361. Growth factors and fibrogenesis: are they good, bad or both?

3231

The role of TNF-related apoptosis inducing ligand (TRAIL) in fibrotic lung disease

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We previously showed a death receptor ligand, TRAIL, accelerates neutrophil apoptosis without associated cell activation (J Immunol 170:1027-33).We studied the role of TRAIL in wild-type and TRAIL-/- mice and investigated the role of TRAIL in patients with IPF.

Mice received intratracheal bleomycin or saline control. BAL at 3,7,16 and 23 days was analysed by cytospin morphology, hemocytometer count and flow cytometry. Collagen deposition, TRAIL expression and TUNEL positive events were also analysed. Samples from IPF patients and controls were examined for TRAIL expression and concentration. Lung function and survival time were retrieved from patient charts.

BAL analysis revealed TRAIL-/- mice had increased neutrophil numbers and reduced neutrophil apoptosis. Collagen analysis revealed a significant difference at 16 days, with TRAIL-/- mice showing increased collagen deposition. At day 23, TRAIL-/- mice had decreased TUNEL positive events. Murine lung sections revealed specific TRAIL expression in BALT and alveolar macrophages. Lung sections from IPF patients revealed an absence of TRAIL expression compared to controls. IPF patients had significantly lower serum levels of TRAIL than controls which inversely correlated with TLCO and positively correlated with survival from diagnosis.

We demonstrated that the neutrophilic inflammatory response to bleomycin is increased in TRAIL-/- compared with wild-type mice and that this finding is associated with increased collagen deposition.We also demonstrated reduced pulmonary and systemic expression of TRAIL in IPF, which correlates with worse pulmonary function and clinical outcome.This data suggests TRAIL may have therapeutic potential in ameliorating IPF.

3232

CCR6 is a receptor for CCL18 expressed on human lung fibroblasts from IPF lungs

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Background: CCL18 is a chemokine released by alternatively activated macrophages binding to a yet unknown receptor. CCL18 induces collagen synthesis by fibroblasts thus indicating that the putative CCL18 receptor is expressed by human lung fibroblasts.

Methods: To identify the receptor we screened a phage display library; the expression of the receptor was confirmed using conventional antibodies. FGF2 Release was measured by ELISA and expression of collagen and α-smooth muscle actin was estimated by quantitative PCR.

Results: Phage-display experiments pointed to the CC-chemokine receptor 6 (CCR6) as CCL18-receptor. Staining lung tissue indicated CCR6 expression by AEC-II and fibroblasts in IPF lungs but not in tumour-free areas of resected lungs from SqC-patients. CCR6 was expressed by lung fibroblast lines derived from IPF lungs but was almost absent in lines from healthy areas of lung tissue from squamous carcinoma (SqC) patients. Spontaneous release of fibroblast growth factor 2 (FGF2) was very low in SqC-fibroblasts (71±25pg/ml) but significantly increased in IPF-fibroblasts (603±463pg/ml). Stimulation of fibroblasts with CCL18 induced a non-significant increase in FGF2 release in SqC-fibroblast lines (143±77pg/ml) but led to a significant increase in IPF lines (1194±601pg/ml) which was blocked by an anti-CCR6 antibody in IPF-fibroblast lines (783±632pg/ml) but not in SqC-lines (112±66pg/ml). Likewise, CCL18 induced an up-regulation of collagen I (coll-1) and αSMA expression. Again, induction of coll-I and αSMA expression was blocked by an anti-CCR6 antibody.

Conclusion: Our data indicate that stimulatory effects of CCL18 on human lung fibroblasts are mediated via CCR6 (patent pending).

3233

Involvement of the Hedgehog signalling pathway in idiopathic pulmonary fibrosis

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Identification of new therapeutic targets in idiopathic pulmonary fibrosis (IPF) is required. We hypothesized that, in the adult lung, SHH pathway plays a critical role during the alveolar repair process and lung fibrogenesis.

Methods: We determined the expression pattern of several members of the SHH pathway in lung biopsies and primary cultured fibroblasts from controls and IPF patients. We also examined the expression profile of SHH pathway after TGF-β stimulation in fibroblasts. We characterized the action of recombinant SHH and cyclopamine, an inhibitor of SHH pathway, on the effect of TGF-β on fibroblast differentiation markers.

Results: In lung biopsies, the ligand SHH, the receptor Patched (PTC), the transmembrane protein Smoothened (SMO) and the transcription factors GLI 1-2-3 were detected in both control and IPF tissues. Compared to controls, PTC was decreased in IPF, both in epithelial cells and fibroblasts. SMO was maintained and GLI2 was localized in the nuclei in IPF. In vitro, there was no difference between control and IPF fibroblasts in the expression of HH signalling components. After TGF-β stimulation, PTC, SMO and GLI3 mRNA were strongly inhibited while GLI2 was increased. Cyclopamine inhibited TGF-β induced expression of myofi-

broblast differentiation markers in control fibroblasts. IPF fibroblasts were less sensitive to cyclopamine treatment. Though, addition of recombinant SHH had no effect.
Conclusions: Our results show that the signaling pathway downstream of SMO is activated in IPF. SMO activity is also necessary to TGF-β induced myofibroblastic differentiation. These data support a pro-fibrotic action of the SHH pathway in IPF.

