491. Molecular: pathology of infectious and inflammatory lung disease

P4745
Induced sputum differential gene expression implicates increased p38 signalling activity in severe asthma
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Rationale: There is an urgent need for further insight into the characteristics and classifications of severe asthma. A thorough understanding of the mechanisms underlying the disease processes is essential to this.

Objective: To investigate the mechanisms of severe asthma through differential gene expression and pathways analysis of sputum gene expression profiles.

Methods: Induced sputum was collected from participants with severe asthma (n=12), defined by the presence of poor asthma control (ACQ>1) or airflow obstruction (FEV1%predicted<80, FEV1/FVC%<70), despite treatment with high-dose inhaled corticosteroid (>1000μg) and long-acting β-agonist, and compared to mild and moderate asthma (uncontrolled (n=21) and controlled (n=21)) and healthy controls (n=13). Gene expression profiles were generated (Illumina Humanref-8 V2) from sputum RNA and analysed using GeneSpring GX11.

Results: In severe asthma, 1236 genes were altered compared to controlled asthma, and 1723 genes were altered compared to healthy controls. Only 48 genes were altered between uncontrolled and controlled asthma. There was enrichment of genes in the p38 signalling pathway associated with severe asthma compared to both controlled asthma and healthy controls. The transforming growth factor-β receptor, interleukin-2 and epidermal growth factor receptor 1 pathways were also enriched in severe asthma when compared to healthy controls.

Conclusions: Severe asthma is associated with substantial differences in sputum gene expression that underlie unique cellular mechanisms that are not just associated with loss of asthma control. The p38 signalling pathway may be important in the pathogenesis of severe asthma.

P4746
Sputum gene expression of mast cell tryptase and carboxypeptidase A3 are increased in eosinophilic asthma
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Rationale: Little is known regarding the presence and activity of mast cells in the sputum of patients with eosinophilic asthma.

Objective: To investigate the gene expression of mast cell tryptase and carboxypeptidase A3 in sputum from patients with eosinophilic asthma.

Methods: Sputum samples were collected from patients with eosinophilic asthma (n=20) and compared to healthy controls (n=10). Gene expression profiles were generated (Illumina Humanref-8 V2) from sputum RNA and analysed using GeneSpring GX11.

Results: Mast cell tryptase and carboxypeptidase A3 were significantly increased in eosinophilic asthma compared to healthy controls.

Conclusions: Mast cell tryptase and carboxypeptidase A3 are increased in eosinophilic asthma, which may have implications for the pathogenesis of asthma.

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airway lumen across asthma inflammatory phenotypes. Sputum gene expression of the mast cell specific proteases may serve as markers of mast cell activation in asthma.

**Objective:** To investigate if sputum gene expression of mast cell specific proteases is associated with asthma inflammatory phenotype.

**Methods:** Induced sputum was collected from participants with asthma (n=59; eosinophilic n=17; neutrophilic n=12; mixed granulocytic n=12; and paucigranulocytic n=18) and healthy controls (n=17). Gene expression profiles were generated (Illumina Human6-V2) from sputum RNA. Mast cell tryptase (TPSAB1), carboxypeptidase A3 (CPA3) and chymase (CMA1) data were extracted and investigated for their relationship to asthma phenotype.

**Results:** Gene expression of tryptase and carboxypeptidase A3 was significantly different among the 5 groups (p<0.0001). There was no difference in chymase expression. Tryptase mRNA was increased (p<0.01) in eosinophilic asthma compared to neutrophilic asthma, mixed granulocytic asthma, paucigranulocytic asthma and healthy controls. Tryptase (r=0.58 p<0.0001) and carboxypeptidase A3 (r=0.59 p<0.0001) expression was strongly correlated with sputum eosinophils.

**Conclusions:** Gene expression of mast cell tryptase and carboxypeptidase A3 is increased in eosinophilic asthma. Airway mast cells are important in eosinophilic asthma and have a unique phenotype associated with expression of tryptase and carboxypeptidase A3 but not chymase.

