined groups.

Conclusions

The miniaturized OCS is a reliably working system to establish the ex-vivo therapies for different indiations. As ex-vivo therapies may need to be applied for more than 4 hours, extended perfusion times and various perfusion solutions are currently investigated.

P53: Respiratory epithelial cells generated from human pluripotent stem cells – new therapeutic approach for (genetic) lung diseases

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

We therefore aim at the differentiation of human embryonic (hESCs) as well as human induced pluripotent stem cells (hiPSCs) into respiratory epithelial cell types. To evaluate the earliest respiratory differentiation steps, we make use of the hESC reporter cell line hES3 NKX2.1-GFP (kindly provided by the lab of A. Elefanty) expressing eGFP under the endogenous promoter of NK2 homeobox 1 transcription factor (NKX2.1), known as the earliest marker in lung development. With our current serum-free monolayer-based differentiation strategy we receive about 70 % definitive endoderm as the first developmental step. Via subsequent anteriorization, FOXA2+/SOX2+ anterior foregut endoderm was induced giving rise to about 15 % NKX2.1-eGFP+ cells. Coexpression of NKX2.1-eGFP with the endodermal marker FOXA2 as well as qRT-PCR analysis indicate a respiratory phenotype of the NKX2.1-eGFP+ cells most likely excluding relation of the NKX2.1 expression to a neuronal or thyroidal cell fate. Additionally, a subset of the NKX2.1-eGFP+ cells coexpressed SOX2 demonstrating specification towards a proximal airway progenitor cell phenotype.

In summary, first steps have been made towards the efficient generation of NKX2.1+ respiratory epithelial progenitor cells. Future work will focus on further optimization of the differentiation strategy and maturation of the cells with regard to cell replacement therapies as well as for disease modeling, drug screening and toxicity tests in vitro.

P54: Developing a score of early postoperative regulatory T cell frequency to predict bronchiolitis obliterans syndrome-free survival at two years after lung transplantation

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Introduction: Regulatory T cells (Treg) have potential to regulate alloantigens and thus may counteract the development of chronic rejection (i.e. bronchiolitis obliterans syndrome, BOS) in lung transplantation. BOS may affect over 60% of lung transplant recipients within five years. Here, we analyzed Tregs in peripheral blood of 120 lung recipients prospectively during the first 2 years and correlated with the onset of BOS at two years.

Materials and Methods: In this study we detected circulating Treg by flow cytrometry in consecutive routine lung transplant recipients before transplantation 3 weeks, 3, 6, 12, 18 and 24 months after transplantation. Treg were defined as CD4+CD25high T cells and were further analyzed for relevant surface as well as intracellular markers such as, amongst others, CTLA4, CD127, FoxP3 and IL-2. Spirometry at 3 weeks, 3, 6, 12, 18 and 24 months after transplantation as well as protocol biopsy results were analyzed. We defined two groups by the development of BOS stage 1 or higher versus BOS stage 0 at two years.

Results: A total of 120 consecutive patients were included into the study. While 97 patients showed a stable clinical course after lung transplantation, 23 patients developed BOS stage 1 or higher within the first 2 years after lung transplantation. As soon as 3 weeks after lung transplantation not only a statistically significant positive correlation could be detected between the frequencies of Tregs and the absence of BOS ($p<0.05$), we also built a score defining a cut off value composed of IL2+/CTLA4+/CD127low and FoxP3+ Treg in peripheral blood at 3 weeks, predicting the probability of BOS development.

Conclusions: Higher frequencies of Treg early after lung transplantation are associated with protection against development of BOS and they may thus have an early predictive function for the ensuing course following transplantation.

P55: hiPSC derived endothelial cell types from scalable cultures for biofunctionalization and tissue engineering

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

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

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

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

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Disease Area: Lung Cancer (Poster 56– 60)

P56: The influence of EGF/HGF signaling crosstalk on therapy resistance in NSCLC cell lines

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths worldwide. Therapeutic treatment of NSCLC includes mainly chemotherapy due to high metastatic spread but recently also targeted therapy using epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) for patients with an EGFR-activating mutation. Although patients benefit from this treatment, the NSCLC cancer develops a resistance against EGFR TKI treatment and recovers after a few months. Besides the gatekeeper mutation T790M in EGFR, the resistance can be mediated by hepatocyte growth factor (HGF) overexpression and amplification of its receptor c-Met. Yet, the mechanisms of this HGF mediated resistance are unknown.

Besides EGFR-TKI resistance lung cancer patients can also develop a resistance against chemotherapeutics like cisplatin. This resistance is also speculated to be influenced by EGF and HGF signaling. Therefore cisplatin resistant cell lines were developed to investigate alterations in signaling occurring after cisplatin treatment.

To obtain a deeper understanding of these mechanisms and provide improved therapeutic options, we analyzed c-Met and EGFR signal pathways MAPK and PI3K-Akt as well as their potential crosstalk in NSCLC cell lines. As model systems three cell lines with alterations in EGFR and c-Met mutation and expression status were selected to gain a broad insight in the individual cellular response. Time-resolved signaling data was acquired using quantitative immunoblotting.

A systems biology approach and modeling based on ordinary differential equations will be applied to this data to describe the interaction of both ligands in a quantitative and time-resolved manner.

P57: Individualisation of radiochemotherapy (RTCT) for locally advanced non-small cell lung cancer (NSCLC)

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Background:

Individualised therapy has not yet found its place in the treatment of stage III NSCLC. Although RTCT can be curative, many tumours progress despite multimodal treatment.
The CTRT 99/97 Bronchial Carcinoma Therapy (BROCAT) study investigated radiotherapy (RT) vs. RTCT after induction chemotherapy. The German Intergroup Lung Trial (GILT) investigated consolidation CT following simultaneous RTCT. Here we present clinical and translational predictors of outcome in these two large randomised trials.

Methods:

We analysed histology subgroups within BROCAT, comparing adenocarcinoma, squamous cell and large cell tumours. We also examined site of first progression (PR), comparing local, central nervous system (CNS), and systemic PR. Within GILT we collected tumour biopsies and established a cooperation within the DZL to analyse potentially prognostic and predictive molecular markers.

Results:

BROCAT (n=214) found longer progression free survival (PFS) with RTCT vs RT after CT, and a trend to longer overall survival (OS).

Site of first PR differed between the study arms ($p < 0.047$), with more CNS and distant metastases after RT (CNS 21%, distant 36%) vs. RTCT (CNS 7%, distant 24%), and more local and thoracic PRs after RTCT (55% vs. 34%).

Histology in BROCAT: 59 adenocarcinoma, 171 squamous cell, 28 large cell, 10 mixed and 35 NSCLC not otherwise specified (NOS). There was a trend to longer OS in all histologies. Squamous cell carcinoma had longer PFS after RTCT, and large cell tumours showed a trend to shorter PFS after RTCT.

The GILT trial (n=279) found no significant OS benefit for consolidation CT after RTCT; however, some subgroups stood out. 27 samples from the GILT trial are being analysed for a panel of molecular markers.

Conclusion:

Further clinical and translational efforts are needed to increase our ability to tailor treatment to the patient and disease in stage III NSCLC.

P58: Role of BAMBI in the regulation of EMT processes in human lung cancer cell lines

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Lung cancer, with its predominant form non-small cell lung cancer (NSCLC), is the leading cause of cancer related-deaths world-wide. One of the hallmarks of lung cancer is the high rate of mutations affecting different signaling pathways including members of the TGFbeta (transforming growth factor beta) signaling cascade. TGFbeta is known to have a dual role in carcinogenesis as it functions as a tumor suppressor by inhibiting cell proliferation and can act as a tumor promoter by inducing epithelial-to-mesinchymal transition (EMT). In lung cancer it has been observed that high TGFbeta levels correlate with poor prognosis and can be used as an independent risk factor for pulmonary metastasis.

The analysis of patient derived lung cancer tissues and tumor-free control samples by immunohistochemistry, transcriptome and array-based epigenetic methylome studies provided evidence for the down regulation of the TGFbeta pseudo-receptor BAMBI expression in tumor tissues. To examine the role of BAMBI in modulating TGFbeta signaling, we restored its expression in lung cancer cell lines using an inducible retroviral vector system. We showed that reconstitution of BAMBI expression resulted in reduced TGFbeta induced SMAD phosphorylation and a marked decrease in the expression of EMT markers at the mRNA and protein level. Furthermore, a reduction of cell motility was observed in a 2D migration assay and 3D collagen invasion assay.

Thus, the absence of BAMBI expression in NSCLC cell lines elevates responsiveness towards TGFbeta signaling and suggests a possible mechanism contributing to progression of lung cancer.

P59: The TGFb pseudo-receptor BAMBI: Possible tumor suppressor in human lung cancer

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Carcinomas of the lung are among the most frequent lung diseases and cause a growing number of fatalities. The majority of tumors develop without symptoms and are therefore usually diagnosed in an already progressed or metastasized state, which results in a 5-year-survival of approximately 16%. Current treatment approaches for NSCLC favor surgery or platinum-based chemotherapy.

Since the transforming growth factor beta (TGFb) pathway is known to be centrally involved in tissue homeostasis as well as carcinogenesis, we aimed with this study to investigate the role in human lung cancer tissues and immortalized cells with special regards to the inhibitory pseudo-receptor BAMBI. >150 patient samples as well as tumor-free lung tissues were screened by immunohistochemistry for the expression of BAMBI, TGFb, SMAD2, SMAD3, SMAD4, SMAD7, SNON as well as pSMAD2 and pSMAD3 as well as EMT Markers. In addition to protein level, gene expression studies applying microarray and qPCR were conducted as well as array-based methylome analyses. Both approaches revealed an abundantly activated TGFb signaling cascade in the tumor tissues compared to tumor-free lungs. The impact on TGFb-inhibition by forced expression of BAMBI was addressed in NSCLC cell lines by transient plasmid transfection as well as retroviral over-expression. Changes in cell viability and downstream differentiation processes were monitored by FACS, immunofluorescence staining and gene expression.

P60: Increased x-ray attenuation in malignant vs. benign mediastinal nodes in an orthotopic model of lung cancer

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Disease Area: Acute Lung Injury (Poster 61 – 64)

P61: Influenza virus impairs fibroblast growth factor receptor 2b dependent epithelial regeneration from a distal airway epithelial progenitor pool

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IV (influenza virus) pneumonia is associated with apoptotic damage of the alveolar epithelial barrier and therefore efficient alveolar repair is crucial for recovery. Lineage tracing studies suggest that the adult lung contains epithelial progenitor cells which proliferate after injury. Fibroblast growth factor 10 (FGF10) plays a major role in lung development and is also known to have reparative, anti-apoptotic potential after injury.

We therefore investigated if FGF10 would support alveolar epithelial repair processes after IV-induced pneumonia.

Following IV infection or naphthalene treatment, epithelial progenitor cells (EpProg), defined as EpCamhighCD49fhighCD104+ Sca-1int showed increased resistance to apoptosis and revealed high proliferation rates. This response was likely mediated by upregulation of the FGF10 receptor FGFR2b on EpProg post IV infection or naphthalene treatment. However, EpProg were found to be primary targets of IV infection, which resulted in reduced FGFR2b upregulation and renewal capacity in the infected compared to the non-infected fraction of EpProg, likely due to virus-induced blockade of the wnt signaling pathway mediating FGFR2b upregulation. Notably, the extent of EpProg infection correlated with the pathogenicity of different IV strains, suggesting that the severity of viral pneumonia might be associated with impairment of FGF10/FGFR2b-mediated epithelial cell renewal. Intratracheal application of recombinant or overexpression of FGF10 increased the reparative response of EpProg, whereas dominant negative FGFR2b overexpression resulted in reduced proliferation rates, sustained alveolar leakage and poor outcome.

We provide evidence that IV-induced blockade of the FGF10/FGFR2b axis may result in reduced epithelial cell renewal capacity and poor outcome and that induction of an FGFR2b-dependent pathway may represent a therapeutic approach to overcome IV-induced impairment of epithelial renewal and to drive tissue repair after injury.

P62: Therapeutic potential of murine bone marrow derived mesenchymal stem cells in influenza virus-induced pneumonia

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Influenza virus (IV) infects the human upper respiratory tract and occasionally spreads to the alveolar compartment causing primary pneumonia. This can lead to acute respiratory distress syndrome (ARDS) with severe alveolar damage, lung oedema and hypoxemia. Antiviral therapies are only effective in the very beginning of infection and specific treatment strategies for IV-induced ARDS are lacking. Recent studies have shown the anti-inflammatory and regenerative potential of mesenchymal stem cells (MSC). MSC display a beneficial role in acute and chronic lung injury, suggesting that MSC delivery may be a promising treatment strategy in IV-induced ARDS [1].

