Aberrant Multiciliogenesis in Pulmonary Fibrosis: Bystander or Driver of Disease Progression?

Idiopathic pulmonary fibrosis (IPF), a chronic and progressive interstitial lung disease of unknown cause, is the most frequent form of pulmonary fibrosis. IPF is characterized by a poor prognosis, with an overall expected survival of 3 to 5 years. Two antifibrotic drugs have been demonstrated to slow the decline in lung function and improve survival. IPF results from repeated insults to an aging alveolar epithelium, leading to alveolar type II cell death. Mesenchymal cells are then recruited to the damaged alveoli, with myofibroblasts contributing to extracellular matrix protein accumulation and the progressive destruction of lung parenchyma. IPF develops in susceptible individuals and is promoted by interaction with environmental agents such as inhaled particles, tobacco smoke, viruses, and bacterial agents. Several signaling pathways involved in lung development are reactivated in IPF, such as Wnt and Shh (Sonic Hedgehog) pathways (1). Genetic factors might also be involved in IPF. For instance, a common variant in single-nucleotide polymorphism for the MUC5B promoter region (rs35705950 variant) was found to be the highest genetic risk factor for sporadic IPF and is associated with an overproduction of Muc5b, specifically in bronchoalveolar junctions, resulting in impaired mucociliary function (2, 3). There is increasing evidence suggesting that bronchiolization of distal airspaces could play a key role during fibrogenesis. This process is characterized by the ectopic appearance of airway epithelial cells in the alveolar space, mainly from basal cell differentiation, and can result in honeycombing when diluted airspaces communicate with proximal airways (4, 5). However, the involvement of the resulting morphological or functional mucociliary abnormalities in IPF is poorly known.

Mucociliary clearance is the first barrier defending the airways from inhaled particles. Thus, mucus stagnation in case of altered mucociliary transport might lead to repeated injury and chronic inflammation in alveolar regions, with progressive replacement of normal parenchyma by scar tissue. In a previous transcriptome profiling study of whole lung tissue, Yang and colleagues identified new molecular subtypes of IPF associated with an increased expression of airway genes such as cilium-associated genes, mucins, and keratins (6). Higher expression of cilium-associated genes was associated with the expression of MUC5B and KRT5, a marker of basal cell airway progenitors. During IPF, Muc5b has been reported to be preferentially expressed in epithelial cells lining honeycomb cysts and in alveolar epithelial type II cells (7). Muc5b was associated with decreased mucociliary transport and enhanced honeycomb-like cyst formation in mice (2). An overexpression of Muc5b mucus was also recently demonstrated in IPF airways (8). Krt5-expressing basal cells are involved in normal airway cell differentiation and repair after lung injury and can differentiate into multiciliated cells through multiciliogenesis. During IPF, Krt5+ progenitors are found in fibrotic areas in which they constitute clusters (Krt5 pods) that could participate in honeycomb-like cyst formation and bronchiolization of distal airways.

In this issue of the Journal, Kim and colleagues (pp. 188–200) report on a study in which they hypothesized that cilium-associated genes might be involved in regenerating alveolar epithelium after injury and that abnormal mucociliary function and architecture might promote fibrogenesis (9) (see Figure 1).

First, the authors found an association in human IPF airway epithelial cells and in total lung between MUC5B expression and two transcription factors required for multiciliogenesis, Myb that acts downstream of Notch signaling and is required for initiating multiciliogenesis, and Foxj1 that is needed for complete multiciliogenesis. Hence, a higher percentage of multiciliated cells was observed in the IPF airway epithelium. This increased multiciliogenesis was associated with cilia structural abnormalities in IPF distal airways, resulting in altered function. These data are consistent with recent results attesting to altered ciliated cell counts and ciliary length in the epithelium of IPF airways (8).

Second, in a bleomycin-induced mouse model of pulmonary fibrosis, the authors studied the dynamics of multiciliated cells in response to bleomycin by labeling cilia at different time points. A decreased staining of cilia with notable deciliated areas in the airways was first found 1–3 weeks after bleomycin challenge compared with saline control subjects. Interestingly, full recovery was observed at 5 weeks, with a significant increase in the number of multiciliated cells that persisted up to 16 weeks, compared with control subjects. The increase in multiciliated cells secondary to lung injury might be driven by the overexpression of cMyb and Foxj1 transcription factors observed from 1 week after injury. Consistent with human data, abnormal cilia structures were observed. These data suggest that an aberrant multiciliogenesis might lead to a mucociliary dysfunction that could contribute to fibrogenesis.

