Immunofluorescence staining revealed a co-expression of PDGFRα-GFP and αSMA in the bronchial compartment as well as in the alveolar space. The expression of ADRP co-localizes with the precursor marker in a subset of cells.
The same pattern of expression could be observed in the constitutive PDGFRa-cre and the conditional PDGFRa-CreER mice.
PDGFRα-GFP mice showed that PDGFRα-expressing precursor cells are differentiating into myo- as well as lipofibroblasts. The constitutive PDGFRa-cre mice revealed restriction of PDGFRα signalling to bronchial smooth muscle cells and alveolar fibroblasts in the lung. Lineage tracing with conditional PDGFRa-creER mice could confirm that postnatal PDGFRα cells derive lipofibroblasts and myofibroblasts.

P3748
Nrf2 is closely related to enhance bleomycin induced airway inflammatory responses caused by diesel exhaust particles in mice
Ying-Ji Li[1], Tkako Shimizu[1], Yukiyo Hirata[1], Hirofumi Inagaki[1], Yusuke Shinkai[2], Ken Takeda[2], Arata Azuma[3], Hajime Takizawa[4], Yamamoto Masayuki[5], Tomoyuki Kawada[1], Shoji Kudoh[6]. *[1]Hygiene and Public Health, Nippon Medical School, Tokyo, Japan; [2]Hygiene Chemistry, Faculty of Pharmaceutical Sciences, Tokyo University of Sciences, Noda, Japan; [3]Pulmonary Medicine/Infection and Oncology, Nippon Medical School, Tokyo, Japan; [4]Pulmonary Medicine, Kyorin University, School of Medicine, Mitaka, Japan; [5]Biochemistry, Tohoku University Graduate School of Medicine, Sendai, Japan; [6]Fukujuji Hospital, Anti-Tuberculosis Association, Kiyose, Japan*

Diesel exhaust particles (DEP) induced oxidative stress play an important role in proinflammatory reaction on airway. Nrf2 is involved in the transcriptional regulation of many antioxidant genes. In the present study, we investigated the effect of DEP on an experimental model of bleomycin (BLM)-induced airway inflammatory responses in both of Nrf2+/+ and Nrf2−/− mice.
BLM was administered IV to Both Nrf2+/+ and Nrf2−/− C57BL/6J mice at a dosage of 80 mg/kg body weight on day zero. Mice were exposed to 1mg/m^3 DEP for 8 hrs/day and 5 days/week. We designed two experimental groups as follows: group 1, BLM alone, clean air; group 2, BLM plus pre-4wks-DEP exposure. Cell populations in BALF were examined at 10 days after BLM injection. We also examined cytokine level in BALF by ELISA.
In the DEP exposed group, the percentage changes from BLM alone group in the total number of cells and macrophages remarkably increased in the both Nrf2+/+ and Nrf2−/− mice. There were DEP-laden alveolar macrophages number were significantly greater in Nrf2-/- than in Nrf2+/+ mice. The percentage changes from BLM alone group in the neutrophils increased in the both Nrf2+/+ and Nrf2−/− mice, the increased neutrophils were significantly greater in Nrf2-/- than in Nrf2+/+ mice. The percentage changes from BLM alone group in the TGF-beta level decreased in the Nrf2-/- than in Nrf2+/+ mice.
These findings suggest that DEP might be an important risk factor on the BLM induced lung injury, and Nrf2 might be an important genetic factor in the determination of susceptibility to BLM induced lung injury caused by DEP via regulating the macrophages defense mechanisms in mice.

P3749

394. Fibrogenesis between epithelial injury and fibroblast proliferation

P3747
Lineage differentiation of pulmonary alveolar fibroblasts
Friederike Klein[1], Werner Seeger[1,2], Annegret Wilde[3], Botond Roska[4], William D. Richardson[5], Robert Voswinckel[1,2]. *[1]Lung Development and Remodelling, Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany; [2]Department of Internal Medicine, University Hospital, Giessen, Germany; [3]Institute of Micro- and Molecular Biology, University of Giessen, Germany; [4]Friedrich Miescher Institute for Biomedical Research, University of Basel, Switzerland; [5]Wolfson Institute for Biomedical Research, University College London, United Kingdom*

Alveolar fibroblasts are key cells to the process of alveolar septation as PDGFRα knock-out prevented alveolar myofibroblast differentiation and completely blocked secondary septa formation. Lipofibroblasts have been proposed to be essential for septation, peak in number during septation and regress significantly thereafter. Whether PDGFRα expressing precursors are deriving both lineages of fibroblasts is not known and transitions in between one or the other fibroblast type are unsolved.
The aims of this work are to analyse the developmental fibroblast lineages, cell-cell transitions and ultimately the role of each cell type and the utility for regenerative septation in adulthood.
To follow lineages, cre-reporter mice (mT/mG) expressing tomato fluorescent protein in the membrane of all cells, which switches to GFP upon cre-recombination, were crossed with constitutive PDGFRa-cre or conditional PDGFRa-creER mice. For identification of active PDGFRa expression, PDGFRα-GFP knock-in mice were used.