We isolated MSC cells from the bone marrow of 8 to 12 weeks old C57Bl6 mice by cell sorting [2]. We characterised their expression markers by flow cytometry and we confirmed their differentiation potential into chondrocytes, osteocytes and adipocytes by microscopy. We co-cultured primary murine alveolar epithelial cells (AECs) with sorted MSC. During influenza infection with PR8 strain, the presence of MSC drastically reduced apoptosis and infection level in AECs. We tested the effect of MSC intratracheal instillation in PR8-infected mice. Similarly to the in vitro experiments, the addition of MSC improved clinical outcomes in comparison with PBS-instilled control mice.

Our experiments show the beneficial role of MSC in PR8-induced injury in vitro and in vivo. Taken together our results support that MSC can be of great value to treat IV-induced lung injury.

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P63: Therapeutic effects of fibroblast grow factor 10 (FGF 10) after hyperoxic lung injury in mice

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Background and aims:

Bronchopulmonary dysplasia (BPD), a chronic lung disease of preterm infants, is characterized by impaired alveolar growth and pathologic vascularization. This project aims to investigate the role of Fibroblast growth factor 10 (Fgf10) in hyperoxic lung injury.

Methods:

1) 10 weeks old Fgf10+/- mice (50% Fgf10 expression compared to WT) in normoxic condition: Lung function and morphometric analysis.
2) BPD model:
    a) Fgf10+/- and Fgf10+/+ mice were exposed to 85% O2 from P0-P8. Morphometric analysis and α-Actin/vWF staining for vessels were performed at P3.
    b) Rosa26rtTA/+;tet(O)Fgf10 (gain-of-function) mice were exposed to 85% O2 from P0-P8. From P9-P45 the pups were exposed to normoxia and fed either with normal food (control) or doxycycline food (experimental) to activate the transgene Fgf10. Morphometric analysis was carried out at P45.
3) Tolerance study: Rosa26rtTA/+;tet(O)Fgf10 and WT mice (both 10 weeks old) were exposed to doxycycline for 2 weeks. Then survival rate, histology, Ki67 and TUNEL staining were performed.

Results:

1) Fgf10+/- mice under normoxic condition have worse lung function and lung structure compared to WT mice.
2) All Fgf10+/- newborn mice die from hyperoxic injury due to increased lung injury and vascular malformation.
3) Overexpression of Fgf10 after hyperoxic injury leads to improvement of lung structure compared to control group without overexpression.
4) Fgf10 overexpression after hyperoxic injury does not increase mortality and side effects (weight loss, mucosal proliferation) are reversible.

Conclusions:

Fgf10 attenuates hyperoxic lung injury, is well tolerated and should be further studied as a potential therapeutic for BPD.

P64: Role of arginase 1 in lung protective immunity against Streptococcus pneumoniae in mice

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Type 2 helper cell (TH2) dominated chronic lung diseases like asthma are associated with an increased risk for bacterial lung infections. However, the underlying mechanisms are poorly defined. Arginase 1 has been suggested to play an important role in the pathophysiology of asthma, and is rapidly induced in lung macrophages by TH2 cytokines, thereby limiting macrophage-derived antimicrobial nitric oxide (NO) production. However, the interplay between TH2 cytokine-dependent upregulation of Arg1 and its effect on lung protective immunity against bacterial infection has not been examined in detail. We here examined the effect of TH2 cytokine-induced upregulation or conditional knockdown of Arg1 in macrophages on lung resistance against Streptococcus pneumoniae. Lung macrophages responded with a profound and specific induction of Arg1 mRNA and protein to treatment with TH2 cytokines both in vivo and in vitro. Increased Arg1 activity was accompanied by both significantly attenuated lung protective immunity in mice challenged with S. pneumoniae and attenuated macrophage killing of S. pneumoniae in vitro. In contrast, conditional knock-down of Arg1 in lung macrophages did not impair lung protective immunity against S. pneumoniae, relative to S. pneumoniae-infected WT mice. Collectively, the data show that TH2 cytokine dependent increased but not decreased Arg1 activity worsens lung protective immunity against major lung-tropic pathogens such as S. pneumoniae. Interventions to limit Arg1 activity in the lung might be a novel immunomodulatory strategy for asthmatic patients to cope with bacterial lung infections.

DZL Platform Imaging (Poster 65- 70)

P65: Correlative three-dimensional observation of lung tissue by different tomographic methods

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Combining light- and electron optical methods on the same sample facilitates the understanding of organ structure by providing both overviews of large samples as well as high resolution insights into very small structural features. We present here the combination of Scanning Laser Optical Tomography (SLOT) with Electron Tomography (ET) to examine mouse lung tissue at low and high magnifications. SLOT allows to analyze, reconstruct and segment a whole mouse lung with a resolution allowing the recognition of structures in size down to single alveoli. The resulting three-dimensional models allow for example the detailed analysis of the conductive blood and airway system architecture.

Insights into subcellular regions are achieved with a resolution in the nanometre scale using ET. In lamellar bodies (the "surfactant" containing organelles in type II alveolar epithelial cells), single lipid lamellae can be observed.

P66: T1-mapping magnetic resonance imaging for the detection of chronic lung allograft dysfunction - initial results

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Background

Bronchiolitis obliterans syndrome (BOS) is the major limiting factor for long-term survival after lung transplantation, However, early markers for the detection are missing.

Aims and objectives

T1 mapping MRI of the lungs is evaluated for the detection of chronic lung allograft dysfunction in patients following double-lung transplantation.

Methods

Fifty-one double-lung allograft recipients were included and gave written informed consent. BOS was classified with spirometry and patients were divided into three groups: BOS 0, BOS0p and late stages (BOS1-3). Coronal T1 maps of the lungs were acquired at room air and 100% oxygen using an

inversion recovery snapshot fast low angle shot sequence at 1.5 T. The coefficient of variation for T1 values under room air as well under oxygen and the oxygen transfer function (OTF) were calculated.

Results

The coefficient of variation for T1 values was significantly higher for BOS 1-3 patients on both the room air (p=0.007) and the oxygen T1 maps (p=0.002) compared to patients with BOS 0 status. The OTF showed a strong trend towards decreased values in the groups with increasing CLAD/BOS stages (p=0.07).

Conclusions

The heterogeneity of T1 values as well as the OTF may be used for early detection of BOS and should be evaluated in future prospective trials.

P67: Surface modification of Carbon Black nanoparticles determine their cytotoxicity on mouse tracheal epithelial cells

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Inhaled nanoparticles can deposit on the airway epithelium and affect the epithelial cells. We are interested in how the effects of nanoparticles on the airway epithelium are changed after surface modification regarding impairment of mucociliary clearance and epithelial cytotoxicity.

We tested the following Carbon Black nanoparticles (CBNP) with similar hydrodynamic diameter: unmodified Printex®90 (CBNP/-), surface-modified Printex®90 with 9-nitroanthracene (CBNP/Na) or benzo[a]pyrene (CBNP/BaP) and an acetylene soot (A. soot). After incubation of mouse tracheae with 10 or 30 µg/ml CBNP for 24 h, their effect on cilia-driven particle transport, apoptosis and cell membrane damage, epithelial integrity and the mRNA expression of cytochrome oxidases (Cyp), mucins and cytokines were determined.
CBNP/- attached to cilia and induce an increase of ciliary beat frequency (CBF) and mucus release. In areas with mucus, particle transport speed (PTS) was decreased but in areas without mucus, PTS was increased. The epithelium remained intact and mRNA expression of cytokines, mucins or Cyp was not increased.

A. soot also led to an increase of CBF, whereas CBNP/Na and CBNP/BaP did not alter CBF. But all three modified nanoparticles caused a decrease of PTS based on induction of apoptosis and cell membrane perforation. Although epithelial cells were lost, the remaining epithelial cells preserved epithelial integrity. In addition, CBNP/Na, CBNP/BaP and A. soot induced mRNA expression of Cyp1a1 and Cyp1b1, but A. soot alone increased mRNA expression of MIP-2, KC, IL-6 and Muc5ac.

Our results indicate that the acute toxicity of CBNP is determined by their surface modification and modified CBNP can impair mucociliary clearance.

P68: Sudan Black B staining is a promising tool for secure localization of alveolar epithelial type II cells and automated proportionator sampling

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By means of stereology it is possible to quantify structural parameters within the lung, e.g. the total number of alveolar epithelial type II cells. When using the "density x reference space design" it is necessary to reduce shrinkage during histological processing, so that initial measurements of the total lung volume before processing still correspond to what is seen under the microscope after processing. We recently compared different types of fixation and embedding and found little shrinkage after primary glutaraldehyde/formaldehyde fixation, postfixation with osmium tetroxide and uranyl acetate and embedding in glycol methacrylate [2]. A proper type II cell quantification, furthermore, requires a secure identification of type II cells under the microscope. The latter, however, can make some difficulties in routine stainings. Therefore, more appropriate staining methods leading to good contrast between type II cells and the surrounding tissue are desirable. Selective immunohistochemistry might be impossible after our proposed method because of impairment of antigenicity by glutaraldehyde, osmium tetroxide or uranyl acetate. Lipid staining with Sudan Black B, however, is cheap, easy to apply in practice and perfectly compatible with our "low shrinkage processing protocol", because lipids (and therefore lamellar bodies) are well preserved during processing and then rather selectively stained by Sudan Black B. Additionally, it appears to be a well suited stain for automated detection of type II cells using the recently developed proportionator sampling [1]. Using thin sections, this approach may even be used for quantitative analysis of lamellar bodies at the light microscopic level.

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P69: Imaging repair processes of small epithelial lesions in the mouse trachea after laser-induced injury

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Small lesions in the airway epithelium occur frequently and offer an entry for pathogens. Therefore injuries have to be repaired to prevent prolonged disruption of epithelial integrity and to maintain a normal airway function. The mechanism of the repair process of small lesions in the airways is largely unknown.

To better understand this repair process of small lesions in the airways we used two-photon microscopy and an ex-vivo model of the mouse trachea. The explanted trachea was cut longitudinally and then imaged with the epithelium facing up. Epithelial lesions were induced by focussing Ti-sapphire femtosecond laser pulses to single epithelial cells for 1-8 seconds. Staining with propidium iodide (PI) allowed identification of damaged cells. Phalloidin was used to stain actin filaments after the experiment.

Damaging of cells in a specific area of the epithelium was possible. Depending on the irradiation time an area of 1-12 epithelial cells was damaged.

Hyperfluorescence around the beam focus and loss of autofluorescence in adjacent cells was observed. Within the lesion nuclei were stained with PI. Small lesions of 1-3 cells were closed within 2-3 h. Lesions of 4-6 cells needed 4-5 h or did not close within the observation time of up to 6 h. Epithelial cells around the lesion changed their shape by stretching. Especially the cells immediately adjacent to the wound margin protracted notably to close the lesion. During this process damaged cells were expelled apically into the lumen. Staining with phalloidin after two-photon microscopy showed a transepithelial actin ring formed in the cells around the wound which participate in closing the lesion.

Healing of small lesions in the airways depends on an active coordinated movement of adjacent epithelial cells. Further studies will focus on the molecular mechanisms of repair that might be impaired in airway diseases.

P70: Improved Diagnosis of Pulmonary Emphysema using in vivo Dark-Field Radiography

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Purpose: The purpose of this study was to assess whether the recently developed method of grating-based X-ray dark-field radiography can improve the diagnosis of pulmonary emphysema in vivo.

Materials and Methods: Pulmonary emphysema was induced in female C57BL/6N mice using endotracheal instillation of porcine pancreatic elastase and confirmed by in-vivo pulmonary function tests, histopathology and quantitative morphometry. Mice were anesthetized but breathing freely during imaging. Experiments were performed using a prototype small-animal X-ray dark-field scanner that was operated at 35 kVp with an exposure time of 5 seconds for each of the 10 grating steps. Images were compared visually. For quantitative comparison of signal characteristics, regions of interest were placed in the upper, middle and lower zones of each lung. Receiver operating characteristic statistics were performed to compare the effectiveness of transmission and dark-field signal intensities and the combined parameter "normalized scatter" to differentiate between healthy and emphysematous lungs.

Results: X-ray dark-field signal and normalized scatter were significantly different between mice with pulmonary emphysema and control mice and show good agreement with pulmonary function tests and quantitative histology. The sensitivity and specificity for identification of emphysema were 50.0% and 90.0% for the transmission signal, 96.7% and 73.3% for the dark-field signal and 96.7% and 96.7% for the combined parameter.

Conclusion: X-ray dark-field radiography is technically feasible in vivo and provides a substantial diagnostic benefit over conventional transmission-based X-ray imaging.

DZL-Platform Biobanking (Poster 71)

P71: DZL Biobanking

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Broad, coordinated access to biomaterials is essential for the translation of research findings into patient therapies. A centrally-organized DZL Biobanking Platform will guarantee that member of the DZL as well as external partners will have easy and direct access to biomaterials from patients with pulmonary disease. The DZL Biobanking Platform will capitalize on existing biobanking structures within DZL sites and will be connected to the Technology and Methods Platform for Network Research in Medicine (TMF e.V.) and Biobanking and Biomolecular Resources Research Infrastructure (BBMRI) catalogues. The biomaterial banks of DZL sites are not homogeneous. They are varying regarding structure and organizational standards methods of biomaterial collection, sample, data and quality management.

