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Authors

Wolters, Paul J
Collard, Harold R
Jones, Kirk D

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Pathogenesis of Idiopathic Pulmonary Fibrosis

Paul J. Wolters[1], **Harold R. Collard**[1], and **Kirk D. Jones**[2]

Paul J. Wolters: paul.wolters@ucsf.edu; Harold R. Collard: hal.collard@ucsf.edu; Kirk D. Jones: kirk.jones@ucsf.edu

[1]Department of Medicine, School of Medicine, University of California, San Francisco California 94143

[2]Department of Pathology, University of California, San Francisco, California 94143

Abstract

Idiopathic pulmonary fibrosis (IPF) is a fibrosing interstitial lung disease associated with aging that is characterized by the histopathological pattern of usual interstitial pneumonia. Although an understanding of the pathogenesis of IPF is incomplete, recent advances delineating specific clinical and pathologic features of IPF have led to better definition of the molecular pathways that are pathologically activated in the disease. In this review we highlight several of these advances, with a focus on genetic predisposition to IPF and how genetic changes, which occur primarily in epithelial cells, lead to activation of profibrotic pathways in epithelial cells. We then discuss the pathologic changes within IPF fibroblasts and the extracellular matrix, and we conclude with a summary of how these profibrotic pathways may be interrelated.

Keywords

pulmonary fibrosis; epithelial-to-mesenchymal transition; unfolded protein response; alveolar type II cell; fibrocytes; myofibroblast; epigenetics

Clinical Features of Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is defined as a chronic fibrosing interstitial lung disease of unknown etiology that occurs in adults and is characterized by the histopathological pattern of usual interstitial pneumonia (1). It is the most common of the idiopathic interstitial pneumonias and one of the most common forms of interstitial lung disease overall, with an estimated prevalence of 50 per 100,000 (2). Importantly, the prevalence of IPF rises dramatically with age; it is virtually absent in patients younger than 50 but is present in an estimated 0.2% of those older than 75 (2).

Among the various forms of interstitial lung disease, IPF has received the most attention because of its uniquely poor prognosis and its unresponsiveness to traditional therapies. Median survival for IPF is just 3 years; progressive breathlessness and eventual respiratory failure are the most common natural history (3). Many patients with IPF experience acute episodes of respiratory worsening that are associated with a high risk of death (4). In approximately half of these acute events, no cause can be found; these idiopathic acute worsenings are termed acute exacerbations (5). It has been hypothesized that acute exacerbations represent intrinsic acceleration of the underlying disease process in IPF,

perhaps triggered by an occult stressor such as viral infection, microaspiration, or ambient pollution (6, 7).

There are no widely accepted therapies for IPF, although the novel antifibrotic agent pirfenidone has been approved for use in some countries (8). Notably, traditional therapy for interstitial lung disease using prednisone and immunomodulatory agents such as azathioprine is ineffective. In fact, a recent randomized controlled trial of combination therapy with prednisone, azathioprine, and the antioxidant acetylcysteine was stopped prematurely for futility and safety concerns in the active therapy arm (9). Because of this lack of efficacy, drug development has focused on targeting fibrogenesis and fibroproliferation, and an increasing number of targeted therapies are in early-phase clinical trials. It seems likely that the therapeutic landscape for IPF will change dramatically in the next decade.

Comprehensive management of the patient with IPF involves establishing an accurate diagnosis through careful multidisciplinary review; management of common comorbidities such as gastroesophageal reflux (GER) disease, obstructive sleep apnea, and depression; vaccination against influenza and pneumococcal infection; structured exercise and education through formal pulmonary rehabilitation classes; and, in progressive or advanced disease, assessment of appropriateness for lung transplantation. In the absence of effective therapies, all patients with IPF should be evaluated for clinical trials of novel drug agents. In patients who do not qualify for a clinical trial, empiric therapy with acetylcysteine and/or proton pump inhibition should be considered (1).

Pathologic Features of Idiopathic Pulmonary Fibrosis

The gross appearance of the lungs in IPF shows a characteristic fibrosis that is distributed along the inferior portions of the lobes with subpleural accentuation. The pleural surface has a bosselated or cobblestone appearance, and on cut section, these regions correspond to areas of airspace enlargement and fibrotic retraction. This pattern of fibrosis has been termed gross honeycombing. The typical microscopic appearance of IPF has been termed usual interstitial pneumonia and is one of spatial (or geographic) and temporal heterogeneity. The spatial heterogeneity of this lesion results in a patchy fibrotic reaction with fibrosis that is prominent in the peripheral portion of the secondary pulmonary lobule and spares the central portion of the lobule. This distribution of fibrosis is also reflected in the temporal heterogeneity of the lesion. As one moves from the peripheral to the central portion of the pulmonary lobule, the lung injury pattern changes from chronic to acute to absent. The regions in the immediate subpleural parenchyma often show advanced scarring and microscopic honeycombing characterized by irregular airspaces lined by bronchiolar epithelium and surrounded by dense fibrosis. The central portion of the pulmonary lobule often shows delicate alveolar septa without significant inflammation or fibrosis (Figure 1). A characteristic finding of usual interstitial pneumonia is the presence of fibroblast foci at the interface between the fibrotic and less involved regions of the lobule (1). These fibroblast foci manifest as proliferations of spindled fibroblasts, often arranged parallel to the alveolar surface, within a lightly basophilic myxoid background (Figure 2). The epithelial surface

above the fibroblast focus often has a cuboidal appearance similar to that seen in in reactive type II pneumocyte hyperplasia.

Usual interstitial pneumonia secondary to idiopathic pulmonary fibrosis is often identified by a paucity of inflammation and a lack of uniform involvement of the lung tissue. Other diseases with similar features often manifest as fibrosis secondary to alveolitis and may yield additional histologic findings that aid in their identification. In autoimmune connective tissue diseases, the lung tissue may have some features of usual interstitial pneumonia, such as peripheral fibrosis and honeycombing; however, there is often additional centrilobular fibrosis in which the less involved lung tissue shows a lacy fibrosis or chronic inflammation (10). In addition, these cases often show prominent lymphoid aggregates or evidence of pleuritis (11). In hypersensitivity pneumonia, the lung may also have peripheral fibrosis with honeycombing; however, many cases also show prominent peribronchiolar fibrosis and may show scattered, poorly formed granulomas (12, 13).

Acute exacerbation of usual interstitial pneumonia shows a pattern of acute lung injury (diffuse alveolar damage) superimposed upon the typical findings of pulmonary fibrosis (Figure 3). These changes are most pronounced in the regions of the lungs that lack significant chronic fibrosis. The fibrotic regions often appear unchanged at the periphery of the lung. The central portions of the lobule show histologic changes typical of diffuse alveolar damage, including diffuse alveolar septal thickening by edema and type II pneumocyte hyperplasia as well as accumulation of airspace fibrin and edema. Other clues of acute exacerbation include nonspecific changes such as hyaline membranes, distal airway squamous metaplasia, and thrombi within small pulmonary arteries.

Pathobiology of Idiopathic Pulmonary Fibrosis

In the following review of the pathobiology of IPF we focus on data obtained from patients with the disease. We do so because there is no animal model that reliably recapitulates the pathologic changes in idiopathic pulmonary fibrosis (14, 15). The bleomycin model of lung injury is commonly used to study molecular pathways of pulmonary fibrosis. However, pathologic changes seen in this model do not recapitulate those seen in idiopathic pulmonary fibrosis. In the bleomycin model, the fibrosis evolves over months, whereas in idiopathic pulmonary fibrosis, the fibrosis evolves over years. In the bleomycin model, the fibrosis is patchy and tends to be airway centric. In idiopathic pulmonary fibrosis, the fibrosis has subpleural accentuation, and the fibroblasts are arranged into a highly organized reticulum (14, 15). These changes are not found in the bleomycin model (15). Therefore, although the bleomycin lung fibrosis model can be used to identify mediators that have potential to cause lung fibrosis, the findings in mice need confirmation in translational studies in fibrotic lung diseases in humans. Where mentioned, data from animal models are used to emphasize specific profibrotic molecular mechanisms and pathways that have been more clearly defined using animal models.

Epithelium

Genetics—Early reports of cases of pulmonary fibrosis occurring in families suggested a genetic predisposition to some forms of the disease (16–18). These cases were followed by

reports that pulmonary fibrosis developed in a subset of patients with defined clinical syndromes such as Hermansky-Pudlak syndrome and dyskeratosis congenita (19–21). Having established that pulmonary fibrosis occurs in patients with defined clinical syndromes enabled investigators to collect cohorts of individuals with uniform disease phenotypes and analyze them for the genetic mutations associated with these diseases. This analysis led to the association of mutations in the *HPS* gene in patients with Hermansky-Pudlak syndrome (22, 23) and mutations in the *DKC*, *TERT*, and *TERC* genes in patients with dyskeratosis congenita (24). These genetic mutations were the first to be linked to syndromes complicated by lung fibrosis and empowered subsequent genetic analysis of pedigrees of families with other forms of lung fibrosis.