Regarding the origin of those multiciliated cells, Krt5+ cells were previously suspected of acting as progenitors (10). After bleomycin insult, Krt5+ cells were found to be increased in number during multiciliogenesis in the airways, but also and mostly in the parenchyma as Krt5 pods. The authors suggest this might be a compensatory mechanism following epithelial deciliation and loss of alveolar type II cells after injury. Cosingtaining of Krt5 and cMyb demonstrated a nuclear expression of cMyb in Krt5+ cells, suggesting multiciliogenesis initiation.

To perturb the multiciliogenesis process during fibrogenesis, the authors used a conditional loss-of-function of IFT88 (intraflagellar transport 88) in Krt5+ cells in the bleomycin model of lung fibrosis. IFT88 is required for cilium biogenesis in mammalian cells, including the nonmotile primary cilium. IFT88 function and primary cilium formation are necessary for critical profibrotic pathways such as Shh signaling (11, 12).
Regarding the mechanism by which Krt5 interfere with ciliogenesis or ciliated cell differentiation (13).

A metalloproteinase that is dysregulated during IPF, has been shown to interfere with normal ciliated cell function after alveolar epithelial injury, thereby linking cilium impairment to abnormalities in lung repair and multiciliogenesis (Figure 1).

In this model, IFT88 deficiency, after bleomycin lung fibrosis, was found to be decreased at 2 and 3 weeks compared with Ift88 wild-type mice. The decrease in fibrosis was associated with a decrease in Krt5 pods in the fibrotic areas. Mice that did not express Ift88 in Krt5+ cells did not show the expected increase in cMyc expression after the bleomycin challenge. Moreover, Ift88 deletion was associated with an inhibition of the Shh pathway in Krt5+ cells. Even though multiciliogenesis was de facto affected in this genetic mouse model, one could argue that the formation of the nonmotile primary cilium was also perturbed, leading to Shh pathway inhibition. In future studies, it will be critical to target a later step of multiciliogenesis than the initial primary cilium formation during lung fibrosis. The molecular mechanisms leading to structural ciliary abnormalities during the repair process and multiciliogenesis after bleomycin-induced fibrosis will also require further investigations.

Conclusions

Mucus hypersecretion and mucociliary impairment involving MUC5B polymorphisms have been of growing interest recently and are believed to participate in epithelial injury and thus drive fibrogenesis. However, this study provides novel insights into the pathogenesis of pulmonary fibrosis, as it is the first to suggest an involvement of multiciliogenesis as an aberrant repair mechanism after alveolar epithelial injury, thereby linking cilium impairment to fibroproliferation and fibrosis (Figure 1). Interestingly, Mmp7, a metalloproteinase that is dysregulated during IPF, has been shown to interfere with ciliogenesis or ciliated cell differentiation (13). Moreover, Krt5+ basal cells have previously been suggested to be relevant actors during the fibrotic process through the constitution of Krt5 pods in the fibrotic parenchyma. These data provide a clue regarding the mechanism by which Krt5+ cells, as progenitors of multiciliated cells, could be involved in fibrogenesis.

Figure 1. Aberrant multiciliogenesis and bronchiolization of distal airways in idiopathic pulmonary fibrosis (IPF). Proposed model for the involvement of primary cilium (IFT88), SHH (Sonic Hedgehog) signaling in basal cells leading to KRT5+ pod formation and aberrant multiciliogenesis after injury. The insert on the left showed immunofluorescence of a primary cilium from an IPF lung section. The axonem of the cilium was labeled with acetylated tubulin (red) and the basal body with pericentrin (green); nuclei (blue) were counterstained with2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI). Defective mucociliary clearance (MCC) as well as defective interactions between abnormal multiciliated epithelial cells (see acetylated tubulin immunofluorescence labeling the cilia in an IPF lung section; nuclei [blue] are counterstained with DAPI) and fibroblasts (leading to fibroproliferation) would promote fibrosis and bronchiolization in distal lung airways (as shown in the hematoxylin and eosin staining on the right). (Scale bar, 10 μm [left panel], 25 μm [middle panel], and 50 μm [right panel].)

Author disclosures are available with the text of this article at www.atsjournals.org.

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