Pulmonary fibrosis: patterns and perpetrators

Paul W. Noble, Christina E. Barkauskas, and Dianhua Jiang

Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, Duke University School of Medicine, Durham, North Carolina, USA.

Pulmonary fibrosis occurs in a variety of clinical settings, constitutes a major cause of morbidity and mortality, and represents an enormous unmet medical need. However, the disease is heterogeneous, and the failure to accurately discern between forms of fibrosing lung diseases leads to inaccurate treatments. Pulmonary fibrosis occurring in the context of connective tissue diseases is often characterized by a distinct pattern of tissue pathology and may be amenable to immunosuppressive therapies. In contrast, idiopathic pulmonary fibrosis (IPF) is a progressive and lethal form of fibrosing lung disease that is recalcitrant to therapies that target the immune system. Although animal models of fibrosis imperfectly recapitulate IPF, they have yielded numerous targets for therapeutic intervention. Understanding the heterogeneity of these diseases and elucidating the final common pathways of fibrogenesis are critical for the development of efficacious therapies for severe fibrosing lung diseases.

Pulmonary fibrosis: a disease spectrum

Pulmonary fibrosis can occur in a variety of clinical settings. An essential component in the evaluation of patients is the determination of whether there is evidence for systemic disease or exposure to environmental irritants (1). The importance of accurate classification of pulmonary fibrosis lies in the fact that the natural history and the potential response to treatment differ depending on the etiology (2). Connective tissue diseases such as rheumatoid arthritis and systemic sclerosis (scleroderma) commonly are accompanied by pulmonary fibrosis, and the diagnosis can often be established with reasonable confidence. However, other connective tissue diseases are less well characterized, and furthermore, the lung can be the first place in which a connective tissue disease manifests. It is important to determine the presence or absence of an underlying connective tissue disorder because some forms of connective tissue disease–related fibrosis are reversible.

Figure 1A shows a chest computed axial tomography (CAT) scan demonstrating traction bronchiectasis and chronic fibrotic remodeling. Surgical lung biopsy shows a homogenous pattern of fibrosis and chronic inflammation with a paucity of normal lung (Figure 1B). No fibroblast foci are identified. This pattern of fibrosis is referred to as fibrotic nonspecific interstitial pneumonia (NSIP). In this case, laboratory evaluation provided evidence of a connective tissue disease with elements of a mixed connective tissue disorder, but there were few fibroblastic foci and microscopic honeycomb changes were not evident (Figure 3, B and C). In addition, there were areas of monocellular inflammation at a distance from established collagen deposition. Connective tissue serologies were not revealing, but immunosuppressive therapy halted disease progression while lung function remained stable and oxygen desaturation with ambulation improved. This type of pulmonary fibrosis has not been investigated rigorously, and this example emphasizes our incomplete understanding of the heterogeneity of pulmonary fibrosis. Thus, the ability to distinguish IPF and UIP from other forms of pulmonary fibrosis is of utmost importance so that clinicians can accurately identify those patients who will benefit from immune-modulating therapy. The mechanisms that lead to reversible pulmonary fibrosis are unknown, and it remains to be seen whether IPF or UIP can be reversed as novel therapeutic approaches are applied.

Idiopathic pulmonary fibrosis

IPF is the most common and most severe form of idiopathic interstitial pneumonia. It is often relentlessly progressive, leading to death from respiratory failure within 2–5 years of diagnosis in the majority of instances (4, 10, 11). Importantly, while the overall prognosis is poor in IPF, it is difficult to predict the rate of progression in individual patients. Over the last decade, random-
ized clinical trials in IPF, coupled with the increased utilization of chest CAT scans has led to the identification of patients at earlier stages of the disease process. However, predicting the clinical course in these patients is particularly challenging. The annual incidence of IPF appears to be rising and is estimated at 5–16 per 100,000 individuals; prevalence is 13–20 per 100,000 (12). IPF is more common in men, and the prevalence rises dramatically with age (7). IPF is sufficiently uncommon under the age of 50 as to mandate an exhaustive search for an underlying connective tissue disease or occult environmental exposure in young patients, particularly women. In contrast, pulmonary fibrosis in patients over the age of 70 is significantly more likely to be classified as IPF (13). Risk factors for IPF include age, male gender, and a history of cigarette smoking (14).