WITHDRAWN

P3750

Ghrelin ameliorates bleomycin-induced acute lung injury by protecting alveolar epithelial cells and suppressing lung inflammation

Shigehisa Yanagi, Yoshifumi Imazu, Kahori Miyoshi, Hironobu Tsubouchi, Nobuhiro Matsumoto, Masamitsu Nakazato. *Division of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, University of Miyazaki, Japan*

Acute lung injury (ALI) is a critical illness syndrome consisting of acute respiratory failure with bilateral pulmonary infiltrates that is refractory to current therapies. ALI is characterized by injury of the alveolar capillary barrier, neutrophil accumulation, and induction of pro-inflammatory cytokines followed by devastating lung fibrosis. Ghrelin, an acylated peptide produced in the stomach, increases food intake and growth hormone secretion, suppresses inflammation, and promotes cell survival. We investigated the pharmacological potential of ghrelin in the treatment of ALI by using a bleomycin-induced ALI model in mice. Ghrelin or saline was given to mice daily starting 1 day after bleomycin administration. Ghrelin-treated mice showed a definitively higher survival rate than saline-treated ones. They also had smaller reductions in body weight and food intake. The amelioration of neutrophil alveolar infiltration, pulmonary vascular permeability, induction of pro-inflammatory cytokines, and subsequent lung fibrosis were notable in ghrelin-treated mice. Additionally, ghrelin administration reduced the injury-induced apoptosis of alveolar epithelial cells. Our results indicate that ghrelin administration exerts a protective effect against ALI by protecting the alveolar epithelial cells and regulating lung inflammation, and highlight ghrelin as a promising therapeutic agent for the management of this intractable disease.

P3751

A longitudinal characterization of lymphangiogenesis in bleomycin-induced pulmonary fibrosis mouse model

Masahiro Yamashita[1], Miyuki Niisato[1], Hitoshi Kobayashi[1], Yuji Shibata[2], Takashi Sawai[2], Kohei Yamauchi[1]. *[1]Respiratory, Allergy and Rheumatology, Iwate Medical University, Morioka, Iwate, Japan; [2]Pathology, Iwate Medical University, Morioka, Iwate, Japan*

Background: The roles of lymphangiogenesis in the pathogenesis of pulmonary fibrosis has been remained to be elucidated, although it has been associated with this condition in human.
Objective: To clarify longitudinal characteristics of lymphangiogenesis in bleomycin-induced pulmonary fibrosis mouse model.
Animal and methods: Pulmonary fibrosis was induced in C57BL/6J female mice by intratracheal injection of 3.0 U/kg of bleomycin. Three mice were sacrificed in 6 individual days, including before the injection and at 7, 14, 21, 28 and 35 days after. Formalin fixed and paraffin embedded lung tissues were used for immunohistopathological and morphometric analyses. Antibodies specific for Vegfr-3, Cd31 and type I collagen were used to detect lymphatics, blood vessels and collagen, respectively.
Results: The dilatation of the existing lymphatics around bronchus and large blood vessels was observed on Day 7, and numerous lymphocytes were organized around them. The lymphatics were newly formed in fibrotic lesions on Day 14, although capillaries were barely detected in the lesions. In Masson Trichrom staining, connective tissue was most prominent on Day 21, and looser after that. The area densities of interstitium and hydroxyproline concentration in lung homogenates were maximally increased on Day 21. The existing and the newly formed lymphatic densities were significantly increased on Day 21, respectively ($p < 0.05$).
Conclusion: In the early stage, the existing lymphatics may play a role in the organization of lymphoid structure, which possibly facilitate the fibrogenesis. In the later stage, the newly formed lymphatics may be associated with the tissue resolution.

P3752

Bioavailability of vascular endothelial growth factor (VEGF)? A role in pulmonary fibrosis?

Shaney Barratt[1], Caroline Jarrett[1], Gavin Welsh[1], David Bates[2], Ann Millar[1]. *[1]Lung Research Group, University of Bristol, United Kingdom; [2]Microvascular Research Laboratories, University of Bristol, United Kingdom*