In addition to implementing the DZL Biobanking portal in order to provide an overview of existing collections and biomaterials, the DZL Biobanking initiative aims to harmonize operating procedures and policies across DZL sites. These harmonization efforts include standardization of informed consent procedures standardization of sample procurement, processing, and handling, as well as the development of harmonized of phenotyping tools and sample management. Member of the platform compiled a forward-looking broad informed consent form allowing for collection, unlimited storage, and unrestricted use of biomaterials and phenotyping data. For a prospective collection of biomaterials and phenotyping data an overarching data management structure was considered including a centralized patient registration and pseudonymization service and a data warehouse for integrating phenotyping, imaging an experimental data.

DZL Others (Poster 72- 79)

P72: Fibroblast-associated lipid bodies in the postnatal and adult mammalian lung

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Pulmonary lipofibroblasts are thought to be involved in crucial aspects of lung physiology (development, regeneration, vitamin A storage, surfactant synthesis). Given the wide range of functions attributed to this cell type, the present study was designed to investigate the presence of lipofibroblasts in a variety of mammalian species (incl. man) and throughout postnatal development of mice, rats and humans.

For this purpose, lung samples from 14 adult mammalian species (Etruscan shrew, mouse, rat, chinchilla, rabbit, dog, seal, goat, human, camel, lama, giraffe, horse and cattle) as well as from postnatal humans and neonatal, 6/7-day-old, 14-day-old and 42-day-old mice and rats were investigated using light and electron microscopic stereology. The volume fraction and the total volume of lipid bodies was estimated and related to the body mass of the animals.

Among the adult animals, lipid bodies were only observed in rodents (mouse, rat and rabbit). In all other species, no lipofibroblasts were observed. Lipid body volume scaled with body mass with an expontent $b = 0.73$ in the power law equation. Throughout mouse and rat postnatal development, the volume of lipid bodies first increased, then declined and persisted at a lower level in the adult animals. We did not observe lipofibroblasts in the postnatal human lung.

In conclusion, among 14 mammalian species lipofibroblasts were only observed in rodents. The great increase in lipid body volume during early postnatal development of the mouse lung confirms the special role of lipofibroblasts during rodent lung development and regeneration. It is evident that the cellular functions of lipofibroblasts cannot be transferred easily from rodents to other species.

P73: Bronchiectasis-Associated Hospitalizations in Germany, 2005–2011: A Population-Based Study of Disease Burden and Trends

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Background: Representative population-based data on the epidemiology of bronchiectasis in Germany are lacking. The aim of the present study was to investigate the current burden and the trends of bronchiectasis-associated hospitalizations and associated conditions in Germany in order to inform patient care and facilitate the allocation of healthcare resources.

Methods: The nationwide diagnosis-related groups hospital statistics for the years 2005–2011 were used to identify hospitalizations with bronchiectasis as any hospital discharge diagnosis according to the International Classification of Diseases, 10th revision, code J47, (acquired) bronchiectasis. Poisson log-linear regression analysis was used to assess the significance of trends. In addition, the overall length of hospital stay (LOS) and the in-hospital mortality in comparison to the nationwide overall mortality due to bronchiectasis as the primary diagnosis was assessed.

Results: Overall, 61,838 records with bronchiectasis were extracted from more than 125 million hospitalizations. The average annual age-adjusted rate for bronchiectasis as any diagnosis was 9.4 hospitalizations per 100,000 population. Hospitalization rates increased significantly during the study period, with the highest rate of 39.4 hospitalizations per 100,000 population among men aged 75–84 years and the most pronounced average annual increases among females. Besides numerous bronchiectasis-associated conditions, chronic obstructive pulmonary disease (COPD) was most frequently found in up to 39.2% of hospitalizations with bronchiectasis as the primary diagnosis. The mean LOS was comparable to that for COPD (10.1 [95% CI 9.8–10.5] days). Overall, only 40% of bronchiectasis-associated deaths occurred inside the hospital.

Conclusions: The present study provides evidence of a changing epidemiology and a steadily increasing prevalence of bronchiectasis-associated hospitalizations. Moreover, it confirms the diversity of bronchiectasis-associated conditions and the possible association between bronchiectasis and COPD. As the major burden of disease may be managed out-ofhospital, prospective patient registries are needed to establish the exact prevalence of bronchiectasis according to the specific underlying condition.

P74: Modulation of immune-mediators from donor lungs using the OrganCareSystem® - a potential mechanism for improved outcome

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Objectives

Release of donor-derived immune mediators (IM), triggering allorecognition and inflammation after transplantation (Tx), may impinge on clinical outcome using warm perfusion of donor lungs (OrganCareSystem®, OCS) or standard cold preservation (SOC). IM were analysed in preservation solutions (PS) and peripheral blood (PB), also clinical outcomes monitored.

Methods

IM were quantified in PS and PB at protein level by multiplex-technology at the end of OCS (n=12) or SOC (n=9). Donor and recipient demographics and midterm outcomes were analysed.

Results

PS concentrations of IL-6, IL-10, IL-16, IFN-g CXCL8, CCL4, Ang-2, PECAM-1 and PDGF-b were significantly higher in OCS than SOC ($p<0.0001$). Inverse distribution was observed for FGF-b ($p=0.005$). High concentrations in PS following OCS correlated with lower concentrations of IM in recipient PB after Tx. OCS vs. SOC median donor/recipient age was 45/55 vs. 46/56, underlying diagnoses: idiopathic/cystic fibrosis (n=6/3 vs. n=5/2), idiopathic pulmonary hypertension (n=0 vs. n=1) and emphysema (n=3 vs. n=1). No significant differences (minutes) of median cross clamp times for right (430 vs. 505) and left lung (569 vs. 641) were seen. Shorter median ICU-stay (3585 vs. 3750) and mechanical ventilation times (795 vs. 1051) were observed in OCS. Significantly higher %predicted FEV1 at discharge (FEV1) (71% vs. 55%, $p=0.04$) and lower PGD-scores at T24 ($p=0.28$) were seen in OCS. Six-month-survival was not different. Correlations between Ang-2 and IL-6 concentrations and FEV1, mechanical ventilation time, paO2/FiO2 and ICU-stay were identified.

Conclusion

IM remained low in PS using SOC probably due to reduced metabolic activity in lung tissue during cold ischemia. During OCS preservation, significantly higher amounts of IM were released into PS which may represent depletion from the organ by accumulation. This 'dialysis' effect was associated with reduced inflammatory conditions after Tx, which had a positive impact on clinical outcome in OCS.

P75: Genome-wide microarray-based screen for FOXJ1-dependent ciliary factors in the murine lung

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Ciliogenesis is crucial for proper organogenesis during mouse embryonic development and homoeostasis of adult tissues. A key regulator of motile cilium formation is the transcription factor FOXJ1 that directs ciliogenesis in various tissues including the respiratory epithelium of the lung and the ependymal epithelium of the brain. Downstream effectors of FOXJ1 are only partially known.

In order to elucidate processes downstream of FOXJ1 that initiate ciliogenesis or enable cilium function, we conducted microarray screens comparing the murine transcriptomes of (1) Foxj1-deficient and wildtype lungs at embryonic day E16.5 and (2) unciliated and ciliated micro-dissected lung epithelia at embryonic day E14.5 and E18.5, respectively. We identified 180 genes deregulated in both screens including both already known regulators of ciliogenesis and novel factors that are now promising candidates for FOXJ1-dependent regulators of ciliogenesis. Many of those candidates exemplary tested by section in situ hybridisation of E18.5 mouse embryos to validate the microarray results are indeed predominantly expressed in the respiratory epithelium as well as in other ciliated tissues. Furthermore, several factors tested display subcellular localisation near the basal body of the cilium of monociliated IMCD3 cells implying ciliary importance.

Currently, we analyse five of these candidates in more detail. For one of our candidates, 1700012B09Rik that is conserved between mouse and human and encodes a short protein of unknown function, we generated a knock-out mouse. We show that this gene is expressed in ciliated tissues including the embryonic node, the developing and adult lung and ependyma and that its expression depends on FOXJ1. The KO mouse neither reveals an apparent motile cilium defect nor a lung phenotype so that it is not yet clear whether 1700012B09Rik is involved in cilium formation or function and if so at what level.

P76: The role of WNT/b-catenin signaling in smooth muscle cells during lung development and repair

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Wnt signaling is important for the formation of different organs during embryonic development. It also plays a key role in lung development, as it is required for branching morphogenesis, proliferation, differentiation and survival of lung progenitors in both the epithelium and mesenchyme. Upregulation of Wnt signaling in the mesenchymal cells is associated with lung pathologies, such as asthma, lung fibrosis, and pulmonary arterial hypertension. After naphthalene injury, an established mouse model of airway epithelial damage, the surviving ciliated airway epithelial cells express Wnt7b. Wnt7b then acts on parabronchial smooth muscle cells (PBSMCs) to induce Fibroblast Growth Factor 10 (Fgf10) expression leading to the expansion of epithelial progenitor cells required for epithelial restoration after injury.

We will further investigate the role of Wnt signaling in PBSMCs (using the SMA-Cre-ERT2 mouse driver line, which we recently validated) by gain and loss of function approaches of Wnt signaling during development and in the context of naphthalene injury. Loss of function of Wnt signaling will be performed by using mouse lines that will allow specific deletion of β-catenin in smooth muscle cells. Gain of function will be performed by using mouse line, which allows expression of stable form of β-catenin in smooth muscle cells. We will also use different reporter lines that will allow us to visualize the smooth muscle cells which underwent Cre activation (Tomatoflox/flox mice) and monitor Wnt signaling (Topgal and Axin2LacZ lines).

P77: CILIA – Conditional immortalization of murine alveolar epithelial cells

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The blood-air barrier is formed by the alveolar epithelium of the peripheral lung consisting of mainly two cell types. The squamous alveolar epithelial type I cells (ATI) cover up to 97% of the total surface area and hence are responsible for the pulmonary gas exchange. However, alveolar epithelial type II cells (ATII) are cuboidal and undertake various functions including synthesis and secretion of surfactant, proliferative capacity and ion transport. Till date there is no murine cell line available that reflects the crucial barrier properties of primary ATI cells, which are functional tight junctions and as a consequence high transepithelial electrical resistance (TEER). The immortalization of alveolar epithelial cells could sustainably diminish the number of animal testing according to the 3R principle ("refine, reduce, replace") and provide the development of in vitro model systems which can be applied for drug delivery and pulmonary diseases studies. To overcome the laborious procedure of isolating primary cells and to enhance the reproducibility of in vitro test systems, we aim at the immortalization of alveolar epithelial cells from mice with different genetic background to generate novel cell lines mimicking the blood-air barrier. We established protocols for immortalization of primary murine alveolar epithelial cells (mAEpC). For this purpose, we lentivirally transduced bona fide immortalizing genes such as the simian virus large T antigen or a set of proliferation genes. Upon infection, cell lines could be established that exhibit a prolonged lifespan and show TEER values comparable with those of primary cells. Currently, these cells are characterized more detailed with respect to the expression of lung specific marker genes. These cell systems could allow standardized toxicity and transport studies for newly developed compounds and be helpful in elucidating infection pathways across the respiratory tract in the context of aerosol transmitted infectious diseases (e.g. swine flu, tuberculosis, etc.).

P78: Intrapulmonary transplantation of macrophage progenitors as novel and long-lasting therapy for hereditary pulmonary alveolar proteinosis

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Hereditary pulmonary alveolar proteinosis (herPAP) is a rare lung disease caused by mutations in the granulocyte/macrophage-colony-stimulating factor (GM-CSF) receptor genes, resulting in disturbed alveolar macrophage differentiation, massive alveolar proteinosis, and life-threatening respiratory insufficiency. So far, the only effective treatment for herPAP is repetitive whole lung lavage, a merely symptomatic and highly invasive procedure. We introduce intrapulmonary transplantation of

macrophage progenitors as a novel, effective and long-lasting therapy for herPAP. In a murine disease model, intrapulmonary transplanted macrophage progenitors displayed selective, long-term pulmonary engraftment and differentiation into functional alveolar macrophages. A single transplantation significantly ameliorated the herPAP phenotype for at least nine months resulting in significantly reduced alveolar proteinosis, normalized lung densities in chest computed tomography, and improved lung function. Importantly, a significant and sustained disease resolution was also observed in a second, humanized herPAP model after intrapulmonary transplantation of human macrophage progenitors. Here, the therapeutic effect was mediated by long-lived, lung-resident macrophages which displayed functional and phenotypical characteristics of primary human alveolar macrophages. Our findings present a novel, effective and long-lasting therapy for herPAP and may serve as a proof-of-principle also for other diseases, expanding current stem cell-based strategies towards potent concepts utilizing the organotropic transplantation of differentiated cells.

P79: Tbx2 Controls Lung Growth by Direct Repression of the Cell Cycle Inhibitor Genes Cdkn1a and Cdkn1b

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A considerable number of diseases in the mature lung can be related to the deregulation of (embryonic) programs that control the balance between proliferation and differentiation of cells.

