Peroxisomal metabolic alterations and their possible involvement in the pathogenesis of pulmonary fibrosis

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Background

Idiopathic pulmonary fibrosis (IPF) is a chronic disease and the most common form of idiopathic interstitial pneumonias. IPF is a progressive, irreversible and usually lethal lung disease of unknown ethiology (Fig.1.2). The main histopathological features of IPF are a heterogenous in appearance with areas of subpleural and paraseptal fibrosis and honeycombing (clustered cystic airspaces with defined walls 3-10 mm in diameter) alternating with areas with less affected or normal parenchyma (Fig.2). Collagen deposition and other ECM proteins characterize and reflect the current ongoing disease. Elevated proinflammatory mediators and reactive oxygen species (ROS) accumulation were suggested as pathogenetic mechanisms of this yet incurable disease.

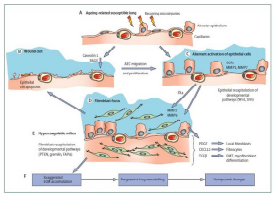


Fig1. Proposed mechanisms involved in the pathogenesis of idiopathic pulmonary fibrosis. Talmadge et al.2011

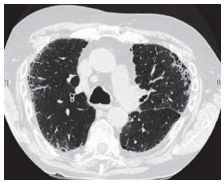


Fig2. Pattern typical of usual interstitial pneumonia on high-resolution CT. Talmadge et al 2011

Introduction

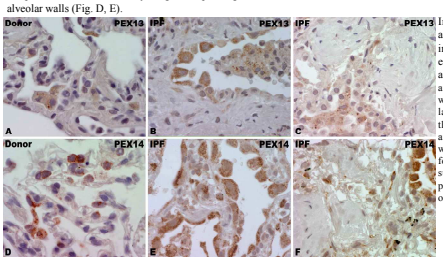
Since patients with peroxisomal biogenesis diseases develop fibrosis of the liver during their first year of life and peroxisomes are involved in the detoxification of ROS and proinflammatory lipid mediators (prostaglandins and leukotriene), we hypothesized that peroxisomal metabolic proteins might be dysregulated in IPF and being one of the reasons leading to the aggravation of the pulmonary pathology. Therefore, we investigated in this study the protein distribution and abundance of peroxisomal enzymes, transporters and biogenesis proteins in pulmonary fibroblasts of control subjects in comparison to IPF patients. Moreover, we investigated the synthesis of collagen, prolyl hydroxylase, peroxisomal enzymes and biogenesis proteins, and HAS2 (Hyaluronan synthase 2) in pulmonary fibroblasts of control subjects after silencing of the PEX13 gene by RNAi.

Methods

Pulmonary fibroblasts of lung donors and IPF patients were obtained from the University of Giessen Lung Centre (UGLC) tissue collection bank. For **Immunofluorescence and Immunohistochemistry** studies fibroblasts of 3 donors (DO) and 3 IPF patients (IPF) were investigated parallel to each other. **Collagen** was measured by using the Sircol soluble collagen Assay (Biocolor).
The cell number was determined by using a Neubauer chamber and counting. **Western blotting**:Whole fibroblast cell lysates of control and PEX13 siRNA transfected cells were made by them. 20 µg protein of whole cell lysates were loaded onto 12.5% SDS gels and blotted to PVDF membranes by semi dry transfer. Immunoblots were done according to Karnati and Baumgart-Vogt 2008, with primary antibody (AB) incubations overnight and detection of antigen-AB-complexes with alkaline phosphatase-labelled secondary antibody and chemiluminescence.**RT PCR**: Peroxisomal genes and collagens were checked by specific primers (Gene bank, NCBI) menitioned in table. As controls the expression of 28SrRNA and the HPRT1 genes were used.

Results

Fig.5. Strong alternations of peroxisomal proteins in lung tissue of patients with IPF;Peroxisomes could be clearly identified in alveolar macrophages and AECII with anti Pex13p and Pex14p antibodies, with highest staining intensity for Pex14p in macrophages. The IPF samples were characterized by irregular expanding and massive fibrosis in between interconnecting air spaces with thickened alveolar walls (Fig. D, E).



Interestingly, Pex13p (Fig. B) and Pex14p (Fig. E) were increased in the transitional epithelial cells of thickened alveolar walls. Pex13p (Fig. B) and Pex14p (Fig. E) protein levels were highly abundant in the single layered flattened epithelial cells of thickened fibrotic alveoli (Fig. B and E), whereas these proteins were strongly decreased in mature foci regions of IPF (Fig. C and F) suggesting an important role of peroxisomes in the pathogenesis of IPF.

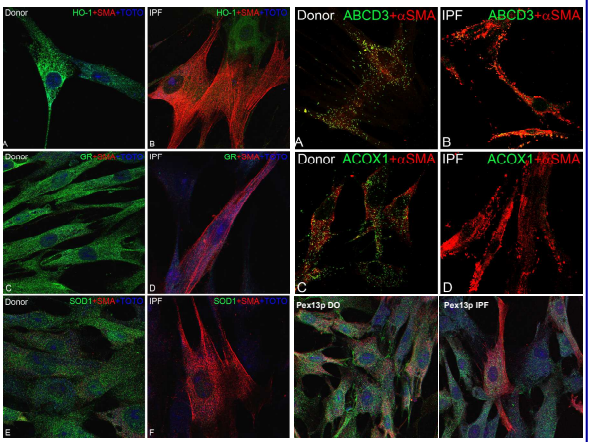
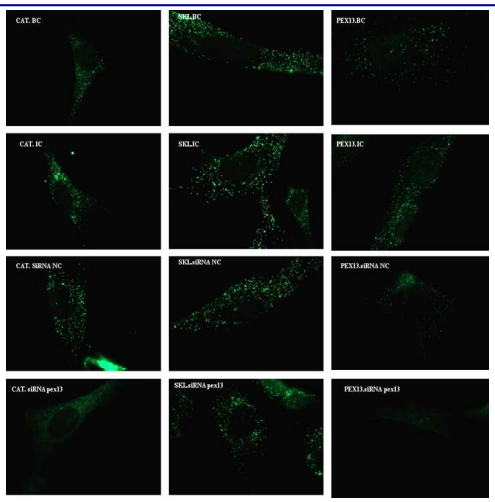