Vascular endothelial growth factor (VEGF) is both a growth factor and permeability factor, involved in tissue repair and as such, has been proposed to have a role in the pathogenesis of pulmonary fibrosis. VEGF is generated as multiple isoforms of two families, $VEGF_{xxx}$ and $VEGF_{xxx}b$ and mediates its effects through specific receptors, VEGFR-1 and VEGFR-2 and co-receptors, neuropilin-1 and neuropilin-2. We hypothesised that these receptors, co-receptors and isoforms would be differentially expressed in normal versus fibrotic fibroblasts.
Normal fibroblasts (NF) and fibrotic fibroblasts (FF) (from patients with proven UIP) were extracted from lung samples using the explant method. Expression of VEGFR-1, 2 and NRP1-1, NRP2 and VEGF isoforms was established at the protein level using western blotting whilst receptor expression was confirmed with immunofluorescence staining of cells in culture.
Both NF and FF expressed VEGFR1 and 2, NRP-1 and 2 and $VEGF_{xxx}/_{xxx}b$ isoforms. No significant differences in VEGFR-2 and NRP-1 expression between NF and FF fibroblast were detected, however both VEGFR-1 and NRP-2 were significantly reduced in FF versus NF ($p<0.05$, non-paired t-test).
VEGF receptors, co-receptors and isoforms are expressed differentially in NF and FF. This suggests a potential role for changes in VEGF bioactivity in the development of pulmonary fibrosis.

P3753

Erythropoietin (EPO) attenuates the expression of its receptor (EPO-R) in bleomycin (BLM)-induced pulmonary fibrosis (PF) in rats

Drosos Tsavlis[1], Anna Tzoumaka[1], Georgia Kokaraki[1], Kokkona Kouzi-Koliakou[1], Anastasia Tektonidou[1], Afroditi Papadopoulou[1], Evangelia Spandou[1]. *[1]Department of Experimental Physiology, School of Medicine, Aristotle University, Thessaloniki, Greece; [2]Department of Experimental Physiology, School of Medicine, Aristotle University, Thessaloniki, Greece; [3]Department of Experimental Physiology, School of Medicine, Aristotle University, Thessaloniki, Greece; [4]Department of Experimental Physiology, School of Medicine, Aristotle University, Thessaloniki, Greece; [5]Department of Experimental Physiology, School of Medicine, Aristotle University, Thessaloniki, Greece; [6]Experimental Physiology, School of Medicine, Aristotle University, Thessaloniki, Greece; [7]Experimental Physiology, School of Medicine, Aristotle University, Thessaloniki, Greece*

Purpose: PF is characterized by apoptosis as well as by inflammation, excessive collagen deposition and fibroplasts' proliferation.The EPO-R is well known to play an important role in the fibrotic-apoptotic pathway. EPO is a multiple functional cytokine with anti-apoptotic, anti-oxidative and anti-inflammatory properties.We looked for the effect of EPO on BLM-induced lung fibrosis, by examining the expression of EPO-R in the lung tissue of rats.
Material and methods: Fifty Wistar rats (300gr) were divided into five groups of 10 animals each:1)control animals,2)intratracheal (i.t) and intraperitoneal (i.p) injection of saline (0.5ml/kg),3)BLM hydrochloride (7.5mg/kg) i.t injection,4)BLM hydrochloride (7.5mg/kg) i.t injection followed by EPO i.p injection (2000 iu/kg),5)saline (0.5ml/kg) i.t injection followed by EPO i.p injection (2000 iu/kg). All rats were sacrified after 14 days.Immunohistochemical evaluation was performed for the expression of EPO-R.A scale of 4 grades was used for the evaluation of the results: 0-25% (A),25-50% (B),50-75% (C),75-100% (D).
Results: In groups 1,2 and 5 (control groups), EPO-R was expressed in the lower grades A (80%) and B (20%). In group 3 (BLM group), EPO-R was expressed in the high grades B (20%), C (70%) and D (10%). In group 4 (EPO group), EPO-R was expressed only in the low grades A (50%) and B (50%). The expression of EPO-R took place in the high grades for BLM group and in the lower grades for BLM+EPO group ($p<0.05$).
Conclusion: BLM injection followed by EPO resulted in significant lower expression of EPO-R compared with BLM group. The protective mechanisms of EPO on PF must be further clarified.

P3754

Rikkunshito ameliorates bleomycin-induced lung injury in mice

Hironobu Tsubouchi[1], Shigehisa Yanagi[1], Seiichi Iizuka[2], Sachiko Mogami[2], Kahori Miyoshi[1], Nobuhiro Matsumoto[1], Masamitsu Nakazato[1]. *[1]Division of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, University of Miyazaki, Japan; [2]Tsumura Research Laboratories, Tsumura & Co., Ibaraki, Japan*