Pediatric pulmonary hypoplasia is a common cause of neonatal death; consequently, a lot of effort has been put into understanding the network of signaling pathways that assure correct lung growth and development. However the mechanisms of cell cycle control during lung organogenesis were only insufficiently understood. Unraveling these is crucial to understand how tissue homeostasis in the lung is achieved and how deregulation of the cell cycle may contribute to pulmonary diseases.

We recently reported on the function of the T-box transcription factor gene Tbx2 in lung development. Tbx2-deficient mice exhibit markedly hypoplastic lungs in combination with reduced branching morphogenesis. Tbx2 mutant lungs feature decreased mesenchymal proliferation accompanied by excessive matrix deposition that indicates premature differentiation of fibroblasts. Downregulation of canonical Wnt-signaling and upregulation of the cell cycle inhibitors Cdkn1a (p21) and Cdkn1b (p27) precede these changes. Genetic depletion of Cdkn1a and Cdkn1b partially restored lung growth in Tbx2 mutant mice. In contrast, misexpression of Tbx2 in the lung mesenchyme of adult mice complementary resulted in hyperproliferation and a loss of Cdkn1a and Cdkn1b expression indicating a direct regulatory function of Tbx2 in control of these cell cycle regulators. We evaluated this presumption by ChIP experiments and showed binding of Tbx2 to the loci of Cdkn1a and Cdkn1b in vivo.

Conclusion:

Tbx2 regulates lung growth and mesenchymal differentiation by direct inhibition of Cdkn1a and Cdkn1b. Hence Tbx2 is a crucial mediator of cell cycle control during organ development in vivo.

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REBIRTH

REBIRTH – Heart (Poster 80- 86)

P80: Epicardial function of canonical Wnt-, Hh-, Fgfr1/2- and Pdgfra-signaling

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The murine embryonic epicardium is a monolayered epithelium that covers the outer surface of the heart. It protects the underlying myocardium and is a crucial source of cells for the developing cardiac fibrous skeleton and the coronary vasculature. Wnt-, Hh-, Fgfr1/Fgfr2- and Pdgfra-signaling pathways were reported to be required for mobilization and/or differentiation in these EPicardium-Derived-Cells (EPDCs) and for the formation of the coronary vasculature. However, the cre lines used for conditional ablation of these pathways might not have been specific for the epicardium. Thus, we aimed to re-evaluate the relevance of canonical Wnt-, Hh-, Fgfr1/Fgfr2- and Pdgfra-signaling in the developing epicardium by the use of a Tbx18cre-mediated conditional approach, which specifically mediates recombination in the epicardium.

We show that the epicardium-specific loss of Ctnnb1 does not affect the mobilization and differentiation of EPDCs, whereas expression of a stabilized version of Ctnnb1 results in formation of hyperproliferative epicardial cell clusters. Epicardial loss of Shh and Smo does not affect cardiac development whereas expression of a constitutively active version of Smo in the epicardium leads to epicardial thickening and loss of epicardial mobilization. Epicardium-specific loss of Fgfr1 and Fgfr2 does not affect cardiac development either, although in contrast, epicardial loss of Pdgfra prevents differentiation of EPDCs into mature fibroblasts.

Our data questions earlier reports on a role of canonical Wnt-, Hh- and Fgfr1/Fgfr2-signaling in murine epicardial development, but supports the notion that Pdgfra-signaling is crucial for differentiation of cardiac fibroblasts from epicardium-derived cells.

P81: New Biodegradable Hydrogels Based on Hyaluronic Acid and Dextran

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The major goal in research of biopolymers is to generate a three-dimensional, biomimetical and biodegradable network. These networks are applied in artificial tissues for skin-transplantation, in vivo drug-release or as artificial muscle fibres.[1]In the case of myocardial infarction a less-invasive injectable biopolymers serve as favourable matrix for cell delivery and redevelopment of damaged regions.[2]

Most of those biopolymers are composed of polysaccharides such as hyaluronic acid, starch and alginates. There are two major strategies to construct a three-dimensional biopolymer network, ether ionic interactions or covalent linkages. However the last approach is favoured due to its stability.

Previous work in the Kirschning group has shown that in situ cross-linkable alginate and hyaluronic acid hydrogels can be applied in tissue engineering. Two cross-linking procedures has been employed, Aldehyde/hydrazine condensation and a strategy basedon metal-free click reactions.

The goal of our research is to oxidize and functionalize dextrane and create novel hydrogels via crosslinking with the alginate and hyaluronic acid. These modifications will create anelastic-viscous and biodegradable three-dimensional networks.

The first oxidations and hydrogelations with and without functionalization, even in nutrient solution, have been carried out successfully. However the functionalization seemed to destabilize the polymer. Mechanistic studies on the pyranose are in progress to get a deep look into the chemistry and stability of dextrane.

Recapitulatory dextrane is a new possibility to create biodegradable und biomimetic cell delivery vehicles. The flexibility related to the functional groups of the dextrane is predestinated to advance the myocardial stem cell therapy.

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P82: Cardiomyogenic differentiation of human pluripotent stem cells (hPSC) in fully-defined suspension culture by the application of small molecules

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Estimations suggest that more than one billion cardiomyocytes per patient will be required for cardiac replacement therapies after myocardial infarction. Human pluripotent stem cells (hPSC) present an attractive cell source to generate these large amounts, not only for the envisioned cell therapies, but also for drug screening and cardiotoxicity tests.

For these purposes an efficient hPSC differentiation process is highly demanded, preferentially in defined and scalable conditions. To this end we and others have successfully developed scalable expansion of hPSCs in suspension culture (Olmer et al., 2010; Zweigerdt et al., 2011).

However, efficient cardiomyogenic differentiation strategies for these cultures were not described yet. Towards this end we have tested chemical compounds including p38 MAPK- and Wnt- pathway modulators in multi well assays and monitored cardiac differentiation by an Nkx2.5eGFP/w reporter line, KDR/PDGFR-α flow cytometry, and cardiac Troponin T-specific immunoflourescence staining. Promising candidate combinations were subjected to hPSC mass suspension cultures in Erlenmeyer Flasks and stirred bioreaktors. The electrophysiology and gene expression of the generated cardiomyocytes were further characterized and successfully applied for cardiac tissue engeneering.

We have achieved an efficient hPSC differentiation process i.e. observed a level of >60% cardiomyocyte induction based on small molecules enabling the derivation of large amounts of functional cardiomyocytes in fully-defined and scalable conditions.

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P83: Residual α-Gal epitope levels in decellularized porcine pulmonary heart valve matrices are dependent on the method of decellularization

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Utilization of decellularized xenogeneic heart valve matrices for heart valve replacement therapy could offer a possible solution for overcoming severe donor shortage surgeons faced using allogeneic matrices. However, implantation of xenogeneic valve matrices into human recipients elicited severe immune responses in the past, mostly ending up into graft rejection. Residual carbohydrate antigens on extracellular matrix proteins, like highly antigenic α-Gal epitopes, are assumed to be main cause for early failure of xenogeneic matrices.

Fresh porcine pulmonary valve conduits were decellularized using different detergent- and enzyme-based decellularization protocols. Subsequent cleavage of remaining α-Gal epitopes using α-Galactosidase was performed on matrix samples of each decellularization group. Resulting tissues, mainly composed from insoluble extracellular matrix proteins, were separated afterwards into pulmonary artery wall pieces and pulmonary valve leaflets, frozen in liquid nitrogen, minced and finally solubilized by protease digestion. Assessment of thus generated solutions concerning α-Gal content was performed using a novel designed lectin-based immunoblot technique.

Decellularization lead to significant reduction of α-Gal content dependently varying among used decellularization protocol between 30 to 50% in comparison to α-Gal contents of native porcine matrix samples. A further reduction of α-Gal in a range of another 15 to 30% could be achieved by additional enzymatic digestion with α-Galactosidase. Combination of decellularization and subsequent enzymatic treatment allowed reductions of α-Gal matrix contents down to levels of those measured in pulmonary valve tissues of 1,3-Galactosyltransefrase-KnockOut pigs.

In summary, residual α-Gal levels can be measured on insoluble matrix components of porcine pulmonary heart valves, which vary in dependence of the decellulariation protocol applied. In

combination with α-Galactosidase the content can be reduced to background levels. Whether this reduction is sufficient for prevention of a hyperacute graft rejection, and has a clinical impact then, has to be tested in an in vivo model.

P84: Optogenetic Control of Bioartifical Cardiac Tissue

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Electrical stimulation is a widely used approach in cardiac tissue engineering for the enhancement of tissue maturation. However, tissue damage due to faradaic reactions might occur during electrical stimulation. We aimed to overcome these limitations by constructing a light-sensitive bioartificial cardiac tissue (BCT) generated from murine induced pluripotent stem cell (miPSC)-derived cardiomyocytes expressing channelrhodopsin, a light-activated cation channel.

Cardiomyocytes were generated from a transgenic miPSC line expressing channelrhodopsin under control of the chicken β-actin promoter. BCTs were prepared by mixing cardiomyocytes and mitotically inactivated mouse embryonic fibroblasts. Light-induced contraction forces were measured in a custom made bioreactor system. Light stimulation of BCTs was performed through a royal blue (470 nm) high power LED. Stimulation triggers were generated by the bioreactor amplifier with software-controlled stimulation duration and frequency. Threshold light intensity required to obtain stable 1:1 pacing was determined. The effect of long-term (14 days) light stimulation of BCT was tested.

Light-inducible BCTs showed spontaneous beating activity, indicating that expression of channelrhodopsin did not result in a leaky membrane current. The magnitude of light-induced contractions was found to depend on two factors: light intensity and stimulus duration. Beating frequency analysis showed that the frequency could be controlled with 1:1 capture up to 300 bpm. Furthermore, no side effects were observed in BCTs after long-term light stimulation.

We have shown light stimulation can be used as an alternative approach to electrical stimulation of cardiac constructs and its potential for improvement of cardiomyocyte maturation and tissue function will be further investigated.

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P85: PNGase F removes glycocalyx structures of decellularized porcine pulmonary heart valve matrices

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Clinical implantation of xenogeneic tissue leads to xenoantigen based graft rejections. So far the galactose α1,3,galactose epitope was identified as strongest xenoantigen which is present on non-human grafts. Not only αGal, but carbohydrates in general are believed to be xenoantigens, since the glycocalyx is species-specific. To address and circumvent the limited availability of human decellularized heart valve matrices for clinical heart valve replacement therapy in future, we investigated the removal of glycocalyx structures of porcine pulmonary heart valves (PPHV) by decellularization and glycolytic treatment.

PPHV were decellularized by different detergent-based protocols (SDS/Na-deoxycholate, SDS/TritonX100, TritonX100 followed by SDS) and enzymatically treated by PNGase F digestion. The potential effect on carbohydrate removal was investigated by histochemical stains on microscopical slides using isolectin B4 (IL-B4) staining αGal epitops, wheat germ agglutinin (WGA) staining N-acetylglucosamines, datura stramonium lectin (DSL) staining β-1,4 linked N-acetylglucosamines and N-acetyllactosamines, and ricinus communis agglutinin I (RCA I) staining N-glycosides. Decellularized only samples served as controls.

All used lectins stained native PPHV tissue, whereas a reduced lectin stain was observed on decellularized PPHV matrices in dependence of the decellularization protocol used. SDS/TritonX100 treatment had no influence on WGA, DSL and RCA I stains, but reduced IL-B4 stain, compared to native tissue. The other protocols reduced IL-B4, WGA and DSL stains. PNGase F digestion abolished IL-B4, WGA and DSL stains almost completely independent on the type of the preceding decellularization process.

Based on our lectin stains, decellularization per se reduced αGal epitopes whereas decellularization by SDS/Na-deoxycholate and TritonX100+SDS, but not by SDS/TritonX100, additionally led to a removal of N-acetylglucosamines. The cleavage of GlcNacβ(1-N)Asn sites by PNGase F eliminates detergent-independent carbohydrate structures like αGal and N-acetylglucosamines. In summary, detergent based decellularization followed by PNGase F treatment resembles an efficient way to remove potentially immunogenic epitopes from PPHV matrices.

P86: Genetic modification of human iPS cells by designer nucleases for myocardial tissue engineering

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Myocardial tissue engineering offers a promising treatment option for regeneration of ischemia affected cardiomyocytes (CM), e.g. after myocardial infarction. This requires a stable cell source of human cardiomyocytes. Most promising is the use of human induced pluripotent stem cells, which have the potential to give rise to cell types of all three germ layers. For clinical translation an enrichment of CM is mandatory, which could be realized by antibiotic selection of transgenic cells.

In the present work, a zeocin resistance gene under control of a specific alpha myosin heavy chain (αMHC) promoter was used for genetic modification of human cord blood iPS cells (huCBiPSC) by designer nuclease-mediated genome editing. A site specific integration of the transgene into the "safe harbor" AAVS1-site, on chromosome 19 of the human genome ensures a stable long-term transgene expression without silencing effects or influence on endogenous genes. Transgenic single cell clones were verified by PCR analysis and checked for expression of pluripotency markers including Oct4, Nanog, Tra-1-60 and SSEA4.