**P4747**

**Coordinate regulation of IL-4 and IL-13 expression in human T cells: 3C analysis for DNA looping**

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Over 5.4 million adults receive treatment for asthma in the UK. Asthma is a chronic allergic disorder characterised by chronic inflammation. Type 2 T helper (Th2) are an important component in the development of the asthma and the response to allergens, producing the cytokines interleukin (IL) -4 and IL-13. In Th2 cells, production of the cytokines IL-4, IL-5, and IL-13 in a co-ordinate manner. Th2 cytokine expression is controlled by cooperation between transcription factors GATA-3 and Nuclear factor of activated T-cells -1 (NFAT1).

The genes encoding these cytokines are located together the same chromosomal region, 5q31.1 in humans. We have investigated the co-ordinated expression of IL4 and IL13 in the Th2 model cell line HUT-78. Using chromatin immunoprecipitation (ChIP) and re-ChIP analysis we have shown that in HUT-78 cells GATA-3 is associated with the IL4 and IL13 promoter regions. Additionally, following stimulation CD3/CD28 antibodies NFAT1 rapidly translocates to the nucleus and becomes associated with the promoter regions and GATA3. Finally, using Chromatin Conformation Capture (3C) we demonstrate looping of DNA at the IL4 and IL13 promoters to bring them into close proximity with each other. This suggests that the formation of a transcriptional hub under the control of GATA-3 and NFAT drives co-ordinate Th2 cytokine production.

**P4748**

**microRNA profiling in pulmonary fibroblasts in COPD**

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COPD is characterized by emphysema with loss of extracellular matrix (ECM), and (small) airways disease with increased ECM deposition and airway wall thickening. How both processes can occur in close proximity in one lung is unknown and needs more attention. Fibroblasts are the principal cells involved in ECM production in the lung. MicroRNAs are small RNAs that can cause downregulation of target protein expression. We hypothesize that microRNA-mediated differences between COPD and healthy fibroblasts, and additionally airway and parenchymal fibroblasts, contribute to the airway and parenchymal changes in COPD.

We profiled microRNA expression in pulmonary fibroblasts from severe COPD patients and controls to investigate effects of COPD, smoking (ex-smokers vs current smokers) and fibroblast type (airway vs parenchymal fibroblast). Using linear models we found 42 microRNAs differentially expressed in COPD patients, 25 between ex-smokers and current smokers and 45 between airway and parenchymal fibroblasts in COPD (p<0.01). Interestingly some of the microRNAs differentially expressed in COPD fibroblasts, i.e. mir-181d and mir-206-5p, are also differentially expressed in lung tissue in relation to emphysema severity (Christenson, S.A. et al. Am J Resp Crit Care Med 2010;181:A2024). COPD and smoking had similar effects on several microRNAs, including mir-181d, mir-206-5p, mir-296-5p, mir-296-1*, mir-23* and mir-202, suggesting a possible mechanism for the link between smoking and COPD development. Furthermore, mir-155 expression was decreased in COPD and within COPD in airway fibroblasts. Given the role of mir-155 in airway remodeling (Rodriguez, A. et al. Science 2007; 316:608-11) this microRNA could be important in the airway changes in COPD.
patients. We have recently identified overexpression of von Hippel-Lindau (VHL) mRNA and protein in skeletal muscles of COPD patients. This would exert a negative impact on muscle capillarization by impairing the signal transduction of hypoxia-induced factor (HIF) to vascular endothelial growth factor (VEGF). It is unclear if VHL overexpression is a primary event induced by cigarette smoke (CS) or if it occurs secondary to development of COPD.

Results: Exposure of 293FT/WT mice to cigarette smoke induced a significant overexpression of HIF-1 mRNA (p<0.03 vs. controls). Likewise, significant VHL mRNA and protein overexpression were elicited (p<0.0003 vs. controls). By contrast, VEGF mRNA expression was not altered (p>0.88).

Conclusion: The current results demonstrate VHL overexpression in skeletal muscles of normal mice exposed to CS. This is in analogy and further confirms our recent findings in skeletal muscles of COPD patients. Thus VHL overexpression appears to be an early primary event related to CS exposure rather than a secondary occurrence to development of the COPD pathology.