Acute lung injury (ALI) is a critical illness syndrome consisting of acute respiratory failure with bilateral pulmonary infiltrates that is refractory to current therapies. ALI is characterized by injuries of the alveolar epithelial barrier, infiltrations of neutrophills into lung parenchyma, and induction of pro-inflammatory cytokines followed by devastating lung fibrosis.
Rikkunshito (RKT), a traditional Japanese medicine, consists of several kind of flavoniods which have been shown to have anti-inflammatory effects. In addition, previous studies have reported that RKT increases plasma level of ghrelin, an acylated and orexygenic peptide, produced predominantly in the stomach. We investigated the pharmacological potential of RKT in the treatment of ALI by using a bleomycin (BLM)-induced lung injury model in mice.
RKT or distilled water was given to mice orally and daily starting from the day of BLM administration. RKT-treated mice showed a definitively higher survival rate than distilled water-treated ones. They also had smaller reductions in body weight and food intake compared to the controls. Additionally, RKT-treated mice showed reduction of pulmonary epithelial permeability, neutrophil alveolar infiltration, and subsequent lung fibrosis.
RKT administration resulted in increase of plasma levels of ghrelin in BLM-treated mice. However, RKT administration also exerted protective effects against BLM-induced ALI response on ghrelin-deficient mice in addition to ghrelin-competent mice.
Our results indicate that RKT administration exerts a protective effects on BLM-induced lung injury in mice independently of the effects of ghrelin, and highlight RKT as a promising therapeutic strategy for the control of the ALI.

P3755

Leukotriene (LT)C_4 aggravate bleomycin-induced pulmonary fibrosis in mice

Masamitsu Tatewaki, Hirokuni Hirata, Masafumi Arima, Takeshi Fukuda.
Pulmonary Medicine and Clinical Immunology, Dokkyo University School of Medicine, Tochigi, Japan Developmental Genetics, Chiba University Graduate School of Medicine, Tochigi, Japan

Background: Synthesis of cysteinyl leukotrienes (cys-LTs) is thought to cause inflammatory disorders such as bronchial asthma and allergic rhinitis. Recent reports have suggested that LTC_4 is an important regulator of pulmonary fibrosis. This study examined the effect of LTC_4 in LTC_4 synthase-overexpressed transgenic (Tg) mice with bleomycin-induced pulmonary fibrosis. We also focused on the function of lung-derived fibroblasts in the Tg mice.
Methods: Prior to administration of bleomycin, pranlukast hydrate, a cys-LT1 receptor antagonist, was intragastrically administered to Tg mice daily from the previous day of the administration. Bleomycin was administrated by intratracheal instillation. Concentrations of IL-4, -13, and TGF-β1 in BAL fluid were measured 14 days after the administration of bleomycin. And lung tissue was examined histopathologically. In addition, lung-derived fibroblasts from Tg and wild-type (WT) mice were cultured for 7 days, and LTC_4 secretion and cell viability were assessed by EIA and MTT assay, respectively. And the expression of TGF-β1 mRNA was measured by real time PCR.
Results: The levels of IL-4, -13, and TGF-β1, and pulmonary fibrosis were greater in Tg than in WT mice. The reduction of LTC_4 function in Tg mice could be decreased both these cytokines and pulmonary fibrosis. Furthermore, continuous LTC_4 secretion from fibroblasts was higher in Tg than in WT mice, while reduction of LTC_4 by pranlukast in fibroblasts from Tg, but not in those from WT mice, decreased cell viability and expression of TGF-β1 mRNA.
Conclusion: These findings first suggest that overexpression of LTC_4 using transgenic mice is responsible for the development of pulmonary fibrosis.

P3756

LSC 2012 Abstract – Wnt11 is identified in 3D human lung tissue model as regulator of distal airway epithelial cell differentiation

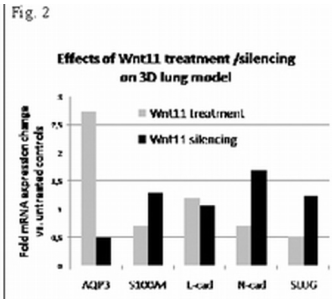
Domokos Bartis[1,2], Veronika Csongei[2], Vijay D'Souza[1], David R. Thickett[1], Judit E. Pongracz[2]. *[1]Respiratory Research Labs, University of Birmingham, United Kingdom; [2]Dept. of Medical Biotechnology, University of Pecs, Hungary*

Alveolar Type II (ATII) cells repopulate the damaged alveolar surface and transdifferentiate into Alveolar Type I (ATI) cells during physiological regeneration. During pathological airway repair excessive Epithelial-Mesenchymal Transition (EMT) might occur, resulting in fibrosis. Mesenchymal signals might contribute to the differentiation and regeneration of pulmonary epithelium stimulating distal airway epithelial differentiation and preventing EMT. To identify these factors, we constructed a 3-dimensional (3D) human tissue model of primary pulmonary cells to mimic epithelial-mesenchymal interactions in the human lung.

Fig 1

Patient	Fold changes of Surfactant protein C expression in Wnt11 treated lung explant cultures
Patient1	2.78 x
Patient2	2.81 x
Patient3	1.51 x
Patient4	22.52 x
Patient5	1.47 x
Patient6	1.66 x

Fig. 2



Distal airway epithelial cells differentiate into ATII-like cells as suggested by morphological changes as well as increased expression of differentiation markers AQP3, SP-A, SP-C in the 3D model. Wnt11 was identified in the model and in human lung explant cultures as one of the main regulators of ATII differerentiation. Added Wnt11 increased the expression of ATII markers, while silencing of Wnt11 resulted in elevated levels of EMT markers N-cadherin and S100A4. We conclude that the 3D lung model is applicable for studying epithelial-mesenchymal interactions in the lung. Our finding may mark Wnt11 as a potential therapeutic target in lung regenerative therapy.

