Editorial

Lungenerkrankungen gehören zu den wesentlichen Erkrankungen weltweit, die WHO erwartet, dass sie in absehbarer Zeit hinsichtlich Morbidität und Letalität die Spitzenposition übernehmen.


Führen chronische Erkrankungen zu einer respiratorischen Insuffizienz bleibt die Lungentransplantation die einzige Option, um das Leben zu verlängern und eine bessere Lebensqualität zu schaffen. Allerdings stehen knapp 400 Lungentransplantationen in Deutschland pro Jahr eine halbe Million „End Stage“ Patienten gegenüber, ein krasses Missverhältnis.


Die nachfolgenden Abstracts geben einen Einblick in den aktuellen Stand der wissenschaftlichen Entwicklung in dieses hochaktuelle Forschungsgebiet.
Disease Area: Asthma & Allergies

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

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-12. 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 reduced 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 species. IL-13 induced airway hyperreactivity in all tested species with a similar methacholine sensitivity. In future studies, PCLS will be used for pre-clinical studies to evaluate the antagonist efficacy.

2 Deregulation of ORMDL3 expression induces stress responses and modulates repair pathways

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 over-expression 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 or Notch signaling 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.

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

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 of the patients were allergic against HDM were analyzed. The allergen Der p 1 and 2 were; 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.

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

“equal contribution: 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 deficiency in μMT mice was analyzed. Allergen Der p 1 and 2 MT mice develop a similar allergic phenotype compared to WT mice and show increased BALF levels in serum and BALF even in IgE absence.
**Introduction:** Recent studies suggest that helper 17 (Th17) cells are important players in the progression of asthma towards a severe phenotype. Characterized by the production of pro-inflammatory cytokines like TNF-α, IL-18, 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 (RORγt), 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 vivo but also of proinflammatory cytokines like IL-17A and IL-17F. 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

**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 angptl4 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 (Prostynat), 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 (U0126), irreversible and selective PPARy antagonist (GW9662), or genistein, a ligand for PPARy, GW9662 did not alter the ability of A1AT to induce ERK1/2 phosphorylation, suggesting that PPARy 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.

**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-inflammation 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, A1AT peptide synthesis, gene expression (RT-PCR), western blots, ELISA, cell adhesion, phagocytosis and viability assays, spectrophotometry, flow cytometry. Results: We have selected systemic 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 A1AT are excellent candidates for the drug development. **Research Support:** Hannover Medical School, German Center for Lung Research (DZL) **References:** Janciauskiene S and Welte T 2013 Cardiovascular & Hematological Disorders-Drug Targets

**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. Surface protein D (SP-D), and its pro-fibrotic immunomodulatory 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, histology 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 wild-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 Pneumologie 2014; 68: 1–25 Georg Thieme Verlag KG Stuttgart · New York
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.

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 [2,3]. 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 COPD 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.


Alveolar epithelial cell types II show a high sensitivity to cigarette smoke extract

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Results: 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.

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, 82 are interested. Physicians engaged in specific health care research projects will recruit target-patients. Over 26,000 patient IDs and the corresponding 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 database for standardized assessments of disease-specific costs, quality of life and for objective outcome evaluation.

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- (“Beobachtungspraxis-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, 82 are interested. Physicians engaged in specific health care research projects will recruit target-patients. Over 26,000 patient IDs and the corresponding 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 database for standardized assessments of disease-specific costs, quality of life and for objective outcome evaluation.
In vitro ciliary development in an epithelial stem 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 M3E3/C3, 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 of ALI and was detectable a few days before the start of the ciliogenesis. The ciliated cells were also detectable by immunoreaction to α-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, instead of the living MC, even without the co-presence of MC, but the morphology of the cilia appears to develop normally. Fibrillar collagen (I) and Matrigel, instead of the living MC, can grow when the ALI conditions are met. These results strongly suggest 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 qPCR. 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 quantitative analysis of lung tissue inflammation. Staining for iNOS activity at the single cell level applied to multiple protease types 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 probe reporters NEMO and LeRoe, the detection of μM-12 activity in FACS was possible. We applied this approach to a double blinded sample set from the Fraunhofer ITEM Hannover (BREATHE). 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.

