472. Interstitial lung disease: from bench to bedside

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Clinical features common to five cases with secondary pulmonary alveolar proteinosis complicated with Behcet disease

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Pulmonary alveolar proteinosis (PAP) is a rare lung disorder characterized by abnormal accumulation of surfactant materials in the lower respiratory tracts. It is classified into three distinct types according to etiology; autoimmune, secondary, and congenital PAP. Secondary PAP (SPAP) comprises ten percent of acquired PAP. Previously, we reported 40 cases of SPAP, in whom more than 70% occurred secondary to hematological disorders, with the majority being myelodysplastic syndrome (MDS). The present study focused clinical features of five patients (four female and one male) who developed PAP during 6 months to 18 years after the onset of Behçet's disease (BD), with underlying trisomy 8-positive MDS in four of them. Oral and cutaneous BD lesions were involved in all cases, but ocular lesions were observed in only one case. Intestinal BD was recognized in three patients who had undergone potent immunosuppressive therapy that resulted in overwhelming sepsis. In the two surviving patients, PAP and BD were managed successfully, although both individuals had a high risk of MDS at diagnosis. Thus, we figure out the common and different points among SPAP patients complicated with BD. The differential diagnoses of SPAP should be ruled out when lung complications are encountered during the course of BD.

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LSC 2012 Abstract – Phenotypic profiling of invading lung fibroblasts in 3D cell culture models

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Rationale: Fibroblasts exhibit an extraordinary capacity to undergo phenotypic changes during development and disease, both in vitro and in vivo. These changes include altered motility, migration or activation. Enhanced migratory capacity of primary lung fibroblasts (lfs) in IPF patients was found in vitro, but the underlying mechanisms remain elusive. The aim of this study was to decipher morphological, molecular and functional differences between invading (i) and non-invading (n-i) lfs in 3D cell culture models.

Methods/Results: We established a high-content 3D invasion model, enabling the separation of i from n-i lfs that allows the comparative analysis of parameters like morphology, invasion depth and protein/mRNA expression levels. Analysis revealed two significantly distinct subtypes. 7.62% of untreated lfs invaded the collagen matrix. Invasion was augmented by TGFβ1 and EGF treatment. Gene expression analysis of i vs n-i lfs demonstrated significantly different expression profiles. Several markers, previously reported to be associated with IPF (MMP13 (ex. ratio=4.47), MMP3 (3.97), Osteopontin (1.45), Pten (0.34)) and genes of unknown function, were found deregulated in i lfs.

Conclusion: Lfs show two distinct subtypes in a 3D cell culture model. Gene expression profiling of i lfs revealed features highly similar to the (myo)fibroblast phenotype found in IPF. Our 3D invasion model constitutes a highly useful tool for high-content pharmacological screenings.

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LSC 2012 Abstract – Age-related changes in the relative expression of functional genes in mesenchymal stem cells

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Age-associated changes increased susceptibility to a variety of lung pathologies. Recently, mesenchymal stem cells or B-MSCs have emerged as a critical reparative response mechanism to lung injury. We had demonstrated an association, in animal models, between age and an increase in the susceptibility to injury and we had identified functional differences between B-MSCs from young and old mice.In the present study, we examined the consequences of aging in the gene expression.

Methods: 3 and 24 months old B6 mice were sacrificed and B-MSC were isolated according to the expression of the surface markers. RNA was obtained after sorting and used for hybridization on Agilent gene expression microarrays. Ingenuity software was used to determine functional enrichment. For statistical analysis we applied significance analysis of microarrays. A qvalue of 5, which corresponds to a 5% false discovery rate, was used as a cutoff of statistical significance. Gene validations were performed by RT-PCR.

Results: We demonstrate that aging induce a decrease in the gene expression on B-MSC. The mechanisms affected by the decrease on gene expression includecellular trafficking, cellular growth and proliferation.

Conclusion: Old B-MSCs have a different expression profile that exhibits a decrease in the expression of genes that control importan B-MSC functions.

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LSC 2012 Abstract – Surfactant protein A in chronic extrinsic allergic alveolitis

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Chronic form of extrinsic allergic alveolitis (EAA) may have common features with idiopathic pulmonary fibrosis (IPF). The aim of presented study was to compare serum SP-A concentrations in IPF and chronic EAA patients and detect possible relationships of SP-A levels, bronchoalveolar lavage fluid (BALF) differential cell counts and high resolution computed tomography (HRCT) patterns in both diseases.

