Revealing the Pathogenic and Aging-related Mechanisms of the Enigmatic Idiopathic Pulmonary Fibrosis

An Integral Model

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Abstract

A growing body of evidence indicates that aberrant activation of alveolar epithelial cells and fibroblasts in an aging lung plays a critical role in the pathogenesis of idiopathic pulmonary fibrosis (IPF). However, the biopathological processes linking aging with IPF and the mechanisms responsible for the abnormal activation of epithelial cells and fibroblasts have not been elucidated. Many of the hallmarks of aging (e.g., genomic instability, telomere attrition, epigenetic alterations, mitochondrial dysfunction, and cellular senescence) have been proposed as essential mechanisms for the development of IPF; however, these disturbances are not restricted to IPF and also occur in other aging-related lung disorders, primarily chronic obstructive pulmonary disease (COPD). Therefore, an unanswered question is why a current/former smoker of about 60 years of age with shorter telomeres, alveolar epithelial senescence, excessive oxidative stress, and mitochondrial dysfunction develops IPF and not COPD; in other words, what makes old lungs specifically susceptible to develop IPF? In this Perspective, we propose an integral model in which the combination of some gene variants and/or gene expression in the aging lung results in the loss of epithelial integrity and consequently in the failure of the alveoli to correctly respond to injury and to face the stress associated with mechanical stretch. Afterward, a distinctive epigenetic "reprogramming" that affects both epithelial cells and fibroblasts provokes, among others, the recapitulation of developmental pathways and the aberrant activation and miscommunication between both cell types, resulting in the exaggerated production and accumulation of extracellular matrix and the subsequent destruction of the lung architecture.

Keywords: pulmonary fibrosis; epithelial cells; aging; genetic susceptibility; chronic obstructive pulmonary disease

Idiopathic pulmonary fibrosis (IPF), one of the most common forms of interstitial lung disease (ILD), is a chronic, progressive, and usually lethal lung disease of unknown etiology and refractory to current therapeutic options (1).

IPF occurs in middle-aged and elderly adults, and the incidence/prevalence increases remarkably with age; most patients are older than 60 years at the time of diagnosis (2). It is important to emphasize that aging impairs the repair capacity and usually worsens the lung fibrotic response of any cause, as observed in bleomycin-induced fibrosis in mice and several human ILDs (3, 4). However, these diseases also occur in young adults and even in children, although they have poorer outcome when they begin in the elderly. In sharp contrast, IPF does not occur in young people, suggesting a mechanistic link between chronological age and this disease.

The Aging Connection

It has been suggested that IPF may result from an "accelerated" aging lung. Supporting this view, a recent study showed that some asymptomatic individuals more than 75 years old present images on high-resolution computed tomography suggestive of ILD (5). However, a number of physiologic, morphologic, and imaging studies indicate that the outcome of lung aging is usually the so-called "senile" emphysema, characterized by distal airspace enlargement with progressive loss of elastic recoil. Moreover, prematurely aged lungs develop emphysematous lesions and not fibrotic lesions. For instance, mice deficient of the "aging suppressor gene" *klotho* develop a syndrome that resembles human aging and, in the lungs, emphysema-like changes (6).

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Therefore, we would expect that an accelerated process of aging, mainly in smokers, should result in emphysema; actually, chronic obstructive pulmonary disease (COPD) is also defined as a disease of accelerated lung aging because it is uncommon before the age of 40 years, and its prevalence increases considerably after 60 years of age (7). Thus, the bridge connecting specifically aging and IPF is presently uncertain.

Is IPF the Result of "Exaggerated" Aging Process(es)?

Aging is characterized by progressive loss of functional integrity, leading to increased susceptibility to disease and death. Recently, nine pivotal hallmarks contributing to the aging process/aging phenotype were proposed (8). These features include genomic instability, telomere erosion, epigenetic changes, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.

Which (and how) of these pathways contribute to the development of IPF is unclear, but emerging evidence indicates that several of them are more affected in subjects with IPF than in age-matched control subjects (9). For example, strong evidence supports that genomic damage (microsatellite DNA instability and loss of heterozygosity) occurs in IPF (10). Likewise, abnormal shortening of telomeres, which are highly susceptible to age-related deterioration; impaired autophagy, an essential process in the turnover of subcellular organelles and proteins; dysfunctional mitochondria; excessive reactive oxygen species production; and mitochondrial-mediated alveolar epithelial cell (AEC) apoptosis have been described in IPF (11–13). Accumulation of senescent cells, characterized by a stable arrest of the cell cycle coupled to a variety of phenotypic changes, is common in aged tissues and has been demonstrated in the alveolar epithelium of IPF lungs (12). Similarly, immunosenescence, an aging-related decline of immunocompetence characterized by the expansion of $CD4^+/CD28^-$ T cells and the presence of autoantibodies, among others, is observed in some patients with IPF (14, 15). Under the mentioned findings, a current concept regarding the pathogenesis of IPF states that excessive and unsolved endoplasmic reticulum stress and mitochondrial dysfunction enhances an apoptotic response of the AECs, which, due to the abnormal shortening of telomeres, have a deficient regenerative capacity, and these processes, together with other aging-related changes, are critical for the development of the disease.