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 and incompletely understood. We hypothesize that in a chronic CS-induced mouse model the pathogenesis of COPD is augmented by an elevated immune response in aged mice. 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 modified 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 5

Evaluation of small molecule FRET reporter for the diagnosis and monitoring of proteolytic activity in a 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 to 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 probe reporters NEMO and LeRoe, the detection of μM-12 activity is now possible. We tested the new approach on a double blinded sample set from the Fraunhofer ITEM Hannover (BREATHE). 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

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 5

Korrekturexemplar: Veröffentlichung (auch online), Vervielfältigung oder Weitergabe nicht erlaubt!
Silencing of mir-148b ameliorates cystic fibrosis-like lung diseases in ßENaC-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 ßENaC-overexpressing (ßENaC-Tg) mice. We performed miRNA array analysis in lung tissue of ßENaC-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. 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 ßENaC-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-mediating knockdown of mir-148b in the lung of ßENaC-Tg mice reduced emphysema formation, goblet cell metaplasia and neutrophilic 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. In- testinal 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 I-BIBO ex vivo (Roth L. 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 Ca2+ (carbachol)-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.

Rescue of function in a cystic fibrosis mouse model by transfer of hematopoietic 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 Cfrd wildtype mice. Therefore, we subjected recipient B6.Cfrd/JpRogoGFPmice 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...
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 model of *P. aeruginosa* lung infection, mice intratracheally injected 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 10^5 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 10^6 cfu/mouse. Within 4 to 8 hours severe signs of infection and lung inflammation were observed. Lethality occurred 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. References: [1] Hauser AR. The type III secretion system of *Pseudomonas aeruginosa*: infection by injection. Nat Rev Microbiol 2009 7: 654 – 665 [2] Davenport C, Winstanley C, Schirmer B, Stelzer T, Tümmler B 1-3, Seifert R 1

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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 endoscope. 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
Downregulation of Hemeoxygenase-1 and Altered Cellular Homeostasis in Cystic Fibrosis Chilippagari SP, Venkatesan S, Garapati V, Mahavadi P, Munder A, Zschauer A, Guenther R, Schneek B, Tümmel B, Henke M 1Philips University Marburg, Marburg; 2Justus-Liebig- Universität Gießen, Gießen; 3Hannover Medical School, Hannover; 4Biomedical Research in Endstage and Obstructive Lung Disease Hannover (BREATH); 5Universities of Gießen and Marburg Lung Center (UGMLC), Member of the German Center for Lung Research (DZL)

Hemeoxygenase-1 (HO-1), an inducible heat shock protein, is upregulated in response to multiple cellular insults via oxidative stress, lipopolysaccharide (LPS) and hypoxia. In this context, LPS activates 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. CFE8410 and 16HBE140 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 CFE8410 cell line and CF mice. Knockdown studies using TLR4-siRNA in 16HBE140 revealed significant decrease of HO-1, confirming the role of TLR4 in HO-1 downregulation. Inhibition of HO-1 using tin protoporphyrin in 16HBE140 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 decreased HO-1 expression. We found that FGF10/FGFR2b upregulation and renewal capacity in the infected compared to the non-infected fraction of EpProg, likely due to virus-induced blockade of the FGF10/FGFR2b axis. However, EpProg post IV infection or naphthalene treatment showed a significant decrease of HO-1 in vitro. Taken together our results demonstrate that downregulation of HO-1 expression in CF is caused due to reduced TLR4 expression and increased intracellular iron and decreased HIF-1α.

Disease Area: Acute Lung Injury

Therapeutic potential of murine bone marrow derived mesenchymal stem cells in influenza virus-induced pneumonia Jankauskaite L, Schmidt C, Lohmayer J, Zimmers KP 1,2, Bellusci S 1,4, Zimmer KP 1,2, Bellusci S 1,4

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 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.