Cardiac differentiation was initiated by aggregating huCBiPSCs in agarose microwells leading to uniform embryoid body (EB) formation, followed by temporal modulation of canonical Wnt signaling in suspension culture. Differentiation resulted in spontaneously contracting EBs, which showed a content of cardiac troponin T positive cells of up to 25%. After 7 days, subsequent zeocin selection provided cardiac bodies (CBs) with a purified CM population of 98%, which expressed the cardiac markers Troponin T, αMHC, βMHC, MLC2a and MLC2v. Selected CBs were directly used for preparation of functional bioartificial cardiac tissue (BCT) which showed synchronous contractility.

In conclusion, we demonstrated that targeting of the AAVS1-site of human iPSCs with a cell type-specific zeocin resistance transgene was successfully implemented for enrichment of CMs and therefore is a promising tool for safe clinical translation of myocardial tissue engineering strategies.

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Rebirth – Blood (Poster 87)

P87: Immunohistochemical analysis of cell populations in teratomas generated by induced human pluripotent stem cells in NSG mice

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Introduction

The discovery of induced pluripotent stem cells (IPSC) was a major advance in the field of regenerative sciences. Pluripotency of the cells is usually investigated by teratoma formation in immunodeficient mice. In studies using murine IPSCs, the development of hematopoietic stem cells and hematopoietic progenitor cells inside the emerging teratomas was observed. This study focused on reproducible teratoma formation from CD34+ derived human IPSC clones, when subcutaneously injected into NSG mice. Further we investigated histogenesis, especially hematopoiesis, by labeling specific surface markers of distinct cell clusters.

Methods

$2x10^6$ cells were injected into the flanks of NOD.Cg-PrkdcscidIl2rgtm1Wjl mice. After teratoma formation, tissue samples were fixed with formaldehyde and paraffin-embedded. Dehydrated sections were stained with Hematoxylin-Eosin (HE) and numerous specific tissue markers. Evaluation was done microscopically.

Results

The injection of hIPSC into immunodeficient mice gave rise to teratomas in 8 weeks time. HE staining of the teratoma sections visualized various areas with cells in early differentiation stages, surrounded by undifferentiated loose united cell structures. Immunohistochemical analysis revealed the presence of tissue from all three germ layers. GFAP expression indicated distributed cells of the central nervous system including glia cells, originating from the ectoderm. Goblet cells, characteristic endodermal cells, were traced by Cytokeratin 18 and 20 immunostaining. Mesodermal descendants, like cartilage and smooth muscle cells, were visualized by HE and Actin staining, latter with increased abundance around vascular and intestinal structures. Vascular structures were labeled by CD34 antibodies. In addition, the presence of hematopoietic cells was demonstrated by human αCD45, αCD49f and αCD90.

Conclusion

This reproducible experimental set-up for teratoma formation with hIPSCs provides a valuable *in vivo* model to profile developmental processes during embryogenesis. The maturation of hIPSCs included the hematopoietic lineage therefore we will apply this model to further pinpoint subpopulations of the hematopoietic niche.

Rebirth – Liver (Poster 88- 89)

P88: Cytokine-directed differentiation of foregut endodermal hPSC-derivatives

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

In our study, we aimed for an efficient protocol that is applicable to specify hPSCs into an endodermal progenitor lineage prior to further terminal differentiation into hepatic cells. We evaluated a cytokine– and small molecule–based protocol activating the WNT pathway by the GSK3beta inhibitor CHIR99021 for an improved definitive endoderm differentiation. Subsequently, we evaluated the inhibition of WNT signalling by sFRP-5 with respect to an enhanced differentiation towards a foregut endoderm cell population. We analysed the effect on WNT target gene expression by an hPSC reporter cell line. The activation of the WNT pathway was investigated by quantitative Western Blots of the active (non-phosphorylated) beta-catenin levels and the sFRP-5 mediated inactivation of WNT signalling by detection of phosphorylated GSK3beta and active beta-catenin, respectively. The endodermal cells' differentiation status was determined by immunocytochemistry and qRT-PCR for the definitive endodermal markers SOX17 and FOXA2 and by qRT-PCR for the foregut endodermal markers GATA4 and AFP, respectively.

In conclusion, our modified protocol allowed the specification of hPSCs into an homogenous endodermal progenitor cell population. Activation of the WNT pathway by the small molecule CHIR99021 supported a cell population expressing endodermal marker genes. Subsequent inhibition of the WNT pathway led to an improved foregut endoderm differentiation of the definitive endoderm cells showing an increased expression of early hepatic markers.

P89: MicroRNA modulation facilitates generation of hepatocytes from human embryonic stem cells

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Currently, hepatic differentiation protocols for human embryonic stem cells (ESCs) require substantial improvements, as the resulting HLCs do not match the therapeutic potential of primary hepatocytes for cell-based therapies. It has been demonstrated that microRNAs (miRNAs), post-transcriptional regulators of gene expression, regulate hepatocyte cell fate during liver development. However, their precise role and underlying mechanisms during hepatocyte differentiation and their utility for the generation of functional hepatocytes remain to be explored further.

The aim of the present study was to identify and to analyze hepatogenic miRNAs for their potential to enhance the hepatic in vitro differentiation of ESCS. Based on miRNA profiling from ESCs, HLCs differentiated from ESCs, fetal liver and adult primary hepatocytes, we chose 20 conserved candidate miRNAs to test their hepatogenic potential. According to the following miRNA screening, we found that inhibition of miR-199a-5p in HLCs facilitates efficient hepatocyte differentiation from mouse as well as human ESCs.

Upon transplantation, miR-199a-5p inhibition in human ESCs-derived HLCs leads to their engraftment and repopulation in the liver of immunodeficient fumarylacetoacetate hydrolase knockout (Fah-/-/Rag2-/-/Il2rg-/-) mice. Furthermore we show for the first time, that human ESCs-derived HLCs are able to engraft and to repopulate the liver of this certain mouse model.

For the elucidation of the underlying molecular mechanism, we identified SMARCA4 and MST1 as two novel targets of miR-199a-5p that contribute to the improved in vitro HLCs generation as well as in vivo liver repopulation. Additionally, we provide first insights into the role of miR-199a-5p during fetal liver hepatoblast development.

Taken as a whole, our findings suggest that miRNA modulation may offer a promising approach to generate more mature HLCs from stem cell sources for the treatment of liver diseases.

Rebirth in Translation (Poster 90- 91)

P90: Characterization of the Rag1-ko rat - A new immuncompromised animal model

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The LEW-Rag1em/Ztm was generated by Rag1 specific zinkfinger-nuclease microinjection into the cytoplasm of zygotes. The recombination activating genes Rag1 and Rag2 code for enzymes, which are essential for the V(D)J-recombination. As a result of this mutation with an autosomal recessive inheritance, the disturbed maturation of B- and T-lymphocytes leads to a highly restricted immune response.

Although the Rag1-ko rat is kept under germfree and specific pathogen free conditions, it develops clinical symptoms including alopecia, reddened or scabby skin, wet and sticky fur, failure to thrive, enlarged or small lymphnodes, pathological changes in lung tissue and an increased number of eosinophils in tissues. These symptoms manifest mostly on day 60 to 120. By characterizing the white blood cells via flow cytometry, we noted a complete lack of B-cells in the Rag1-ko rat. Additionally, the results displayed significantly lower values of T-lymphocytes (CD3+/CD4+ and CD3+/CD8+) and an increase of CD4+/CD8+ double positive cells in these animals. Alterations of the number of cells positive for the surface markers CD25, CD161 (NKR), TCR$\alpha\beta$ and TCR$\gamma\delta$ were also observed.

The phenotype so far observed resembles tightly the human Omenn Syndrome (OS), which is in 90% of cases caused by a defect of the Rag1 or Rag2 gene. It was first described in 1985 and belongs to the severe combined immunodeficiencies (SCID). OS is characterized by oligoclonal activated T-cells in the peripheral blood and a lack of B-cells leading to severe and recurrent infections with ubiquitous pathogens with generalized erythrodermia, alopecia, hypereosinophilia and lymphadenopathy.

As there are broad similarities between the Rag1-ko rat and the human OS, our aim is to clarify the suitability of this rat model for research in OS. Furthermore we want to evaluate its use in research for other autoimmune diseases and transplantation studies.

P91: Preclinical assessment of improved lentiviral vectors for gene and cell therapy of pulmonary alveolar proteinosis

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Congenital pulmonary alveolar proteinosis (PAP) caused by mutations in GM-CSF receptor α chain (CSF2RA) represents a rare, life-threatening disease characterized by the accumulation of phospholipids and proteins in the lungs due to a functional insufficiency of alveolar macrophages. So far, therapy options are limited, but recent data suggest that a gene therapy approach based on the intratracheal application of gene-corrected macrophages may be feasible.

To this aim, we have generated SIN-lentiviral constructs expressing the codon-optimized human CSF2RA-cDNA in combination with EGFP (Lv.EFS.CSF2RA.EGFP) or the inducible suicide gene iCaspase9 (Lv.EFS.CSF2RA.iCASP) from an EFS1a-promoter sequence. BaF3 cells transduced with these vectors showed stable and longterm (>3 month) expression of CSF2RA (CD116) as detected by flow cytometry. Furthermore, these cells survived in an hGM-CSF dependant proliferation analysis even at low concentrations of GM-CSF (5 ng/ml) confirming the formation of functional hybrid receptors with the murine GM-CSF receptor ß-chain by the transgene. Further characterization of GM-CSF receptor downstream signalling revealed 5- to 6-fold increased STAT5 phosphorylation by Western blot analysis in response to hGM-CSF (over control cells). In addition, administration of a chemical inducer of dimerization (AP20187) to activate the iCaspase9 suicide switch led to time- and concentration dependent apoptosis of Lv.EFS.CSF2RA.iCASP transduced cells.

Imposingly, conferring the vector to patient-derived CSF2RA-deficient CD34+ cells rescued hGM-CSF dependent colony formation and allowed for effective granulocyte and monocyte differentiation. Furthermore, healthy CD34+ samples transduced with the vector exhibited no aberrations in colony formation or in vitro differentiation towards macrophages analysed by surface marker expression of CD11b, CD68 and CD163.

Thus, we generated suitable vectors for a cell-based gene therapy approach for CSF2RA deficiency PAP, establishing functionality and safety in BaF3 as well as primary hematopoietic stem cells. Given its iCaspase9 safety switch in particular the CSF2RA.iCASP construct appears suitable for further evaluation towards first clinical approaches.

Rebirth – CARPUD (Poster 92- 93)

P92: Generation of Clara cells from murine pluripotent stem cells - a new tool to explore airway epithelial regeneration

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

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

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

P93: Congenital Pulmonary Alveolar Proteinosis iPS-derived hematopoietic progenitor cells (HPCs) reveal functional defects upon GM-CSF administration

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Hereditary Pulmonary Alveolar Proteinosis (herPAP), caused by a mutation in the GM-CSF receptor β-chain (Csf2rb), is an extremely rare lung disease resulting from the inability of alveolar macrophages to clear the alveolar spaces from surfactant phospholipids. Since current treatment options are extremely limited, we here investigate the suitability of a gene therapy approach based on hematopoietic cells derived from induced pluripotent stem cells (iPSC). Studies were performed in a murine model for Csf2rb-deficiency (Csf2rb-/-). Therefore iPSCs were generated from lin- bone marrow cells of Csf2rb-/- mice utilizing lentiviral overexpression of the standard Yamanaka-factors OSKM. Generated PAP-iPSCs displayed all major pluripotency criteria such as SSEA-1 expression, alkaline phosphatase activity, endogenous Sox2, Oct4, Klf4, Nanog reactivation, as well as three germ layers differentiation capacity assessed by teratoma formation.

Following an eight-day embryoid-body based differentiation protocol, the PAP-iPSCs gave rise to CD41+ hematopoietic progenitor cells (HPCs) that were capable to differentiate into granulocyte-, monocyte-, and erythrocyte-containing colonies comparable to HPCs derived from control iPSCs. However, upon differentiation with GM-CSF, PAP-iPSCs - in contrast to control iPSCs - were unable to form GM-type colonies, recapitulating the defect found in primary lin- bone marrow cells of Csf2rb-/- mice. Furthermore, the obtained HPCs form both control and PAP-iPSCs were differentiated into macrophage-like cells in the presence of M-CSF. iPSC-derived macrophages expressed CD45, CD11b and F4/80, exhibited typical chemokine secretion, and activated the transcription factor STAT5 in response to IL-3 and GM-CSF in a similar manner to bone marrow-derived macrophages.

In summary, we generated murine Csf2rb-deficient iPSC lines, which upon hematopoietic differentiation recapitulated GM-CSF dependent functional defects characteristic of PAP. These cells – upon genetic correction - appear as a promising source to test future cell and gene therapy strategies.