However, COPD, another aging-associated lung disorder, but phenotypically very different from IPF (particularly noted in the presence of hyperplastic/hyperactivated AEC phenotypes in IPF) show many of these alterations. Telomere attrition, alveolar cell senescence, loss of heterozygosity, microsatellite instability, excessive reactive oxygen species production, and apoptosis have been also revealed in AECs from patients with COPD (16–18). Moreover, mice with telomerase mutation and sequential shortening of telomeres develop emphysematous lesions, although they appear to be protected from bleomycin-induced lung fibrosis (19, 20). Likewise, increased $CD4^+/CD28^-$ T cells and circulating autoantibodies are often found in patients with COPD (21, 22).

Therefore, the crucial question here is why does a current/former smoker$\sim$60 years old with shortened telomeres, epithelial senescence, mitochondrial dysfunction, etc., develop IPF and not COPD? In other words, what makes old lungs specifically susceptible to IPF?

Here we propose that a network of interrelated and often overlapped biopathological processes converge, leading to the development of IPF (Figure 1).

Loss of Epithelial Integrity at the Crossroads of IPF and Aging

Susceptibility to IPF is likely related to a complex genetic architecture characterized by a combination of gene variants and transcriptional changes that result in the loss of epithelial integrity.

A recent case-control genome-wide association study (GWAS) that included 1,616 patients with fibrotic idiopathic interstitial pneumonias, mostly IPF, and 4,683 control subjects identified several gene variants, critical for the epithelial integrity, as important risk factors (23). Thus, strong association was found with variants in the *DSP* gene, which encodes desmoplakin, an essential protein for normal desmosomal adhesion. Desmosomes are intercellular junctions that provide strong adhesion between cells and play a crucial role in the maintenance of epithelial integrity mainly in tissues that are under mechanical forces, such as the lung. Gene variants from other epithelial cell adhesion components were also found as risk factors, including the catenin cadherin-associated protein α3, which interacts with β-catenin, and dipeptidyl peptidase 9, which seems to be implicated in cell adhesion and migration. Additionally, GWAS also identified common variants in *TERT* and *TERC* and in oligonucleotide/oligosaccharide-binding fold containing 1 (*OBFC1*) that appear to function in a telomere-associated complex required for telomere length homeostasis. These polymorphisms may contribute to telomere attrition, a hallmark of aging. In another study, novel genetic variants residing in toll-interacting protein (TOLLIP), an important regulator of innate immunity, were identified as risk factors (24). Interestingly, TOLLIP interacts with smad 7, suppressing transforming growth factor (TGF)-β signaling and, among other effects, inhibits epithelial-to-mesenchymal transition (25). In both GWAS studies, the MUC5B rs35705950_T allele that encodes for a fundamental macromolecule involved in mucociliary clearance was identified as a strong risk factor.

Of note, GWAS studies in COPD have revealed a very different profile. Thus, four susceptibility loci have been found in COPD, including 4q31 (hedgehog interacting protein [HHIP]), 15q25 (α-nicotinic receptor [CHRNA 3/5]), 19q13 (egl-9 family hypoxia-inducible factor 2 [EGLN2]), and 4q22 (family with sequence similarity 13, member A [FAM13A]) (26, 27). In contrast, 10 genetic risk loci were identified in IPF: TERT (5p15), MUC5B (11p15), 3q26 region near TERC, FAM13A (4q22), DSP (6p24), OBFC1 (10q24), ATP11A (13q34), DPP9 (19p13), and chromosomal regions 7q22 and 15q14-15 (23).

