Maladaptive TGF-β Signals to the Alveolar Epithelium Drive Fibrosis after COVID-19 Infection

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To the Editor:

Many people with coronavirus disease (COVID-19) show persistent symptoms after clearance of the infection. The extent of postacute sequelae of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (PASC; aka long COVID) is still being characterized and is a focus of current research. However, one clear manifestation of PASC is chronic lung fibrosis, particularly among those recovering from severe COVID-19 (1).

Studies of fibrotic lung disease as a sequela of COVID-19, herein referred to as PASC lung fibrosis, have been limited. A recent study found that up to 11% of patients hospitalized with COVID-19 had persistent lung abnormalities identified by computed tomography scan (2). Autopsy studies have identified early fibrogenic signals in the lungs that appear to represent a trajectory of dysregulated lung repair (3, 4). However, the mechanism(s) driving PASC lung fibrosis (characterized by nonspecific interstitial pneumonia–like features [1]) cannot be fully elucidated by autopsy studies, because acute lethality from COVID-19 is typically from acute respiratory distress syndrome (ARDS), with findings of diffuse alveolar damage (3, 5).

To investigate potential mechanisms driving PASC lung fibrosis, we performed single-cell RNA sequencing (scRNA-seq) of lung explants and compared our data to other scRNA-seq datasets prepared from control and idiopathic pulmonary fibrosis (IPF) lung explants (Figure 1A). All code and methods are available at https://github.com/ivonyao/PASC. We identified a decrease in the alveolar type 2 (AT2) cell fraction in fibrotic conditions, with compensatory increases in representation of other epithelial cell types (Figure 1B). Moreover, we found a decrease in the FABP4$^+$ (alveolar) macrophage population in PASC conditions (Figures 1C and 1D).

We compared the module scores of the top dysregulated pathways between conditions in all major lung epithelial populations and found a similar pattern of upregulation among several profibrotic pathways between all epithelial cell types of both PASC lung fibrosis and IPF, compared with controls (Figure 2A). We and others have observed that transitional cells (KRT8$^+$, KRT17$^+$, KRT5$^-$) are increased in lung explanted from those undergoing transplantation for chronic lung dysfunction after SARS-CoV-2 infection (1). These transitional cells have gained attention, as they are found in both ARDS and IPF (5, 6). As such, we focused our attention on the transitional cell population and observed striking similarities between PASC and IPF in the upregulation of fibrogenic signals such as TGF-β, p53, and cellular senescence (Figure 2A). Our analyses also revealed that transitional cells having a cellular senescence signature, absent in control lungs but progressively increasing from PASC to IPF conditions, suggest that cellular senescence may be a prerequisite for fibrosis. Accordingly, transitional cells observed in lungs of those who succumbed to COVID-19 and non–COVID-19 ARDS, where there is a lack of organized fibrosis, lacked signatures of cellular senescence.

Dysregulated TGF-β signaling affects the immunologic response to acute SARS-CoV-2 infection and correlates with the severity of COVID-19 (7, 8). The important role of TGF-β in driving lung fibrosis is well documented (1), and considering the association between the development of PASC lung fibrosis and severity of COVID-19, we further evaluated the magnitude of TGF-β signaling in transitional cells observed in patients with PASC (Figure 2A). We created an inferred communication network of the aggregate TGF-β signals to understand potential intercellular communications driving lung fibrosis (Figure 2B). In all conditions, TGF-β signals originated and emanated from macrophages. Interestingly, the target of TGF-β signals switched from solely being plasmacytoid dendritic cells (pDCs) in controls to an equal distribution between pDCs and transitional cells in IPF and were largely concentrated on the transitional cell population in PASC lung fibrosis. Loss of the FABP4$^+$ macrophages (Figure 1D) appears to be the primary driver of reduced TGF-β signaling between macrophages and pDCs (Figure 2C). Moreover, the probability of these putative TGF-β signaling networks between macrophage subpopulations and transitional cells was highest in PASC lung fibrosis compared with IPF (Figure 2C).

