The Integrated Stress Response Links Muc5b to Pulmonary Fibrosis

Pulmonary fibrosis is a chronic lung disease characterized by the destruction of the lung parenchyma and excessive deposition of the extracellular matrix, resulting in increased work of breathing, impaired gas exchange, and eventual death from respiratory failure. Although the molecular mechanisms underlying the development of pulmonary fibrosis are incompletely understood, a multicellular model of pulmonary fibrosis pathology is emerging. Genetic association studies in humans implicate failed repair of the alveolar epithelium as the initiating event in pulmonary fibrosis, acting upstream of the recruitment of profibrotic monocyte-derived alveolar macrophages from the circulation and activation of alveolar fibroblasts (1, 2). By far, the most common genetic factor associated with idiopathic pulmonary fibrosis is a mutation in the promoter region of a gene encoding *MUC5B* (epithelial mucin) (3). Mutations in other genes expressed only in the alveolar epithelium, for example, *SFTPC* (surfactant protein C), have also been associated with an increased risk of pulmonary fibrosis (4). Furthermore, investigators have used murine models to localize the profibrotic effects of mutations that reduce telomere length and cause the Hermansky-Pudlack syndrome to the alveolar epithelium (5, 6).

In this issue of the *Journal*, Dobrinskikh and colleagues (pp. 62–74) hypothesized that the MUC5B promoter mutation in humans induces proteostatic stress in the lung epithelium that activates the integrated stress response (ISR), precluding lung repair after injury (7). Using a combination of single nucleus RNA sequencing and RNAscope, Dobrinskikh and colleagues found an association between *MUC5B* expression and upregulation of ISR genes in the airway and alveolar epithelial cells of idiopathic pulmonary fibrosis donors. To better understand the interplay between increased *MUC5B* expression and the ISR in the pathogenesis of pulmonary fibrosis, the authors engineered a mouse that overexpresses *Muc5b* in the alveolar epithelium. Upon bleomycin challenge, these transgenic mice showed enhanced epithelial ISR activation and worsened fibrosis compared with control subjects. Critically, a small molecule inhibitor of the integrated stress response (ISRIB) developed by Peter Walter's laboratory ameliorated fibrosis in transgenic mice (8).

These findings raise the question: "How does activation of the ISR in the epithelium promote the development of fibrosis?" Single-cell RNA sequencing analysis of mice recovering from lung injury provides a plausible answer to that question. (9–11). Using this technology, several groups of investigators identified a population of epithelial cells that emerged during recovery from lung injury that was absent in the normal adult lung. While these cells have been referred to by many names, we call them transitional alveolar epithelial cells, reflecting their intermediate state between AT1 and AT2 cells. These cells also express high concentrations of cytokeratins and genes associated with cell cycle arrest (9–11). A homologous population of cells was identified in single-cell RNA sequencing data generated from patients with pulmonary fibrosis and lethal severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia (12–14). Importantly, these cells have also been observed during murine lung development, suggesting they represent a bottleneck in AT2 to AT1 differentiation during fibrosis rather than the emergence of a new cell type (15). Consistent with the findings of Dobrinskikh and colleagues, Watanabe and colleagues implicated activation of the ISR in this bottleneck. They found that ISRIB administration attenuated bleomycin and asbestos-induced lung fibrosis. Using a lineage tracing system to label AT2 cells before the injury, they show that ISRIB reduced the number of transitional epithelial cells and increased the number of lineage-labeled AT1 cells (16).

The findings of Dobrinskikh and colleagues raise important questions about why the ISR is activated during AT2 to AT1 differentiation and how it becomes pathologic rather than adaptive. Activation of the ISR by one of four kinases results in the phosphorylation of eIF2α, which induces conformational changes that prevent its release from the guanine exchange factor eIF2B, resulting in inhibition of GTP hydrolysis and thereby reducing protein synthesis. ISRIB attenuates the ISR by stimulating the guanine exchange activity of eIF2B even in the presence of phosphorylated eIF2α, restoring protein synthesis. By restoring global protein translation, ISRIB also inhibits the translation of specific mRNAs, including the transcription factor *Atf4*, which are selectively translated when global translation is inhibited (8). It is, therefore, not clear whether the ability of ISRIB to accelerate alveolar epithelial repair and improve fibrosis is a consequence of the restoration of protein translation or the inhibition of gene transcription induced by ATF4 or other transcription factors, for example, IRE1 (induced by ISR activation in response to endoplasmic reticulum stress) (8). Interestingly, increased protein concentrations of ATF4 and its downstream target DDIT3 have been observed in hyperplastic alveolar epithelial cells of idiopathic pulmonary fibrosis lungs (4). Consistent with the findings of Watanabe and colleagues, Dobrinskikh and colleagues found that attenuation of lung fibrosis after ISRIB administration was associated with decreased expression of *Atf4* and *Ddit3*. Understanding the role of the ISR in AT2 differentiation will be critical to developing pharmacologic strategies targeting it in patients with pulmonary fibrosis. ■

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