Genetic predisposition
Several studies have identified familial forms of pulmonary fibrosis (15). The precise contribution of genetic transmission to IPF is difficult to ascertain because of the late onset of disease presentation, but it appears to be responsible for 5% of IPF cases. Transmission is autosomal dominant, with variable and reduced penetrance. A number of different genetic mutations have been associated with the development of pulmonary fibrosis, some in genes expressed only in epithelial cells in the lung and others in more ubiquitously expressed genes. Disease-linked mutations in the surfactant protein C gene (SFTPC) were first identified in adults by Loyd and colleagues (16), and subsequently, mutations in the surfactant protein A2 (SFTPA2) gene were identified in a distinct cohort of familial pulmonary fibrosis patients (17). As both proteins are expressed by alveolar epithelial cells, these important discoveries have generated new insights into pathogenesis (discussed below) and have provided a contextual basis for the observation that fibrosis only occurs in the lungs in IPF.

The most common mutations identified in familial pulmonary fibrosis are those in the genes encoding telomerase (TERT and TERC) (18, 19). Interestingly, unlike surfactant proteins A and C, telomerase is expressed by cells outside the lung, particularly in stem cells and progenitor cells, raising intriguing questions regarding the role of these mutations in alveolar epithelial cell renewal and the development of pulmonary fibrosis that require further study (Figure 4).

More recently, a common polymorphism in the promoter of the MUCSB gene has been identified in both familial pulmonary fibrosis and sporadic IPF (20). This SNP was identified in 34% of subjects with familial interstitial pneumonia and in 38% of subjects with IPF (20). MUCSB is expressed in bronchiolar epithelium and not by alveolar epithelial type 2 cells (AEC2s), under physiologic conditions, and the functional consequences of this polymorphism remain to be elucidated. Nevertheless, the prevalence of the polymorphism is much greater than that of surfactant mutations, which supports an emerging theme of abnormalities in proteins uniquely expressed in the lung contributing to IPF pathogenesis.

Pathogenesis
The paradigms of pathogenesis of IPF have evolved as the description of the radiographic and pathologic patterns have been refined over the last two decades (21). The ability to distinguish between immune-driven pulmonary fibrosis, which is fairly obvious in the settings of rheumatoid arthritis and scleroderma but much more
subtle in the context of undifferentiated connective tissue disease processes, has improved. However, diagnostic tools to distinguish between IPF and other forms of pulmonary fibrosis remain rudimentary relative to other diagnostics, such as those utilized in oncology. The conceptual transition of IPF pathogenesis from an inflammatory-driven process to a primarily fibrotic one has been well documented (22–24), largely based on the paucity of inflammatory infiltrates at the leading edge of fibrosis on lung tissue biopsy and the poor clinical response of IPF to systemic corticosteroid therapy. These observations, coupled with the identification of mutations in surfactant protein genes and evidence of alveolar epithelial cell apoptosis using histochemistry and electron microscopy analysis from patient biopsies, suggest that AEC2s are involved in the pathogenesis of fibrotic lung disease.

The vulnerable alveolar epithelium in IPF

Several lines of evidence have emerged implicating a combination of environmental, age-related, and genetic factors that coalesce to create an alveolar epithelium that is susceptible to injury from either unknown endogenous factors or exogenous insults such as viral infection or microaspiration (25–27). The mutations in surfactant proteins C and A2 that have been identified in familial pulmonary fibrosis result in abnormalities in surfactant protein folding (28). Misfolded proteins are recognized by the cell, and in order to limit their deleterious effects, the cell activates the unfolded protein response (UPR) (29). The UPR is an adaptive response designed to halt protein production and augment production of chaperone proteins in order to facilitate proper protein folding. The accumulation of misfolded proteins and activation of the UPR creates a challenge to the cell, referred to as ER stress, which can also be induced by viral infection (30). If unabated, ER stress activates a program that leads to apoptosis (Figure 4).

