Cellular Mechanisms of Tissue Fibrosis. 7. New insights into the cellular mechanisms of pulmonary fibrosis

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PULMONARY FIBROSIS IS A GENERAL term used to describe an increased accumulation of extracellular matrix in the distal lung, rendering the lung stiff and compromising its ability to facilitate normal gas exchange. Patients typically present with the insidious onset of shortness of breath with exertion as the disease often goes unnoticed in its early stages. Pulmonary fibrosis can be associated with a number of underlying diseases (such as connective tissue/rheumatologic disease) or environmental exposures (asbestosis), or it can be idiopathic in nature. Idiopathic pulmonary fibrosis (IPF) is the most common form of fibrotic lung disease with a prevalence of 14.0–42.7 cases per 100,000 individuals in the United States (depending on the case definition used) and a median survival of 2.5–3.5 yr (64). It is viewed as a disease of aging, with the median age at diagnosis being in the mid-60s. There are few effective therapies for IPF short of lung transplant (52). Because a pharmacologic therapy capable of halting or at least slowing the progression of the disease has been elusive, there are intense efforts to better understand the factors that trigger and perpetuate this disease. As we begin to unravel this mystery, it is becoming clear that important clues lie in the complex cross talk that exists between the alveolar epithelium and the many cell types in the neighboring mesenchyme.

IPF arises in the alveolar regions of the lung, a region that consists of two kinds of epithelial cells, type 2 alveolar epithelial cells (which make large amounts of surfactant) (AEC2s) and type 1 cells (through which gas exchange occurs) (AEC1s), as well as a number of mesenchymal cell types (Fig. 1). It is hypothesized that cross talk between the alveolar epithelium and its associated mesenchyme is dysregulated in IPF pathogenesis, and this leads to the unchecked proliferation of extracellular matrix-producing cells. There is strong evidence, at least from genetic analysis of rare familial cases of IPF, that defects that incite the development of the disease can originate in the alveolar epithelium.

In this article, we review our current knowledge of triggers and/or perpetuators of pulmonary fibrosis with special emphasis on the alveolar epithelium. We present new ideas from mouse models and lineage-tracing studies that address the potential cell types responsible for generating the histology characteristic of IPF. We review current knowledge of the origins of pathologic myofibroblasts in the lung and provide some additional hypotheses. Throughout the article, we raise a number of questions to highlight critical voids in our current understanding of the pathogenesis of this disease.

Defects in Alveolar Epithelial Cells Underlying Disease Pathogenesis: a Historical Review

It is now generally accepted that insults to the alveolar epithelium or derangement of alveolar epithelial cells can serve as a trigger of this disease. This hypothesis is supported by evidence that has accumulated over a number of years. The first line of evidence is the presence of hyperplastic AEC2s overlying fibroblastic foci, both characteristic histologic hallmarks of the disease (34). The abnormal morphology of hyperplastic AEC2s in IPF is striking, as is the location of these cells (i.e., usually overlying fibroblast foci, the presumed “active” lesions in IPF). In light of clear evidence that AEC2s self-renew and give rise to AEC1s following bleomycin-induced lung injury in the murine lung (68), we hypothesize that the hyperplastic morphology of AEC2s in IPF relates to cellular stress and failure to regenerate AEC1s lost by injury or wear and tear. The inability of defective AEC2s to cover basement membrane denuded by loss of AEC1s may perpetuate the development of fibroblastic foci.
Fig. 1. Proposed model for the development of pulmonary fibrosis in human lung. The distal human lung consists of distal bronchioles (<3 mm in diameter) containing basal cells, Scgb1a1+ secretory cells, ciliated cells, and some mucus-producing cells [commonly MUC5B+, occasionally MUC5AC+ (71)]. The distal bronchioles lead to alveolar ducts that are lined by poorly defined cuboidal epithelium. The alveolar ducts ultimately open to the alveoli that contain Sftpc+ type 1 and 2 alveolar epithelial cells (AEC1s and AEC2s) in close association with a number of different mesenchymal cell types (top). At steady state when tissue turnover is low, AEC2s will occasionally self-renew and differentiate into AEC1s. Note the close approximation of AEC2s and the niche-comprising resident fibroblasts in the alveolar space.