Thirteen patients with chronic EAA and 7 patients with IPF were enrolled. All subjects underwent evaluation of SP-A serum concentrations, HRCT of the chest and BALF. HRCT alveolar and interstitial scores (HRCTa, HRCTi) were assessed according to Gay S[1].

EAA patients had significantly higher HRCT alveolar score then IPF group (p=0,003). Chronic EAA group exhibited positive correlation between HRCTi and BALF eosinophils (p<0,01) [Fig. 1]. Serum SP-A concentrations did not differ between both groups.

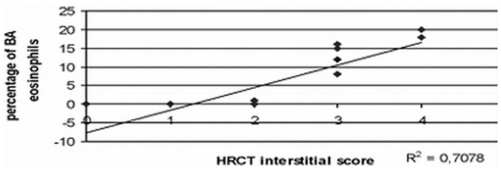

Figure 1. Positive correlation between HRCTi and BALF eosinophils in chronic EAA patients.

Our study shows that SP-A serum concentrations do not differ between chronic EAA and IPF patients and thus should not be used as a biomarker for IPF detection. Prognostic value of serum SP-A concentrations in chronic EAA patients should be the aim of further studies.

Reference:

[1] Gay SE, et al. Idiopathic pulmonary fibrosis: predicting response to therapy and survival. Am J Respir Crit Care Med 1998; 157:1063-72.

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LSC 2012 Abstract – The cell-penetrating P1pal-12 pepducin limits pulmonary fibrosis in the murine bleomycin model

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Idiopathic pulmonary fibrosis is the most devastating fibrotic diffuse parenchymal lung disease which remains refractory to available pharmacological therapies.Therefore, novel treatment options are urgently required. Protease-activated receptor (PAR)-1 is a heptahelical G protein-coupled receptor that mediates critical signaling pathways in pathology. Interestingly, bleomycin-induced lung fibrosis was shown to be diminished in PAR-1 deficient mice. We thus hypothesized that pharmacological PAR-1 inhibition may be an interesting therapeutic approach to combat pulmonary fibrosis. Consequently, we explored the effect of P1pal-12 (a pepducin blocking the PAR-1/G-protein interaction) during the development of lung fibrosis induced by intranasal instillation of bleomycin. We show that once daily treatment with 0.5, 2.5 or 10 mg/kg P1pal-12, reduced severity and extent of fibrotic lesions in a dose-dependent manner (2.5 and 2 fold reduction with 2.5 and 10 mg/kg). These findings correlated with significant decreases in fibronectin, collagen and α-SMA mRNA expression levels in treated mice. Moreover, fibrin deposition in the lungs was reduced by 26% ± 3% ($p<0.05$) in 2.5 mg/kg treated mice compared to untreated controls. Finally,P1pal-12 reduced bleomycin-induced IL-6 and MCP-1 levels in lung homogenates by 65±3% ($p<0.01$) and 36±3% ($p<0.05$) respectively. Overall, our data show that P1pal-12 limits lung fibrosis suggesting that targeting PAR-1 may be a promising therapeutic strategy for pulmonary fibrosis.

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Effect of nintedanib on silica-induced lung inflammation and fibrosis in mice

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Introduction: One-year treatment with the receptor tyrosine kinase inhibitor nintedanib (BIBF 1120) specific for vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR) was associated with a 68.4% reduction in the rate of decline of forced vital capacity in patients with idiopathic pulmonary fibrosis (IPF) versus placebo, which approached statistical significance.
Aim: To further explore its mode of action, nintedanib was tested in a mouse model of silicosis displaying ongoing pulmonary inflammation and fibrosis.
Methods: Within 30 days a single intranasal administration of silica caused a robust lung inflammation with a significant increase in macrophages, neutrophils and lymphocytes in the BALF, increased IL-1 beta, CXCL1/KC and TIMP1 production, and increased collagen deposition in the lung. Histologic analysis revealed chronic inflammation with granuloma formation and fibrosis demonstrated by collagen staining.
Results: Nintedanib administered by gavage at 30 and 100 mg/kg/day significantly reduced neutrophil and lymphocyte counts, but had no effect on macrophage counts in the BALF.
Furthermore, IL-1 beta, CXCL1/KC, TIMP1, collagen in lung and lung inflammation with granuloma and fibrosis were drastically reduced.
Conclusion: Nintedanib effectively reduced silica-induced chronic inflammation and fibrosis in mice. The anti-inflammatory and anti-fibrotic features of nintedanib may impact the progressive course of fibrotic lung diseases like IPF or silicosis.