In 2001, Nogee et al. (25) described a family with interstitial lung disease. Using a candidate-gene approach, they identified a heterozygous mutation in the surfactant protein C (SPC)-encoding gene that causes skipping of exon 4 and deletion of the terminal 37 amino acids (SPC ^{exon4}). This report indicates that mutations in SPC, a major protein manufactured and secreted by alveolar type II cells, could lead to interstitial lung diseases. Shortly thereafter, Thomas et al. (26) reported a Q188L missense mutation of the C-terminal portion of SPC in a large kindred of patients with familial pulmonary fibrosis (FPF). Subsequent reports have identified an additional six mutations in the *SPC* gene that are associated with pulmonary fibrosis (27). Most of these mutations occur in the BRICHOS domain (28), which serves a chaperone function for SPC that prevents the aggregation of SPC while aiding its insertion into membranes (28). Although these studies provide strong evidence for linkage of SPC mutations to specific familial cases of pulmonary fibrosis, these mutations reportedly occur in only 1% of sporadic cases of pulmonary fibrosis (Figure 4) (29, 30).

Whole-genome linkage analysis on a large kindred of individuals with pulmonary fibrosis was performed using a single-nucleotide polymorphism (SNP) panel of more than 6,000 markers (31). The analysis identified chromosome 10q22 as the affected region. The genes encoding surfactant proteins A1, A2, and D, which reside in this region of the chromosome, were identified as candidate genes. Sequencing these genes identified a missense mutation predicting a G231V missense mutation in surfactant protein A2 (31). Subsequent sequencing of the surfactant protein A genes in other FPF kindreds identified a second missense mutation (F198V). Both mutations predict instability of the translated protein, and the mutated proteins are abnormally retained in the endoplasmic reticulum (ER) and fail to form higher-order oligomers.

The observation that patients with dyskeratosis congenita, a disease of premature aging, develop pulmonary fibrosis provided the first clue that mutations in age-related genes may predispose to pulmonary fibrosis (20, 32). This association led to the sequencing of *TERT* and *TERC* as candidate genes in 73 probands of FPF kindreds (33). Mutations in *TERT* or *TERC* were identified in 6 of the 73 probands, establishing that these mutations are associated with cases pulmonary fibrosis independently of other features of dyskeratosis congenita. Similarly, a whole-genome linkage scan of two large FPF kindreds displayed linkage to chromosome 5p15 with a logarithm of odds (LOD) score of 2.8. Sequencing the *TERT gene*, which resides in this region of the chromosome, revealed a missense R865H

mutation and a frameshift V747fs mutation in the probands of the two kindreds (34). Sequencing additional families identified four additional *TERT* mutations and one *TERC* mutation in affected probands. Similarly, mutations in *TERT* or *TERC* have been identified in individuals with sporadic IPF (35, 36). Interestingly, although mutations in *TERT* or *TERC* are identified in a small minority of sporadic IPF patients (Figure 4), a significantly higher proportion of affected patients have short telomeres when measured in either their peripheral blood (36) or alveolar epithelial cells (AECs) (35). Collectively, these studies show that mutations in the aging-related genes *TERT* and *TERC* are associated with up to 15% of FPF cases and rare sporadic IPF cases. The questions of why patients with IPF have short telomeres despite having no *TERT* or *TERC* mutation and how these mutations or shortened telomeres contribute to the development of lung fibrosis have yet to be answered.

In 2011, a large genome-wide association study reported the linkage of a SNP in 82 families with FPF (37). The strongest linkage occurred in chromosome 11: marker D11S1318, with a LOD score of 3.3. Finer mapping of this region located a minor allele of the SNP rs35705950, located 3 kb upstream of the *MUC5B* gene, that was present in 34% of individuals with FPF. Further analysis found this SNP in 38% of individuals in a cohort of sporadic IPF patients and 9% of healthy controls. The rs35705950 allele was associated with lung expression of MUC5B that was 37.4 times higher than in unaffected subjects. Independent analysis confirmed association of the rs35705950 SNP with sporadic IPF in two additional cohorts (38): The SNP was present in 34% of patients with IPF and only 11% of healthy controls. Although these data provide strong evidence for a linkage of *MUC5B* polymorphisms to IPF, the molecular consequences of the SNP and how increased MUC5B protein expression may contribute to development of lung fibrosis require further explanation.

Collectively, analysis for genetic mutations and polymorphisms in patients with FPF or sporadic IPF has yielded significant advances in understanding the genetic predisposition to development of lung fibrosis. A common theme to all the mutations identified to date is that they affect genes that are either expressed in lung epithelial cells (e.g., *SPA*, *SPC*, *MUC5B*) or lead to identifiable molecular changes in AECs (*TERT, TERC*). For example, telomeres are short in the AECs of nearly all patients with sporadic IPF (35). As discussed below, this knowledge has centered the understanding of the pathology of IPF around the role that the epithelial cell, and its dysfunction, plays in the development of lung fibrosis.

Endoplasmic reticulum stress—The ER is the cellular compartment where secreted and membrane proteins are made, folded, and matured for packaging and trafficking via the Golgi complex. ER stress occurs when there is an imbalance between cellular demand for protein synthesis and the ER's capacity to synthesize, process, and package the requisite proteins (39). In response to this stress, the cell activates a cellular response termed the unfolded protein response (UPR) that leads to activation of biochemical pathways designed to match the protein production capacity of the ER. If the UPR cannot match the demand, a terminal UPR is activated and the cell sacrifices itself through apoptotic pathways (39, 40). Mammalian cells contain three sensors of unfolded proteins—IRE1α (inositol-requiring enzyme 1α), PERK (protein kinase RNA-like ER kinase), and ATF6 (activating transcription factor 6)—which, when activated, initiate the UPR. Of these sensors, IRE1α is

a key mediator of the apoptotic switch (41, 42). When the ER is under stress, IRE1α senses the unfolded proteins associated with such stress and undergoes *trans*-autophosphorylation. This phosphorylation signals the activation of proapoptotic pathways (41). In addition, IRE1α has an RNase that, when active, splices XBP1 (X-box-binding protein 1) mRNA to produce the transcription factor XBP1s, whose targets enhance ER protein-folding capacity (43). Thus, two markers of ER stress and the UPR are phosphorylation of IRE1α and splicing of XBP1.

Studies have reported that markers of UPR activation are elevated in alveolar type II cells of patients with IPF (44–46). AECs in IPF lungs express increased levels of p50ATF6 (the processed form of ATF6) (45, 47), CHOP [CCAAT/enhancer-binding protein (C/EBP) homologous protein], BiP (binding immunoglobulin protein), and IRE1α (44–46). In addition, type II cells isolated from IPF lungs demonstrated increased levels of active phosphorylated of IRE1α, XBP1s.

Many possible causes of ER stress have been identified in patients with IPF. Herpesvirus protein expression colocalizes with markers of the UPR in AECs from patients with IPF (46), suggesting that ectopic production of viral proteins may activate the UPR. As mentioned above, mutations in SPC and SPA have been identified in patients with lung fibrosis. When the SPC mutants SPC ^{exon4} or L188Q are expressed in lung epithelial cells, they activate mediators of the UPR, as evidenced by increased levels of BiP, EDEM (ER degradation–enhancing α-mannosidase-like protein), p-eIF2α (phosphorylated - eukaryotic initiation factor 2 alpha), and XBP1s (48-50). Similarly, expression of the G231V or F198S mutant of SPA in epithelial cells leads to increased levels of BiP and XBP1s (51). These potential causes of UPR activation in IPF lungs cannot be the only ones, because surfactant mutations account for no more than 5% of cases of familial or sporadic IPF (Figure 4), and herpesviruses have been identified in patients with sporadic IPF (46, 52). Therefore, if UPR activation is a central pathway promoting development of IPF, other triggers of UPR activation need to be identified.

TGF-β activation—Levels of active transforming growth factor β (TGF-β) are increased in the lungs of patients with IPF. All three isoforms (TGF-β1, TGF-β2, and TGF-β3) of inactive TGF-β are synthesized and secreted bound to the latency-associated peptide (LAP) (53). Under normal conditions, TGF-β is bound in its inactive form to LAP, which is disulfide-linked to latent TGF-β-binding proteins, cross-linked to the extracellular matrix. During the development of lung fibrosis, AECs express increased levels of the integrin $α_vβ_6$ (54), which can bind to the arginine-glycine-aspartate (RGD) sequence of LAP (55). Binding of $α_vβ_6$ is restricted to the TGF-β1 and TGF-β3 isoforms because the TGF-β2 isoform lacks an RGD sequence. Activation of epithelial cells expressing $α_vβ_6$ through binding of mediators such as thrombin (55b) and the phospholipids sphingosine 1-phosphate (56) and lysophosphatidic acid (57) to their receptors induces actin/myosin-mediated contraction of the epithelial cell. This contraction pulls on the latent TGF-β tethered to the epithelial cell via binding of LAP to the integrin $α_vβ_6$. This retraction induces TGF-β activation and signaling in localized, spatially restricted lung regions because the active TGF-β does not appear to be released into a soluble form. Whether $α_vβ_6$-mediated TGF-β activation contributes to IPF has yet to be proved, but possible profibrotic processes

associated with TGF-β activation include inhibition of AEC proliferation, differentiation of fibroblasts to myofibroblasts (59), and activation of programming that promotes mesenchymal transition of epithelial cells (60).