Monocyte-derived macrophages are profibrotic and a key source of the TGF-β that drives lung fibrosis (9, 10). Notably, monocyte-derived macrophages with a prominent TGF-β signature accumulate in the lungs of those who succumb to COVID-19 ARDS (4). Together with our findings in PASC lung fibrosis (Figures 2A–2C), these data suggest TGF-β expression by macrophages targets transitional cells to promote fibrosis. Indeed, TGF-β has a biphasic action in the regeneration of the alveolar epithelium, representing an early event required for AT2 cells to assume a transitional cell state, yet blocks differentiation into AT1 cells (5). Accordingly, activated macrophages may play a central role in providing the TGF-β stimulus to forestall wound healing and promote fibroproliferation through interrupting AT2-to-AT1 differentiation with concomitant accumulation of profibrotic transitional cells.

Herein, we provide a focused evaluation of epithelial and immune cells in PASC lung fibrosis and identify a putative

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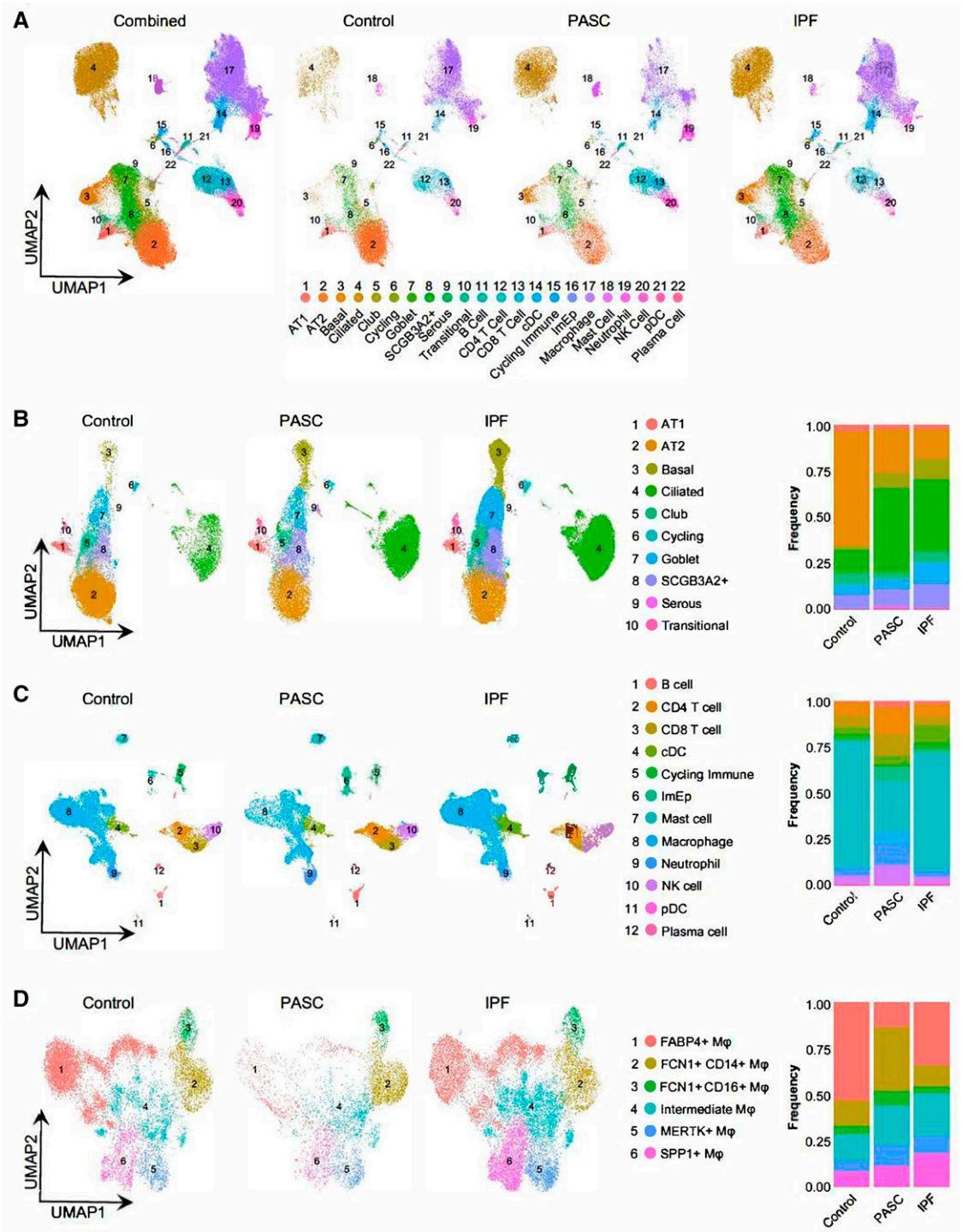