Following injury or "wear and tear" to the alveolar epithelium in otherwise normal lung, dead alveolar epithelial cells are replaced by descendants of AEC2 stem cells that self-renew and differentiate into AEC1s. We also hypothesize that Scgb1a1+ cells and/or basal cells serve as a source of AEC2s following injury. This hypothesis is based on murine data revealing that bronchiolar epithelial cells in the mouse differentiate into both AEC2s and AEC1s following injury (68, 81, 92, 93). Note that in mouse bronchioles basal cells are not normally present. These repair processes effectively cover denuded basal lamina, and fibrosis does not develop. If injury occurs to alveolar epithelium that is already stressed or defective (as is hypothesized to be the case in IPF), the AEC2 stem cells cannot differentiate to effectively cover denuded basal lamina and profibrotic signals are likely released (middle). Based on the detailed description of human IPF histology, we hypothesize that the persistently denuded basal lamina is eventually covered via one of 2 proposed mechanisms (bottom). Both of these mechanisms result in the destruction of delicate alveolar architecture and deposition of significant amounts of extracellular matrix. The first ineffective repair model (bottom left) stems from human IPF histology data revealing the unusual presence of pseudostratified epithelium (with many mucus-producing cells) in distal airways and lining "honeycomb cysts" of IPF patients. At least 2, nonexclusive, hypotheses can explain this finding. First, conditions may be such that Scgb1a1+ cells in the distal bronchioles, differentiate predominantly into mucus producing goblet cells rather than AEC2 cells. Second, alveolar duct cells may change their phenotype and resemble bronchiolar epithelium, including basal cells. In the second ineffective repair model (bottom right), we hypothesize that stressed AEC2s are unable to differentiate into AEC1s but they retain some capacity to self-renew and cover the denuded basal lamina with a hyperplastic Sftpc+ cuboidal epithelium that lines the airspace. This epithelial type is associated with fibroblast foci. This model is speculative but based on extensive lineage-tracing of epithelial cells in the mouse (5, 68, 81, 93) and on detailed analysis of human IPF histology (71).
Also supporting the notion that IPF is driven by dysfunction of the alveolar epithelium are human genetic studies revealing associations between mutations in genes encoding surfactant proteins and the development of familial forms of pulmonary fibrosis (11, 24, 60, 73, 78). Supporting work has been done with gene-targeted mouse lines, reinforcing the human data and revealing that misfolded surfactant proteins can lead to enhanced endoplasmic reticulum stress and activation of the unfolded protein response (55, 56). These processes enhance fibrotic remodeling in the murine lung (41) and are upregulated in human IPF lung (39, 42).

Another line of evidence suggesting that pulmonary fibrosis is related to epithelial cell dysfunction is the correlation between certain latent viral infections, such as EBV, CMV, HHV-7, and HHV-8, and human IPF (17, 18, 35, 42, 77, 85, 90). Such viruses are known to infect and remain latent in pulmonary epithelial cells (75), thereby potentially rendering them less able to repair following subsequent infection. Injury may also potentially change the transcriptional program of these cells so that they begin to secrete profibrotic factors and recruit inflammatory cells and/or fibrocytes (cells of bone marrow origin that express both hematopoietic and stromal cell markers) to the lung. These hypotheses have been assessed in murine models of fibrosis. Established pulmonary fibrosis, induced by intratracheal FITC administration, can be exacerbated by latent y herpesvirus infection (51), a viral infection that maintains its persistence in lung epithelial cells (75). Such viral latency can augment fibrotic insult in the murine lung. This is secondary to an increase in production of the profibrotic factor transforming growth factor-β1 (TGβ1) and other factors that recruit circulating fibrocytes and potentially promote fibrosis via direct effects on mesenchymal cells (84).

Some of the most compelling evidence that points towards an etiology of IPF is human genetic data identifying mutations in telomerase and telomere-related genes in a subset of patients with IPF (3). Telomerase is an enzyme that synthesizes new telomere DNA, whereas telomere-related mutations. Moreover, IPF patients, on average, have shorter telomeres than age-matched controls in leukocytes and in alveolar epithelial cells (1, 10). Because short telomeres, even in the presence of telomerase, limit renewal capacity of high-turnover tissues (such as bone marrow, intestines, and testes) (25), it has been hypothesized that short telomeres in alveolar epithelial cells render these cells unable to effectively repair and cover denuded basement membrane following an injury, thereby leading to generation of profibrotic signals and perpetuation of fibrosis (1, 3).

