472. Interstitial lung disease: from bench to bedside

4526 Clinical features common to five cases with secondary pulmonary alveolar proteinosis complicated with Behçet 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, in whom more than 70% occurred secondary to hematological disorders, with the majority being myelodysplastic syndrome (MDS). The present study focused on clinical features of five patients (four female and one male) who developed PAP between 6 months to 18 years after the onset of Behçet’s disease (BD), with underlying trilogy 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 investigate 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.

Methods/Results: We established a high-content 3D invasion model, enabling the separation of lfs from n-i lfs that allows the comparative analysis of parameters like morphology, invasion depth and protein/RNA 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 lfs vs n-i lfs demonstrated significantly different expression profiles. Several markers, previously reported to be associated with IPF [MMPI1 (ex. ratio=4.47), MMP3 (3.97), Osteopontin (1.45), Pten (0.34)] and genes of unknown function, were found deregulated in lfs.

Conclusion: Lfs show two distinct subtypes in a 3D cell culture model. Gene expression profiling of 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.

4527 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: We found a high-content 3D invasion model that enables to differentiate primary lung fibroblasts in vitro. Analysis of lfs vs n-i lfs revealed different expression profiles. Several markers, previously reported to be associated with IPF [MMPI1 (ex. ratio=4.47), MMP3 (3.97), Osteopontin (1.45), Pten (0.34)] and genes of unknown function, were found deregulated in lfs.

Conclusion: Lfs show two distinct subtypes in a 3D cell culture model. Gene expression profiling of 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.

4528 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 pathogens. Recently, mesenchymal stem cells or 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. Microarray expression analysis was performed. Results: We demonstrate that aging induce a decrease in the gene expression on B-MSC. The mechanisms affected by the decrease on gene expression include cellular 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.

4529 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.

Chronic EAA patients had significantly higher HRCT alveolar score then IPF group (p<0.003). Chronic EAA group exhibited positive correlation between HRCTal and BALF sp-A levels (p<0.01) [Fig. 1]. Serum SP-A concentrations did not differ between both groups.

Idiopathic pulmonary fibrosis is the most devastating fibrotic parenchymal lung disease which remains refractory to available pharmacological therapies. Therefore, novel treatment options are urgently needed. 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 expression levels in treated mice. Moreover, collagen 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. Our data show that P1pal-12 limits lung fibrosis suggesting that targeting PAR-1 may be a promising therapeutic strategy for pulmonary fibrosis.

4532
Telomere (TL) shortening is associated with disease severity in scleroderma (SSC) associated interstitial lung disease (ILD)

Introduction: Telomere shortening plays a role in the pathogenesis of chronic lung disease, which may be related to the decreased proliferation capacity of alveolar epithelial cells, leading to an increase in the rate of age-related fibrosis. Thus, we hypothesized that telomere shortening is associated with disease severity in SSC-ILD.

Methods: We measured telomere length (TL) by Southern Blot and quantitative PCR in 79 SSC-ILD patients and 30 healthy controls. A regression analysis was performed to determine the relationship between TL and different parameters of disease severity.

Results: In SSC-ILD patients, shorter TL was associated with higher disease activity score (p=0.01) and a lower performance status (p=0.04). A logistic regression analysis showed that TL was independently associated with disease severity (p=0.01).

Conclusion: Telomere shortening is associated with disease severity in SSC-ILD and may represent a potential biomarker for disease activity and progression.

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