Epithelial-to-mesenchymal transition—Epithelial-to-mesenchymal transition (EMT) is the process by which epithelial cells acquire molecular and cell physiologic features commonly associated with mesenchymal cells following activation by specific growth factors, of which TGF-β is the prototype (60, 61). This molecular reprogramming, which causes epithelial cells to express mesenchymal cell–associated genes, occurs in three biologic contexts: development, cancer, and fibrosis (62). Many molecular pathways for these three subtypes of EMT overlap regardless of the biologic context. For example, growth factor activation of epithelial cells triggers upregulation of a specific set of proteins (e.g., slug, FSP1, αSMA) and downregulation of others (e.g., E-cadherin, syndecan 1) (62). These changes in protein expression cause epithelial cells to lose their polarity and tight junctions and to become more mobile (62).

Normal tissue homeostasis does not require EMT (62, 63); rather, EMT programming is activated during conditions of tissue injury and remodeling (62, 63). The evidence that alveolar cells exhibit EMT in IPF patients is based on immunohistochemical studies colocalizing epithelial cell– and mesenchymal cell-associated proteins within IPF lungs (60, 64, 65). In one report, the alveolar type II cell markers TTF-1 (thyroid transcription factor 1) and prosurfactant protein B colocalized with αSMA (α smooth muscle actin) in the majority of AECs in IPF lungs (65). Similarly, immunostaining colocalized the alveolar type II cell marker prosurfactant protein C and the mesenchymal proteins N-cadherin, αSMA, and calponin 1 in IPF lungs (60, 64). These proteins did not colocalize in normal lungs. mRNA analysis confirms that epithelial cells express mesenchymal proteins in IPF lungs. Epithelial cells isolated by laser capture express higher levels of mRNA for the mesenchymal proteins type I collagen, αSMA, and calponin 1 (64). This finding was confirmed in AECs isolated from IPF lungs, which also demonstrated elevated mRNA levels for type I collagen, αSMA, and calponin 1 as well as the transcription factor SLUG (64), which has been shown to promote EMT.

Although evidence indicates that epithelial cells acquire mesenchymal features in IPF lungs, the contribution of these mesenchymal changes to the fibrotic process remain unresolved. One ongoing controversy is whether epithelial cells acquire sufficient mesenchymal characteristics that they can be classified as fibroblasts (61, 66). In humans, the only datum supporting this possibility is the finding that fibroblasts isolated from IPF patients express the epithelial cell protein keratin 18 (67). However, this finding does not prove that fibroblasts are derived from epithelial cells, because it is possible that fibroblasts express this epithelial cell–associated protein in isolation or are undergoing a mesenchymal-to-epithelial transition (68). Although it is difficult to resolve whether fibroblasts are derived from epithelial cells in human studies, elegant lineage tracing studies have shown that the transition of alveolar type II cells accounts for 30–40% of cells expressing the mesenchymal proteins vimentin or S100A4 in models of lung fibrosis (60, 69). Similar lineage tracing studies have reported that myofibroblasts, in contrast, are rarely derived from epithelial cells in the bleomycin lung fibrosis model (66, 69). Whether AECs ever express a sufficient

number of mesenchymal markers to identify them as fibroblasts in IPF lungs remains unresolved. Resolution of this issue is complicated by the lack of definitive, cell-specific markers for fibroblasts. Although it may be important to understand whether epithelial cells become fibroblasts in IPF patients, what is more important is how activation of EMT programming, or production of mesenchymal proteins by epithelial cells, contributes to the fibrotic process. This may be determined through additional studies using genetically modified mice that lack either mesenchymal proteins or signaling mediators of EMT in epithelial cells in models of lung fibrosis.

Relationships between the genetic predisposition to idiopathic pulmonary fibrosis, epithelial-to-mesenchymal transition, endoplasmic reticulum stress, AND TGF-β—The UPR, TGF-β, and EMT are activated in patients with IPF (Figure 5), but how this activation occurs remains largely undefined. Genetic predisposition may explain the activation of these processes in some patients. As outlined above, surfactant mutations lead to activation of the UPR. Although there is strong evidence for activation of the molecular pathways of the UPR in the AECs of IPF patients, whether this activation is sufficient to lead to development of lung fibrosis remains unresolved. For example, ectopic expression of the L188Q mutated form of SPC in the type II cells of mice for 6 months did not lead to fibrosis despite activation of the UPR (49). However, these mice demonstrated increased apoptosis and exaggerated lung fibrosis in response to bleomycin (49). These data show that activation of the UPR for 6 months is not sufficient to promote development of lung fibrosis in mice, but that such UPR activation alters the epithelium to a phenotype that, when subjected to a second external stimulus, is more prone to activation of profibrotic pathways. Whether chronic UPR activation in the alveolar epithelium over many years— which may be the situation in humans—is sufficient to cause lung fibrosis needs further study.

Mutations of the SPA protein lead to UPR activation in epithelial cells (51, 70). Patients heterozygous for the G231V SPA mutation have higher levels of TGF-β in their bronchoalveolar lavage fluid than healthy control subjects do (70). These observations suggest a link between activation of the UPR and production of TGF-β. In support of this relationship, epithelial cells heterologously expressing the G231V or F198S SPA mutations express higher levels of TGF-β and of the TGF-β-binding proteins LTBP-1 and LTBP-4, which are required for TGF-β secretion (70). This increased TGF-β secretion is at least in part dependent on activation of the UPR, because siRNA gene silencing of IRE1α, PERK, or ATF6 significantly decreases mutant SPA–mediated TGF-β secretion. This UPR-mediated TGF-β secretion appears limited to specific genetic mutations, because not all the examined SPA or SPC mutations mediate TGF-β secretion. Overall, these results suggest that, in some circumstances, activation of the UPR may contribute to development of pulmonary fibrosis by enhancing TGF-β secretion.

A relationship between activation of the UPR and EMT has also been reported (50, 71). When AECs are exposed to the ER stress inducers thapsigargin, tunicamycin, or the SPC ^{exon4} mutation, the cells increase expression of the ER chaperone BiP and XBP1s, change morphology to a shape more consistent with mesenchymal cells, decrease expression of E-cadherin and zonula occludens 1, and increase expression of αSMA (71). This ER

stress–induced EMT depends on Src kinase, because inhibition of Src using PP2 [4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4]pyrimidine] blocks the ER stress–mediated EMT (71). Similarly, stable expression of the L188Q mutated form of SPC leads to decreased expression of E-cadherin and zonula occludens 1, increased expression of S1004A and αSMA, and a morphologic change of the epithelial cells to a mesenchymal shape (50). The TGF-β inhibitor SB431542 or siRNA knockdown of the TGF-β signaling molecule Smad2 blocks EMT changes. Further, these changes depend on UPR activation, because siRNA knockdown of IRE1 blocks Smad2/3 phosphorylation (50). Collectively, these reports suggest that ER stress and activation of the UPR could contribute to fibrotic remodeling in the lungs, in part via TGF-β activation and EMT-mediated pathways.

External stressors in idiopathic pulmonary fibrosis—The sections above outline genetic mutations predisposing to the development of lung fibrosis and how these mutations may lead to profibrotic molecular changes originating within epithelial cells. In these examples, the primary pathologic change is a genetic mutation that causes the epithelial cells to become intrinsically abnormal. These genetic abnormalities lead to activation of molecular pathways such as the UPR, TGF-β secretion, and EMT, and ultimately to lung fibrosis (Figure 5). Although in theory these mutations could lead directly to lung fibrosis, there are no data to support this possibility. For example, the telomerase-deficient mice have not been reported to develop lung fibrosis (72, 73). Similarly, when the L188Q mutated form of SPC is overexpressed in mouse alveolar type II cells, the mice do not develop lung fibrosis despite activation of the UPR and increased apoptosis of alveolar type II cells (49). Why the genetic mutations do not lead directly to the development of lung fibrosis in mouse models remains unresolved. One possibility is that mice, because of their short life span, are not exposed to the effects of the mutations long enough to cause fibrosis, whereas humans are exposed to the effects of the mutations for decades before lung fibrosis develops. An alternative explanation for the lack of confirmation that genetic mutations lead directly to fibrosis is that exposure to additional environmental insults in genetically predisposed individuals is required for development of lung fibrosis. For example, aged mice develop more severe lung fibrosis after exposure to bleomycin or murine gammaherpesvirus infection (74–76). In the case of gammaherpesvirus infection, the predisposition to more severe fibrosis in aged mice is associated with greater activation of the UPR and increased apoptosis (76). Similarly, mice expressing the L188Q mutated form of SPC develop lung fibrosis after administration of bleomycin in doses that do not cause fibrosis in control animals (49). Collectively, these observations suggest that genetic mutations may not be sufficient to cause lung fibrosis; rather, a second event—that is, an environmental exposure—may act in concert with a genetically predisposed epithelium.