Rebirth – NIFE (Poster 94- 98)

P94: Investigation of the effect of different flow rates on the cell viability of fresh carotid arteries in vitro

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Introduction

Peripheral arterial disease leads to the damage of the blood vessels, currently replaced with vascular grafts, which are not able to regenerate in vivo. Tissue engineered vascular substitutes using decellularised equine carotid artery (ECA) seeded with the patient´s own cells and mechanically stimulated in vitro represent an attractive alternative. The aim of this study was to investigate the optimum hydrodynamic conditions for maintaining the viability of fresh ECA in vitro.

Methods

ECAs were isolated and disinfected in antibiotics prior to loading in a bioreactor. The optimum duration of the antibi-otic treatment was assessed through MTT assay and sterility test at 30min, 1hour and 2hours. 3D fluid-structure interaction (FSI) simulations, based on stress-strain data from ECAs, were conducted on LS-DYNA to determine the wall shear stresses (WSS) at two steady volume flow rates of 265ml/min (physiological) and 132.5ml/min. The samples were then cultured statically and dynamically at the above mentioned flow rates, for 1day, and assessed with MTT as-say, H&E stain and immunofluorescence using CD31.

Results

The optimum time to disinfect the ECAs without affecting their viability was 1hour. The WSS for the physiological and half-physiological flow rates used was 5.6 and 2.9Pa, respectively. These were rather larger than the physiological val-ues reported by experimental studies (2.5-5Pa). The samples conditioned for 1 day at both flow rates showed signifi-cantly higher levels of viability compared to the samples cultured statically and to the negative control (decellularised ECA). The histological and immunofluorescence analysis revealed the integrity of the ECM, for both the static and dy-namic samples, and the presence of endothelial cells.

Conclusion

The results suggested that dynamic conditioning improves cells viability. This study provided the basis for optimising the culture of the cell-seeded carotid arteries in vitro to generate peripheral arterial-like tissue.

P95: Biocompatible Medical Devices: Modification of Polymer Surfaces

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The treatment of patients with adult respiratory distress syndrome (ARDS) is challenging. To bridge the time until the transplantation of the deficient lung with a donor lung is possible, medical devices are needed. An oxygenator is a device allowing the gas exchange (O2, CO2) of the blood through a poly-4-methylpent-1-ene (PMP) membrane. One major problem is the foreign body response of the organism towards the membrane surface after blood contact. This can induce blood coagulation which leads to blocking of the surface and challenging the gas exchange.

To prevent these problems, we are modifying and humanizing the surface. One approach is to covalently attach RGD-peptides to the PMP surface promoting cellular adhesion. The RGD-modified blood oxygenator can be seeded with lung endothelia cells which stealth the surface from the blood stream.

In a first step the activation of surfaces is performed with open-air plasma. This technology enables the chemical derivatization of surfaces in increasing wetability or introducing reactive groups. Linked with a PEG spacer, it is possible to bind an oxanorbornadiene to the surface. With this system a ligation of azide-labelled RGD-peptides or other bioactive components is performed in a copper-free "Click"-reaction.

An alternative approach to bind specific proteins to the surface is followed using the high affinity of biotin to streptavidin. A biotinylated surface will allow the immobilization to a variety of Strep-tagged proteins.

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P96: Development and characterization of a decellularised xenogeneic mitral valve scaffold

Marisa Granados[1,*], *Lucrezia Morticelli*[1,3], *Pavel Yablonski*[1], *Andres Hilfiker*[1], *Igor Tudorache*[1], *Sergei Cebotari*[1], *Axel Haverich*[1,3], and *Sotirios Korossis*[1,3]

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Introduction

Mitral valve regurgitation is the second most common cause of surgery of the heart valves. Current treatment options are imperfect, requiring re-operations or lifelong anticoagulation therapy. The aim of this work was to develop and characterize a decellularised mitral valve scaffold for mitral valve replacement.

Methods

Mitral valves from 6 month old pigs were disinfected, placed in hypotonic buffer and treated with SDS and sodium de-oxycholate for 36 hours, followed by extensive washing and nucleic acid digestion. Radial sections comprising annulus, leaflets, chordae tendinae, and papillary muscle were analyzed histologically by H&E and DAPI, immunohistochemically by collagen IV, and by alpha-gal fluorescence staining. DNA was extracted from the annulus, anterior leaflet, and chordae, and quantified using a NanoDrop spectrophotometer. Sections of the treated leaflets were analyzed under transmission electron microscopy (TEM), whereas fresh and treated leaflet strips were subjected to uniaxial tensile loading to failure.

Results

Following decellularisation, no cell nuclei were observed under H&E or DAPI. There was also no change in the presence of collagen IV. The treatment resulted in a significant decrease of alpha-gal. DNA content was significantly reduced compared to the native tissue. TEM showed cell-free decellularised tissue, with a conserved histoarchitecture. The decellularised tissue demonstrated a grossly-maintained mechanical integrity.

Conclusion

A protocol that effectively removed cells and DNA, whilst maintaining the native valve histoarchitecture and mechanical integrity was developed. Although some alpha-gal was still detectable after decellularisation, the reduction observed was encouraging. The presence of alpha-gal could potentially be overcome in the clinical setting by the use of alpha-gal knockout porcine tissue. However, analyzing the effect of decellularisation on alpha-gal in wild-type porcine tissue could provide an insight on whether other sugars, also potentially immunogenic, are removed. Future work will focus on optimizing the protocol in order to further decrease the alpha-gal content.

P97: Guided Functional Re-Engineering of the Mitral Valve Leaflets

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Introduction

Mitral valve regurgitation represents the second major valvular disorder in the western world, whereas current strategies for mitral valve reconstruction are imperfect. The aim of this study was to develop a tissue engineered substitute for mitral valve leaflet reconstruction using acellular porcine pericardium seeded with porcine mesenchymal stem cells (pMSCs).

Methods

Porcine pericardial scaffolds were decellularised as described previously. pMSCs were cultured on the mesothelial sur-face of the scaffolds (3cm diameter) under static conditions, using 3 different cell densities (2×104, 1×105 and 2×105 cells/cm2). The seeded scaffolds were analysed by scanning electron microscopy (SEM), H & E and live/dead staining at 1, 3 and 7 days. Following 3 days of static culture, samples seeded with 1×105 cells/cm2 were cultured dynamically (10% strain) for 1 day in a biaxial strain bioreactor. Following dynamic conditioning, samples were assessed for cell viability with live/dead staining and MTT assay, and for extracellular matrix (ECM) integrity with H&E.

Results

The optimum seeding density for acellular pericardial samples was 105 cells/cm2. Samples seeded with this density and maintained statically for 3 days, prior to dynamic conditioning, showed the best cell penetration without a significant disruption in the ECM. Seeded samples conditioned dynamically for 1 day showed similar levels of viable cells to seeded samples cultured statically for 1 day. Cell alignment was also obvious in the dynamically conditioned samples.

Conclusion

Acellular pericardium was shown to be an optimum material for cell repopulation. Reseeded scaffolds were viable after 1 day under 10% dynamic strain. This study provided the basis for optimising the mechano-stimulation of cell-seeded pericardial scaffolds in vitro in order to generate heart-valve like tissue.

P98: Patient-specific computational modelling of the mitral valve

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Current approaches to valve repair/replacement are based to one-size-fits-all. As such there is a big drive towards more personalised surgical interventions. The aim of this project is to develop a novel prognostic/forecasting computational simulation tool that will provide patient-specific pre-operative optimisation of mitral valve (MV) replacement and repair. MicroCT images of porcine MV were segmented in a commercial software (Simpleware) and the whole MV apparatus was reconstructed. In the preliminary computational models, the leaflets were simplified as membranes and the chordae as tension strings. Anterior and posterior leaflet samples, together with samples from the two main chordae tendineae types (strut & commissural) were tested under uniaxial tension to obtain the regional biomechanics of the MV. The stress-strain data obtained was imported in the computational model to specify the regional material properties of the MV. The model was imported into the commercial software LS-DYNA, where a pressure driven (max 120 mmHg) MV closure was simulated for one cardiac cycle. The MV apparatus demonstrated significant regional and directional mechanical anisotropy. The anterior leaflet demonstrated significant directional anisotropy, whereas the posterior showed a rather isotropic behaviour. Significant differences in the mechanical properties were also found between the different types of chordae tested. The computational simulations predicted regions of both leaflets with elevated stress concentration during the cardiac cycle, in accord with failure regions observed clinically. Moreover, the simulations indicated variable loading of the different chordae during the cardiac cycle. This study has indicated that different components of the MV experience different levels of stress and strain, which has an implication in the selection of appropriate repair materials for MV reconstruction. Future work will focus on developing blood-structure interaction models and incorporating the left ventricle, aortic valve and aortic arch anatomy from clinical scans.

Rebirth – Enabling Technologies (Poster 99- 104)

P99: Combined ultra highfield MRI and SPECT-CT are promising tools in interdisciplinary research on animal models of human lung diseases

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Animal models of lung disease have provided extraordinary information to understand human disease. They are powerful tools that enable the study of the mechanisms and natural history of such diseases. Nevertheless anatomic and immunologic differences between mice and humans mean that those models have limitations that must be considered when interpreting the results. Magnetic resonance imaging (MRI) is a promising tool especially for serial studies, because of the absence of ionizing radiation. The detailed representation of interstitial structures remains the strength of CT while SPECT-CT adds the functional and physiological aspects into a molecular imaging setup.

The ongoing development of computed tomography, SPECT and magnetic resonance imaging has markedly improved the imaging of lung diseases. With the optimization of the technology new demands on the interdisciplinary collaboration come up.
The well-known technical problems of lung MRI (low spatial resolution, motion artifacts, low signal-to-noise ratio of the lung parenchyma) have been reduced by recent technical advances.

Concerning chronic infiltrations CT scanning remains the superior imaging modality due to the inferior spatial resolution of MRI.

There are ongoing research projects involving the applied technology of MRI, biophysical measurements using MR e.g., combined T1 and T2 mapping using efficient imaging sequences, using radial scans with ultra short TE enabling the visualization of detailed lung structures without using expensive hyperpolarized helium techniques and the use of MR to longitudinally monitor tissue parameters.

We are on the move in developing and using in-vivo ultra-high field MRI techniques in combination with SPECT-CT to study aspects of lung diseases and lung transplantation. The increased sensitivity and enhanced contrast mechanisms at these high magnetic field strengths in multimodal applications might provide insight into yet unsolved problems.

P100: Generation of CF-patient derived iPS cells and efficient footprintless designer nuclease-based gene targeting

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The ability to genetically modify human induced pluripotent stem cells (hiPSCs), including the correction of gene defects by means of homologous recombination (HR) is of great interest regarding their potential for ex vivo gene therapy, especially in terms of rare pulmonary diseases like Cystic Fibrosis (CF). Genetic engineering of hiPSCs via customized designer nucleases has been shown to be significantly more efficient than conventional gene targeting, but still typically depends on the introduction of additional genetic selection elements.

For the generation of CF patient-specific iPS cells, endothelial cells from the peripheral blood or fibroblasts from skin biopsies of CF-patients were isolated and reprogrammed through lentiviral overexpression of pluripotency factors. The CF-iPS cells, homozygous for F508del mutation, morphologically resemble human embryonic stem cells, express pluripotency markers and could be differentiated in vitro into derivatives of all three germ layers. For gene targeting approaches we developed a protocol for the establishment of efficient non-viral and selection-independent gene targeting in hESCs and hiPSCs. The protocol was applied to target the endogenous safe harbour locus AAVS1. Here, by using ZFNs and TALENs, targeting efficiencies of up to 1.6% were demonstrated for one hESC and two hiPSC lines, and stable transgenic PSC lines were generated by FACSorting. The high targeting efficiencies obtained allowed for direct PCR screening of correctly targeted clones by applying TALENs together with short single stranded oligonucleotide donors without any pre-selection (Merkert et al.). Targeting the underlying genetic defect in our CF iPSCs revealed targeted integration of the missing base pairs, as demonstrated in cell pools. The establishment of single cell clones is currently ongoing.

The established targeting protocol will enable footprint less gene correction and transgene-independent isolation of mutation-corrected CF-iPSC clones which will facilitate disease modelling, drug screening and, ultimately, the generation of clinically useful transgenic iPSC derivatives.

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Merkert, S., Wunderlich, S., Bednarski, C., Beier, J., Haase, A., Dreyer, A.-K., Schwanke, K., Meyer, J., Göhring, G., Cathomen, T., and Martin, U. (2014). Efficient designer nuclease-based homologous recombination enables direct PCR screening for footprint less targeted human pluripotent stem cell clones. Stem Cell Reports 2, 107-118.

P101: Hydrogels for Two-Photon Polymerization and Vascular Tissue Engineering

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Hydrogels are able to mimic the basic three-dimensional (3D) biological, chemical, and mechanical properties of native tissues. Hyaluronic acid (HA) is one of the components of human extracellular matrix (ECM) and represents an extremely attractive starting material for the fabrication of scaffolds for tissue engineering. Due to poor mechanical properties of hydrogels, structure fabrication in this material class remains a major challenge. Two-photon polymerization (2PP) is a promising technique for biomedical applications, which allows the fabrication of complex 3D microstructures by moving laser focus in the volume of a photosensitive material. A chemical modification of hyaluronan allows application of the 2PP technique to this natural material and, thus, precise fabrication of 3D hydrogel constructs. To create materials with tailor-made mechano-chemical properties, HA was combined and covalently cross-linked with poly(ethylene glycol) diacrylate (PEGDA) in situ. 2PP was applied for the fabrication of well elaborated 3D HA and HA–PEGDA microstructures. For enhanced biological adaption, HA was functionalized with a human epidermal growth factor.