P3757

LSC 2012 Abstract – Activation of Wnt/β-catenin signaling promotes lung epithelial repair in emphysema

J. Callegari, K. Mutze, M. Königshoff. *HMGU, Comprehensive Pneumology Center, Munich, Germany*

Emphysema is characterized by airspace enlargement, tissue destruction and reduced Wnt signaling. Wnt/β-catenin activation attenuated experimental emphysema. Here, we aim to elucidate the mechanism of Wnt/β-catenin induced alveolar epithelial cell repair in vitro and in vivo.
Alveolar epithelial type II cells were isolated from untreated (ATIIc) or elastase treated (ATIIe) C57BL/6 mice at day 3, 7 and 14 with similar purity (>94%) and viability (>92%). ATIIe cells depicted increased cell numbers (i.e. 6,07±1,62x106 ATIIe and 3,39±0,86x106 ATIIc, day14), and significantly reduced expression of the Wnt target genes Axin2, LEF1 and LRP6 (i.e. LRP6 0,29±1,42 ATIIe vs. 2,38±0,56 ATIIc, day7) as determined by qRT-PCR. Freshly isolated ATIIe cells exhibited increased apoptosis susceptibility (Annexin V/PI staining). Live cell imaging of cultured ATII cells revealed altered ATIIe cell morphology and migratory behavior. Wnt activation of ATII cells by rWnt3a led to increased expression of Wnt target genes (i.e. Axin2 -2,15±0,12 rWnt3a for 24h vs. -5,18±0,29 control), epithelial markers SPC, TJP1, and Occludin, and increased proliferative capacity (BrdU).
Primary ATIIe cells exhibited reduced Wnt/β-catenin activity and altered functional capacity. Wnt/β-catenin activation led to increased epithelial marker expression and stabilized ATII cell monolayers. Thus, activation of Wnt/β-catenin is a suitable tool to increase alveolar epithelial cell repair capacity in pulmonary emphysema.

P3758

LSC 2012 Abstract – Imaging Wnt/beta-catenin signalling in an ex vivo tissue culture model of lung repair

Franziska Uhl, Melanie Königshoff. *HMGU, Comprehensive Pneumology Center, Munich, Germany*

Emphysema is a pathophysiological hallmark of COPD and characterized by airspace enlargement and impaired alveolar repair processes. Recently, decreased Wnt/β-catenin signalling has been linked to emphysemateous changes in human disease and animal models thereof. Here, we aim to further decipher the underlying mechanisms and image structural changes involved in lung repair processes.
We applied an ex vivo tissue culture model from wildtype (C57Bl/6) and Wnt reporter mice (Bat-/TopGal) subjected to elastase treatment or PBS. Tissue slices (300 μm) generated using a vibratome were viable in culture for up to 7 days ex vivo (WST-1: d3 211±68% vs. d7 241±67%). Structural integrity of control (C) and emphysemateous (E) lung slices was determined by qPCR and immunofluorescence for lung specific cellular markers as well as live tissue imaging. Functional Sftpc was decreased in E lung slices compared with control and further decreased over time shown by ELISA (d1: 135±19 ng/ml C vs. 82±17 ng/ml E). Wnt signalling activation by LiCl led to an increase in Sftpc expression accompanied by increased β-galactosidase staining in alveolar regions and upregulated target gene expression, such as Axin2, Dkk2 or Lef1.
Emphysemateous tissue slices closely reflect COPD-like changes in vivo. Lung slices are viable up to 7 days ex vivo allowing determination of structural changes and cell fate in the diseased lung upon signal pathway modification.

P3759

Proliferation of alveolar type II pneumocytes is stimulated by Jagged-1 in vitro

Rainer Zembacher, Susanna Desole, Caroline Linhart, Kathrin Watzinger, Christian M. Kähler. *Department for Internal Medicine I - Pneumology, Medical University Innsbruck, Austria*