Therapeutic effects of fibroblast growth factor 10 (FGF 10) after hyperoxic lung injury in mice Chao CM, Al Alam D, Schermuly R, Ehrhardt H, Zimmer KP, Bellusci S

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 FGFFR2b on EpProg post IV infection or naphthalene treatment. However, EpProg were found to be primary targets of IV infection, which resulted in reduced FGFFR2b 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 FGFFR2b upregulation. Notably, the extent of EpProg infection correlated with the wnt10a and the pal gene expression of different IV strains, suggesting that the severity of viral pneumonia may 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 FGFFR2b overexpression resulted in reduced proliferation rates, sustained alveolar leakage and poor outcome. We provide evidence that IV-induced blockade of the FGFR/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.

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: 10 weeks old Fgf10−/− mice (50% Fgf10 expression compared to WT) in normoxic condition: Lung function and morphometric analysis. BDP model: Fgf10−/− and Fgf10+/+ were exposed to 85% O2 from P0-P8. Morphometric analysis and α-Actin/WF staining for vessels were performed at P3. Rosato25TAT4−/− tet-O:Fgf10 (gene-deletion) mice were exposed to 85% O2 from P0-P8. From P9-P4 the mice 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. Tolerance study: Rosato25TAT4−/− 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: Fgf10+/− mice under normoxic condition have worse lung function and lung structure compared to WT mice. All Fgf10+/− newborn mice die from hyperoxic injury due to increased lung injury and vascular malformation. Overexpression of Fgf10 after hyperoxic injury leads to improvement of lung structure compared to control group without overexpression. Fgf10 overexpression after hyperoxic injury does not increase mortality and side effects (weight loss, mucosal proliferation) are reversible. Conclusions: Fgf10 attenuates hypoxic injury.
peroxic lung injury, is well tolerated and should be further studied as a potential therapeutic for BPD.

**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 knockdown-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.

**Results:**

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 architecture 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.
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, LC3B or LC3BII and the ratios did not develop significantly more fibrosis than wild type animals. However, inactivation of beta-catenin in the mesenchyme impairs the entry of the mesenchymal progenitors into the SMC lineage both in vitro and in vivo. Furthermore, we have reported that inhibition of beta-catenin signaling in the mesenchyme leads to the loss of amplification of the mesenchymal progenitor cells and increased 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. An overall increase in CMA was observed. We conclude that different autophagy pathways are differentially regulated in IPF and in bleomycin injured mouse lungs.

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 downstream targets from 14 days post injury, expressed Fgf10 and downstream targets were significantly decreased. In contrast, wild type mice undergoing spontaneous repair after bleomycin injury, expressed Fgf10 and downstream targets 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
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 vascular 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 substantial 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 levels of 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.
Deficient Autophagy in Hermansky-Pudlak Syndrome associated lung fibrosis

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Deficient autophagy seems to play an important role in Hermansky-Pudlak syndrome (HPS) lung fibrosis. HPSII lungs show enlarged alveolar type II cells (AECII) with giant lamellar bodies. We previously reported lung fibrosis in a HPS 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 HPS 1/2 mouse data. Here, we aim to analyze autophagy, an important lysosomal degradation pathway, under HPSII conditions. Immunohistochemistry was performed on lung explants. Tests 1, 3, 7, 14 days after lung instillation with bleomycin and surfactant accumulation and alveolar epithelial cell death analysis were performed. Immunohistochemistry showed decreased staining for LC3B and increased staining for LC3II on the day 14 post-instillation. Immunogold labelling for LC3B was performed in lung explants. Prior to the onset of collapse induration (D7), the lungs were explanted. Prior to the onset of collapse induration (D7), the lungs were explanted. The lungs were then analyzed for autophagy markers (LC3B, p62 and BECN1). We observed a decrease in LC3B and an increase in beclin-1 expression in the HPS1/2 lungs compared to WT lungs. Electron microscopy results confirm the qualitative observation of increased 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 in AECII of HPS 1/2 compared to WT mice. Our results point towards defective autophagy in AECII under HPSII conditions both in mice and men. In depth analysis of this pathway is underway to further understand the role of defective autophagy in the development of HPSII.

Role of Bcl-xL in hepatocyte growth factor-elicted 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-elicted, pro-survival pathways have yet not been investigated in detail in lung epithelial cells. Based on literature, our study is focused on Bcl-xL, a pro-survival protein involved in mitochondrial control of apoptosis. Analysis of IPF lung 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 glutaraldehyde. Immunohistochemistry revealed that the AECII of HPS 1/2 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 in AECII of HPS 1/2 compared to WT mice. Our results point towards defective autophagy within AECII under HPSII conditions both in mice and men. In depth analysis of this pathway is underway to further understand the role of defective autophagy in the development of HPSII.

