Global Gene Expression by Fibroblasts Derived from IPF-Lung: Role of CCL8 in IPF

Objective: The aim of this study is to understand the complex molecular mechanisms and develop biomarkers, exploration of global gene expression in IPF-lung tissues.

Materials and Methods: We performed gene expression profiling of fibroblasts obtained from surgical biopsy specimens of 12 lung samples (8 IPF and 4 controls) using Illumina HumanHT-12 BeadChip. To validate CCL8 as a candidate gene, the mRNA expression by fibroblasts was measured using RT- and Real-Time PCR. CCL8 protein was measured in BALF using ELISA. Immunofluorescent stain was applied to localization of CCL8.

Results: 233 genes were differently expressed between the two groups. Ten-fold or greater change was observed in 15 genes. Among them, CCL8 was expressed 22.8 folds higher in IPF. CCL8 mRNA of the IPF (n=14) were expressed higher than controls (n=10) on RT- and Real-Time PCR (p=0.022). The concentration of CCL8 protein in BALF was significantly higher in IPF (n=86) than controls (n=35, p=0.001). A cut off value (1.31pg/μg) possessed 73.6% accuracy with 45.7% of specificity and 84.9% sensitivity for the diagnosis. IPF-subjects with CCL8 levels higher than 6.837pg/μg showed shorter survival period compared with those with lower levels (p=0.03). CCL8 levels showed good correlation with numbers of BAL neutrophils (r=0.297, p=0.01). In IPF-lungs, CCL8 was co-localized on α-SMA positive cells in interstitium.

Conclusion: The transcriptome analysis identified new sets of genes to be possibly involved in IPF. Among them, CCL8 may be a noble genetic biomarker for diagnosis of IPF and prediction of survival.

An integrative analysis of DNA CpG methylation and mRNA expression profiles in primary fibroblast of patients with idiopathic pulmonary disease

Epigenetic mechanisms are known to be associated with pathogenesis in idiopathic pulmonary fibrosis (IPF). Due to characterized cell-type specificity of the transcriptomic and epigenetic marker, we aimed to investigate a relationship between CpG methylation and gene expression profiles in primary fibroblast of IPF. We performed genome-wide DNA methylation and gene expression profiling in fibroblasts derived from the lung of 8 IPF and 4 control subjects using the Illumina HumanMethylation450 BeadChip and Illumina HumanHT-12 BeadChip. Global DNA methylation and RNA expression were analyzed in an integrative manner to identify IPF-specific genes governed by DNA methylation. We performed function validation of one of the genes identified by our analysis. We identified 88 methylation loci on 42 genes which were associated with significant changes in transcriptome levels, in which S100 Calcium Binding Protein A4 (S100A4) showed the most significant correlation. The differential methylation in the 3 CpG loci within the S100A4 gene were confirmed in IPF fibroblasts using bisulfite sequencing. S100A4 mRNA and protein expression levels were significantly higher in fibroblasts of IPF than those of control groups. And we found that S100A4 was intensely stained in alveolar macrophage, fibroblast, smooth muscle cell and lymphocyte in interstitial spaces of IPF lungs. The candidate genes showing significant correlation between the DNA methylation and RNA expression, especially S100A4, may play roles in pathophysiology of IPF, and could be promising biomarkers diagnosing IPF.