43. New insights into the pathogenesis of idiopathic pulmonary fibrosis and sarcoidosis

194
Defective p53-mediated pro-apoptotic pathway in idiopathic pulmonary fibrosis

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Cell death by apoptosis represents a crucial factor in lung homeostasis, repair and remodeling in a variety of pulmonary pathologic processes. Therefore, apoptosis within the lung is becoming a growing area of interest. Idiopathic pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP) is a progressive lung disease characterized by fibroblast proliferation, extracellular matrix deposition and progressive lung function impairment. Because effective therapeutic strategies still remain limited research has been directed toward new lung cell putative targets for novel therapeutic options. Attention has been focused on lung cell functions as fibroblast phenotype, proliferation and activity. Cell nucleus contains several substructures as nuclear bodies which include critical regulators of cell proliferation, apoptosis and genome stability. Homeodomain-interacting protein kinase HIPK2 represents a transiently recruited protein that contributes to p53 foforilation that lead to cell cycle arrest and apoptosis.

In our study we analyzed HIPK2 gene dysfunction in human lung fibroblasts micro-dissected from fibroblastic foci of IPF/UIP using molecular biology and immune-histochemical techniques. We demonstrate that fibroblasts from informative IPF/UIP patients have the allelic deletion (LOH) of microsatellites inside HIPK2 gene as compared to bronchial epithelial cells micro-dissected from the same patients. Furthermore, low level of HIPK2 protein was detected in human lung fibroblasts from IPF/UIP patients in comparison with normal ones. These results suggest that altered IPF/UIP fibroblasts growth and function may be also related to defective apoptosis-regulatory genes.

195
Nanoparticle-assisted gene delivery in a bleomycin-injured lung fibrosis model

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Pulmonary fibrosis (PF) is a progressive and usually fatal disease for which no specific therapy exists to date. Increasing evidence suggests that changes present in IPF result from sequential alveolar epithelial injury and abnormal alveolar wound repair. Complex cell interactions, mediators, and growth factors are involved in this process. Hepatocyte growth factor (HGF) has been implicated in the prevention and treatment of PF. We have previously demonstrated that gene transfer of hHGF through *in vivo* electroporation attenuated PF. For future clinical applications, gene therapy applications require safe and efficient methods for gene delivery. Biocompatible NP may therefore represent an efficient, safe, yet minimally invasive method for gene delivery in the respiratory tract. Polyvinylalcohol (PVA) coated super-paramagnetic iron oxide NPs (PVA-SPIONs) were fluorochrome-labelled and unilaterally administered to the left lung 7 days after bleomycin-induced lung injury in a rat model. After 24 hours NP deposition in fibrotic lung tissue was evaluated by confocal microscopy. In preliminary proof-of-concept studies, confocal microscopy showed homogenous NP deposition in the lung preferentially confined to the alveolar epithelium and macrophages without overt histological evidence for alveolar inflammation. Further EM studies are performed for a detailed characterisation of PVA-SPION-cell interactions. Thus initial experiments showed a preferential deposition of NP to alveolar epithelial cells that are a target for novel gene therapies in lung fibrosis. Engineered SPIONs may therefore provide a novel approach for gene delivery in pulmonary fibrosis.

196
Expression of angiogenic growth factors and stromal cell derived factor-1/CXC chemokine receptor 4 (SDF-1/CXCL12 – CXCR4) biological axis in idiopathic pulmonary fibrosis: a lung tissue study

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Background and aim of the study: Idiopathic pulmonary fibrosis (IPF) is associated with aberrant repair, persistence of collagen deposition, and the development of vascular remodeling. However, the role of angiogenesis in the pathogenesis of IPF is still undetermined. The aim of this study was to evaluate the combined mRNA expression of vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2 (FGF2), insulin-like growth factor 1 (IGF1) epidermal growth factor (EGF) and its receptor (EGFR) in lung tissue. In addition, we have investigated the expression of chemokine CXCL12/stromal cell-derived factor-1 (SDF-1) and its receptor, CXCR4, in order to identify alterations that maybe implicated in the pathogenesis of IPF.

Methods: Expression analysis of the aforementioned growth factors and biological axis CXCL12/CXR4 analysis was performed using real-time RT-PCR. The subjects studied consisted of two distinct groups: patient with IPF (n = 25) and subjects (control) undergoing thoracic surgery for reasons other than interstitial lung disease (n = 10).