Clinicians should be cognizant of the fact that telomere-mediated pulmonary fibrosis may actually be the presenting sign of dyskeratosis congenita, a syndrome that is also associated with extrapulmonary manifestations including mucocutaneous disease, bone marrow failure, and liver disease (e.g., cryptogenic cirrhosis) (86). Why is lung disease the predominant phenotype associated with telomere dysfunction in humans when cells in all organs are affected by the mutation? Perhaps the answer to this lies in subtle differences in the intrinsic regenerative capacity of stem cells in low vs. high turnover tissues? Perhaps the effect of telomere dysfunction extends beyond the epithelium and affects the mesenchymal cells in the alveolar niche? Further work is needed to answer these questions.

There are now limited data to suggest that derangements in bronchiolar epithelial cells (and not just alveolar epithelial cells) can predispose a patient to develop IPF. A rather common genetic variant in mucin 5B (MUC5B) is associated with the development of both familial and sporadic IPF (72, 91). The precise mechanism by which this genetic variant leads to fibrosis has not yet been elucidated, but there are several hypotheses. The first hypothesis is that variant MUC5B alters the local epithelial cell environment, perhaps by altering mucosal host defense and/or local cytokine production (72), and that these changes affect the capability of bronchiolar or alveolar progenitor cells to self-renew and differentiate in response to injury. A second, nonexclusive hypothesis is that the variant MUC5B leads to excessive mucus production that may provide a physical barrier, compromising normal epithelial repair of denuded basal lamina (72). Interestingly, a recent retrospective study of survival in two cohorts of patients with IPF revealed that the MUC5B polymorphism is significantly associated with improved survival and that there was evidence of a possible interaction between interferon-γlb treatment and MUC5B genotype (61). It is unclear why IPF patients with the MUC5B polymorphism fare better, but it is intriguing to consider that the disease is perpetuated by a unique mechanism that potentially leads to a more benign course or to a more robust response to therapy.

Further supporting the notion of an epithelial defect driving development of fibrosis are data suggesting that targeted ablation of AEC2s in the murine lung results in histologically apparent fibrosis (74). In these experiments, diptheria toxin receptor (DTR) expression was driven in AEC2s by the murine SPC promoter; intraperitoneal diptheria toxin (DT) was administered daily for 14 days. Because murine cells do not normally express DTR, only AEC2s expressing DTR could be destroyed following DT administration. Daily destruction of AEC2s in this injury model resulted in fibrosis. We conducted a similar experiment with a slightly different mechanism of AEC2 destruction that did not result in fibrosis (5). In our injury model, AEC2 death was induced once every 2 wk (as opposed to daily) through the administration of tamoxifen to mice carrying an inducible SftpcCreER allele and a ROSA locus containing a loxp-flanked stop sequence followed by the same coding region of the catalytic subunit of DT. Approximately 50% of AEC2s are destroyed with each tamoxifen dose, and the lungs was allowed to heal between episodes of AEC2 ablation. After four rounds of AEC2 ablation, we saw no clear histologic evidence of fibrosis in the lung parenchyma.
These separate but related AEC2 ablation experiments highlight the fact that different AEC2 ablation methods lead to different histologic changes in the lung. Daily ablation of AEC2s results in fibrotic change, likely due to persistence of denuded basal lamina and continued disruption in epithelial-mesenchymal cross talk. On the other hand, intermittent ablation allows for efficient repair and coverage of denuded basal lamina and absence of significant fibrosis. The discrepant results in these two injury models may also be due to differences in the specificity of diphtheria toxin-induced ablation. While SftpcCreER activity is active specifically in AEC2s in adult mice, it is possible that the constitutively active SPC-DTR transgene (expressed during embryonic development in Sftpc+ progenitors that may ultimately give rise to AEC1s) leads to some DTR expression on AEC1s in adult mice, thereby leading to ablation of AEC2s and AEC1s in this model. In either case, these experiments suggest that the mechanism and duration of epithelial injury are important when it comes to the development of fibrosis.