3234
Heat Shock Protein 27 modulate mesothelial and epithelial to mesenchymal transition (EMT)
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Introduction: Pulmonary fibrosis (PF) has currently no treatment. We have shown that adenoviral gene transfer of TGF-β1 (AdTGF-β1) to the pleura induces a severe pleural fibrosis that invades the parenchyma. In this process, mesothelial cells differentiate into myofibroblastes (α-SMA positive cells) through an EMT-like process suggesting a key role of mesothelial cells in PF. Heat Shock Protein 27 (HSP27), is a chaperon for actin. Its role in fibrogenesis is unknown.
Methods: Sprague Dawley rats received intrapleural injection of AdTGF-β1 or AdDL (empty vector). Mesothelial Met-5A and A549 cells were treated with rTGF-β1.
Results: *in vitro:* 1) mesothelial cells are susceptible to rTGF-β1 induced EMT 2) HSP27 is strongly linked to α-SMA during EMT (colocalisation and co-immunoprecipitation). 3) HSP27 overexpression induces an EMT and siRNA mediated HSP27 inhibition blocks TGF-β1 induced EMT and mesothelial cell migration 4) HSP27 modulates the TGF-β1/SMAD pathway. Data were reproduced in A549 epithelial cells.
In vivo: 7 days after AdTGF-β1 injection, HSP27 and α-SMA are overexpressed and colocalize in fibrotic sub-pleura areas. AdTGF-β1 rats treated by intrapleural injections of OGX427 (AntiSens Oligonucleotide, ASO, directed against HSP27) have a strong decrease in HPS27, α-SMA expression, mesothelial cells migration into the parenchyma and fibrosis compare to AdTGF-β1 rats treated with control ASO.
Conclusion: HSP27 plays a major role in EMT and could be a key target to inhibit EMT in PF and others diseases involving EMT.
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3235
Long-term effect of hepatocyte growth factor in the normal lung: A stereological assessment
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Background: Hepatocyte growth factor (HGF) gene transfer attenuates bleomycin induced lung fibrosis. Being a multifunctional pleiotropic factor, HGF is a potent mitogen for alveolar epithelial cells and shows antiapoptotic properties. Thus, we hypothesize that HGF might lead to remodelling processes within the alveolar walls in normal rat lungs.
Material and methods: Adult male Fisher rats F344 were instilled with 350μl of pSpChHGF plasmid (Human HGF under control of surfactant protein C promoter), followed by extracorporal electroporation at eight electric pulses of 200v/cm, at 10ms interval. One month after HGF gene transfer, animals were sacrificed and the tissue samples were collected. Stereological assessment was performed on light and electron microscopic level to study structural changes of lung architecture and septal wall tissue. Untreated normal adult male rat lungs served as controls.
Results: Stereology revealed that hHGF transfer increased alveolar surface area and decreased the blood-gas barrier thickness (0.48±0.06μm vs. 0.35±0.04μm, $p = 0.0159$), mainly attributed to a decrease of total basal membrane volume (118.99±11.74mm^3 vs. 35.63±8.32mm^3, $p < 0.01$) and interstitial cell volume (209.15±16.31mm^3 vs. 108.04±12.17 mm^3, $p < 0.01$). Total volumes of collagen and elastin within septal wall were unchanged.
Conclusion: Structural parameters with respect to alveolar surface area and blood-gas barrier suggest a further augmentation of gas exchange capacity after HGF-gene transfer, being a consequence of a septal wall remodelling leading to a decrease in total volume of interstitial cells and basement membrane within the septal walls. An emphysema-like phenotype was not observed.

3236
HGF expressing stem cells in the human fibrotic lung originate from the bone marrow
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Background: Pulmonary fibrosis (PF) is a progressive disease of unknown etiology. Abnormal alveolar epithelial wound repair after injury may result in pulmonary fibrosis. We hypothesize that stem cells have a healing capacity by migrating to the site of injury and secreting hepatocyte growth factor (HGF) which supports alveolar epithelial repair, therefore contributing in reduction of fibrosis.
Methods: Immunohistochemistry using paraffin lung sections from patients with two histological lung fibrosis pattern, usual interstitial pneumonia (UIP) and non-specific interstitial pneumonia (NSIP) (both n=5) was performed with several stem cell markers.
Results: Specific cells in the lung parenchyma were stained positive for HGF in UIP and NSIP. They were mainly located in the fibrotic areas. These HGF-positive cells did not co-stain for markers of alveolar epithelial cell (Surfactant protein C) or fibroblasts (vimentin). However, HGF-positive cells showed strong co-staining for the mesenchymal stem cell markers CD44, CD29, CD105, and CD90, indicating that HGF positive cells in the lung are of stem cell origin. The HGF-positive cells were also positive for CXCR4, suggesting that the HGF-positive cells in UIP and NSIP are recruited from the bone marrow.
Conclusion: HGF-positive stem cells with the origin of the bone marrow (CXCR4 positive) can be detected in both UIP and NSIP, indicating a crucial role in the development or resolution of pulmonary fibrosis. These data indicate a possible role for stem cell therapy of patient with fibrotic lung disease in the future.