Notch is an ancient cell-signaling system that regulates the specification of cell fate. Recently, Notch was found to confer antigen presenting cell function on mast cells, induce histamine release in human basophils and regulate migration and survival of eosinophils.
In acute lung injury, alveolar type II cells activate macrophages, secrete soluble mediators, migrate and spread in response to the injury. Additionally, Notch stimulated myofibroblast differentiation and migration of cultured RLE-6TN cells. However, until now, nothing is known on the role of Notch activation regarding proliferation of rat alveolar type II cells.
Rat alveolar type II cells (RLE 6TN) were obtained from the American Type Culture Collection (ATCC no. CRL-2300; Manassas, VA, USA) and were cultured in DMEM/Ham's F12 containing 10% fetal calf serum and L-glutamine. Cell proliferation was measured by direct cell count and the fluorometric proliferation assay EZ4U basing on tetrazolium salt reduction. Cells were incubated with the test substances in medium containing 0.5% fetal calf serum for 24h at 37°C and 5% CO_2.
Jagged-1 significantly stimulated proliferation of alveolar epithelial cells within a wide concentration range [5μg/ml to 100pg/ml]. The maximum effect was observed at 100ng/ml. To show specificity of the observed effect, rat alveolar type II cells were preincubated (45 min) and co-incubated with the specific gamma secretase inhibitor DAPT [10^{-4} M] which completely abolished the effect of Jagged-1 [ng/ml].
Herewith, we report for the first time that the Jagged-1/Notch signalling pathway is affecting rat alveolar type II cell proliferation in vitro.

P3760

Wnt11 inhibits epithelial-mesenchymal transition induced by TGFb1 in human type II alveolar epithelial cells

Domokos Bartis[1], Vijay D'Souza[1], Judit E. Pongracz[2], David R. Thickett[1]. *[1]Respiratory Research Laboratories, University of Birmingham, United Kingdom; [2]Department of Medical Biotechnology, University of Pecs, Hungary*

Increased activity of TGFβ1 plays a crucial role in the pathogenesis of idiopathic

pulmonary fibrosis (IPF). Alveolar type II cells (ATII) undergo EMT expressing mesenchymal markers when exposed to a high concentration of TGFβ1 in both in vitro and in vivo models. Mesenchymal signals might contribute to the differentiation and regeneration of pulmonary epithelium. Wnt11 is a secreted glycoprotein known to be expressed in the mesenchyme of the embryonic lung.
We constructed a 3-dimensional (3D) human tissue model of primary human pulmonary cells to mimic epithelial-mesenchymal interactions in the lung. Our results indicate that human lung fibroblasts are a source of Wnt11 in the lung tissue model. ATII cells isolated from human lung samples were treated with recombinant TGFβ1 and/or Wnt11. Expression levels of the EMT markers N-cadherin, Vimentin, alpha smooth musle actin (αSMA) and SLUG were determined by qPCR and immunofluorescence. We found that Wnt11 inhibits EMT induced by TGFβ1 in ATII monolayers and in 2D and 3D ATII+fibroblast co-cultures. Wnt11 treatment resulted in decreased expression of EMT markers compared to TGFβ1 treated cell cultures.

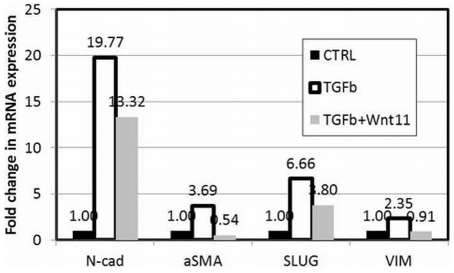


Figure 1. Human ATII cells subjected to TGFβand Wnt11 treatment.

We propose that the pulmonary mesenchyme might contribute to the homeostasis of epithelial cells by secreting Wnt11. The finding that effects of TGFβ1 can be antagonized by Wnt11 may mark it as a potential therapeutic target in the fibrotic diseases of lung.

P3761
Epithelial Pten controls acute lung injury and fibrosis by regulating intercellular junctional integrity and EMT
Shigehisa Yanagi, Kahori Miyoshi, Hironobu Tsubouchi, Nobuhiro Matsumoto, Masamitsu Nakazato. *Division of Neurology, Respirology, Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, Japan*

Injury to alveolar epithelial cells (AECs) and its repair process are integral to the pathogenesis of acute lung injury (ALI) and idiopathic pulmonary fibrosis (IPF). Disruption of AECs integrity and its reconstitution are crucial for ALI progression. In addition, myofibroblasts, key effector cells in IPF, partially originate from AECs through epithelial-mesenchymal transition (EMT). However, the regulation mechanisms of AECs integrity remains unclear. We explored the role of epithelial Pten in lung injury by generating a postnatally, and lung epithelium-specifically Pten-null (SOPten) mouse strain. Sixty percent of SOPten mice died of hypoxia, whereas all control mice survived after bleomycin insult. SOPten mice demonstrated aggravated ALI and lung fibrosis with enhanced disruptions of intercellular junctional complexes of AECs and degradation of basement membranes. Epithelial-derived myofibroblasts were increased in epithelium-specific Pten-deficient mice. Lungs of bleomycin-treated SOPten mice showed increased pAkt, pS6K, Snail and MMP expressions, and decreased claudin-4, E-cadherin, and laminin-beta1 expressions. Systemic Akt inactivation definitively saved SOPten mice through amelioration of ALI and aberrant EMT. Finally, we detected reduction of PTEN expression and hyperactivation of AKT in the AECs of human IPF lung. Our results indicate the pivotal role of EMT process for the progression of ALI and lung fibrosis. They also highlight epithelial Pten as an essential gatekeeper controlling ALI and lung fibrosis by modulating intercellular junctional integrity and EMT, and the Pten/PI3K/Akt pathway as a potential therapeutic target in these intractable diseases.