In summary, these data suggest that dysfunctional and/or damaged epithelial cells are critical players in the development and perpetuation of pulmonary fibrosis and support the concept that alveolar epithelium in IPF is vulnerable to insults (59). Much work needs to be done to elucidate the specific pathways that directly link epithelial cells with the behavior of specific mesenchymal cell types. It is likely that “stressed” epithelial secrete cytokines and/or growth factors that serve as profibrotic signals? If so, which are the key cytokines and on which specific cell types do they have an effect? Does the epithelium also lose its ability to secrete anti-fibrotic factors? Are there other regulatory/inhibitory factors from epithelial cells that keep stromal cell behavior in check? Does the epithelium secrete constitutive factors that promote self-renewal? If the primary problem is a deficiency in epithelial stem cell function, can we identify specific pathways that may be targeted to enhance stem cell capacity? These are all questions that have yet to be addressed but will be important in tackling as we try to identify more specific therapeutic targets to halt progression of fibrotic lung disease.

Mesenchymal Cells and Fibrosis Development and Progression

Are the types of epithelial cell abnormalities described above enough to explain the pathogenesis of pulmonary fibrosis? Or must there exist additional abnormalities in underlying stromal cells as well in order for fibrosis to develop and continue unchecked? Alternatively, can intrinsic alterations in mesenchymal cell types alone lead to the development of disease? To begin to answer these questions, we need to understand and be able to identify the different mesenchymal cell types that reside in close proximity to the alveolar epithelial cells. The stromal milieu is complex, and we need to precisely define cell types to better understand how the many cell types interact. There is a growing body of evidence that describes and supports the role of endothelial cells and immune cells (in particular macrophages) in the pathogenesis of pulmonary fibrosis, but an in depth assessment of the functions of these cell types is beyond the scope of this particular review.

The mesenchymal cell type most often associated with the development of pulmonary fibrosis is the myofibroblast, a cell type that typically expresses vimentin and aSMA (20). These fibroblasts are believed to synthesize the bulk of the excess collagen and other extracellular matrix components that are deposited in the fibrotic lung. This cell type is also implicated as the dominant source of collagen in normal wound healing (13, 22, 27), in invasive cancers (14, 69, 83), and in the fibrosis of other organs as well, including the kidney (6, 29, 46) and liver (37, 49, 63). While there exist many data to support the role of myofibroblasts in the pathogenesis of fibrotic disease, we are only beginning to understand the origin of this cell type in adult organisms. Understanding the origin and these cells is of utmost importance because inhibition of the development of these myofibroblasts from their precursor cells could potentially be efficacious from a therapeutic standpoint as IPF would then be targeted at a critical point in its pathogenesis and ongoing progression.

There are a number of theories regarding the origin of myofibroblasts, with sources ranging from the bone marrow to the epithelium. Recent work in kidney fibrosis has revealed that the majority of myofibroblasts derive from resident tissue fibroblasts (~50%) and from bone marrow-derived cells (~35%) with a minority from endothelial-to-mesenchymal transition (10%) and epithelial-to-mesenchymal transition (5%) (44). There is debate in the renal literature as to whether pericytes are a source of myofibroblasts and fibrosis. Earlier lineage-tracing work using a FoxD1-CreERT2 mouse line suggested that pericytes are a predominant source of myofibroblasts (29). However, more recent lineage-tracing work using both NG2-Cre and Pdgfrb-Cre suggests that only 3–6% of all myofibroblasts derive from pericytes (44). These disparate results highlight the need for a more nuanced understanding of the many cell types or cell subtypes that are involved in fibrosis progression.