Wnt-Heparan Sulfate Proteoglycan interaction in fibrotic lung disease

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Introduction: Idiopathic pulmonary fibrosis (IPF) is a lethal lung disease of unknown etiology. Recent findings indicate that Wnt signaling pathways, such as Wnt/β-catenin and the Wnt/β-catenin activation 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 was stained for Wnt/β-catenin.
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, 6-0-DS Heparin treatment of A549 cells did not decrease WNT signaling, while binding of WNT3a to 6-0-DS Heparin was not altered, thereby suggesting less inhibition of WNT signaling upon binding to 6-0-DS Heparin. **Conclusion:** Taken together, our findings revealed that HS sulfation and degradation modulate WNT/β-catenin signaling upon binding to 6-O-DS Heparin.

**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 Tia1 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 Tia1 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 Tia1 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.

**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 focusing 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 (MFs) 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 02 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, MFs 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 littersmates. Other stress response systems were not found to be significantly regulated in contrast to findings from...
The reprogramming of the cellular protein quality control system in the neonatal lung shows a role in changes in fetal versus 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 hypoxia. Ongoing experiments using different markers and functional assays will characterize this response in more detail.

Disease Area: Pulmonary Hypertension

**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) in hypoxia compared to wild-type 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, echocardiography, 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 hypoxic conditions 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.

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 (C57BL/6J) 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, Phase smoke-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 regulation 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.

Disease Area: Endstage Lung Disease

**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 Ster-
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 adherence 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 Titaniaoxides (TiO2) deposited on PMP using PVCAPD as an effective coating technique for enabling the endothelialization was assessed.

Methods: PVCAPD 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 24 h. 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.

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 followed by application of cíclosorine (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

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 not develop in allografts treated with vehicle instead of LPS nor in pulmonary isografts. Altogether and autoreactive antibodies have been detected in BOS patients but their pathogenic role is disputed. Here, we investigate B cell infiltration, cytokine expression, 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 cíclosorine (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

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 can be treated with new drugs whilst undergoing normothermic perfusion and ventilation. Here, we present data from our first three lung transplants with the aim of proving 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 bronchi. Reperfusion was performed with 1.0–1.2 l/min and ventilation was started aiming for a tidal volume of 250–300 ml in respect to patient’s 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, a high degree of visible differences 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.

Changes in local alpha-1-antitrypsin expression during the pathogenesis of experimental bronchiolitis obliterans syndrome Siebers K, Atanasova S, Reichert M, Padberg W, Grau V
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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 is still associated with survival rates of 53% after 5 years. Necrosis/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. Isogenic transplantation provoked a graft-specific decrease in AAT mRNA-expression, which was transiently reversed by therapy. Control allograft recipients, however, showed AAT induction. 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.

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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, 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 homebox1 (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 the endoderm and NKX2.1 expressing cells. Based on the results, the hiHSC, 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.
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-correlated 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.

Miniaturized 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 harvested 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 7.0 mmol/l, while the two others indicated lactate levels up to 1.0 mmol/l. Pathological work up revealed no significant morphological changes, except for focal atelectasis. There were no definable differences in between the examined groups. Conclusions: The miniaturized OCS is a reliably working system to establish the ex-vivo therapies for different indications. As ex-vivo therapies may need to be applied for more than 4 hours, extended perfusion times and various perfusion solutions are currently investigated.

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 homebox 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 anteriorisation, 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.

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 cytometry 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. Spriometry 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.
Applications like full endothelialisation of gas exchange membranes in extracorporeal membrane oxygenation (ECMO) devices for improved hemocompatibility, cell therapy of pulmonary hypertension 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 fluorochrome reporter/antibiotic resistance under the control of EC specific promoters (VEcadherin or CD31) for monitoring of differentiation and selection/quantification 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. References: 1 Olmert R, R, et al., Suspension culture of human pluripotent stem cells in controlled, stirred bioreactors. Tissue Eng Part C Methods, 2012. 18(10): p. 772 – 84. 2 Orlova, V.V., et al., Functional identity of endothelial cells and pericytes from human pluripotent stem cells demonstrated in cultured vascular plexus and zebrafish xenografts. Arterioscler Thromb Vasc Biol, 2014. 34(1): p.177 – 86.