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Telomere (TL) shortening is associated with disease severity in scleroderma (SSC) associated interstitial lung disease (ILD)

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Attrition of TL is associated with the development and progression of pulmonary fibrosis. TL length has been shown to be reduced in individuals with SSC *vs* healthy controls. ILD develops in over 25% of individuals with SSC. We hypothesized that TL shortening is an important mechanism driving the pathogenesis of SSC-ILD.
Methods: Whole blood was collected from SSC-ILD patients (n=132). SSC-ILD was defined as extensive or limited disease (Goh et al, AJRCCM 2008;177). DNA, isolated using a Promega extraction kit, was analysed using quantitative real time PCR. TL length was calculated using the method described by O'Callaghan and Fenech (Biol Proced. 2011; 31).
Results: Mean±SEM TL length in SSC cohort was 65.1±4.7 kb/diploid genome. In limited disease (n=100, 74 female, age 53.2±1.1 yrs) mean TL length was 77.0±5.5 kb/diploid genome (see figure 1). In extensive disease (n=32, 18 females, age 46.5±3.5 yrs), the mean TL length was 24.8±3.5 kb/diploid genome($p<0.0001$). TL length correlated with extent of fibrosis on CT ($p<0.001$). TL typically shorten with age. In our cohort TL length increased with age($p=0.02$) perhaps reflecting a trend towards more extensive disease in younger subjects.
Conclusion: TL length is significantly associated with disease extent in SSC-ILD.

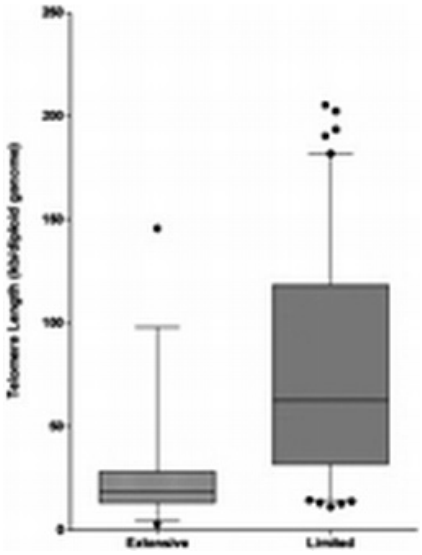

Figure 1. Comparison of telomere length in individuals with limited and extensive SSC associated ILD. Box and whisker plot demonstrating mean and 95% confidence intervals with outliers.

Our observation suggests an important role for premature cellular senescence in the pathogenesis of SSC-ILD.
HA is an ERS-Fellow.

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HSP47siRNA targeting to myofibroblasts attenuates bleomycin-induced pulmonary fibrosis

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Rationale: The 47-kDa heat shock protein 47 (HSP47) plays a role in the processing of procollagens as a collagen molecular chaperone. The HSP47-positive cells increase in lung fibrosis, which suggests an important role of HSP47 in the pathogenesis of lung fibrosis. It is expected that to control the expression of the HSP47 may reduce the lung fibrosis. Lung myofibroblasts are speculated vitamin A (VA)-storing cells in the lungs like hepatic stellate cells. Based on these properties of myofibroblasts, we prepared a VA-coupled liposomes carrying HSP47siRNA. The goal of this study is to investigate whether the inhibition of HSP47 for the myofibroblasts with siRNA influences to lung fibrosis.
Methods: Sprague-Dawley male rats were treated with intra-tracheal bleomycin (BLM) or PBS. We injected VA-coupled liposomes carrying HSP47siRNA into rat tail vein three times in a week. We made 5 groups; PBS intratracheally & PBS intravenously, BLM & PBS, BLM & VA-HSP47siRNA, BLM & HSP47siRNA, and BLM & VA-randomRNA, respectively. We measured the contents of lung hydroxyproline, BAL cells counts, HSP47 expression, and cytokines.
Results: Western blotting showed the expressions of HSP47 were reduced in rats treated with VA-coupled liposomes carrying HSP47siRNA. The liposomes with HSP47siRNA significantly improved lung fibrosis morphologically and the increases of hydroxyproline contents in the lungs, the inflammatory cytokines, and the number of BAL cells.
Conclusions: These results suggest that HSP47siRNA improves bleomycin-induced lung fibrosis and this drug delivery system is useful methods.