Similar investigations to identify the origin of myofibroblasts have been conducted in the lung. While earlier work suggested that alveolar epithelial cells undergo epithelial-to-mesenchymal transition in the setting of fibrotic lung disease (36, 87), our more recent work has shown no appreciable contribution of lineage-labeled AEC2s to myofibroblasts after bleomycin injury to the murine lung (68). We reported a remarkable heterogeneity of stromal cell types in bleomycin-induced fibrosis in the mouse lung and in human IPF. In our murine model, we demonstrated the proliferation of lineage-labeled pericyte-like cells following bleomycin. These cells were labeled following activation of NG2-Cre (nerve/glial antigen 2 BAC transgenic line; Ref. 30) with tamoxifen administration. These lineage-labeled cells, for the most part, did not express aSMA and therefore were not thought to be a major source of myofibroblasts. In our human work, we reported the presence of abundant pericyte-like cells in IPF that, for the most part, did not express aSMA. These data suggest that pericyte-like cells proliferate and contribute to fibrotic remodeling but do not give rise to myofibroblasts.

More recently, different (but not necessarily conflicting) results related to whether pericytes can give rise to myofibroblasts in the lung have been reported by a group using a Foxd1-Cre line to label a stromal cell population during embryonic development (30). These cells express pericyte markers and following bleomycin injury, give rise to myofibroblasts (as assessed by the coexpression of lineage label and aSMA). So why do pericytes labeled with the Foxd1-Cre give
rise to myofibroblasts but those labeled with the NG2-CreER do not? One factor to consider is that the NG2-CreER was induced in the adult and only labeled 14 ± 4.9% of the pericytes/stromal cells (68). In addition, it is possible that pericytes of the Foxd1+ lineage do not express NG2 in the adult lung.

Additional work has been done to evaluate the contribution of bone marrow-derived progenitor cells in pulmonary fibrosis. While bone marrow-derived cells were apparent in fibrotic regions following intratracheal bleomycin, these cells did not express aSMA, suggesting that these cells were also not a source of myofibroblasts (26). Other reports suggest that circulating fibrocytes are trafficked to the lung following fibrotic stimulus and contribute to the cellularity of disease. Similarly, these cells do not clearly contribute to the myofibroblast burden in the fibrotic lung (62).

So from where do myofibroblasts that contribute to pulmonary fibrosis arise? If lung fibrosis mirrors kidney fibrosis in response to epithelial damage, a majority of myofibroblasts likely derive from tissue resident fibroblasts. What is the precise identity of those tissue resident precursor cells? Are all tissue resident fibroblasts capable of making this transition? Are all resident fibroblasts the same? Recent data suggest that a subset of resident fibroblasts expressing platelet-derived growth factor receptor alpha (Pdgfra) exist in close proximity to alveolar epithelial cells and are major components of the alveolar epithelial cell niche (5). Our previous work demonstrated that Pdgfra+ cells (identified based on the expression of a GFP reporter from a Pdgfra-H2B:GFP mouse line) proliferate following bleomycin lung injury (68). It is enticing to hypothesize that this abundant cell type is a source of myofibroblasts and a prime candidate for a major stromal mediator in the epithelial-mesenchymal cross talk that governs alveolar homeostasis. Another potential source of myofibroblasts is mesenchymal cells derived from the (transcription factor) Tbx4 lineage. Tbx4-derived cells in bleomycin treated murine lung co-express a variety of stromal cell markers, including aSMA (79). Rigorous lineage-tracing experiments to follow the fate of these cell types specifically will need to be done to address this question with some clarity. Once the identities of myofibroblast precursor cells are more specifically understood, more detailed analyses of the pathways that govern their proliferation and differentiation can be undertaken, with the goal of identifying therapeutic targets that can ameliorate lung fibrosis.

Finally, we raise the question of whether aSMA+ myofibroblasts are the only cells capable of producing extracellular matrix components and contributing to scar formation in the lung. We have provided examples of other stromal cell types that proliferate in the lung following bleomycin instillation. These cell types (e.g., pericytes and/or pericyte-derived cells, resident fibroblasts, and fibrocytes) are, for the most part, not aSMA expressing, but do they still contribute to generation of stiff extracellular matrix? How should we define these stromal cell subsets? How, if at all, do these individual cell subtypes interact with epithelial cells? What gene regulatory programs are responsible for their behavior? Much in vitro IPF work has been done with aSMA-expressing myofibroblasts (often collected and grown based on their ability to grow efficiently on plastic). It may be time to develop strategies to identify and isolate other stromal cell types to better understand their potential roles in the pathogenesis of fibrosis.