P4752 BAMI B—— A TGF-β pseudoreceptor with possible functional involvement in COPD and NTHI infection

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TGFB-β signaling in non-malignant pulmonary diseases to date is still controversially discussed. Nontypeable Haemophilus influenzae infection (NTHI) may play a role in the pathogenesis of chronic obstructive pulmonary disease (COPD) as an infectious trigger. Complementary new data are available regarding the influence of acute and persistent infection on tissue remodeling and repair factors such as transforming growth factor (TGF)-β. Here we show that the TGF-β pseudoreceptor BAMI is expressed in the human lung.

NTHI infection was analyzed in lung tissues obtained from COPD patients and controls utilizing a human ex vivo and in vitro models. Detection of NTHI was achieved by in situ hybridization (ISH). For characterization of TGF-β signal- ing molecules, immunohistochemistry (IHC), ISH and RT-PCR were performed. Expression of the TGF-pseudoreceptor BMP and Activin Membrane-bound Inhibitor (BAMI) was analyzed using immunohistochemistry (IHC), ISH and RT-PCR.

BAMI mRNA and protein expression were evaluated in lung tissue and cell culture using ELISA. An infection with NTHI was detected in vivo in 38% of the COPD patients in contrast to 0% of controls. Transcriptome arrays showed no significant changes of TGF-β receptors 1 and 2 and Smad-3 expression, whereas a strong expression of BAMI with upregulation after in vitro infection of COPD lung tissue was demonstrated. BAMI was expressed ubiquitously on alveolar macrophages (AM) and included in alveolar capillaries (ACE).

We show for the first time a considerable expression of the TGF pseudoreceptor BAMI in the human lung. BAMI is upregulated in response to NTHI infection in COPD lung tissue in vivo and in vitro.

P4753 Elevated osteoprotegerin in COPD is potentially regulated by oxidative stress dependent glycosamin glycan synthase kinase-3β and β-catenin signalling

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We recently reported that spumus osteoprotegerin (OPG) in COPD is higher than that in controls and is a potential biomarker in COPD (To M et al. CHEST 2010 Dec 2 [Epub ahead of print]). However, the molecular mechanism involved in OPG elevation in COPD was not clarified yet. Here we investigated the role of glycosamin glycan synthase kinase-3β (GSK-3β)- and β-catenin in OPG transcription, especially under oxidative stress.

AsA49 cells, an alveolar epithelial cell line, were treated with cigarette smoke condensate (CSCM) and β-catenin protein was determined by western blot. CSCM induced OPG release (measured by ELISA) with a maximum induction of 147% and also increased β-catenin protein expression in a concentration-dependent manner (295±0.8%). CSCM also induced maximum induction of 161% in A549 cells, which were transfected with β-catenin siRNA using lipofectamine for specific knockdown (KD) of β-catenin, and IL-1β-stimulated OPG production by the A549 cells was measured by ELISA. β-catenin KD led to lower OPG production by 90% in A549 cells compared to baseline with IL-1β stimulation compared to non-transfected controls (46±13.1 pg/ml in β-catenin KD and 1712.4±55 pg/ml in wild type without IL-1β, and 1231.3±37 pg/ml in β-catenin KD and 2669.1±165 pg/ml in wild type under IL-1β stimulation). The inhibition of β-catenin expression significantly increased OPG production with a maximum induction of 161% and with EC50 of 1.2 mM.

Thus, defect of GSK-3β causes β-catenin expression, leading upregulation of OPG. This will be a new insight on molecular mechanism of COPD pathogenesis.

P4754 Overexpression of chitinase 3-like 1 (YKL-40) in lung-specific IL-18-transgenic mice, smokers and COPD