Synthetically modified fibrin hydrogel was applied for culturing of vasculogenic cells under perfused conditions. Fibrinogen was covalently linked with PEG-NHS in order to improve its degradability resistance and physico-optical properties. We have studied the influence of degree of fibrinogen PEGylation, as well as, the concentration of enzyme thrombin, used for hydrogel preparations, on cellular responses. After evaluation of optimal gel composition and PEGylation ratio, the hydrogel was applied for investigations of vascular tube formation within a perfusable microfluidic device. The morphological development of this co-culture within perfused hydrogel was monitored over 12 days, demonstrating the creation of interconnected HUVECs-ASCs network. Analysis of co-localization of HUVECs with ASCs indicated that ASCs were localized in the vicinity of HUVEC tubes.

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P102: Laser-generated nanomaterials for biomedical applications on cellular level

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To address biomedical research on the cellular level, nano-scaled tools feature optimal properties to transport effective cargo molecules, to release antibacterial ions and drugs, to act as proliferative platform and to visualize target structures at the area of interest. In general, nano-scaled tools are based on spherical nanoparticles from materials such as gold, silver or iron, which are equipped with biological functions for specific action or which can be embedded into a polymer composite to form

bioactive implants. However, the chemical fabrication of nanoparticles often limits their biocompatibility by the adoption of critical precursors or the creation of distinct reaction by-products.

In this context, the technology of pulsed laser ablation in liquids represents an establishing alternative for the fabrication of ultrapure mono- and multimaterial nanoparticles within seconds. The method is profiting from the adoption of high purity targets and solvents in combination with laser light exclusively, therefore delivering colloids with maximal material purity. Nanoparticle functionalization with biomolecules may further be accomplished in a single-step, in situ process during laser fabrication, resulting in highly biocompatible and functional nanobiohybrids with remarkable surface loadings.

Various biomedical applications of such nanobiohybrids will be presented, covering (I) the crossing of advanced biological membranes as nanocarriers, (II) the precise immunolabeling of cellular structures as nanomarkers, (III) the magnetic cell separation by nanomagnets, (IV) their applications in solid-phase-assays as highly specific and sensitive nanosensors for allergens and (V) their ability to scavenge reactive oxygen species (ROS) as nanocatalysts. Further, the antibacterial and proliferative properties of polymer nanocomposites and the suitability of ceramic nanocomposites as bone-replacement materials will be showcased.

P102a: Biological Laser Printing – 3D patterning cells and biomaterials

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A fundamental understanding of interactions between different cells and their environment is essential for cell-based therapies, like stem-cell-therapies in regenerative medicine. Common ex vivo cell studies in two-dimensional cell cultures have significant limitations and are not appropriate to simulate the complex interactions in 3D tissue and cell–microenvironments in vivo. It has become obvious that cell behavior differs dramatically in 3D. Thus, printed 3D cell patterns could bridge the gap between common cell culture conditions in vitro and animal models. Innovative 3D cell models could provide new insights in understanding of cell behavior, tissue functions and regeneration, and for analyzing the effect of agents like pharmaceuticals or cosmetics ex vivo.
Biological laser printing is a promising technique to arrange different biological components in a well-defined 3D pattern. Cells and biomolecules are embedded in a hydrogel as a supporting structure for scaffold-free generation of 3D cell models or tissue. It has been proven previously that different cell types survive the printing and that they are not affected in their behavior by the laser printing process; the phenotype and differentiation potential of stem cells is not influenced.
Different applications of biological laser printing for studying cell behavior and for scaffold-free tissue engineering will be presented, a mono-cellular 3D stem cell graft, a multicellular 3D skin equivalent, and a defined 3D spot array for microscopically observation of cell-cell and cell-environment interactions.

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P103: Perfusion feeding improves suspension culture of human pluripotent stem cells in controlled, stirred bioreactors

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

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P104: A novel mouse model for the investigation of adaptive immune responses upon de novo antigen expression in lung epithelial cells

Mathias Riehn[1,*], *Marcin Cebula*[1], *Aaron Ochel*[1], *Pratibha Gaur*[1], *Hansjörg Hauser*[1], and *Dagmar Wirth*[1]

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Upon infection, viral antigen presentation is accompanied with activation of innate immunity which shapes the adaptive immune response and in particular the T cell response. We aimed at developing a mouse model to induce immune mediated lung damage to better understand the mechanisms involved in lung regeneration.

For this purpose, we mated SPCCre mice in which the CreERT2 recombinase is expressed in lung alveolar type II epithelial cells (Rock et al., 2011) to RosaOva transgenic mice (Sandhu et al., 2011). In the resulting double transgenic mice Tamoxifen induced Cre recombination results in de novo OVA expression in about 50% cells. We employed a reporter mouse model SPCCre x ROSALuc to monitor the time course of activation of the Cre recombinase specifically in vivo in lung. To evaluate the T cell response upon antigen expression, we generated SPCCre x ROSA Ova x OT-I mice. In this model, the intracellular neo-antigen Ova is induced in alveolar type II cells of the lung in presence of Ova antigen specific CD8 T cells (OT-I). Upon Tamoxifen mediated induction of antigen we observed massive infiltration of lymphocytes as documented by histological analysis. Interestingly, this T cell infiltration was transient, suggesting OT-I cell mediated destruction of alveolar type II cells followed by regeneration of lung tissue. These findings indicate the potential of the model to investigate T-cell response, antigen clearance and reconstitution of respiratory tissue independently of the innate immune induction in the full-fledged adaptive immune system.

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REBIRTH Others (Poster 105- 112)

P105: Core/Shell electrospun fibers as biodegradable scaffolds for sustained drug delivery in Wound Healing applications

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Aim.

During the process of wound healing it is really important to keep the area safe from bacteria infections and treat possible inflammation incidents. Model antiseptic and anti-inflammatory agents can be encapsulated into a drug delivery carrier for combined treatment. Electrospinning (E-Spin) has been acknowledged as a versatile technique for the production of biodegradable fibrous scaffolds to encapsulate therapeutics for wound healing.

Methods.

Both single jet and coaxial jet E-Spin were used as a method to produce fibers. Polycaprolactone (PCL), Benzoin (BZ) and Acetyl Salicylic Acid (ASA) were dissolved in 99,8Vol.% 2,2,2-Trifluoroethanol (TFE) for the single jet E-Spin. For the coaxial jet E-Spin the same solution was used for the core while a solution of Poly-lactic acid (PLA) or PCL in the same solvent was used as the sheath solution. Morphology of the fibrous scaffolds as well as fiber diameter and pore size were examined by Scanning Electron Microscopy (SEM). After incubation in PBS and acetate buffer at 37 oC inside a water bath the absorbance was measured using a UV-Vis spectrophotometer to evaluate the cumulative release of the drugs and the release mechanism.

Results.

The coaxial approach resulted in fibers with an average diameter of 1.83 & 0.68 μm and an average pore size of 16.11 & 9.09 μm2 for BZ and ASA respectively. Furthermore, the amount of drug released in the first 8 hours was reduced from 65.1% to 11.65% for BZ and from 58.32% to 33.14% for ASA while the encapsulation efficiency increased from 87.5% to 97.1% for BZ and from 34.36% to 86.13% for ASA, in contrast to single jet electrospun fibers, following a Fickian diffusion in all cases.

Conclusions.

Biodegradable scaffolds from PCL core-shell fibers can be considered as promising drug delivery carriers for sustained release of antiseptic and anti-inflammatory agents in wound healing applications.

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P106: Chitosan/Polycaprolactone electrospun biodegradable scaffolds for Cardiovascular Tissue Engineering

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Introduction.

Polycaprolactone (PCL) and chitosan (CS) are polymers with attractive properties (excellent biocompatibility & degradation). However their mechanical properties seperately do not satisfy the needs for cardiovascular tissue engineering. In the present work we aimed to optimize electrospinning parameters to obtain a flexible PCL/CS polymeric scaffold, with combined nano- and micro-fiber architecture and appropriate mechanical properties for cardiovascular tissue engineering in regenerative medicine.

Methods.

PCL and CS were dissolved in acetic acid (AAC) and 2,2,2-trifluroethanol (TFE) using different concentrations. Electrospinning was performed at a custom made apparatus, at room temperature. Morphology of the fibrous membranes were examined by SEM. Cyclic sinusoidal uniaxial mechanical tests were performed with an electroforce dynamic tensile testing system. Rectangular, 15x10 mm strips were cut and tested at 0-30% strain, 1 cycle/sec, RT, dry conditions. The applied force and the local principal strain were monitored and stress/strain data was computed. Mechanical properties like Young's modulus (the elastic modulus at linear portion of stress/strain curve) was evaluated. For biophysical characterization FTIR spectroscopy and contact-angle studies were performed.

Results.

SEM observations showed a micro fibrous (2 μm) structure in PCL scaffolds and a combined nano/micro- (0.25-2 μm) arrangement in PCL/CS blend scaffolds. Young's modulus showed a significant drop from 25 MPa (PCL) to 5-6 MPa for AAC and 12 MPa for TFE with increasing CS concentration, (0-30% total strain). The hydrophillicity of the scaffolds was significantly higher with the addition of CS.

Discussion.

From preliminary results it seems that the concentration of CS plays an important role in structural appearance of electrospun PCL fibers. A combination of small porous nanofibers with a greater porous microfibers arrangement was obtained in polymer blends, suitable for potential cell seeding. Elastic modulus of polymer blends, especially in higher CS concentration, was close to properties measured in soft cardiovascular tissues.

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Dimosthenis Mavrilas et al. Dynamic mechanical characteristics of intact and structurally modified bovine pericardial tissues. Journal of Biomechanics, Volume 2005;38:761-768

P107: Young versus aged cell sources – genetic aberrations in ips-cells and the effect of primary cell proliferation on reprogramming efficiency

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The quality of iPSCs derived from somatic cells of aged individuals may be critical for the production of clinically useful cell products. Indeed, the proliferation of primary cells from aged individuals is typically lower than in juvenile cells. Additionally, genomic and mitochondrial mutations appear to accumulate over time and have been suggested to contribute to aging and cancer formation.

Therefore, our aim is to compare reprogramming efficiencies and frequency of mutations in iPSCs from old versus young sources. To possibly exclude cell-type-specific effects, we focused on endothelial cells (ECs) derived from different sources including cord blood and umbilical cord, as well as peripheral blood and saphena veins of elderly donors.

Population doublings were determined in early passage ECs. Microarray analysis of primary cells was performed. Cells were reprogrammed with lentiviral vectors expressing Thomson or Yamanaka factors. Three methods for detection of genetic aberrations were applied: karyotyping, aCGH and exom sequencing. Detected SNPs will be confirmed by PCR.

ECs from blood of adult donors could be isolated in a very small portion of samples. Higher proliferation and reprogramming rates in cells from younger sources were observed. In contrast, reprogramming of cells from aged sources with both lentiviral constructs was 10-100 fold less efficient. Many genes related to cell cycle and genome repair were upregulated in cells with high reprogramming efficiency. Karyotyping revealed chromosomal aberrations in considerable portion of iPSC from elderly patients, whereas iPSC clones from young sources had normal karyotype. In contrast, preliminary results from aCGH didn't demonstrate any significant differences between groups. Bioinformatic analysis of exom sequencing data is ongoing.

Our preliminary data indicate that young cell sources are easier to reprogram and iPSCs derived from juvenile cells may be of superior quality compared to iPSCs from elderly patients. These features make them promising candidates for future therapeutic applications.

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P108: REBIRTH-active – an exercise intervention to improve workability and endogenous regeneration

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The REBIRTH-active study group is an initiative of the REBIRTH Cluster of Excellence and comprises scientists from several departments including: Cardiothoracic, Transplantation and Vascular Surgery, Cardiology and Angiology, Paediatrics, Dental Prosthetics, Sports Medicine, Gynaecology, Clinical Pharmacology, Biometrics, Hannover Medical School's Human Resources, REBIRTH Business management. The major aim of REBIRTH-active is the improvement of daily activity and physical exercise to increase endogenous cellular regeneration, work ability and exercise capacity.

In two prospective REBIRTH-active studies, effects of physical training on cellular ageing, exercise capacity, dental, cardiac and psychosocial health as well as on work-ability will be investigated.

In the first REBIRTH-active study, 67 inactive middle-aged employees of MHH underwent a six-month exercise programme involving 30 minutes of physical exertion per day. Exercise training was individually organized, and the employees achieved an average of 190 minutes per week.