Using In Vitro Models to Understand the IPF Myofibroblast Phenotype

As mentioned above, a significant amount of work has been done to characterize the identity and behavior of the presumed pathogenic fibroblasts in IPF. We will give a brief overview of the types of experiments that have been conducted, the hypotheses that have been generated, and the limitations and caveats associated with these experiments, all with the goal of demonstrating the current scope of our understanding of the identity and behavior of these cells. Before proceeding, however, it is important to point out that the human fibroblasts used in the work described below were typically obtained from the tissue based on their ability to grow out of tissue explants onto tissue culture dishes. We are aware of few, if any, attempts to identify and characterize the subsets of fibroblasts and/or other stromal cell types that are isolated under these conditions. Whereas much of the murine in vivo work described previously/being done is becoming more focused on specific stromal cell types, the human stromal cells studied in vitro have remained less well characterized.

IPF fibroblasts possess a malignant phenotype with an increased capacity for invasion. It has been proposed that fibroblasts in the IPF lung acquire a phenotype that is reminiscent of malignant cells. Nearly two decades ago, it was shown that fibroblasts from the IPF lung display enhanced migratory capacity when assessed in a chemotaxis chamber with platelet-derived growth factor (PDGF) as the chemoattractant. Interestingly, fibroblasts from tissues with more dense fibrosis displayed capacity for migration compared with fibroblasts isolated from earlier stage disease (76). Subsequent in vitro work has shown that IPF fibroblasts, compared with fibroblasts from normal human lung, display slower growth rates, higher rates of apoptosis, and a profibrotic secretory phenotype (65). In addition, fibrotic lung fibroblasts, unlike normal fibroblasts and more consistent with cancer-derived cells, are able to survive in the absence of attachment and interaction with extracellular matrix and neighboring cells, displaying anchorage-independent growth in soft agar (80). These results, when taken together, suggest that IPF fibroblasts demonstrate a malignant phenotype.

In following up on these observations, experiments have been conducted to more specifically determine the capacity of human IPF fibroblasts to migrate and invade matrix and to identify the regulatory functions that govern this behavior. Li et al. (45) have shown, through the use of an invasion assay in which fibroblasts isolated from human lungs are evaluated for their ability to spontaneouly invade Matrigel (a basement membrane matrix; BD Biosciences), that IPF fibroblasts display increased invasive capacity compared with fibroblasts from normal lungs and that this phenotype is regulated by hyaluronan synthase 2 (HAS2) and CD44.

IPF fibroblasts demonstrate impaired mechanosensitive signaling. It has long been viewed that myofibroblasts, with their contractile properties, are key effector cells in wound healing. After facilitating wound closure, these cells typically disappear from granulation tissue, presumably via a de-differentiation mechanism (37), a clearance mechanism (21, 40), or a combination of both. In IPF, myofibroblasts are believed to persist inappropriately, leading to progressive fibrosis. But what drives their persistence? It has been shown that mechanical
stimuli (e.g., stiff extracellular matrix with myofibroblasts generating high contractile forces) can be converted to fibrogenic signals \( \text{e.g.}, \text{liberation of TGF-\(\beta\)} \), which, in turn, maintains the myofibroblastic phenotype \( \text{88} \). More recently, it has been shown that there exists an intrinsic mechanotransduction mechanism that promotes myofibroblast differentiation. This mechanism is regulated by nuclear translocation of MKL1 \( \text{myocardin-related transcription factor-A, a mechanosensitive transcription factor that is involved in activating the fibrotic gene program} \) and results in stiff matrix-promoting aSMA gene expression by normal lung fibroblasts \( \text{28} \). These experiments were done by comparing (myo)fibroblast behavior on polyarylamide hydrogels of differing stiffness. In following up on this work, a series of ex vivo and in vivo experiments by Zhou et al. \( \text{94} \) have demonstrated that this intrinsic mechanotransduction is mediated by the Rho kinase (ROCK) pathway and that this pathway regulates myofibroblast contractility, differentiation, and survival. Significantly, these experiments also demonstrated that preexisting myofibroblasts can be shuttled to an apoptotic fate if their contractile properties are disrupted.