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In this study, we analyzed the lung mRNA expression profiles of murine model of COPD, lung-specific IL-18-transgenic mice using microarray analysis. In the IL-18-transgenic mice, the expression of 608 genes was found to fluctuate more than 2-fold in comparison with control WT mice, and was clustered into 4 groups. The expression of 140 genes was constitutively increased at all ages, that of 215 genes increased gradually with aging, that of 171 genes decreased gradually with aging, and that of 82 genes decreased temporarily at 9 weeks of age. Interestingly, the levels of mRNA for the chitinase-related genes chitinase 3-like 1 (Chi3l1), Chi3l3, and acidic mammalian chitinase (Chi3l4) were significantly higher in the lungs of transgenic mice than in control mice. The level of Chi3l1 protein increased significantly with aging in the lungs of IL-18 transgenic, but not WT mice. Previous studies have suggested that Chi3l3 and AMC are IL-15-driven chitinase-like proteins. However, IL-13 gene deletion did not reduce the level of Chi3l1 protein in the lungs of IL-18 transgenic mice. Furthermore, the protein level of YKL-40, the human homolog of Chi3l1, was significantly (P<0.01) higher in Remin samples from smokers (318±37.0 pg/ml, n = 28) and COPD patients (268±9.3±32 ng/ml, n = 45) than in those from non-smokers (177±8.2±22 ng/ml, n = 30). In COPD patients, there was a significant negative correlation between serum level of YKL-40 and%FEV1. Chitinase-related genes may play an important role in establishing pulmonary inflammation and emphysema changes.

Rationale: Insufficiency in repair capacity of fibroblasts might contribute to this phenomenon. In this study, the stimulatory effect of TGF-β1 on proliferation and elastin production was investigated in human primary lung fibroblasts.

Methods: The subjects were classified into COPD and control group according to GOLD criteria. Fibroblasts were cultured from lung tissue. Proliferation was measured with AlamarBlue method. mRNA and protein level for elastin was measured using real-time RT-PCR and Fastin Elastin assay respectively. The percentage of increase of proliferation and elastin production with 1ng/ml TGF-β1 was calculated for fibroblasts from each subject.

Results: Totally 18 subjects were included, with 8 in control group and 10 in COPD group. There were no significant differences between the two groups in age, gender or smoking history. FEV1 for COPD group and control group was 1.89±0.3 vs 1.74±0.2 (p=0.01) in smoking and 1.21±0.2 vs 1.07±0.1 (p=0.05) in nonsmokers. COPD patients showed a slower proliferation than control fibroblasts, with a reduced response to TGF-β1 treatment (the percentage of increase being 123±2.5% vs 174±1.7%, p=0.0001).

The percentage of increase in proliferation was positively correlated with FEV1 (p=0.009). Despite an increase in the mRNA expression and protein secretion for elastin, the promotive effect of TGF-β1 on elastin synthesis which was observed in fibroblasts from control subjects was diminished in fibroblasts from COPD patients.

Conclusions: Our findings suggested that COPD fibroblasts have a decreased response to TGF-β1 in both proliferation and elastin secretion, supporting the impaired repair mechanism in the development of COPD.
P4757
Adrenergic receptor blocker reverses cardiac metabolic remodeling and improves right ventricular function in experimental pulmonary hypertension
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Albeit right ventricular failure (RVF) is a major complication in patients with pulmonary arterial hypertension, the pathobiology of RVF is still incomplete and RV-targeted therapies are practically non-existent. Since a metabolic switch has been reported for left heart failure, we sought to describe any potential changes in cardiac fuel metabolism and mitochondrial dysfunction in RVF. PAH was induced in S-D rats by a combination of a VEGF-receptor blocker followed by 4 weeks of 10% hypoxia (SuHx). Gene expression was evaluated by RT-PCR and confirmed by western blot. T Tests were performed to determine whether any of the protein fragments generated following neutrophil activation or the addition of neutrophil elastase were significantly different in the treatment group, yet unchanged in the placebo status (base deficit) approached the baseline values of normoxic animals. Molecu

Results: 12 spectra of the EXACTLE patients. T Tests were performed to determine whether any of the protein fragments generated following neutrophil activation or the addition of neutrophil elastase were significantly different in the treatment group, yet unchanged in the placebo status (base deficit) approached the baseline values of normoxic animals. Molecu

Conclusion: Our data underpin the importance of miR-20a in the BMP signaling pathway and emphasize the need for further studies to address the therapeutic potential of antagonists for the human disease.

P4758
Plasma protein profiling in alpha-1-antitrypsin deficiency
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Autophagy is the major cellular route for degradation of long-lived proteins and plays an important role in the survival of different cells. It is classically described as a process of mitochondrial self-degradation. In some settings, however, autophagy is known to be an important mechanism in the cellular response to stress and it is involved in the pathogenesis of many diseases. In this study, we investigated the role of autophagy in the response of lung fibroblasts to hypoxia in idiopathic pulmonary fibrosis (IPF), a chronic, severe, and life-threatening lung disease that affects more than 400,000 Americans, and is characterized by an excess of fibroblasts in the lungs.