Several groups have documented that the UPR/ER stress pathway is activated in IPF (28, 31). However, at present, the significance of this response in IPF is unknown (Figure 4). In an attempt to link misfolded proteins with pulmonary fibrosis, efforts have been made to generate transgenic mice that express the mutated surfactant protein C in AEC2 cells (28). Although these mutations do induce ER stress in murine lungs, a second insult such as low-dose bleomycin or a viral infection is still necessary to produce fibrosis (32–34). However, the threshold for bleomycin-induced fibrosis is markedly reduced, supporting the concept that activation of the UPR is a marker for a vulnerable alveolar epithelium. Further support for such a concept has been the identification of herpes virus DNA in lung tissue from IPF patients (35). Although efforts to identify an active infection in patients with progressive disease have been difficult (36, 37), previous infection could add an additional component of vulnerability to the alveolar epithelium. Future efforts need to focus on generating novel genetic models that target specific downstream mediators of the UPR/ER stress response in AEC2 cells to more precisely define the link with fibrosis.

Epithelial-mesenchymal crosstalk

Pathologic hallmarks of UIP/IPF are the destruction of basement membrane and presence of hyperplastic alveolar epithelial cells (38). This has been suggested to create an environment of "frus-
Following lung injury, a constellation of events is set into place as the lung attempts to limit the extent of damage and restore function. Epithelial cells and innate immune cells (particu-
larly macrophages) are the first responders charged with assessing the severity of the insult and mounting a host response. Diffuse alveolar damage occurs at focal sites of injury, with hyaline mem-
brane formation and a fibronectin-rich provisional matrix as the coagulation cascade is locally activated in the context of increased alveolar permeability. The coagulation cascade has been suggested as a therapeutic target in IPF (51, 52), but a recent trial evaluating Coumadin (warfarin) was terminated for lack of efficacy (25, 53). However, more directed approaches such as targeting factor Xa, which has been shown to be expressed in IPF tissue and may contribute to TGF-β activation through interaction with the PAR-1 receptor, could be more fruitful (52).

The interplay between epithelial cells and macrophages in orchestrating the recruitment of fibroblasts is an area of active investigation. Both populations are capable of releasing a variety of mediators that have chemotactic properties for fibroblasts (54–56), but the relative contributions of these cell types has been difficult to discern. Recently, lysophosphatidic acid (LPA) has been shown to be present following lung injury, and its cognate receptor, LPAR1, is necessary for the recruitment of fibroblasts to the lung and the development of fibrosis following lung injury (55). Based on these data, an antagonist of LPAR1 is in early clinical development for IPF (57).

As noted above, activation of TGF-β is undoubtedly a critical ingredient for fibrogenesis. Several studies have suggested that fibrosis is abrogated in the absence of TGF-β signaling in the epithelium (58, 59). The integrin αvβ6 has been shown to be upregulated on epithelium following lung injury and to be necessary for activation of latent TGF-β (60). Anti-β6 antibodies are being studied in a Phase II clinical trial for IPF (52, 61). The precise and fundamental signals generated by epithelium that promote mesenchymal expansion have not been elucidated, but a recent study in acute kidney injury suggested that epithelial cell cycle arrest may generate signals that promote fibrosis (62). Interestingly, this process appeared to be mediated by JNK, and fibrosis was abated with a JNK inhibitor. Of note, a JNK inhibitor is in clinical development for IPF (63, 64).

Mesenchymal expansion

Patients suffering from IPF succumb to respiratory failure due to the inexorable accumulation of gas-exchanging regions of the lung that are obliterated by extracellular matrix. Analysis of lung tissue shows advancing regions of fibroblast accrual and destruc-
tion of the normal interstitices of alveolar tissue. The source of mesenchymal expansion in IPF and experimental models of lung fibrosis have been an area of active investigation. In addition, the source of the pathologic hallmark of IPF — the emergence of α-SMA—staining myofibroblasts juxtaposed to vulnerable alveolar epithelium — remains unexplained.