Utility and Limitations of Animal Models in the Study of IPF

A perennial topic of debate in the field of IPF is the utility of animal models in the study of the disease. Bleomycin, a chemotherapeutic agent used in the treatment of certain human cancers, has been the most commonly used agent to induce pulmonary fibrosis in animal models of the disease. Bleomycin can be administered through a variety of routes including intratracheal \( \text{most common} \), intraperitoneal, oropharyngeal aspiration, and via osmotic pump. It induces DNA strand breaks \( \text{48} \) and oxidative injury \( \text{70} \), thus leading to epithelial injury, inflammation, and ultimately fibrosis.

The pathology generated by intratracheal bleomycin is not fully representative of IPF histology. The diagnostic criteria for IPF \( \text{usual interstitial pneumonia} \) are threefold: 1) nonuniform pattern of disease involvement with normal lung interspersed with diseased lung, 2) architectural distortion \( \text{honeycomb change and/or scar} \), and 3) presence of fibroblast foci, presumed to be indicative of current ongoing disease. These structures are covered by hyperplastic AEC2s \( \text{33} \). While not a diagnostic criterion, human IPF specimens also typically include areas of alveolar collapse with incorporation of basa lamina \( \text{57} \). While experimental bleomycin fibrosis can recapitulate alveolar collapse and cystic air spaces 14 days after intratracheal instillation \( \text{54} \), it is also typically characterized by significant neutrophilic inflammation and there rarely exist examples of the hyperplastic AEC2s that are pathognomonic for the human disease \( \text{15, 54} \).

Unlike IPF, however, the fibrosis generated after intratracheal bleomycin is not progressive. This is one of the most common criticisms of this experimental model. Following intratracheal bleomycin, collagen content \( \text{as assessed by hydroxyproline assay} \) peaks around 21–28 days postinjury \( \text{31} \). Recent reports and our own personal experience with this model suggest that the fibrosis induced by a single exposure to bleomycin is self-limited and can display some resolution/reversal during the weeks following the injury \( \text{7, 43, 68} \).

Investigators have tried to optimize the bleomycin fibrosis model to better replicate the histology associated with human IPF. In one such study, a repetitive bleomycin model was developed in an attempt to recapitulate the recurrent alveolar injury that is hypothesized to drive IPF pathogenesis. Degryse et al. \( \text{15} \) describe a model in which they administered intratracheal bleomycin biweekly up to eight times. The histology from this repetitive injury model revealed prominent hyperplastic AEC2s in areas of fibrosis as well as more of a temporally heterogeneous pattern of lung injury \( \text{i.e., fibrotic scar next to hyperplastic AEC2s next to normal tissue} \). Further, the fibrosis that developed seemed to persist until at least 10 weeks after the last bleomycin dose. While the histological results of this model do seem more consistent with human IPF, the time-intensive nature of this model may limit its applicability in the laboratory.

Despite its imperfections, the bleomycin model of pulmonary fibrosis remains the most common in the study of fibrotic lung disease. Other fibrosis generating models include the following \( \text{reviewed in Ref. 53} \): granulomatous inflammation \( \text{32} \), fluorocine isocyanate \( \text{38, 67} \), irradiation-induced \( \text{50} \), adenosine deaminase deficiency \( \text{8} \), and murine gamma-herpesvirus \( \text{which is typically used to augment a fibrotic response to another stimulus} \) \( \text{23, 47} \). While many investigators are now designing experiments with human IPF tissue/cells, the field at large still relies heavily on murine models of the disease. A murine model of IPF that recapitulates the disease more faithfully than bleomycin would be most welcome.

New Models/Hypotheses for the Development of Human IPF

By combining the data outlined above, we have created a cellular model for the development of pulmonary fibrosis with special emphasis on epithelial cell defects and proposed mechanisms of ineffective epithelial repair. In our model \( \text{Fig. 1} \), we depict normal distal bronchioles and alveoli at steady state. Under these circumstances, AEC2s self-renew and differentiate to AEC1s in response to normal tissue wear and tear. Secretory cells in the distal bronchioles or the poorly defined cells of the alveolar duct may also self-renew and under some conditions may differentiate to AEC2s as well. This hypothesis is supported by murine work demonstrating that distal bronchiolar cells that express Scgb1a1+ can give rise to AEC2s and AEC1s after bleomycin injury and H1N1 influenza infection \( \text{68, 81, 93} \). In a normal lung, alveolar epithelial injury \( \text{whether it be infectious, environmental, or otherwise} \) is efficiently repaired by an increase in self-renewal and differentiation of AEC2s to replace dead epithelial cells and to cover denuded basal lamina. In pulmonary fibrosis, however, the AEC2s are unable to respond to epithelial injury and the basal lamina remains denuded. This is hypothesized to cause release of profibrotic mediators and proliferation and/or recruitment of fibroblast cell types to the mesenchymal space as the lung attempts to heal the wound. In this scenario, epithelial repair is ineffective and hallmark IPF pathology results.