Figure 1. Cell type identification of epithelial cells and immune cells in single-cell RNA sequencing (scRNA-seq) evaluation of lungs from control, postacute sequelae of severe acute respiratory syndrome coronavirus 2 (PASC) lung fibrosis, and idiopathic pulmonary fibrosis (IPF). (*A*) We performed scRNA-seq of lung explants from five patients with PASC lung fibrosis undergoing lung transplantation, four of which had samples from the apical and basal segments of the lung independently processed for cell capture and barcoding, providing a total of nine scRNA-seq samples. These data were merged with scRNA-seq data from GSE146981 and GSE135893 that contain lungs from control subjects ($n = 21$) and patients with IPF ($n = 30$) using the Harmony R package for batch correction and Seurat R package for analysis. As part of our quality control, we removed cells that were doublets, low quality, stressed, and had mitochondrial genes <10%, which yielded 38,321 control, 40,324 PASC, and 62,365 IPF cells. All the sequencing data, together with their associated metadata, have been deposited in the GEO database under accession code GSE224955. (*B–D*) UMAP and frequency of (*B*) epithelial cell clusters, (*C*) immune cell clusters, and (*D*) macrophage populations. AT1 = alveolar type 1 cell; AT2 = alveolar type 2 cell; pDC = plasmacytoid dendritic cells.