The long-standing belief that the source of mesenchymal expansion and myofibroblast accumulation was derived from resident lung fibroblasts has been challenged over the last several years by data suggesting that either a bone marrow–derived cell or alveolar epithelium could be the source of mesenchymal expansion. Several studies have suggested that fibrocytes — circulating mesenchymal cells — can be recruited to the lung following injury and potentially contribute to the burden of fibroblast accumulation and tis-

tue remodeling (65–67). In addition, fibrocytes have been identified in the blood of patients with IPF (68, 69) and might be useful as a clinical marker for disease progression (70). Difficulties have

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**Figure 5**

Proposed mechanisms of severe lung fibrosis. Injured AEC2s attempting to repair damage release growth factors, cytokines, coagulants, and other substances. These factors promote mesenchymal expansion and activation, leading to the accumulation of matrix-producing and invasive fibroblasts/myofibroblasts. Figure adapted with permission from the American Journal of Respiratory Cell and Molecular Biology (2).
arisen in the interpretation of these studies because of challenges with identifying and following the course of fibrocyte trafficking, due to the lack of specific markers and alterations in cell surface expression of fibrocyte markers in lung tissue.

Recently, a great deal of interest has been generated by the hypothesis that AEC2 cells may be a major source of fibroblast accumulation in experimental models of lung fibrosis and in IPF through epithelial-to-mesenchymal transition (EMT). EMT is an accepted biological process in cancer progression and is an appealing concept in IPF because of the unique features of fibroblastic foci. In addition, EMT has been suggested to be an important contributor to kidney fibrosis, although it remains an area of controversy (41, 71). Studies suggested that stimulating isolated AEC2 cells in vitro with TGF-β would lead to the expression of fibroblast markers and loss of AEC2 cell markers (72). In addition, immunohistochemistry suggested that there might be co-localization of AEC2 and fibroblast markers in IPF (72), but these observations have not been confirmed in all studies (73). To test the hypothesis that EMT contributes to experimental pulmonary fibrosis in vivo, two groups independently performed AEC2 cell lineage tracing experiments using a fragment of the human surfactant protein C (SFTPC) promoter driving β-galactosidase as a lineage label (74, 75). Either repeated doses of bleomycin or adenoviral delivery of active TGF-β were used to cause pulmonary fibrosis (74, 75). Both studies suggested that AEC2 cells are a significant source of mesenchymal cells in experimental pulmonary fibrosis.

An alternative approach of in vivo genetic lineage tracing was designed to explore the putative role of EMT in mesenchymal expansion and myofibroblast accumulation following lung injury (76). An Sftpc-CreER;2 knock-in allele was generated to induce the heritable expression of a fluorescent lineage tag in mature AEC2 cells. Lineage tracing of adult Sftpc-positive AEC2s revealed no evidence to suggest that they give rise to any type of stromal cells after bleomycin administration (76). Interestingly, this study did suggest that pericytes, a population of cells associated with blood vessels that has been implicated as a source of myofibroblasts in other organs (77, 78), are a source of stromal cell expansion following lung injury (76).

Two independent mouse lines, NG2-Cre-ER and forkhead box J1–CreER (FoxJ1-CreER), were used to induce expression of a heritable fluorescent tag in pericyte-like cells in the lung and to follow their fate after bleomycin treatment (76). Pericyte-like cells were found to proliferate and expand in areas of fibrosis, but they did not express high levels of α-SMA, suggesting that a yet-undefined stromal cell type might be the source of the expanding population of myofibroblasts following injury. This study significantly questions the relevance of in vitro studies examining AEC2 expression of fibroblast markers in vivo. Immunohistochemistry and confocal microscopy using a panel of stromal markers in fresh biopsy samples from patients with IPF recapitulated the heterogeneity of stromal markers described in the mouse and failed to demonstrate co-localization of NG2 or α-SMA in fibroblastic foci (76). Further studies are needed to delineate the significance of the variety of fibroblast populations and the source of myofibroblast expansion in both the bleomycin mouse model and in human IPF.

**Emergence of pathologic mesenchymal phenotypes**

In addition to the challenges in identifying the sources of fibroblast expansion, there have also been difficulties in elucidating the properties of fibroblasts that promote unrelenting tissue remodeling rather than normal repair. One hypothesis to explain the failure of fibrosis to resolve in the setting of IPF is that IPF fibroblasts may be resistant to apoptosis (79, 80). This hypothesis is appealing, but in vivo evidence supporting an apoptosis-resistant phenotype driving severe fibrosis has been elusive. The unrelenting nature of progressive fibrosis in IPF could be considered analogous to tumor cell growth, although fibroblasts isolated from IPF patients are not monoclonal or transformed (81–83). The pathologic hallmarks of IPF include matrix deposition, basement membrane destruction, and expansion of the mesenchyme. These properties suggest that fibroblasts acquire functions that overlap with tumorigenesis, such as invasive potential and insensitivity to growth inhibitory signals.