What such key external exposures are in IPF patients remain to be established. One possibility is that typical environmental exposures such as pollution, dust, or smoke may contribute to development of lung fibrosis in genetically predisposed individuals. Evidence for this possibility comes from epidemiologic studies reporting that smoke and dust exposure are found more often in patients with IPF than in healthy controls (77). An exposure that has received considerable attention is GER (and associated microaspiration). Patients with IPF are more likely to have GER than are control subjects (78, 79). Treatment

of GER with either proton pump inhibitors or surgical fundoplication is associated with slower disease progression and better survival (80, 81), suggesting that GER and microaspiration may contribute to disease pathogenesis and/or exacerbation in genetically vulnerable individuals. What environmental exposures activate profibrotic molecular programming in the epithelium of genetically predisposed individuals and whether an exposure exceeding routine everyday exposures of the lung need further study.

Mesenchyme

Given that the epithelium is abnormal in IPF, the relationship between epithelial cell dysfunction and deposition of excess collagen within remodeled lung tissue needs explanation. The simple view—that subjecting epithelial cells to ER stress, leading to TGF-β activation and transition of epithelial cells to collagen-secreting mesenchymal cells, is a major source of the pathologic deposition of collagen—is likely inadequate. Limitations of this view are noted above. More likely, the development of lung remodeling and fibrosis depends on an interaction between (*a*) an epithelium that is dysregulated by the underlying genetic mutations, UPR activation, and/or EMT and (*b*) fibroblasts, the professional collagen-secreting cells of the lung. Whether this relationship is simply stimulation of fibroblasts by TGF-β that is activated by the epithelial cell integrin $\alpha_v\beta_6$ or whether there are more complex interactions between the epithelium and mesenchyme is the subject of ongoing research. The following section highlights recent advances in understanding the relevant interactions between epithelial and mesenchymal cells.

Fibrocytes—Fibrocytes are bone marrow-derived mesenchymal cells that can be identified in the circulation or in tissues by their coexpression of the hematopoietic precursor markers CD34 or CD45 and the mesenchymal cell protein type I collagen (82). Fibrocytes are proposed to contribute to development of lung fibrosis by directly producing extracellular matrix proteins such as type I and type III collagen, by differentiating into fibroblasts or myofibroblasts, or by producing cytokines that induce collagen deposition (83, 84). Fibrocytes have been found both in circulation (85–87) and in the lung parenchyma (85) of patients with IPF. An increase in the percentage of circulating fibrocytes correlates with disease exacerbations in IPF patients (87). These elevated fibrocyte levels return to baseline after the exacerbation resolves. Furthermore, IPF patients whose fibrocytes represent >5% of their total circulating blood leukocytes have a worse prognosis than do those whose fibrocytes represent <5% of total circulating blood leukocytes. These epidemiologic studies suggest that fibrocytes are significant contributors to lung fibrosis.

The alveolar epithelium may play a role in recruiting fibrocytes to IPF lungs. Fibrocytes express the chemokine receptors CXCR4 (84, 85) and CCR2 (88). The alveolar epithelium in IPF lung expresses CXCL12, a ligand for CXCR4 (85, 86). Furthermore, circulating levels of CXCL12 are increased in the plasma of IPF patients (85, 86). Similarly, the alveolar epithelium of IPF patients expresses CCL2 (89), the ligand for CCR2. These observations suggest that circulating fibrocytes may be recruited to IPF lungs through a CXCR4/CXCL12 or CCR2/CCL2 axis and that expansion of fibrocytes in the lungs may contribute to IPF.

Fibroblasts—Epithelial cell dysfunction and aberrant epithelial-mesenchymal signaling lead to the activation of fibroblasts and the deposition and remodeling of matrix. This chronic activation appears to lead to profibrotic, pathologic changes in IPF fibroblasts (90–94). The myofibroblast is the classic pathologic fibroblast phenotype described in IPF lungs (59). Several mediators, including TGF-β, can elicit the differentiation of fibroblasts to myofibroblasts. Compared with resident lung fibroblasts, myofibroblasts secrete excessive amounts of matrix, including type I collagen (95). This excess matrix deposition may lead to pathologic lung fibrosis and remodeling. The myofibroblast phenotype may not be durable and appears to require ongoing activation by myofibroblast differentiating factors (e.g., TGF-β), because the phenotype can be reversed by withdrawal of a differentiating stimulus such as myoD (myogenic differentiation 1) or by stimulation with factors such as FGF (fibroblast growth factor) (96, 97). Additional phenotypic changes of fibroblasts that could contribute to the development of lung fibrosis include resistance to apoptosis and acquisition of an invasive phenotype (Figure 6) (90, 98). These phenotypic changes appear durable; they persist after the fibroblasts are removed from patients.

During normal wound healing, unneeded fibroblasts are removed through the activation of apoptotic pathways (99). This clearance mechanism for fibroblasts limits ongoing matrix deposition and fibrosis. Unlike normal fibroblasts, IPF fibroblasts resist apoptosis when exposed to FAS ligand (91) and have greater proliferative capacity when grown on polymerized collagen (94, 100). Molecular changes of IPF fibroblasts that potentially protect them from apoptosis include SPARC (101) or survivin production (102), increased STAT-3 signaling (103), and lower levels of the prostaglandin E2 generator cyclooxygenase 2 (COX-2) (104) and of the transcriptional activator of apoptosis-inducing proteins forkhead box 03a (FoxO3a) (100). IPF fibroblasts interact with polymerized collagen via the $\alpha_2\beta_1$ integrin (94). The levels of this integrin are abnormally low in IPF fibroblasts, resulting in pathologic activation of the PI3K (phosphoinositide 3-kinase)/Akt pathway due to inappropriately low PTEN (phosphatase and tensin homolog) function (94). This leads to high levels of FoxO3a, which enable IPF fibroblasts to escape the negative regulation of proliferation normally exerted by a type I collagen–rich environment (100). Activation of these prosurvival pathways in IPF fibroblasts may lead to their retention in IPF lungs, allowing the fibroblasts to deposit collagen in an ongoing manner, which ultimately leads to pathologic levels of lung remodeling and fibrosis.

It has been commented that IPF has features similar to those of cancer. Most notable is the relentless progression of IPF, which is similar to that of cancer, and the appearance of foci of fibroblasts, which are reminiscent of tumorlets (105). Although the foci of fibroblasts are not monoclonal and are more accurately described as reticula of fibroblasts (105), their anatomic appearance suggests the possibility that fibroblasts isolated from IPF patients may be able to invade the extracellular matrix much like metastatic cancer cells do (90, 93, 98). Significantly, IPF fibroblasts invade artificial basement membranes more readily than control fibroblasts do (90, 93, 98). The mechanisms for this enhanced invasion are poorly understood; however, the invasive properties correlate with levels of αSMA expression. Furthermore, the invasive phenotype of lung fibroblasts can be induced by the $\alpha_5\beta_1$ integrin and negatively regulated by $\alpha_4\beta_1$ integrin signaling (98). Additional clues to regulation of

the invasive phenotype of IPF fibroblasts come from studies reporting that overexpressing hyaluronan synthase 2 (HAS2) in myofibroblasts results in a more severe fibrotic phenotype following bleomycin-induced lung injury (90). The development of this fibrosis depends on the hyaluronan receptor CD44, because the invasive phenotype and degree of fibrosis are diminished in CD44-deficient mice and in those treated with a CD44-blocking antibody. Similarly, siRNA knockdown of HAS2 or treatment with CD44 blocking antibodies limits the invasive capacity of IPF fibroblasts (90). These studies demonstrate that IPF fibroblasts have an enhanced invasive phenotype and that this invasive phenotype is regulated by a range of mediators, including CD44 and HAS2.

Epithelial-mesenchymal cross talk—In regions of normal or IPF lung, epithelial cells and fibroblasts are in close apposition. The best evidence of this association is fibroblast foci, which have a layer of epithelium overlying the fibroblasts (Figure 2). At these regional sites, epithelial cells may signal directly to fibroblasts by releasing soluble mediators that can act in *trans* to activate adjacent fibroblasts. One example is the release of TGF-β by epithelial cells (106) and its activation by the epithelial integrin $\alpha_v\beta_6$, which could signal to fibroblasts, converting them to αSMA-expressing myofibroblasts. Another cytokine IPF AECs produce in increased amounts is PDGF (platelet-derived growth factor) (107), which can contribute to lung fibrosis by promoting fibroblast proliferation (108). This possibility is currently being tested in a clinical trial examining whether a tyrosine kinase inhibitor that targets a several receptors, including PDGF receptor, slows the progression of IPF (109).