Fig.3. HO-1, GR, SOD1 and antioxidant enzymes were reduced in fibroblasts of IPF patients, even though the effect on antioxidant enzymes and the peroxisomal compartment was not dependant on the myofibroblast phenotype in the strongly heterogeneous cell cultures from donor and IPF patients.

Fig.4. Peroxisomal acyl-coenzyme A oxidase 1, is a β-oxidation enzyme. This enzyme was strongly reduced in fibroblasts of IPF patients. ABCD3 was also downregulated in IPF. Similar to the mentioned peroxisomal metabolic proteins, also Pex13p was reduced in IPF samples, suggesting that in addition to peroxisomal metabolism also peroxisomal biogenesis was impaired.



In **Fig.6** is the distribution and intensity of catalase, SKL and Pex13p in PEX13 siRNA transfected donor fibroblasts are depicted. Catalase was mistargeted to cyoplasm in PEX13 KO, revealing a targeting defect whereas only small part of SKL proteins targeted to the cytoplasm, suggesting that the targetting defect was not complete. PEX13KO is presented in this figure also.

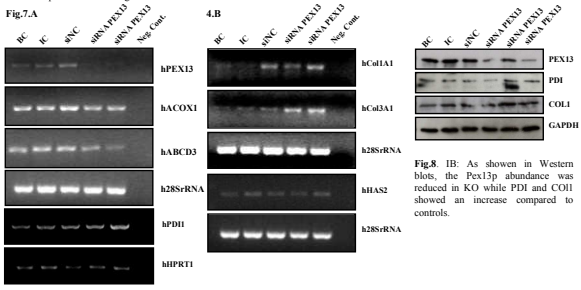


Fig.7.A.B.RT-PCRs depicting expression of peroxisomal biogenesis genes, peroxisomal enzymes, prolyl hydroxylase gene, hyaluronan synthase 2 (HAS2), collagen 1 and collagen 3 genes. The expression of 28SrRNA and of the HPRT1 gene were used as controls. Prolyl hydroxylase, collagen 1 and collagen 3 mRNAs were upregulated in siRNA treated cells. HAS2 was slightly upregulated, whereas ABCD3 and ACOX1 were slightly downregulated in PEX13 siRNA treated cells.

Fig.8. IB: As shown in Western blots, the Pex13p abundance was reduced in KO while PDI and COI showed an increase compared to controls.

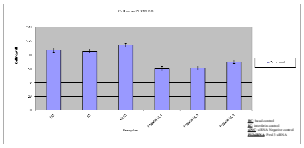


Fig.9. The cell number was reduced about 30% in PEX13 siRNA treated fibroblasts. Mean number of cells per squares in each different samples are depicted.

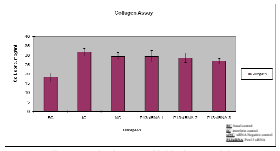


Fig.10. Collagen production was highly increased in PEX13 siRNA treated fibroblasts even though the number of cells was highly reduced. Collagen production was high in IC, NC due to stress induction while cell number even increased.

Discussion

The oxidant-antioxidant imbalance was suggested to play a major role in the pathogenesis of IPF. Therefore, alterations of antioxidant enzymes in distinct subcellular compartments may be an important reason also for IPF progression. Our results revealed that HO-1 of the endoplasmic reticulum as well as cytoplasmic GR and SOD-1 were strongly down-regulated, whereas catalase the peroxisomal enzyme with strongest capacity to degrade hydrogen peroxide was only milder affected in fibroblasts of different IPF patients. Interestingly, catalase is an enzyme that is present in highest abundance in peroxisomes in type II alveolar epithelial cells and in ciliated bronchiolar epithelial cells in mice and man (Karnati and Baumgart-Vogt 2008, 2009) and an effect of catalase deficiency on pulmonary fibrosis has been observed (Odajima et al 2010). In contrast to catalase, peroxisomal lipid metabolizing enzymes and transporters, as well as the biogenesis protein Pex13p are more equally distributed in peroxisomes of distinct cell types of the lung (Karnati and Baumgart-Vogt 2008) and are indeed strongly down-regulated in IPF fibroblasts, suggesting significant alterations in peroxisomal eicosanoid degradation.
Moreover, collagen production was increased and fibrotic markers were upregulated in cells with a PEX13 knockdown, suggesting a role of peroxisomes in protection against fibrosis. Finally prolyl hydroxylase (procollagen-proline dioxygenase) an enzyme involved in the production of collagen, (converting proline to hydroxyproline) was upregulated in PEX13KO fibroblasts.
We speculate that the strong down-regulation in peroxisomal eicosanoid degradation might lead to the accumulation of proinflammatory lipid mediators, prolongation of inflammatory reactions and stress-induced release of profibrotic mediators in IPF fibroblasts, aggravating the molecular pathogenesis of IPF.

References

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