⌒ The Bloom Is in Bud for Interstitial Lung Diseases

We, as clinicians, often try to fit human disease into a box. We identify a constellation of signs and symptoms, name a disorder, and then try to find treatments that might be effective. However, when it comes to interstitial lung diseases (ILDs), such an approach may need to be reconsidered. ILDs are heterogeneous in origin. Treatments predominantly focus on limiting the initial injury, suppressing the triggered immune response, and, more recently, targeting the common fibrotic processes independent of diagnosis (1). In this issue of the *Journal*, Furusawa and colleagues (pp. 1430–1444) were able to demonstrate that two separate ILDs—idiopathic pulmonary fibrosis (IPF) and chronic hypersensitivity pneumonitis (CHP)—likely share common final pathways in their pathologic "roots" (2). Their study also suggests that differences between IPF and CHP may be indicative of inciting injuries and etiologies, along with their differing immunologic responses.

IPF is a chronic, progressive fibrotic ILD of unknown etiology, characterized by a usual interstitial pneumonia (UIP) pattern that results in respiratory failure and death (3). CHP is a clinically variable syndrome that results from repeated inhalation of a variety of antigens, typically causing a granulomatous response, but may also lead to UIP, progressive fibrosis, and poor outcomes (4). Distinguishing the two clinical entities can be difficult (5), although it is of clinical importance as antigen identification might improve the prognosis of CHP (6). By comparing and contrasting RNA-sequencing expression profiles in large data sets, Furusawa and colleagues were able to highlight similarities and differences between CHP and IPF.

What sets this study apart? The story begins 15 years ago, when gene expression arrays were starting to show much promise. In a study by Selman and colleagues (7), biopsy profiles were obtained from a dozen patients with CHP and compared with cases of IPF and nonspecific interstitial pneumonia. At its infancy, gene expression profiles were able to classify ILD and, more importantly, began to provide insights into its molecular mechanisms. Selman showed that the CHP gene expression signature was enriched for inflammation, T-cell activation, and immune responses—in contrast to IPF, which exhibited tissue remodeling and myofibroblast gene activation (7). But with only a dozen patients in the largest study group, and no validation cohort, uncertainty of those discoveries remained. The current study represents an impressive 10-fold increase in size and power, and its thoughtful and methodical approach supports the validity of its findings.

The study used a mix of samples from the University of California, San Francisco, National Jewish Health, and the Lung Tissue Research Consortium, for a total of 288 cases—with 82 cases for CHP and 103 cases each for IPF and unaffected controls. The samples came from both explants and surgical lung biopsies. Understandably, such a large-scale RNA-sequencing effort did present some challenges. The authors found that gene expression differed significantly by batch effect and between explants and standard lung biopsy samples, likely owing to differences in severity of disease stage. Wisely, the authors elected to conduct the study separately for the two sets of samples defined by acquisition method. Although this reduced the potential power for discovery, the added stringency of focusing on the overlapping data sets helped to focus the selection of genes of interest.

The authors used a series of standard analyses to help with a systems-biology understanding of the shared and contrasting molecular features. The study identified more than 400 genes commonly up- and downregulated in both IPF and CHP, as compared with the controls. Some such examples were upregulation of CXCL13, S100A2, and a novel gene, SPRRA1, and downregulation of ITNL2 and BTNL9 in both entities. In contrast, genes specific to CHP were BGN, CXCL9, and CHIT1. But the most informative results lay in the pathway analyses. Shared upregulated pathways included collagen catabolic process, collagen fibril organization, and cell adhesion, whereas downregulated pathways included calcium ion transmembrane transport and angiogenesis. CHP-specific upregulation was noted in chemokine-mediated signaling pathways and immune responsiveness pathways, whereas downregulation was seen in steroid metabolic processes and angiogenesis.

Furthermore, gene set enrichment analysis was used to highlight a survey of large gene sets. The study noted several developmental pathways common to IPF and CHP, such as pathways involved in epithelial cell development and extracellular matrix–receptor interaction. Notably, the PI3K–Akt pathway, a prosurvival/antiapoptotic pathway previously shown to be of importance in IPF pathogenesis (8), was found to be enriched in CHP, suggesting that PI3K–Akt signaling might represent a plausible therapeutic target for both diseases.

A myriad of clinical traits, including scoring pathology for fibrosis, were also included in the study, using Weighted Correlation Network Analysis to better delineate which genes and pathways in CHP might be acting as drivers for a specific phenotype. For instance, CHP genes involved in adaptive immune responses and B-cell receptor signaling showed positive correlation with FEV, whereas expression of genes involved in epithelial development negatively correlated with $D_{L_{CO}}$.

The commonalities between CHP and IPF were best evident in shared MUC5B expression. MUC5B is a gene that encodes a mucin precursor protein (9), and its promoter variant rs35705950 is a risk factor for development of both IPF and CHP (10, 11). In this study, the minor allele frequency for MUC5B was similar in both patients with IPF and those with CHP and was higher than in the

control cohort. Additionally, MUC5B status and expression correlated with a UIP pattern on histology and computed tomography, suggesting its role in mediating a particular type of fibrosis independent of disease etiology.

So what can we take away from this avalanche of gene expression data? Clinically, CHP can be difficult to distinguish from IPF. The contrasting elements in this study provide more evidence that molecular classification of these difficult-to-diagnose entities will be possible and that we need to continue to move in that direction. Although "a rose by any other name might smell as sweet," for ILDs, it may be more important to understand their shared features in order for targeting therapies to have the broadest effect, while using their distinguishing features to help define them. ∎

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A New "TYK" Tok Era for the Study of Long Noncoding RNAs in Pulmonary Hypertension

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by increased pulmonary arterial pressure and pulmonary vascular resistance, ultimately leading to right heart failure and death. This increased vascular resistance leads to pulmonary vascular wall thickening and remodeling via phenotypic changes in proliferation and apoptosis in pulmonary arterial smooth muscle cells (PASMCs), pulmonary arterial endothelial cells (PAECs), pericytes, and fibroblasts (1). Over the past decade, appreciation has increased regarding the pervasive importance of noncoding RNA biology in controlling pulmonary vascular function and the pathogenic progression to PAH (2). Though studies of microRNAs in PAH have dominated the literature, the biologic roles of long noncoding RNAs (lncRNAs) increasingly are emerging as pathogenic hubs of disease (3).

TYKRIL and lncRNA Biology

Tens of thousands of lncRNA transcripts are encoded by the human genome. They are transcripts over 200 nucleotides long without predicted protein-coding potential. lncRNAs typically bind either proteins or other RNA molecules to enact epigenetic, transcriptional, and posttranscriptional regulation of gene expression, affecting a wide range of biological processes ranging from cell proliferation, apoptosis, and differentiation (4). lncRNAs have dynamic and specific expression patterns, are expressed in both the nucleus and cytoplasm, and are released at detectable and reproducible quantities into the circulating plasma (5). A crucial challenge in the study of these molecules is their poor sequence conservation across mammalian species, thus making analysis of their *in vivo* mechanisms of action particularly challenging.

Though a number of lncRNAs have been reported as dysregulated in tissue and plasma of subjects with PAH, the