Additional soluble mediators produced by epithelial cells that may signal adjacent fibroblasts are Wnt proteins. Wnts are secreted glycoproteins that play key roles in lung development, cancer, and fibrogenesis. Lipid modification of Wnts makes them relatively insoluble, which suggests that they may be deposited in the extracellular matrix and act locally after release (110). In humans, 19 Wnt glycoproteins and 10 receptors for Wnts have been identified (111). Microarray screens revealed that Wnts, including Wnt2 and Wnt5a, and the Wnt receptor Fzd7 are overexpressed in IPF lungs. A more focused analysis of Wnt expression in IPF alveolar epithelium revealed that Wnt1 and Wnt3a are produced by IPF alveolar epithelium (112). Further, Wnt3a stimulates type I collagen production by fibroblasts. The downstream canonical Wnt signaling molecule β-catenin has been localized in nuclei of fibroblast foci in IPF lungs, suggesting that IPF fibroblasts may respond to Wnt activation (113, 114). Thus, Wnts produced by epithelial cells are candidate mediators of profibrotic signaling between epithelial cells and the mesenchyme in IPF.

Propagation of Lung Fibrosis

A key issue to resolve to understand the pathobiology of IPF is why the disease has a relentlessly progressive clinical course. A common explanation is that IPF progresses due to repetitive, often microscopic injury to the alveolar epithelium (115). The term injury implies an external force that causes harm to the lung. There is little clinical or histopathologic evidence to support this claim. In contrast, histopathologic analysis of IPF tissues commonly demonstrates normal lung epithelium adjacent to remodeled lung (Figure 1). The possibility that the epithelium is preferentially injured at the deepest recesses of the lung is hard to confirm. In contrast, the injury hypothesis is supported by histopathologic findings in

patients suffering an acute exacerbation of IPF (Figure 3). In these cases, patients have the pathologic hallmarks of lung injury—hyaline membranes, alveolar filling with proteinaceous material, and organizing pneumonia—diffusely throughout their lungs (116). Because the pathologic changes of an acute exacerbation of IPF are diffuse and occur in a minority of patients with IPF (5, 117), other explanations for the relentless progression of IPF should be considered in patients who do not exhibit such acute exacerbations.

Alternative explanations for IPF progression outlined in the preceding sections include the genetic mutations associated with IPF and the phenotypic changes identified in IPF fibroblasts. Genetic mutations are durable, irreversible, changes that could contribute to disease progression via persistent activation of the UPR or secretion of TGF-β. However, the surfactant mutations are the only genetic mutations with defined molecular pathways that can explain their contribution to lung fibrosis. The absence of an understanding of molecular mechanisms leading to pulmonary fibrosis in patients with *MUC5B*, *TERT*, or *TERC* mutations or in individuals with no identifiable mutation means that nongenetic explanations for the relentless progression of IPF must be considered. Another possible explanation is that the durable phenotypic changes of IPF fibroblasts lead to IPF progression. However, these changes have yet to be proved relevant in in vivo model systems.

Role of the extracellular matrix—The extracellular matrix is extensively modified in IPF lungs. One possible explanation for IPF progression is that the pathologically remodeled extracellular matrix includes molecules that signal mesenchymal cells to release additional extracellular matrix. If a critical threshold of profibrotic matrix is deposed, there may be a feed-forward, autoamplified loop of matrix deposition and remodeling. A recent study used proteomic analysis identified more than 40 proteins that are over- or underrepresented in decellularized IPF matrix (118). Many of these proteins are known to activate signaling pathways on the cells that engage them. Further, decellularized IPF matrix is significantly stiffer than normal controls (118). When fibroblasts are cultured on stiff matrices or the stiffer IPF matrix, they adopt an activated myofibroblast phenotype (118– 120) that includes increased αSMA and decreased COX-2 expression, which are features of IPF fibroblasts. Thus, it is possible that a change in the composition or stiffness of the extracellular matrix can contribute to the propagation of lung fibrosis. How these changes in extracellular matrix proteins relate to epithelial cells, and whether they are sufficient to explain the phenotypes of IPF fibroblasts, requires further study.

Epigenetics—Regulation of gene expression by epigenetic influences has recently been recognized as an important mechanism by which environmental or other stresses can induce lasting changes in the phenotype of a cell, tissue, or organism (121). Epigenetic changes include alterations in DNA methylation, histone modification, or microRNA expression (121, 122). Exposure to environmental stresses such as tobacco smoke (123, 124), air pollution (125), and aging (126) can lead to epigenetic DNA changes. In a study of epigenetic changes in IPF lung, microRNA microarray analysis revealed that levels of 18 microRNAs, including let-7d, were decreased in IPF lungs (127). The let-7d microRNA localized to the alveolar epithelium, and the decreased let-7d levels in epithelial cells were associated with increased expression of the mesenchymal proteins N-cadherin, vimentin,

and αSMA. Similarly, genome-wide methylation analysis identified a large number of differentially methylated genes in IPF lungs (128, 129). Changes in DNA methylation corresponded to mRNA expression for numerous genes, some of which have known roles in IPF, such as regulation of apoptosis and biosynthetic cellular processes (128–130). These findings suggest that DNA methylation and/or other epigenetic changes are important in the pathogenesis of IPF and that their enduring influences on gene expression could in part explain the relentless progression of the illness.

Subclassification of Patients with Idiopathic Pulmonary Fibrosis

IPF has specific clinical, radiologic and histopathologic features that are required for diagnosis. Within the clinical entity of IPF, subgroups of IPF patients with distinct cellular or molecular phenotypes predicting differences in rates of progression are beginning to be distinguished. For example, histopathology can be used to sub-categorize patients with IPF. IPF patients with greater numbers of fibroblastic foci have more rapid progression of IPF (131) and patients with greater numbers of lung mast cells have slower progression of IPF (132). Similarly, genetics have been used to sub-categorize IPF patients. IPF patients with the TT or GT *MUC5B* genotype have slower rates of progression than IPF patients with the GG *MUC5B* genotype (133). IPF patients carrying the minor allele rs5743890 in the *TOLLIP* gene are at increased risk for mortality from IPF (134). Finally, circulating molecular markers have been used to sub-phenotype patients with IPF. Measured markers include MMP7 (135), CCL18 (136), KL-6 (137, 138), and SPD (139), all of which have been reported to predict poor survival when elevated in IPF patients. These studies demonstrate that patients meeting clinical criteria for the diagnosis of IPF may have distinct cellular or molecular subtypes. Whether these subtypes represent distinct diseases, different phases of the same disease or can be used to predict response to therapy requires further research.

Conclusions

IPF is a progressive lung disorder of increasing prevalence that is a major cause of death for individuals suffering from the disease. Recent advances in the mechanistic understanding of IPF pathogenesis permit the consideration of a plausible sequence of events that lead to the development of pulmonary fibrosis. This sequence of events can be subdivided into three pathophysiologic stages (Figure 7). The predisposition stage includes genetic mutations or variations (i.e., SNPs) that predispose individuals to develop lung fibrosis; the chronic epithelial cell turnover during the lifetime of an individual, which leads to shortened telomeres; and environmental exposures. These factors ultimately lead to epithelial cell dysfunction. Not all individuals in this stage will necessarily develop clinically relevant disease; whether they do depends on the degree and duration of exposure to these factors. In initiation stage, molecular mediators of epithelial cell dysfunction such as ER stress; excessive TGF-β activation; and growth factor, chemokine, or Wnt secretion lead to EMT, fibrocyte recruitment, and fibroblast differentiation. These lead to the progression stage, during which pathologic mesenchymal cells release abnormal types and quantities of matrix proteins, which remodel and scar the lung. The pathologically remodeled matrix or epigenetic changes within fibroblasts may lead to a feed-forward loop of mesenchymal cell

activation and progressive fibrosis. Some patients with IPF may bypass some of these stages. To develop therapies for IPF, a clearer understanding is needed of the relationships between the specific molecular pathways that are activated during each stage of IPF. This understanding will inform which stage of the disease therapies target as well as whether targeting one stage of the disease is sufficient to slow progression or whether simultaneous targeting of molecular pathways in each stage of disease is required for effective therapy.