Results: IGF-1, EGF and FGF2 mRNA levels are significantly decreased in the patients with IPF compared to the controls (P=0.028, P=0.023 and P=0.009). CXCL12 TR1 and CXCL12 TR2 transcript levels were significantly lower in patients compared to controls (p=0.017 and p=0.001). EGFR and CXCR4 mRNA levels were not significantly different between patients and controls.

Conclusion: Our results show an important downregulation in angiogenetic mechanisms in IPF.

197
Expression of the biological axis VEGF-CXCL12/CXCR4 in bone marrow mesenchymal stem cells (BM MSCs) in patients with idiopathic pulmonary fibrosis (IPF)

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Objective: The source of lung fibroblasts is a critical question in the pathogenesis of fibrotic lung disorders. Recent evidence indicates that a significant proportion of the mesenchymal cells may be derived from extrapulmonary sources. A hypothesis could be the possibility that the mesenchymal stem cells (MSCs) may be derived from circulating fibrocytes.

Aim: This study investigates the reserve and function, the molecular and proteomic profile of BM MSCs and the expression of the biological axis CXCL12/CXCR4 in patients with Idiopathic Pulmonary Fibrosis (IPF).

Methods: We evaluated the frequency of MSCs in the BM mononuclear cell fraction using a limiting dilution assay. We have also assessed the molecular and proteomic characteristics in terms of inflammatory cytokine gene and protein expression of BM MSCs (VEGF). Total RNA isolated from differentiated MSCs (RNeasy mini kit;QIAGEN, GmbH,Hilden,Germany) was reverse transcribed (SUPERSCRIPT II;Gibco) and amplified by PCR (RT-PCR).

Results: MSCs from IPF patients (n=10) and from age-/sex-matched healthy individuals (n=10) were similar in frequency, differentiation potential, survival, immunophenotypic characteristics, and protein profile. However, a significant increase in the mRNA expression has been detected in both CXCL12 and CXCR4. In addition, no statistical difference was found in the VEGF expression between patients and controls.

Conclusion: Our results are in agreement with the hypothesis that MSCs stem cells may be involved in the pathogenesis of IPF, via activation of the biological axis CXCL12/CXCR4.

198
NIR spectroscopy of penetrating light and its diagnostic value in idiopatic pulmonary

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We describe new method of noninvasive evaluation of lung parenchyma by NIR spectroscopy of penetrated light. Our technical solution consists of external source of NIR emission and NIR spectroscope with flexible cannula containing spectroscopic probe which is inserted into the working channel of the bronchoscope. Spectroscopic readings from the probe taken during bronchoscopic procedure bring information about the quality of lung parenchyma. Such an approach seems to be feasible in our preliminary study elucidating the NIR spectroscopy differences in UIP versus healthy persons. Study population consisted of 12 healthy voluntiers and 7 patients with UIP. Table 1. shows mean differences between the groups for evaluated parameters and its statistical power.

Table 1

	Healthy		Pathological		t-Test	
	Y1–	σ^2(Y1)	Y2–	σ^2(Y2)	Y1– - Y2–	P(H1:Y1– - Y2–)≠0
τn–	0,80	0,0008	0,547	0,061	0,253	0,045
Δmaxτn	0,44	0,0052	0,763	0,152	-0,319	0,092
σ^2τn	0,115	0,0008	0,203	0,0075	0,108	0,048

τn–: mean value of standardized transmittance. Δmaxτn: maximal difference of standardized transmittance. σ^2τn: variance of standardized transmittance.

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Results show good diagnostics value of the method in discrimination between normal and UIP afflicted tissue. Abbreviations: NIR – near infrared.