While epithelial dysfunction is undoubtedly a driver of IPF, it is unlikely that epithelium-specific abnormalities are the only contributors to disease progression. We hypothesize that IPF develops because epithelial injury and/or cellular stress is met by a dysregulated mesenchymal response. This allows for the establishment of what is likely to be a self-perpetuating cycle of stromal cell proliferation, extracellular matrix deposition, and scar formation, all of which may then further inhibit
normal epithelial repair (Fig. 2). By better understanding the normal epithelial-mesenchymal cross talk in the lung and by parsing out how these pathways become dysregulated in IPF, we may ultimately be able to address the treatment of this disease in a novel fashion.

**Therapeutic Perspectives**

At present, there are still no widely effective therapies for IPF. We anticipate 2014 to be a watershed year for clinical trials and drug development for IPF as the results of several large trials will become available: the INPULSIS trial, the TOMORROW trial, and the ASCEND trial. The INPULSIS trial consists of two phase 3 trials comparing the safety and efficacy of the multikinase inhibitor Nintedanib (BIBF 1120) in two cohorts of well-characterized IPF patients. The phase 3 TOMORROW trial, which follows a promising phase 2 trial suggesting that Nintedanib may slow the loss of lung function in IPF patients over 1 yr (66), includes a total of 1,066 patients who were randomized 3:2 to receive Nintedanib or placebo for a total of 1 yr; this trial has a primary endpoint of rate of decline in forced vital capacity. The phase 3, double-blind, placebo-controlled ASCEND trial has been designed to evaluate the safety and efficacy of pirfenidone, a small molecule with antifibrotic properties whose benefit was originally suggested nearly a decade ago (4) and that has subsequently been shown in the CAPACITY trials to reduce the decline in lung function of a well-characterized cohort of IPF patients at 1 yr (58). In addition to these landmark studies, several phase 2 trials involved interesting biological targets are enrolling patients (recently reviewed in Ref. 59). Although the last decade

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**Fig. 2.** Potential pathways involved in the development of pulmonary fibrosis. The biology underlying the pathogenesis of pulmonary fibrosis is complex. This figure illustrates known and hypothesized relationships between two components of the alveolar region of the lung, the epithelium and the mesenchyme. We hypothesize that dysregulated cross talk between these two components leads to development of the disease. As depicted here, while there may be an inciting factor or event that predisposes an individual to developing the disease (e.g., chronic cellular stress or epithelial injury), a host of other abnormal responses (e.g., abnormal proliferation of fibroblasts and excessive stiffening of the mesenchyme) are likely to be integral in the progression of fibrosis. Concepts written in italics within the concept boxes in the figure are hypothesized and yet to be experimentally proven in the precise context presented. ER, endoplasmic reticulum; SNP, single-nucleotide polymorphisms; TGF-β1, transforming growth factor-β1.
has witnessed a number of failed clinical trials in IPF, the current and future landscape holds considerably more promise.

Summary

We are beginning to better understand the complex cellular relationships that affect the development of pulmonary fibrosis. While progress has been made to understand the specific cell types that drive disease progression and the mechanisms through which multiple cell types interact, many questions still remain. We have proposed several models for the development of disease and have highlighted specific areas of ambiguity. As the outstanding issues begin to be addressed, we may move even closer to identifying effective therapeutic targets in the treatment of IPF.

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AUTHOR CONTRIBUTIONS

Author contributions: C.E.B. prepared figures; C.E.B. drafted manuscript; C.E.B. and P.W.N. edited and revised manuscript; C.E.B. and P.W.N. approved final version of manuscript.

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