Acknowledgments

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Summary Points

1. Epithelial cell dysfunction is a central cause of IPF.

2. Genetic mutations predispose individuals to develop pulmonary fibrosis.

3. Pulmonary fibrosis appears to be due to gene-environment interactions.

4. IPF fibroblasts have distinct phenotypic alterations that contribute to the development of lung fibrosis.

5. The remodeled matrix may provide a feed-forward loop of profibrotic signaling.

Future Issues

1. Establish how MUC5B, TERT or TERC mutations lead to profibrotic molecular changes within the lung.

2. Define additional genetic mutations that predispose to the development of lung fibrosis.

3. Define which environmental factors contribute to IPF and how they relate to the molecular pathogenesis of IPF.

4. Establish novel animal models of pulmonary fibrosis that more reliably recapitulate the human disease.

5. Define molecular mechanisms that explain how short telomeres and aging lead to pulmonary fibrosis.

6. Establish molecular links between defined biologic processes that occur in each stage of IPF.

7. Define the profibrotic molecular pathways of IPF that can be subject to effective therapeutic intervention.

8. Determine whether IPF consists of a group of disorders that share certain phenotypic features but reflect distinct subgroups of patients, characterized by different pathogenetic molecular mechanisms and clinical phenotypes.
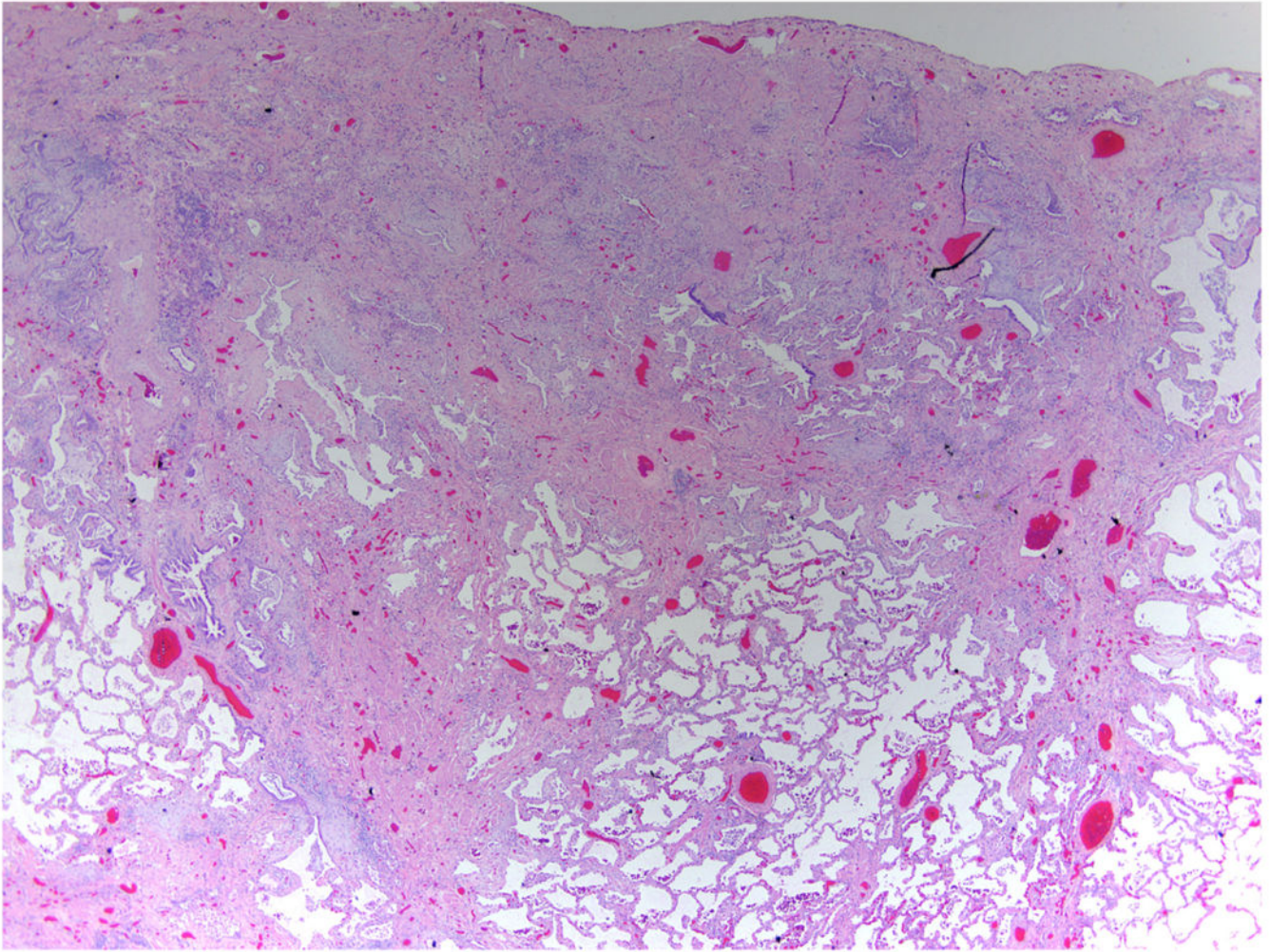
Figure 1.
Pathologic changes in usual interstitial pneumonia. This low-power image of usual interstitial pneumonia shows the typical subpleural accentuation of dense fibrosis with irregular airspaces and central delicate alveolar septa. Stained with hematoxylin and eosin; magnification of 20×.
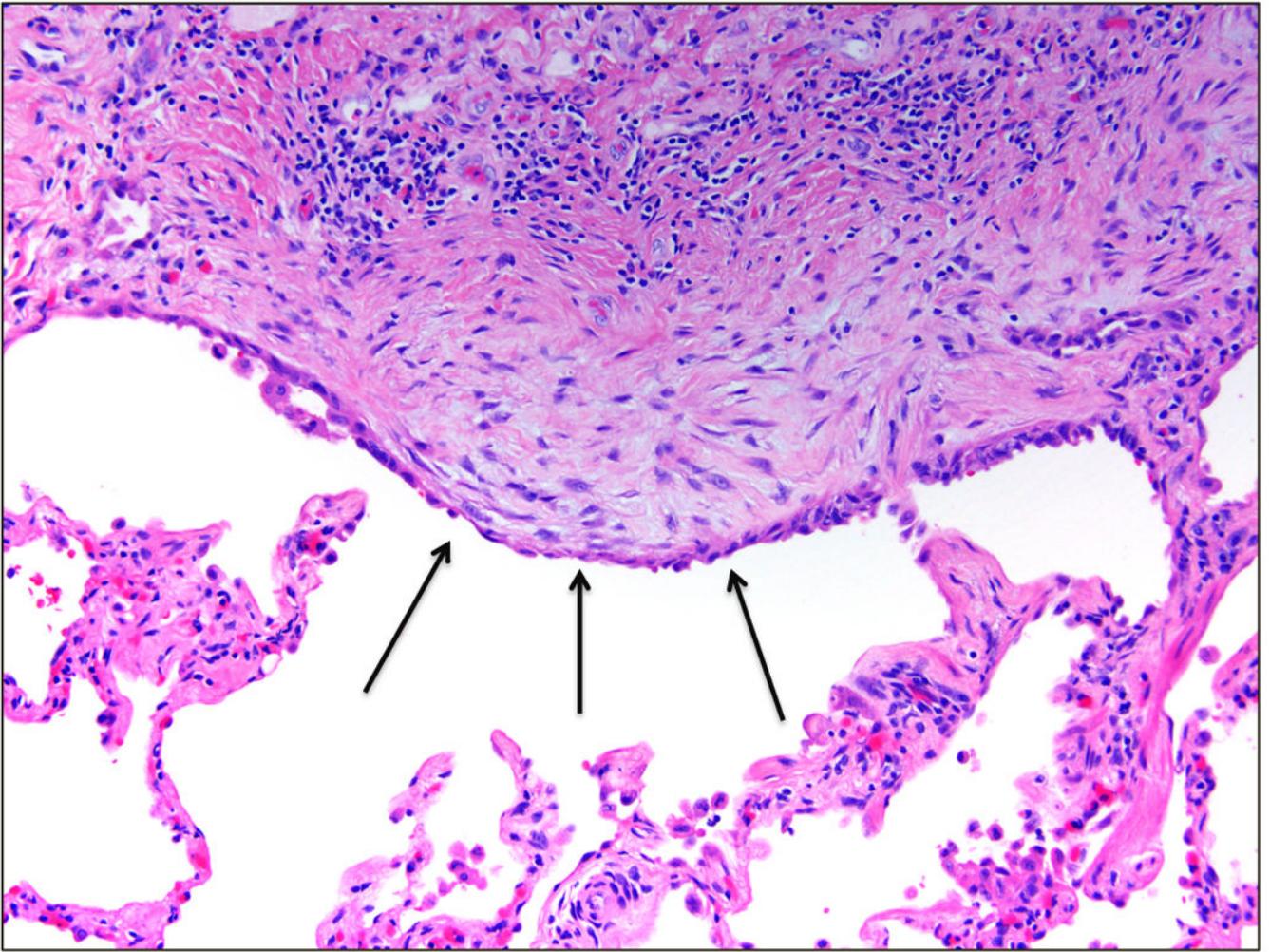
Figure 2.
Fibroblast foci. The fibroblast focus shows a proliferation of spindled fibroblasts within a myxoid matrix, producing a bulge of tissue into the airspace. The overlying epithelium often shows plump reactive-appearing cells (*arrows*). Stained with hematoxylin and eosin; magnification of 200×.
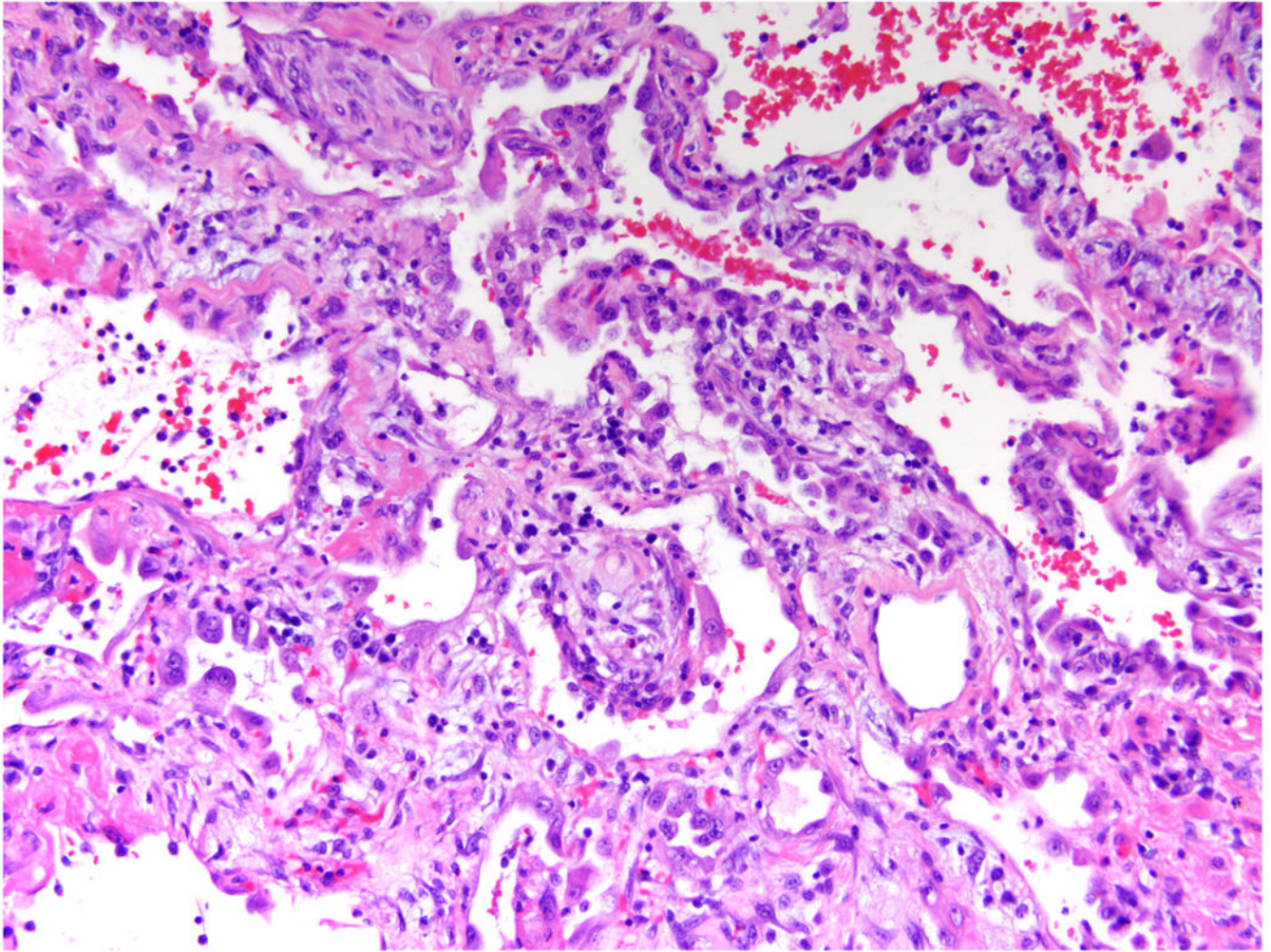
Figure 3.
Pathologic changes in acute exacerbation of idiopathic pulmonary fibrosis. In acute
exacerbation of usual interstitial pneumonia, the central lung tissue shows histologic
changes of diffuse alveolar damage including edematous alveolar septa, type II pneumocyte
hyperplasia, and hyaline membrane formation. Stained with hematoxylin and eosin;
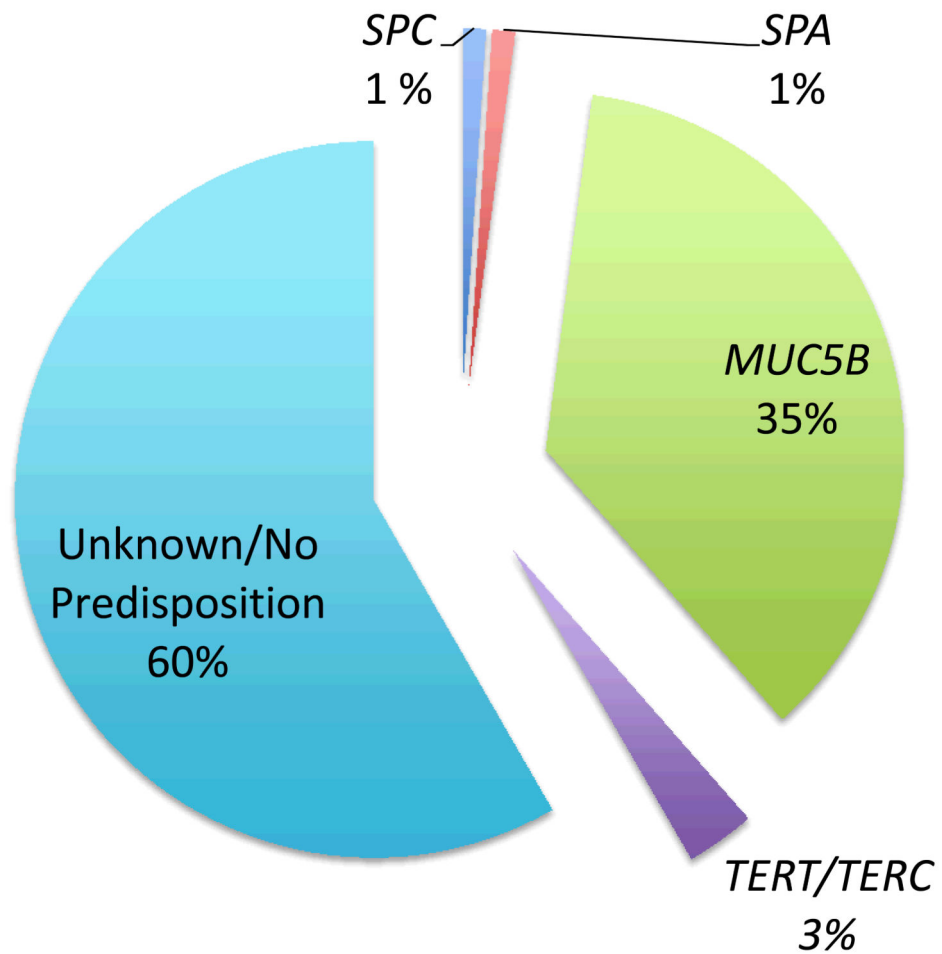magnification of 200×.