**Disease Area: Lung Cancer**

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

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**Results:** There was a trend to longer OS in all histologies. Squamous cell carcinoma had longer PFS after 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 carcinoma, 28 large cell, 10 mixed and 35 NSCLC not otherwise specified (NOS).

**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 CITR 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.
allowing the recognition of structures in size down to single alveoli. The resulting three-dimensional models allow for the detailed ana-
lysis of the conductive blood and airway system architecture. Insights into subcellular regions are achieved with a resolution in the nanometre scale using ET. In lamellae bodies (the "surfactant" containing organelles in type II alveolar epithelial cells), single lipid lamellae can be observed.

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 limit-
ing 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, BOS 0 p and late stages (BOS 1 – 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 was calculated.

Results: The coefficient of variation for T1 values was sign-
ificantly 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.

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-nitroanthrone (CBNP/Na) or benzylpyrene (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 integ-
ty and the mRNA expression of cytotoxic enzymes (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 with-
out 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 pre-
served 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 Musac. Our results indicate that the acute toxicity of CBNP is determined by their surface modification and modified CBNP can impair mucociliary clearance.

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 quantitatively determine the 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 measure-
ments 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 shrink-
age after primary glutaraldehyde/formaldehyde fixation, post-fixation with osmium tetroxide and uranyl acetate and embedding in glycol methacrylate [2]. A proper type II cell quantification 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 tetro-
oxide or uranyl acetate. Linking particles 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 devel-

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 io-
dide (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 les-
ions 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 pro-
truded 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.

**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 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 values were significantly different between mice with pulmonary emphysema and control mice and showed 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.

**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 and an exponent 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.

**DZL-Platform 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.

**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 were 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 males. 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-of-hospital, prospective patient registries are needed to establish the exact prevalence of bronchiectasis according to the specific underlying condition.

**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 = 6 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 PCT-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 FEGI, 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 in PS which may represent depletion from the organ by accumulation. This ‘dialysis’ effect was implicative for improved outcome in OCS.

**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. Despite the plethora of FOXJ1 effectors in the lung and the ependyma, the mechanism of FOXJ1 is 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 16.5 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 dependent on both the cilium and the basal body of the cilium of monocolonial 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 ependymal node, the developing and adult lung and ependyma and that its expression depends on FOXJ1. KO mouse embryos never reveals an apparent motile cilium defect nor a lung phenotype so that it is not yet clear whether 1700012B09Rik is involved in ciliogenesis or formation or function and if so at what level.

**The role of WNT/β-catenin signaling in smooth muscle cells during lung development and repair**

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Wnt signalling 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. Uregulation of Wnt signalling in the mesenchymal cells is associated with lung pathologies, such as asthma, lung fibrosis, and pulmonary arterial hypertension. After naphthalene injury, an established model of airway epithelial damage, the surviving ciliated airway epithelial cells express Wnt7b. Wnt7b then acts on the fibroblastic cells and smooth muscle cells. We will also use different reporter lines that will allow us to visualize the smooth muscle cells which underwent Cre

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 57% 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 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 lung 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 (mATEpC). 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 life-span 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 aiming at elucidating mechanisms of cell proliferation. Upon infection, cell lines could be established that exhibit a prolonged life-span 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 aiming at elucidating mechanisms of cell proliferation.

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 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 iCas9 gene therapy approach which can be linked to the 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 iCas9 gene therapy approach in particular the CSF2RA,iCas9 construct appears suitable for further evaluation towards first clinical approaches.

