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Research paper

Transcriptomic evidence of immune activation in macroscopically normal-appearing and scarred lung tissues in idiopathic pulmonary fibrosis

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a fatal lung disease manifested by overtly scarred peripheral and basilar regions and more normal-appearing central lung areas. Lung tissues from macroscopically normal-appearing (IPFn) and scarred (IPFs) areas of explanted IPF lungs were analyzed by RNASeq and compared with healthy control (HC) lung tissues. There were profound transcriptomic changes in IPFn compared with HC tissues, which included elevated expression of numerous immune-, inflammation-, and extracellular matrix-related mRNAs, and these changes were similar to those observed with IPFs compared to HC. Comparing IPFn directly to IPFs, elevated expression of epithelial mucociliary mRNAs was observed in the IPFs tissues. Thus, despite the known geographic tissue heterogeneity in IPF, the entire lung is actively involved in the disease process, and demonstrates pronounced elevated expression of numerous immune-related genes. Differences between normal-appearing and scarred tissues may thus be driven by deranged epithelial homeostasis or possibly non-transcriptomic factors.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a distinct disorder within the broad group of diseases termed interstitial lung diseases (ILD), an inclusive group of lung disorders characterized by inflammation and/or fibrosis of the lung parenchyma [1–3]. As the most severe form of ILD, IPF causes substantial patient morbidity and mortality, has a median survival of approximately three years, and has limited proven efficacious therapies. Lung transplantation remains the only viable intervention in end-stage lung disease due to IPF.

As a fibrotic lung disease, IPF histologically demonstrates a pattern of lung injury termed usual interstitial pneumonia (UIP), which is characterized by dense regions of scarring, interspersed regions of relatively normal lung architecture, fibroblastic foci, patchy inflammatory cell infiltration, and honeycomb (cystic) change. The fibroblastic foci contain alpha-smooth muscle actin (α-SMA)-expressing myofibroblasts. These histologic observations support the commonly accepted paradigm that IPF is a disease of excess extracellular matrix (ECM) accumulation and dysregulated mesenchymal cell proliferation [4].

Despite intense research effort over the past several decades, the pathobiological mechanisms of IPF are not fully understood. As part of this effort, numerous transcriptomic profiling studies of lung tissues from patients with IPF have been performed using several approaches, including serial analysis of gene expression (SAGE) [5], microarray analysis [6–12], RNASeq [13–16], and single-cell RNASeq [17]. These studies have revealed a wealth of phenomenological information with important mechanistic implications, stimulating and focusing research on several specific pathophysiological mechanisms of IPF, such as disturbances in expression of genes associated with extracellular matrix, inflammation and immunity, and pulmonary epithelia. Subsequent studies have more specifically focused on contributions from epithelial disturbances [17], including those affecting expression of surfactants, cilium-associated genes, and mucins, including MUC5B [11,18]; matrix metalloproteinases, including MMP7 [8,19]; and immune inflammation involving T cells, B cells, macrophages [20–23], and numerous