Figure 4.
Estimated frequencies of genetic mutations predisposing to idiopathic pulmonary fibrosis (IPF). This pie chart illustrates the estimated prevalence of mutations in surfactant protein C (*SPC*), surfactant protein A (*SPA*), mucin 5B (*MUC5B*), and telomerase (*TERT*, *TERC*) that predispose patients to develop sporadic IPF. All percentages are estimates derived from reports in specific patient cohorts.
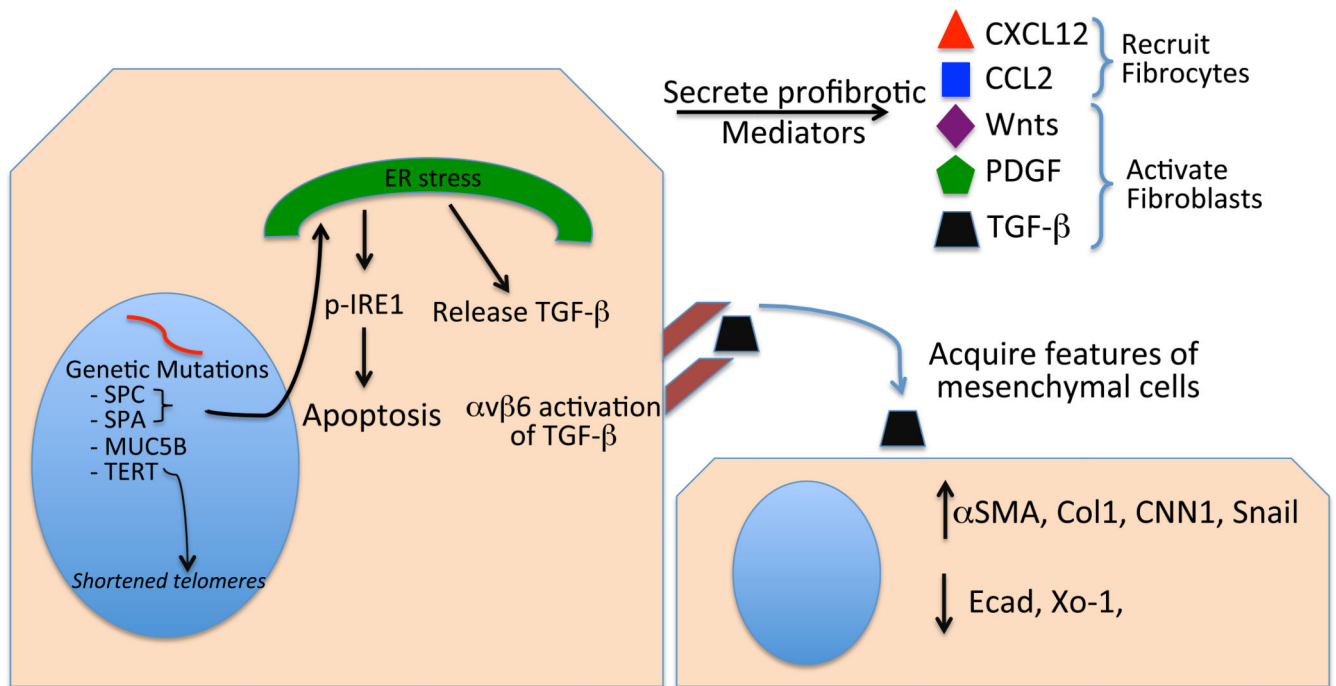
Figure 5.
Profibrotic attributes of epithelial cells in idiopathic pulmonary fibrosis lungs. Genetic mutations in epithelial cell–associated proteins predispose to the development of lung fibrosis by leading to the development of short telomeres or endoplasmic reticulum (ER) stress. ER stress may be profibrotic by causing apoptosis or release of TGF-β. Epithelial cells release profibrotic mediators such as Wnts, PDGF, and TGF-β, which activate fibroblasts, or CXCL12 and CCL2, which recruit fibrocytes to the lung. Epithelial cells produce the integrin $α_vβ_6$, which activates TGF-P and may thereby cause epithelial cells to undergo epithelial-to-mesenchymal transition.
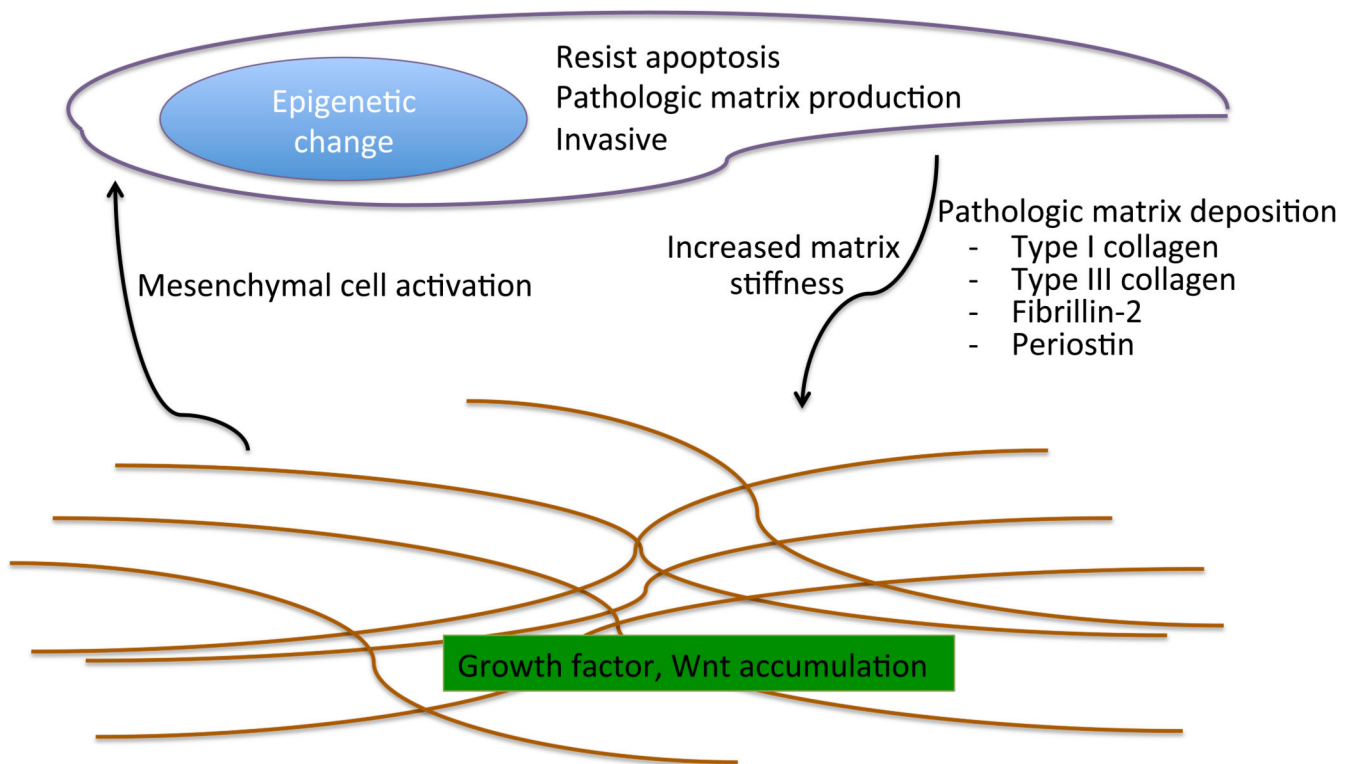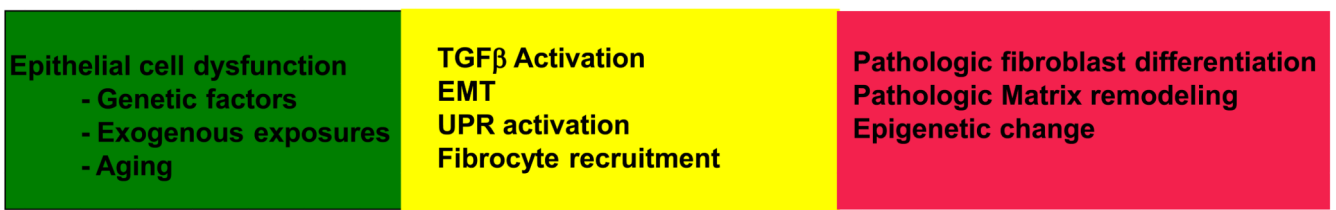
Figure 6.
Mechanisms by which mesenchymal cells or matrix contribute to lung fibrosis. Idiopathic pulmonary fibrosis fibroblasts acquire pathologic, profibrotic traits such as resistance to apoptosis, production of excess matrix, and an invasive phenotype. The remodeled matrix is abnormally stiff, and the composition of the extracellular matrix is extensively altered compared with that in normal lungs. In turn, these pathologic changes within the matrix may further promote profibrotic traits within fibroblasts.

1. Predisposition

2. Initiation

3. Progression

Epithelial cell dysfunction
- **Genetic factors**
- **Exogenous exposures**
- **Aging**

TGFβ Activation
EMT
UPR activation
Fibrocyte recruitment

Pathologic fibroblast differentiation
Pathologic Matrix remodeling
Epigenetic change

Figure 7.
Pathophysiologic stages of idiopathic pulmonary fibrosis (IPF). The pathogenesis of IPF can be delineated into three stages. The initial predisposition stage includes processes such as genetic mutations, environmental exposure (routine or otherwise), and aging that predispose an individual to develop lung fibrosis. The second initiation stage includes defined profibrotic processes, such as TGF-β activation, fibrocyte recruitment, epithelial-to-mesenchymal transition (EMT), and activation of the unfolded protein response (UPR), that, when engaged, accelerate profibrotic processes. The final progression stage includes molecular processes that lead directly to fibrosis, such as pathologic fibroblast differentiation, matrix deposition and remodeling, increased matrix stiffness, and profibrotic epigenetic changes within fibroblasts and epithelial cells. Events in the latter stage may bypass the first two stages, leading to persistent mesenchymal cell activation and matrix remodeling.