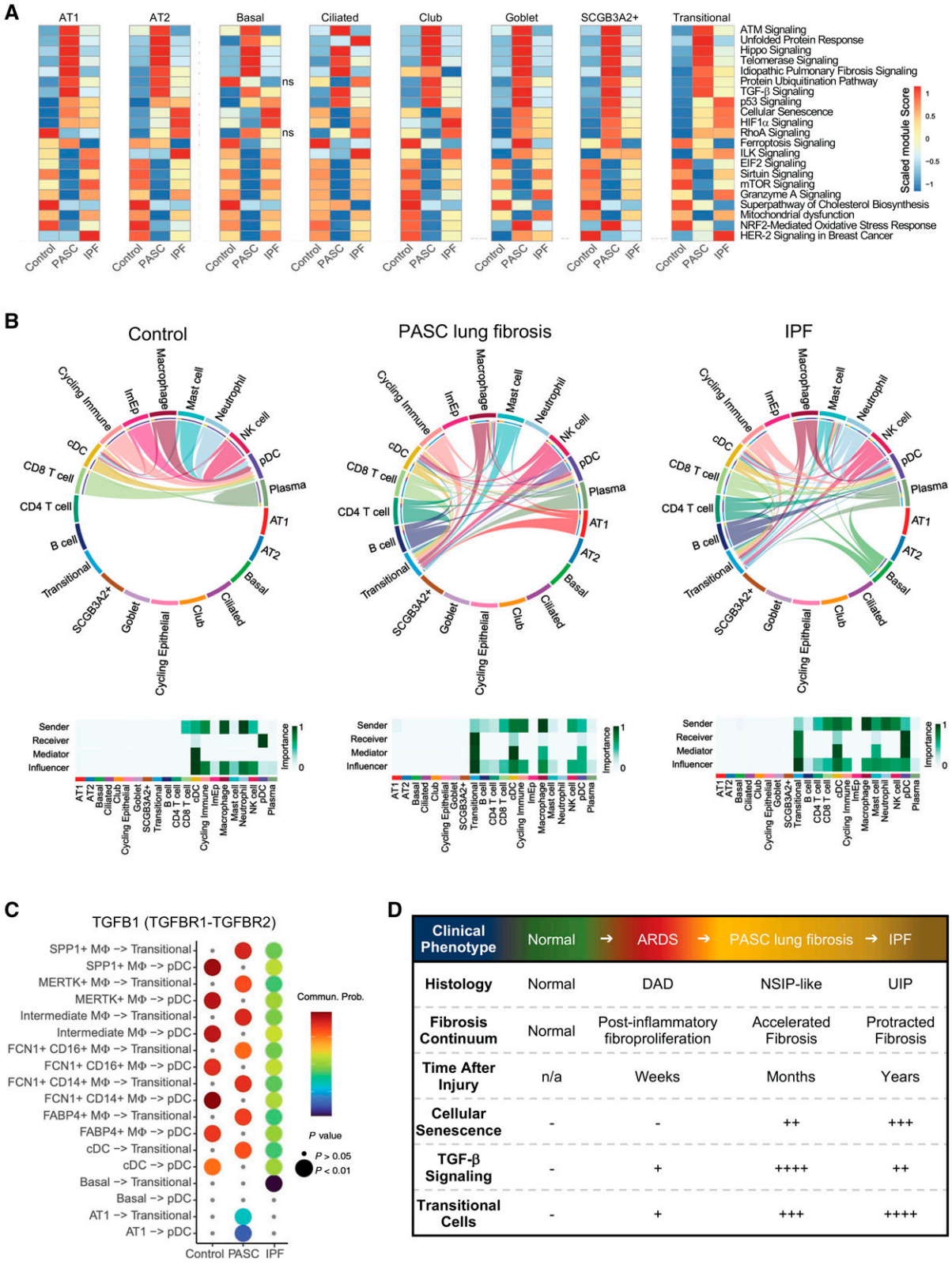

Figure 2. Cells in postacute sequelae of severe acute respiratory syndrome coronavirus 2 (PASC) lung fibrosis develop a profibrotic signaling network like that in idiopathic pulmonary fibrosis (IPF). (*A*) Differentially expressed genes (DEGs) were determined within each epithelial cell type between each condition (control, PASC, IPF). Ingenuity pathway analysis was used to identify the canonical pathways that were represented by the DEGs between conditions, and the top pathways that were common to all evaluations were used to generate a module score. Except for those that

maladaptive communication network that interlinks the profibrotic macrophage with the activation and/or development of transitional cells in lung fibrosis. This analysis is a first step in understanding the mechanisms of PASC lung fibrosis, and future directions would expand into other cellular compartments (e.g., mesenchymal cells). We propose a concept where PASC lung fibrosis is localized along a spectrum of fibrotic lung diseases, with one end anchored by a resolving fibroproliferation present in ARDS and the other end anchored by IPF (Figure 2D). The accelerated appearance of PASC lung fibrosis from an overwhelming viral-mediated inflammatory stimulus results in a nonspecific interstitial pneumonia-like pathology. In contrast, the development of IPF appears to involve a more protracted timeline, which could contribute to a very different histopathological finding (i.e., usual interstitial pneumonitis). Only time will reveal whether SARS-CoV-2 infections produce a progressive destruction of the lungs, such as in IPF. However, emerging research, including what we present here, demonstrates that the underlying pathogenic mechanisms driving fibroproliferation in PASC lung fibrosis have common features with IPF. Hence, studies into PASC lung fibrosis could provide novel insights into the maladaptive mechanisms that have broader implications across many types of chronic and progressive interstitial lung fibrosis. ■

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Figure 2. (*Continued*). are designed as "ns" (nonsignificant), all comparisons were significant by a Kruskal-Wallis test with an FDR < 0.05 (nearly all were highly significant, with FDR < 0.0001). (*B* and *C*) Inferred communication network of all ligand–receptor interactions for the TGF-β signaling pathway was determined. Using CellChat, we measured weighted-directed networks, including out-degree, in-degree, flow betweenness, and information centrality, to respectively identify dominant senders, receivers, mediators, and influencers for the intercellular communications. In a weighted directed network with the weights as the computed communication probabilities, the out-degree, computed as the sum of communication probabilities of the outgoing signaling from a cell group, and the in-degree, computed as the sum of the communication probabilities of the incoming signaling to a cell group, can be used to identify the dominant cell senders and receivers of signaling networks, respectively. (*B*) The chord diagram visually represents inferred cell–cell communication network of the TGF-β pathway, and the heatmap visually represents the data network centrality scores, dominant senders, receivers, mediators, and influencers in the intercellular communication network by computing several network centrality measures. (*C*) The probability of the communication score of ligand–receptor pairs (TGFB1 with TGFBR1 and TGFBR2) from sender cell groups to receiver cell groups. (*D*) Schematic of our proposed concept for a continuum of lung fibrosis. ARDS = acute respiratory distress syndrome; AT1 = alveolar type 1 cell; AT2 = alveolar type 2 cell; DAD = diffuse alveolar damage; n/a = not applicable; NSIP = nonspecific interstitial pneumonia; pDC = plasmacytoid dendritic cells; UIP = usual interstitial pneumonitis.