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 (Lx.EFS.CSF2RA.EGFp) or the inducible suicide gene iCas9 (Lx.EFS.CSF2RA.iCas9) from an EFS.1a-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 dependent 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 β-chain by the transgene. Further characterization of GM-CSF receptor downstream signaling 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 iCas9 suicide switch led to time-dependent reduction of cell viability. With the aim to establish a mouse model of pulmonary alveolar proteinosis, we have generated homozygous CSF2RA null mice (Csf2r−/−) reconstituted with wild type hematopoietic stem cells. Using the rec-Breast-Cancer-2 acid-labile (rBC2aL) promoter, we were able to reconstitute the bone marrow of Csf2r−/− mice with wild type hematopoietic stem cells, enabling the formation of MPS-1 and -2 in the lungs of mutant mice. To this aim, we have established a Csf2r−/− mouse model that reflects the crucial barrier properties of primary ATI cells, which are representative of the blood-air barrier. We established protocols for immortalizing primary murine alveolar epithelial cells (mATEpC). 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 life-span 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 aiming at elucidating mechanisms of cell proliferation.

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 regeneration 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 species, we have differentiated iPSCs into Clara cells in vitro, mimicking the blood-air barrier (Toppgal/LacZ lines). We evaluated 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 iCas9 gene therapy approach, the CSF2RA,iCas9 construct appears suitable for further evaluation towards first clinical approaches.
Clara cell reporter mouse strains enabled identification of generated Clara cells. iPSCs from CCSP-rtTA-s-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 dexmethasone, 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 immunodefficient 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 VFPpos cells using the DCI supplemented ML protocol.

**Confrontal 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.
Development and characterization of a decellularised xenogeneic mitral valve scaffold
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Introduction: Mitral valve regurgitation is the second most common cause of heart valve disease. The incidence of mitral valve regurgitation has increased in recent years due to improving medical treatment and the overall aging population. The aim of this study was to develop and characterize a decellularized 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 deoxycholate for 36 hours, followed by extensive washing and nucleic acid digestion. Radial sections comprising annulus, leaflets, chordae tendineae, 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 decellularization, 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 decellularized tissue, with a conserved histoarchitecture. The decellularized tissue demonstrated a graft-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.

Generation of CF-patient derived iPSCs and efficient footprintless designer nuclease-based gene targeting
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Introduction: The ability to genetically modify human induced pluripotent stem cells (iPSCs), including the correction of 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 patient-specific iPSCs, encephalial 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-iPSCs, 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 ZFN and TALENs, targeting efficiency of up to 15% was 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 (Merkt 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 hiPSCs from 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. References: Merkt, S., Wunderlich, S., Bednarski, C., Beier, J., Haase, A., Dreyer, A.-K., Schwanke, K., Meyer, J., Gohring, M., 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.

A novel mouse model for the investigation of adaptive immune responses upon de novo antigen expression in lung epithelial cells
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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 generated SPCCre mice in which the CreERT2 recombinaise 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% of cells. We employed a reporter mouse model SPCCre x ROSA+Luc 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 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. References: Rock, J. R. and B. L. Hogan (2011). “Epithelial progenitor cells in lung development, maintenance, repair, and disease.” Annu Rev Cell Dev Biol 27: 493 – 512. Sandhu, U., et al. (2011). “Strict control of transgene expression in a mouse model for sensitive biological applications based on RMCE compatible ES cells.” Nucleic Acids Res 39(1): e1.

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. At this aim of the 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 (3 cm 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 conditioned samples. Conditioned samples 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 mecha-no-stimulation of cell-seeded pericardial scaffolds in vitro in order to generate heart-valve like tissue.

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 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 acetic 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 34.96% 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. References: A. Szentivanyi et al. A review of developments in electrospinning technology: New opportunities for the design of artificial tissue structures. Int J Artif Organs 2010;34:986 – 997 A. Szentivanyi et al. Electrospun cellular microenvironments: Understanding controlled release and scaffold structure. Advanced Drug Delivery Reviews 2011;63:209 – 220

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 polymer 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-trifluoroethanol (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, 15 x 10 mm strips were cut and tested at 0 – 30% strain, 1 cycle/s, 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 hydrophilicity 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. References: Szentivanyi A. et al. Electrospun cellular microenvironments: Understanding controlled release and scaffold structure. Advanced Drug Delivery Reviews 2011;63:209 – 220. Dimosthenis Mavrilas et al. Dynamic mechanical characteristics of intact and structurally modified bovine pericardial tissues. Journal of Biomechanics, Volume 2005;38:761 – 768

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