199

A possible role for toll-like receptor (TLR) 5 in fibrotic pulmonary sarcoidosis

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Rationale: Sarcoidosis is a systemic disease of unknown aetiology. Bacteria, such as *Mycobacterium tuberculosis*, might play a role in disease pathogenesis. The innate immunity receptor Toll-like receptor (TLR) 5 recognizes flaggelin and seems important in the immune response against bacteria (Hayashi et al, *Nature. 2001 Apr 26;410(6832):1099-103*). We speculate that genetic differences altering TLR-5 function play a role in disease pathogenesis and are more prevalent in (subgroups of) sarcoidosis patients.
Methods: We studied the frequency of three single nucleotide polymorphisms (SNPs) in the TLR-5 gene in 160 sarcoidosis patients and 194 healthy controls, all Caucasian (Rs 5744168, Rs2072493 and Rs5744174). Sarcoidosis patients were patients with Löfgren's syndrome (n=57) or non-Löfgren sarcoidosis (n= 103), stratified as acute/self-remitting disease (n=45) or chronic disease without (n=35) and with fibrosis (n=23) according to radiographic evolution over a 4-year follow-up period.
Results: In the group without Löfgren's syndrome, patients with fibrotic sarcoidosis had a significant decrease in T allelic carriage of the polymorphism Rs5744174 compared to patients without fibrosis and healthy controls (T carriage= 52%, 85% and 84% respectively, p= 0.007 and 0.003). The prevalence of T carriage in patients with Löfgren's syndrome was 75% (p=0.17) Furthermore, no differences were found in allelic distribution of the other two SNPs.
Conclusion: These results suggest that a TLR-5 polymorphism may contribute to developing fibrosis in sarcoidosis. The next step is to determine the functional impact of this polymorphism on the immune response against TLR-5 agonists, such as *Mycobacterium tuberculosis*.

200

Chemokine mRNA expression profiling in pulmonary sarcoidosis

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Chemokines play a crucial role in the recruitment of inflammatory cells into the lung in sarcoidosis, a Th1 cell-mediated inflammatory disease characterised by a CD4+ lymphocyte alveolitis. To date, most chemokines has been investigated individually and there are no data on the chemokine network that likely operates in sarcoidosis and/or specific clinical phenotypes in sarcoidosis. We, therefore, used quantitative RT-PCR to investigate simultaneous mRNA expression of 9 chemokines (CCL2, CCL5, CCL15, CCL19, CCL22, CCL24, CXCL9, CXCL10, CXCL11) and two other chemoattractant molecules (osteopontin, chemerin) in bronchoalveolar lavage (BAL) cells from sarcoidosis patients (n=61) and control subjects (n=17). Of the chemokines studied, sarcoid BAL cells expressed higher mRNA levels of CCL5 (p=0.0001), CXCL9 (p=0.001) and CXCL10 (p=0.0001) than control. Cells from patients with acute sarcoidosis expressed lower mRNA levels of all of the chemokines that were up-regulated in cells obtained from patients with chronic disease ($p<0.05$). CCL2 mRNA expression was higher in patients with more advanced disease, defined by chest radiography (CXR-stage I vs. stage II: p=0.04; CXR-stage I vs. stage III: p=0.02). The mRNA expression of chemerin and osteopontin was neither up-regulated in sarcoidosis as a whole nor in any of the studied clinical phenotypes ($p>0.05$). In conclusion, our data provide further evidence that the chemokine profile changes during disease course in sarcoidosis. Grant support: IGA MZ CR NR/9037, MSM6198959205.

201

Frequency of beryllium and mycobacterial specific blood CD4$^+$ T-cells in patients with sarcoidosis

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Background: Sarcoidosis is a granulomatous disease of still obscure aetiology, characterized by an exaggerated T helper 1 immunophenotype.
Objective: To test the specific CD4 T-cell response to several antigens (beryllium, Mycobacterium tuberculosis), which are known to cause granulomatous inflammation, in subjects with sarcoidosis (S), tuberculosis (TB), berylliosis (B) and controls (C).
Methods: The prospective study included 27 subjects (20 males), aged 21-76 (mean 45.7±18.8), with recent onset of S (8), B (1), TB (10) and 8 healthy volunteers. Isolated peripheral blood mononuclear cells were stimulated with either beryllium, PPD, ESAT6 or CFP10 in the presence of αCD49d, αCD28 and Brefeldine for 6h. The frequencies of specific IFN-γ, IL-2 and TNF-α producing CD4$^+$ T-cells were calculated by flow cytometry. The cut-off value was set at 0.05% cytokine-producing CD4$^+$ T-cells.
Results: Using this method, we could clearly show that patients with TB have had an increased number of specific IFN- γ producing CD4$^+$ T-cells following the stimulation with ESAT6, CFP10 and PPD. In addition, the patient with B displayed an increase in the percentage of specific INF- γ producing CD4$^+$ cells following the stimulation with beryllium. A half of patients with S showed a specific CD4$^+$ T-cell response following PPD, ESAT6 and CFP10 stimulation.
Conclusions: We successfully established a method based on flow cytometry which clearly detects beryllium and mycobacteria specific CD4$^+$ T-cell responses. This is a promising time-saving method to exclude B in patients with S. However, the method does not allow the differentiation of mycobacterial infection and S. Further studies are ongoing.

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