Methods: Mass spectrometry was used to identify proteolytic fragments in plasma following neutrophil activation by ionophore or the addition of neutrophil elastase (NE). 72 pairs of plasma samples from the EXACTLE trial, taken at baseline and 6 months, were subsequently analysed using weak cation exchange beads and MALDI-TOF-MS, to determine whether any of the protein fragments generated in vitro were present in clinical samples and changed significantly in response to augmentation.

Results: Following neutrophil activation or the addition of NE to plasma 325 peptides from 32 proteins were identified. 207 peptide peaks were detected in the spectra of the EXACTLE patients. T Tests were performed to determine whether any of the peak intensities of the peptides changed with treatment. Of these, 12 were significantly different in the treatment group, yet unchanged in the placebo group. The most significant of these had a p value of 2.08E-05.

Conclusion: Changes in the plasma peptidome can be found in patient plasma after the addition of calcium ionophore and NE. Some of these changes can be detected in plasma pre/post augmentation. We aim to use more in depth proteomic approaches to further characterise changes in the plasma peptidome, and determine clinical utility as a guide to monitoring and treatment in ATD.

P4759
TRPA1 expression and characterisation in patients with chronic cough
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Aim: The TRPA1 ion channel is thought to have an important role in the cough reflex. It has been shown that inhalation of cinnamaldehyde, a specific agonist of TRPA1, induces cough in normal human volunteers. We wanted to determine the expression and characterisation of the TRPA1 receptor in human lung tissue.

Methods: Bronchial biopsies were obtained from patients with the Cough Hypersensitivity Syndrome on fibreoptic bronchoscopy. Lung resection samples were obtained from patients undergoing lung resection for lung cancer. Dorsal root ganglia were used as a positive control. These tissue samples were analysed by immunohistochemistry with a specific TRPA1 antibody.

Results: Samples from 14 patients (11 males, mean age 56 years) with the Cough Hypersensitivity Syndrome and 10 lung resection samples (6 males, mean age 68 years) were obtained. All the tissue samples stained for TRPA1. The TRPA1 stain was avidly taken up by the bronchial epithelium, smooth muscle bundles and nerve tissue.

Conclusion: We have described the distribution of TRPA1 ion channel in lung tissue. TRPA1 is agonised by several environmental irritants and endogenous mediators of inflammation. The presence of TRPA1 ion channel in bronchial mucosal nerves and epithelium suggests an important role in the cough reflex. The localisation of these ion channels on smooth muscle could suggest a role in asthmatic inflammation as well.

P4760
Expression of the Bcl-2 binding protein beclin 1 in human idiopathic pulmonary fibrosis
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Introduction: Alpha-1-antitrypsin deficiency (ATD) results in an imbalance of proteases and antiproteases with proteolytic destruction of lung parenchyma, and subsequently early onset emphysema. We hypothesised changes in plasma peptidome with emphysema.

Methods: Mass spectrometry was used to identify proteolytic fragments in plasma following neutrophil activation by ionophore or the addition of neutrophil elastase (NE). 72 pairs of plasma samples from the EXACTLE trial, taken at baseline and 6 months, were subsequently analysed using weak cation exchange beads and MALDI-TOF-MS, to determine whether any of the protein fragments generated in vitro were present in clinical samples and changed significantly in response to augmentation.

Results: Following neutrophil activation or the addition of NE to plasma 325 peptides from 32 proteins were identified. 207 peptide peaks were detected in the spectra of the EXACTLE patients. T Tests were performed to determine whether or not the peak intensities of the peptides changed with treatment. Of these, 12 were significantly different in the treatment group, yet unchanged in the placebo group. The most significant of these had a p value of 2.08E-05.

Conclusion: Changes in the plasma peptidome can be found in patient plasma after the addition of calcium ionophore and NE. Some of these changes can be detected in plasma pre/post augmentation. We aim to use more in depth proteomic approaches to further characterise changes in the plasma peptidome, and determine clinical utility as a guide to monitoring and treatment in ATD.