P3762
LSC 2012 Abstract – Mitochondrial metabolism controls lung fibroblast activation in vitro and in vivo
J. Schwarz, O. Eickelberg. *HMGU, Comprehensive Pneumology Center, Munich, Germany*

Background: Fibroblasts exhibit an extraordinary capacity to undergo fibroblast-myofibroblast activation in association with diseases such as pulmonary fibrosis. Little is known regarding the role of mitochondrial function in fibroblast biology in general and phenotypic switching in fibrosis in particular.
Aims and objectives: To demonstrate the interplay of mitochondrial metabolism with fibroblast-myofibroblast activation.
Methods and results: We demonstrate that stimulatΨon of primary lung fibroblasts with the profibrotic cytokine TGFβ1 results in an enhanced mitochondrial membrane potential ($\Delta\Psi_m$). Moreover, proliferation, ECM deposition, and motility of these cells are increased compared to fibroblasts with low$\Delta\Psi_m$. Interestingly, TGFβ1-stimulated cells displayed increased expression of profibrotic markers (αSMA, FN1 and Col1), demonstrating fibroblast-myofibroblast activation. To further corroborate these findings, we sorted primary lung fibroblasts for high and low resting $\Delta\Psi_m$ ($\Delta\Psi_m$H/L). While $\Delta\Psi_m$H fibroblasts are morphologically indistinguishable, they demonstrate increased myofibroblast activation compared with $\Delta\Psi_m$L fibroblasts. Finally, total cell homogenates derived from fibrotic, bleomycin-treated mice demonstrated increased $\Delta\Psi_m$ compared with cell homogenates from saline-treated mice.
Conclusion: Our data suggest that mitochondrial metabolism plays an important role in the phenotypic activation of lung fibroblasts, both in TGFβ1 stimulated cells in vitro and bleomycin-induced fibrosis in vivo.

P3763
An extract from a traditional Chinese medicine polyherbal formula and its main active ingredients regulate cAMP signaling: A potential treatment for lung fibrosis
Zhiping Fu, Beixian Zhou, Dunqiang Ren, Minhui Chen, Xiang Jie, Yansheng Wang, Jun Xu. *The State Key Lab of Respiratory Disease, Guangzhou Medical University, Guangzhou, Guangdong, China*

Rationale: Traditional Chinese Medicine has developed polyherbal formulations in which multiple agents contained in one formula act synergistically. *Tianlongkechunling* (*TLD*), one such formula mainly composed of Ford nervilia, Tussilago farfara, schisandra chinensis, Monkshood and Pinellia Tuber, has long been used for treating chronic pulmonary disease in south of China. It has been found that the main active ingredients in *TLD* formula are flavonoids and other bioactive compounds. We examined *TLD* extract for its potential effects on anti-fibrosis.
Methods & results: Using an Epithelial Mesenchymal Transition(EMT) model, immunoblotting demonstrated up-regulation of E-cadherin and suppression of Fibronectin expresson by *TLD* extract, thereby reversal of EMT in the treated A549 cells. Additional experiments displayed that *TLD* can inhibit hyper-expression of p-ERK 1/2, p-Akt, p-STAT3, NF-κB and α-SMA in lung fibroblasts of nonspecific interstitial pneumonitis(NSIP) and HMGB1-stimulated up-regulation in those of normal or NSIP, concomitant with reduced levels of inflammatory cytokines. Alzet osmotic minipumps application of *TLD* extract to Bleomycin(BLM)-challenged mice significantly decreased acute inflammation and pulmonary fibrosis. Mucosal application of *TLD* extract to native mouse trachea by Ussing chamber recordings suggested activation of Cl^- secretion by increasing cAMP. These effects were mimicked by a mix of isolated components.
Conclusion: We suggest that the extract from *TLD* may provide a novel and effective treatment for interstitial lung fibrosis through multi-ingredient synergistic regulation of cAMP signaling.

P3764
Chemokine profiles of A549 human alveolar epithelial cells that underwent epithelial-mesenchymal transition by TGF-b and/or TNF-a
Masafumi Horie[1], Yasuhiro Yamauchi[1], Akira Saito[1], Taisuke Jo[1], Yu Mikami[1], Hajime Takizawa[2], Takahide Nagase[1], Tadashi Kohyama[1]. *[1]Department of Respiratory Medicine, University of Tokyo, Japan; [2]Department of Respiratory Medicine, Kyorin University School of Medicine, Tokyo, Japan*