Peak oxygen consumption rose by more than 20% after six months, corresponding to normal values in men 15 years younger. In addition, telomere length in peripheral blood mononuclear cells as a marker of cellular regeneration increased. Psychosocial indicators of burnout were reduced. The work ability index significantly improved, and the number of sick days taken by the active employees was reduced by 40%. Cardiovascular and echocardiographic parameters were enhanced as well. Dental health was also assessed before and after the trial period showing that severe periodontitis may reduce the benefits of long-term exercise training.

In the ongoing second REBIRTH-active study, the effects of a comparable worksite intervention on work ability, cellular regeneration, oxidative stress, cardiovascular function and dental health will be investigated in 300 women. The effect of the hormonal status with regard to the pre- and postmenopausal stage will be evaluated as well.

P109: LINE1-mediated Retrotransposition and its Consequences for the Genomic Stability of Human Pluripotent Stem Cells

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Human pluripotent stem cells (hPSCs) are considered as favourite cell source for regenerative medicine. Recent findings indicate that potentially tumorigenic chromosomal abnormalities and mutations arise in hPSCs during their generation, expansion and differentiation. Such mutations could be induced by human non-LTR retrotransposons (LINE1, Alu, SVA). It has been reported that the reprogramming process towards hiPSCs might enhance the activation of LINE1s and that similar levels of active LINE1s were found in hESCs. Aim of this study is to investigate whether LINE1 mobilization may also affect the genomic integrity of hPSCs and their differentiated derivatives. Using a novel retrotransposition reporter assay, optimized for the use in hPSCs and for stable integration into the human AAVS1 safe harbour locus, we will assess whether LINE1 activity may cause genetic aberrations in hPSCs. In this regard, LINE1 expression levels are assessed using immunoblottings, immunofluorescence stainings and qRT-PCR. LINE1 mediated genomic destabilization and preferential integration sites will be analysed by Array-CGH and high-throughput sequencing. By now, we were able to stably integrate hPSC-specific LINE1 reporter vectors into the AAVS1 locus with an efficiency of approximately 1,25%. Additionally, we successfully proved reporter expression and retrotransposition of the LINE1 reporter transgenes in hiPSCs using qPCR as well as via expression of retrotransposition dependent G418 resistance. Moreover, we detected a high number of LINE1 de novo insertions in hPSCs which surprisingly did not increase during long term cultivation. Also, no karyotypic abnormalities were found during approximately 30 passages after LINE1 reporter integration. Experiments are in process to evaluate retrotransposition rates and integration preferences in hPSCs during long term culture. Further ongoing analyses will demonstrate whether LINE1 retrotransposition may induce any genetic aberrations and if not, which cellular mechanism are responsible for curtailing LINE1 mobilization.

P110: Molecular characterization of hiPSCs from a CDG-Ia patient revealed glycosylation and response to mannose supplementation indistinguishable from hESCs

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PMM2-CDG, former known as Congenital Disorder of Glycosylation-Ia, is the most abundant form of CDG-diseases mainly affecting N-glycan synthesis [1]. N-glycosylation is crucial for protein folding, stability and localization. Sitting at the cell surface, N-glycans form key structures for inter- and intracellular signaling. A total loss of N-glycans is lethal [2]. Clinical symptoms of PMM2-CDG are broad and involve many organs and currently no treatment options exist and only little is known about the relationship of the defective PMM2 enzyme and the disease phenotype [2].

In order to better understand the impact of a reduced PMM2 activity on early human development, human induced pluripotent stem cells (hiPSCs) from patient-specific PMM2-CDG-fibroblasts were generated by lentiviral gene transfer of the four transcription factors OCT4, SOX2, KLF4 and c-Myc (OSKM).

These cells were positively tested for genomic integrity, expression of classical pluripotency associated factors and differentiation capacity. Deep-sequencing revealed that the transcriptome of PMM2-CDG-iPSCs is comparable to human embryonic stem cells (hESCs) and a control iPS cell line. N-glycomic analyses by capillary gel electrophoresis with laser-induced fluorescence detection (CE-LIF) showed a comparable N-glycan repertoire in PMM2-CDG-iPSCs compared to "healthy" hiPSCs and hESCs with predominantly high-mannose type N-glycans. By GNA-lectin staining, detecting mannosylated structures, it was demonstrated that PMM2-CDG-iPSCs exhibit comparable glycosylation intensity as control cell lines. Of note, mannosylation could be increased not only in control cell lines but also in PMM2-CDG-iPSCs by supplementation of 10 mM mannose to culture media. This observation underlines the recent finding that dietary mannose supplementation in mice during pregnancy is of therapeutic importance for CDG-Ia phenotypic outcome in offspring.

The PMM2-CDG-iPSC model has the potential to generate a platform to understand the role of glycosylation on the stem cell level and during early human development.

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P111: Immunohistochemical characterization of teratomas induced by pluripotent stem cells

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The pluripotency of stem cells is proven by the formation of a teratoma, growing after injection of putative pluripotent cells in mice with a dysfunctional immune system (teratoma assay by Wesselschmidt). After reaching a sufficient size the tumor is removed and subjected to histopathological analysis. Although this assay has never been standardized it is used regularly in stem cell research.

Teratomas are multi-layered tumors that contain cells derived from all three embryonic germ layers, namely entoderm, mesoderm and ectoderm. If the morphology of the teratoma is premature, the diagnosis of the germ layers has to be assisted by immunohistochemisty.

Aim of the project is to establish a novel immunohistochemical antibody panel, that can be used in diagnosis of immature teratomas and to give a statement about the possibilities of standardization in terms of number of cells implanted, graft site and maturation time.

Induced pluripotent stem cells of mouse and primate origin were subjected to the teratoma assay by subcutaneous implantation. The mice were sacrificed at different time points and underwent histopathological analysis for determination of the stage of differentiation and tumor size. Slides were stained by hematoxylin and eosin as well as by antibodies against differentiated tissues. Additionally several markers of undifferentiated cells were established for the immunohistochemical stainings, i.e. alpha-fetoprotein as it is only expressed in the fetal liver and in the yolk sac, brachyury, an early mesoderm marker, and myo-D1 as a marker for immature muscle cells.

Using an immunhistological marker panel will likely help to standardize teratoma assays. If the markers enable to prove presence of all three embryonic germ layers even in morphologically undifferentiated teratomas, duration of teratoma assays can be reduced, contributing considerably to the 3R concept of animal welfare.

P112: Generation of induced pluripotent stem cell lines for establishment of preclinical large animal models

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The prerequisite of transferring in vitro developed regenerative therapies based on pluripotent stem cells (PSC) into clinics is the establishment of large animal models to analyze cell fate and behavior in vivo. For cardiac replacement studies sheep as well as pig models are existing and well-established for preclinical testing. Induced pluripotent stem cells may represent an ideal cell source for future regenerative therapies. In the last years the generation of ovine (oviPSC) and pig induced pluripotent stem cells (piPSC) has been reported.

Using HIV-derived reprogramming vectors encoding for human OCT4, SOX2, NANOG, KLF4, and C-MYC, we were now able to generate oviPS-like cell clones from endothelia cells. Resulting oviPS-like cells show typical characteristics of pluripotent stem cells and could be maintained under standard human embryonic culture conditions. OviPS-like cells stain positively for pluripotency markers such as OCT4, SOX2 and NANOG and differentiate in vitro into derivatives of all three germlayers. However, characterisation of the OviPS-like cells revealed dependency of transgenic reprogramming factors as demonstrated by high expression of all exogenous factors and relatively low expression of endogenous factors, which was similar to the recently generated porcine iPSC-like cells.

Interestingly, our data indicate that despite persisting transgene expression, the generated oviPS-like cells can not only be cultured for a prolonged time, but differentiate into various cell types of the three germ layers. Nevertheless, persistent dependency on the transgenic reprogramming factors representing a common phenomenon in "reprogrammed" cells of pig and sheep indicate incomplete reprogramming. More research is clearly required to obtain further insight into basic requirements for pluripotency in both species and for the development of more suitable culture and reprogramming techniques with the final aim to obtain true pluripotent stem cells form pig and sheep.

Industry Input (Poster 113- 116)

P113: Options to determine the human disease link of drug targets

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In the drug making process it is a challenge to set up appropriate cellular systems and animal models which provide a clear link to human disease and can be used for evaluation of drug potency and efficacy.

To compare gene signature in specific animal models with cells from normal and disease tissue various experiments were performed. To understand the relevance of distinct signaling pathways in lung fibrosis, gene expression profiling can be used. Data generated from samples of rodent bleomycin models, normal human lung fibroblasts (NHLFs) and human fibrotic lung biopsies will be discussed. All samples showed deregulation of fibrosis related pathways. In contrast to the human fibrotic tissues inflammation related pathways were upregulated in the rodent bleomycin treated lung tissue samples.

Furthermore, the efficacy of a steroid was tested in an organotypic cell culture model. The precision cut lung slice technology was set up to establish a culture system reflecting the three-dimensional tissue architecture of the lung using primary cells. Here, slices of rat lungs were challenged with the pro-inflammatory stimulus LPS *in vitro*. Gene signatures were determined 24 hrs after the challenge and revealed an upregulation of various inflammation relevant genes, e.g. IL1b, TNF□, IL6 and KC. These cytokines also showed an increased release and changed tissue content upon LPS challenge as assessed by ELISA. In this setting, the cytokine expression and release was strongly reduced by treatment with the steroid dexamethasone. These data obtained in the organotypic setting reflect the broad anti-inflammatory efficacy of a steroid.

P114: Advances in RNA-Seq Analysis for Target Research

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RNA-Seq is an approach to transcriptome profiling that uses deep-sequencing technologies, whereby millions of RNA fragments are sequenced and analyzed to discover and estimate the relative molecular concentration of individual transcripts. It has over the last 5 years rapidly become a trusted technique to measure gene expression in both human samples and animal models. RNA-Seq provides a relatively unbiased and absolute measurement of the levels of transcripts and their isoforms compared to other methods like DNA microarrays. Furthermore its relevance to target research is increasing with widespread use and growing clinical relevance of RNA-seq measurements.

The pace of RNA-seq data generation both in-house and from the public databases is increasing, thus computational analysis pipelines must be upgraded to keep up. We apply the latest RNA-seq tools to achieve gains in processing speed, storage savings, and measurements of transcriptome features. The

performance gains allow us to regularly update the in-house gene expression database with the latest genome annotations to facilitate future target discovery. Alongside these improvements, we have also expanded on the quality metrics used to assess the success of an RNA-seq experiment. The new pipeline has been applied to projects requiring target transcript validation for the characterization of new animal models and in differential gene expression analysis of treatments versus controls.

P115: A HCA-based assay to quantify fibroblast-to-myofibroblast transition

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Fibrotic diseases are largely incurable, irreversible, and contribute to significant mortality. Characteristics of fibrosis are a net accumulation of extracellular matrix (ECM) proteins and the disruption of normal tissue architecture. Fibroblast-to-Myofibroblast transition (FMT) is thought to be a key pathological process in Idiopathic Pulmonary Fibrosis (IPF). Myofibroblasts are contractile cells expressing a-smooth muscle actin, and contribute to excessive deposition of ECM proteins, e.g. fibronectin, collagen and laminin.

The development of high-throughput assay for FMT screens are important to support drug discovery, target identification and pathway expansion projects. High Content Analysis (HCA) represents a straight forward technology for automated, multiplexed image acquisition and analysis, allowing the quantitative evaluation of cellular and morphological parameters down to the single-cell level. In the present study, we describe the development of a HCA assay, in which normal human primary fibroblasts (NHLF) are induced to differentiate into myofibroblasts by TGFβ1 stimulation. The degree of myofibroblast differentiation is determined by quantitative analysis of newly synthesized α-smooth muscle actin (αSMA) assembled into actin fibers. The characterization of a HCA FMT is described.

P116: Recombinant AAV vectors as a tool to study New Drug Concepts in rodent animal models

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Adeno-associated virus (AAV) is a parvovirus, consisting of only capsid protein and a single-stranded DNA genome. Recombinant AAV (rAAV) is created by replacing the rep and cap gene with an expression cassette and is widely used for gene delivery in animal models for the following reasons: It is non-pathogenic in humans or animals, shows a very low immunogenicity, is unable to replicate and leads to episomal long-term expression in non-dividing or slowly dividing cells.

Numerous AAV capsid sequences were isolated from human and nonhuman primate tissues, cloned to generate recombinant AAV vectors and characterized in multiple tissues in vivo. In the past production and purification protocols for the major serotypes and variants thereof, namely AAV1, 2, 5, 6, 6.2, 8 and 9, were established in our group. Furthermore, tissue specificities were optimized through a combination of the selected AAV serotype, the route of administration and the use of tissue-specific promoters.

The potential applications of AAV/mediated gene expression include:

1. Target characterization by overexpression or down-regulation of a target gene to mimic pharmacological activation or inhibition of a potential target
2. Development of disease related animal models for compound testing, e.g. induction of disease related phenotype by overexpression of cytokines
3. Generation of "humanized" animals by overexpressing the human gene in order to test compounds lacking cross-reactivity

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