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Active transcription in the vascular bed characterizes rapid progression in idiopathic pulmonary fibrosis

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Main text

Idiopathic Pulmonary Fibrosis (IPF) is the most common manifestation of interstitial lung disease (ILD), with a median survival of 3-5 years after diagnosis. IPF is characterized by progressive fibrosis with the development of fibroblastic foci in the interstitium. Despite the short median survival, there is a striking variance in the clinical course of IPF; with some patients characterized as stable (SP) versus rapid progressors (RP) based on their decline in lung function and loss of forced vital capacity (FVC)(1). While the presence of these two patient groups is well known, the biological mechanisms defining this dichotomy are poorly understood. To investigate these differences, we collected lung biopsies from IPF subjects at the time of diagnosis and followed their disease progression (IRB #: PRO0032158, Table S1). We identified 5 SP and 4 RP at time of diagnosis based on loss of at least 10% FVC within the first year, as well as 3 terminal-stage IPF subjects delineated for transplant (TP) and 3 age-matched normal lung tissues (NC) (Clear margins from excision for pulmonary hamartoma). We investigated the presence of key transcriptional signatures using spatial transcriptomics (2).

Briefly, sequential cuts of lung biopsies were stained with Trichrome, H&E, and immunofluorescently labeled to identify nuclei, fibroblasts (α smooth muscle actin, αSMA), and the vascular bed (CD31). Using a combination H&E and immunofluorescent staining, regions of interests (ROIs) were selected for transcriptional analysis (see Supplemental methods).

First, we sought to investigate if transcriptional signatures of ROIs showing key features of IPF (collagen deposition and presence of fibroblastic foci) were sufficient to discriminate between SP and RP (Figure 1A-B). SP showcased increased expression of immunoglobulin genes (Figure 1C), while RP had increased expression of surfactant proteins A1, A2, and C, for which genetic variants have been associated with poorer prognosis in patients with IPF (3). Further, CCN1, a protein expressed in fibroblasts at homeostasis and highly induced in dysregulated fibrosis (4), was found to be significantly increased in RP.
While these differences were present in the overall transcriptome, they were not fully reflected in the specific cell populations (Figure S1A), prompting a closer analysis of the different cell types. Unlike the general areas and the fibroblastic foci (Figure S1B-D), the vasculature in the RP (Figure 1D) was more transcriptionally active with an increased proportion of differentially expressed genes compared to SP and to TP (Figure 1E-G and S1E-G), but similarly active compared to NC (73 upregulated genes in NC and 70 in RP), suggesting a novel avenue to investigate the role of pulmonary vasculature in the progression of IPF. At the transcriptional level, upregulated genes in the vascular bed of RP compared to SP, TP and NC, including upregulation of LRRC38, LUM, COA5, and ITM2A, were mainly under the control of TBX3 (Figure S1H), a transcription factor overexpressed in models of lung fibrosis (5) (Figure 1H and S1I). These differences were also corroborated by comparison with publicly available single-cell RNAseq datasets that included endothelial cells (Table S2), and TBX3 expression at the protein level was confirmed by immunofluorescence (Figure S1J).

Given the increased transcriptional activity found in the vasculature of the RP group, we investigated whether there could be a transcriptional crosstalk between the vascular bed and the fibroblastic foci. In the fibroblastic foci (Figure S1D), distinct HLA-DQ haplotypes were expressed in the SP and RP groups, suggesting differential immune responses in the two groups. Further, MMP7 was highly expressed in the RP group compared to SP and TP (Figure S1D and S1I), strengthening its role as a biomarker for rapid FVC decline. The role of MMP7 in the reorganization of the extracellular matrix is well known. However, MMP7 can serve as a crosstalk mediator between the secreting cells and the endothelium. Indeed, MMP7 indirectly promotes angiogenesis via the cleavage of the soluble VEGF receptor 1, and the cleavage of CCN2, resulting in the increased bioavailability of VEGF and re-activation of its angiogenic activity (6). While our data suggest a crosstalk between fibroblasts and vascular bed, future studies will be needed to better understand other targets and interactions between cell types.
involved in lung fibrosis, including the effect of VEGF in the interaction between the capillary endothelial cells, the epithelium and the inflammatory cells.

In conclusion, this study suggests a key role of the vascular bed in the progression of IPF, warranting further investigations. Given the preliminary nature of this study there are some limitations that will need to be addressed in a larger cohort, including a larger size control group with minimal lung disease to allow for statistically robust multivariate analysis. Further, these results show similarities with publicly available datasets that performed single cell transcriptional analyses, they also highlight key differences. This could be attributed either to the technical aspect of the transcriptional profiling or to a different selection of patients. Future studies will be needed with a controlled patient cohort to discern the true variability between gene profiling by sc-RNASEq and spatial transcriptomics. Nevertheless, these data highlight distinct pathways of crosstalk between the fibroblasts and the vascular bed, which could prove central to the pathophysiology of the disease. Targeting such pathways and modulating the interaction between the vasculature and the fibroblasts may usher the development of future therapeutics to limit the progression of IPF.

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References

Figure 1. Transcriptional profile of the IPF lung shows unique signatures. (A) Lung biopsies from SP and RP IPF patients stained by H&E (left) and Trichrome stain (right). (scale bar = 250µm). (B) Immunofluorescence staining and ROI selection (scale bar = 500µm). (C) Differential gene expression analysis (SP = 5 subjects, RP = 4 subjects). (D) Immunofluorescence staining and ROI selection for vascular bed (scale bar = 500µm). (E-F) Number of upregulated genes. (G) Differential gene expression analysis of vascular bed. (H) Unique upregulated genes in the rapid progressor vascular bed. Significance for differential gene expression: log2 fold change 1 or -1, p-value = 0.05.