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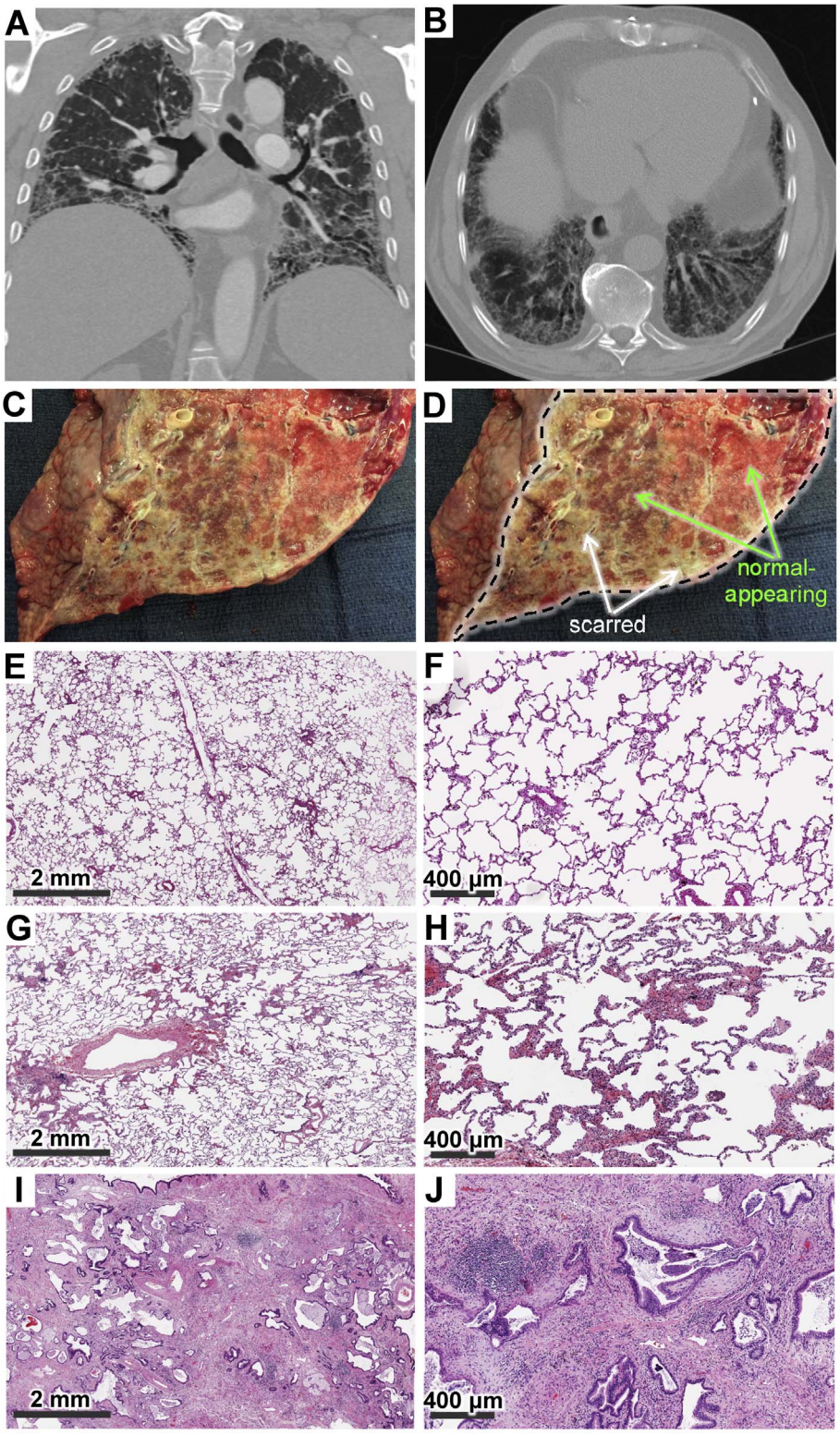

Fig. 1. Representative radiology (A, B), gross pathology (C, D), and histology (E–J) findings in patients with IPF (A–D, G–J) and healthy controls (E, F). A, B. Radiologic images in the IPF patients demonstrate reticulation, honeycombing, traction bronchiectasis, and volume loss in a predominantly peripheral and basilar distribution, consistent with advanced pulmonary fibrosis. C, D. Gross appearance of a sagittally cut lung explant from a patient with IPF (C) and the same image with markings superimposed (D). The cut plane revealing the internal parenchyma of the lung is demarcated by the black dotted line in panel D, whereas selected macroscopically normal-appearing and macroscopically scarred areas are indicated with green and white arrows, respectively. Also note the cobblestone appearance of the pleural surface on the left side of panels C and D outside of the dissection area. E, F. Low- (E) and high-magnification (F) histologic images of normal lung parenchyma from HC lung tissue. G, H. Low- (G) and high-magnification (H) histologic images from macroscopically normal-appearing IPF lung areas (IPFn) demonstrate largely preserved pulmonary microarchitecture, but scattered areas of organizing pneumonia and non-specific interstitial pneumonia are also present. I, J. Low- (I) and high-magnification (J) histologic images from macroscopically scarred IPF lung areas (IPFs) demonstrate dense areas of scarring, collapse of secondary lobules, architectural remodeling, honeycombing (cysts lined with ciliated respiratory epithelium and goblet cells), fibroblastic foci, and lymphocyte aggregates.

cytokines and chemokines [24]. The majority of molecular studies have focused on the most scarred areas of the lung since these areas are usually more accessible by standard surgical biopsy. However, the heavily scarred areas likely represent late stages of disease in which initial pathobiological mechanisms have dissipated.

IPF is characterized by scarring, but the IPF lung consistently demonstrates substantial geographic heterogeneity. Radiologic and gross pathology observations often show severe scarring of the lung in predominantly peripheral and basilar areas, whereas central and apical areas appear normal and seemingly unaffected. We recently analyzed and quantified histologic findings in macroscopically normal-appearing lung tissue in patients with IPF [25] and found that these areas exhibited patterns of lung injury termed organizing pneumonia ([OP], characterized by basophilic-staining deposits of ECM containing spindle-shaped fibroblasts or myofibroblasts), and nonspecific interstitial pneumonia ([NSIP], characterized by diffuse interstitial