Several studies have demonstrated an abnormal proliferative capacity of IPF fibroblasts and have focused on the interactions with extracellular matrix (83–86). Polymerized collagen is naturally found in tissues and acts as a negative regulator of fibroblast proliferation (86). In contrast, monomeric collagen that is present during tissue injury supports fibroblast proliferation (86, 87). This physiologic process that accompanies normal wound repair is aberrant in IPF fibroblasts and is mediated by pathological integrin signaling (86). On normal fibroblasts, β1-integrin interacts with polymerized collagen to activate the negative growth regulator tumor suppressor phosphatase and tensin homolog. In IPF fibroblasts, this negative feedback mechanism is defective, facilitating the circumvention of the negative feedback signals to inhibit proliferation (86). This is accompanied by aberrant activation of the PI3K/Akt/S6K1 signaling pathway in IPF fibroblasts (86, 88). There is also evidence of genome-wide derangements in translational control in IPF fibroblasts, detectable after nine generations in culture and resulting in ribosomal recruitment of proteins that promote tissue remodeling (89).

Additional evidence of a pathologic mesenchymal phenotype driving unrelenting pulmonary fibrosis derives from the examination of the invasive capacity of IPF fibroblasts (90, 91). One of the hallmarks of IPF is the destruction of basement membrane in alveolar tissue leading to collapse of alveoli (92). IPF fibroblasts/myofibroblasts have been shown to spontaneously invade tissue in vitro models (93). The mechanisms that regulate myofibroblast functions in vivo remain poorly understood, although recent evidence suggests that NADPH oxidase 4 is essential for TGF-β-induced differentiation of fibroblasts to myofibroblasts in vitro and for bleomycin-induced pulmonary fibrosis in vivo (94). When hyaluronan synthase 2 (HAS2) was transgenically overexpressed by myofibroblasts in vivo, a severe fibrotic phenotype followed bleomycin-induced lung injury (95). The development of this fibrosis was dependent on the hyaluronan receptor CD44 (95). Hyaluronan is a glycosaminoglycan produced in great abundance during noninfectious lung injury and regulates the inflammatory response (95, 96). Mice with deletion of HAS2 in mesenchymal cells fail to develop the same degree of fibrosis as wild-type mice (95). Knockdown of HAS2 or treatment with anti-CD44 antibodies inhibits the invasive capacity of IPF fibroblasts (95).

Mice deficient in β-arrestins are protected from bleomycin-induced pulmonary fibrosis (97). β-Arrestins regulate G-protein-coupled receptor signaling, and a deficiency in fibroblasts thwarts the invasive capacity of both murine and IPF fibroblasts (97). Lysyl oxidases modify extracellular matrix and have been suggested to contribute to tumor metastasis (98). Inhibition of lysyl oxidase-
like 2 has been shown to blunt the development of lung and liver fibrosis (99), and this approach is in early clinical development for IPF therapy (100, 101). Collectively, these studies address components of fibroblast/myofibroblast biology and suggest that the unrelenting nature of severe pulmonary fibrosis may relate to processes that promote tumor-like qualities in the expanding mesenchyme (Figure 5). Elucidating the key regulators of these properties, such as microRNAs (102–105) or epigenetic processes (106–108), are important areas of future investigation.

Summary
Severe pulmonary fibrosis is a complex process involving the interplay of a variety of cell types contributing to architectural distortion and loss of gas exchange function. Recognizing the complexities and heterogeneity of fibrosis in clinical settings is necessary for optimal clinical care and clinical trial enrollment. An understanding of how communication between the alveolar epithelium and mesenchyme leads to pathological phenotypes may unlock the mysteries of progressive pulmonary fibrosis and lead to the development of efficacious therapies for IPF.

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Address correspondence to: Paul W. Noble, Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, Duke University School of Medicine, 106 Research Drive, Durham, North Carolina 27710, USA. Phone: 919.684.0355; Fax: 919.684.5266; E-mail: paul.noble@duke.edu.

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