Rationale: Epithelial-mesenchymal transition (EMT) is supposed to be implicated in the pathogenesis of lung fibrosis through enhanced TGF-b1 signaling. TNF-a has also been implicated in tissue fibrosis. Recently, TGF-b induced EMT is augmented by TNF-a, suggesting that enhanced EMT by TNF-a may be important in the pathological processed of lung diseases. However, the mechanism of enhanced EMT by TNF-a has not been fully elucidated. Therefore, we evaluated the cytokine/chemokine profiles of the cells that underwent EMT by TGF-b and/or TNF-a.
Methods: A549 cells were incubated for 48 hours with 5 ng/ml of TGF-b1, 10 ng/ml of TNF-a, or TGF-b1 and TNF-a to undergo EMT. After 48 hours incubation, growth medium was changed to serum-free medium, and the supernatants were collected at 48 hours. Cytokine/chemokine array was performed using Ray Bio human cytokine array V. Relative values were quantified by densitometry, and normalized to that obtained in non-treated sample.
Results: TNF-a induced production of inflammatory chemokines, such as RANTES and MCP-1. TGF-b induced production of inflammatory chemokines, including GM-CSF, GRO, GRO-a, MCP-2, and MCP-3. Simultaneous stimulation with TNF-a and TGF-b induced production of GM-CSF, GRO, GRO-a, IL6, MCP-1, RANTES, and these effects were enhanced on the production of GRO, GRO-a, IL-8, and MCP-1.
Conclusion: The cells treated with TGF-b1 or TNF-a have different chemokine profile, and the effect on chemokines production was enhanced by the combination with TGF-b1 and TNF-a. This study might contribute to understanding mechanism of EMT enhanced by TNF-a and pathogenesis of lung fibrosis.

P3765

Let us try pirfenidone in paraquat poisoning: Effect of pirfenidone on pulmonary fibrosis due to paraquat poisoning in rats

Soroush Seifirad[1,2], Farzad Masoudkabir[1], Shervin Taslimi[2]. *[1]Endocrinology and Metabolic Research Institute, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran; [2]Pulmonary and Critical Care Research Center, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran*

Background: This study investigated the effectiveness of pirfenidone (PF) compared with antioxidants, in the prevention of pulmonary fibrosis and increasing the survival in acutely paraquat (PQ) poisoned rats.
Methods: Fifty rats equally randomized into 5 groups. Three groups poisoned with intraperitoneal injection of 15 mg/kg PQ. From these three groups one also received vitamin C (500 mg/kg, IP), E (200mg/kg, IP) and N-acetylcysteine (250 mg/kg, IV). Two others received either Normal Saline (NS) or PF (200 mg/kg-IV). Two groups were not poisoned and received NS or PF (200mg/kg, IV). All injections except PQ repeated for another 4 times in consecutive days. On the 15th day of study or the corresponding day of death a semi-quantitative determination of lung fibrous was done using Ashcroft staging criteria on the lung sections.
Results: PF decreased PQ induced lung fibrosis ($P<0.001$) while antioxidants did not decrease the lung fibrosis ($p=0.413$). Life expectancy decreased in PQ+NS (11 95% CI 7.94 to 14.05) and PQ+antioxidant (11 95%CI 7.77 to 14.23) groups. The increase in the survival of rats in PQ/PF group was insignificant (13.4 95% CI 11.13 to 15.67).
Conclusion: This study showed that PF is able to decrease pulmonary fibrosis following PQ poisoning.

P3766

LSC 2012 Abstract – Endothelial progenitor cells and the endothelin system

Susanna Desole, Florian Albrecht, Helene Vogelsinger, Katharina Cima, Christian Kähler. *Pneumology/USPH Innsbruck, Department of Internal Medicine I, Medical University Innsbruck, Austria*

Bone-marrow–derived endothelial progenitor cells (EPCs) might play a key role in the formation of new vessels. Endothelin-1 (ET-1) is known to modulate different stages of neovascularisation. We investigated a potential link between the ET system and EPCs in pulmonary hypertension (PH).
EPCs were isolated from Sprague-Dawley rats and rat pulmonary artery (paECs) endothelial cells served as positive control. ET-A and B receptor expression and detection of prepro-ET and ET converting enzyme (ECE) mRNA were performed by RT-PCR.
In calcium (Ca2+) flux assays EPCs loaded with FURA-2 were exposed to ET-1 [10^{-6}M and 10^{-8}M]. For selective inhibition of receptor subtypes, EPCs were pre-incubated with ETRA (BQ123) or ETRB (BQ788) antagonists for 20 min before stimulation with ET-1.
EPCs express both ET-receptor subtypes. Both prepro-ET-1 and ECE encoding mRNA could be detected in EPC. In Ca2+ flux experiments addition of ET-1 elicited a significantly increased intracellular Ca2+ flux which could be inhibited by BQ123 (96%) and BQ788 (45%).
We proved for the first time the expression of both ETRA and ETRB and detected mRNA of prepro-ET and of ECE on EPCs. We also found that ET-1 activates Ca2+ flux in EPCs. In summary, our data reveal for the first time a link between EPC and the ET system.