Other approaches that have examined individual proteins involved in epithelial cell integrity support a critical role for the loss of epithelial integrity in IPF. CD151 is a tetraspanin expressed at the basolateral surface of AECs and is important to

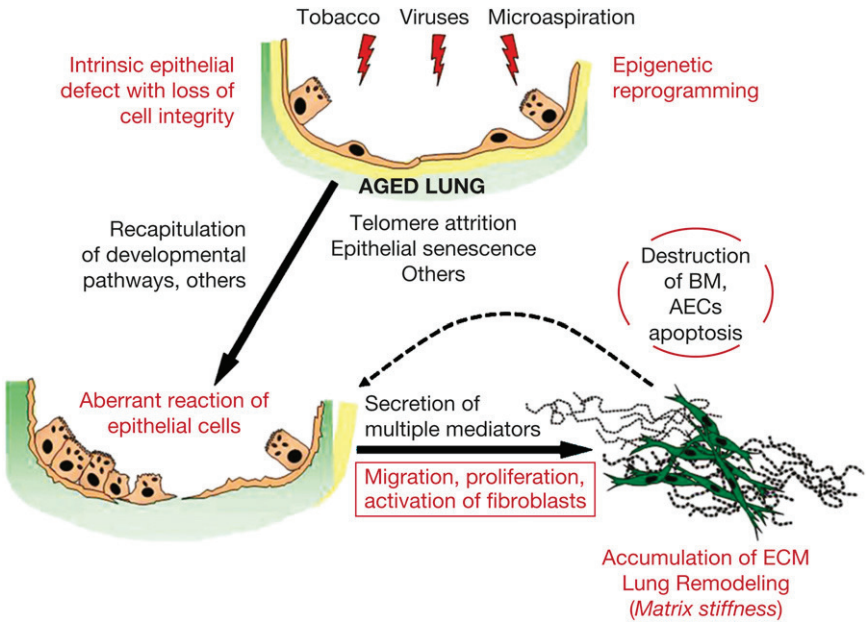

Figure 1. The proposed integral model involved in the pathogenesis of idiopathic pulmonary fibrosis. The initial "mark" in the genetic architecture is characterized by a variable combination of gene polymorphisms and transcriptional changes that result in the loss of epithelial integrity and spatial orientation. As consequence, epithelial cells are progressively unable to respond appropriately to repetitive microinjuries and to the stress associated with ventilation mechanical stretch. These processes take place in aging lungs characterized, among other things, by abnormal telomere shortening, mitochondrial dysfunction, cellular senescence, and impaired autophagy. Also, a distinct aging-related epigenetic reprogramming, involving DNA methylation, histone tails modification, and a dysregulation of microRNAs expression, takes place. The conjunction of loss of epithelial integrity and the epigenetic changes leads to an aberrant reaction of the epithelium, mediated at least partially by the sustained reactivation of a variety of embryological pathways. "Hyperactive" epithelial cells secrete a variety of growth factors, chemokines, matrix metalloproteinases, and procoagulant mediators, among others, inducing the expansion of the fibroblast/myofibroblast population, the disruption of basement membrane (BM), and the exaggerated extracellular matrix (ECM) production. In turn, fibroblasts/myofibroblasts secrete several mediators and enzymes that provoke additional epithelial/basement membrane damage. During this chaotic lung remodeling, increased matrix stiffness generates microenvironmental cues affecting the behavior of fibroblasts and epithelial cells and enhancing the frenzied and finally irreversible lung remodeling and honeycombing. AEC = alveolar epithelial cell. Modified by permission from Reference 73.

maintain epithelial integrity via firm adhesion to the basement membrane. A recent study showed that the expression of CD151 is down-regulated in AECs from IPF lungs (28). Importantly, CD151-deficient mice spontaneously developed age-related lung fibrosis, whereas lung injury worsened AEC disintegrity and provoked a severe fibrotic reaction.

Lung epithelium in IPF shows decreased expression of phosphatase and tensin homolog (PTEN) (29). In mice, the inactivation of *Pten* provokes the disassembly of tight junctions with disruption of AEC integrity and destruction of the basement membrane, which results in exacerbated lung fibrosis after injury. Interestingly, miR-21, a microRNA that is significantly increased in IPF, targets PTEN, suggesting that epigenetic mechanisms may be operating in the loss of epithelial integrity (30, 31). Likewise, alveoli epithelia-specific Shp2-deficient mice exhibit distorted alveolar architecture and develop spontaneous pulmonary fibrosis without inflammation (32). Shp2 is a tyrosine phosphatase implicated in fibroblast growth factor–induced lung branching morphogenesis and seems to play a pivotal role in the alveolar homeostasis and epithelial repair program.

Recent evidence demonstrated that the elongation of long-chain fatty acids family member 6 (Elovl6) is down-regulated in IPF lungs and that lack of Elov16 in mice is associated with spontaneous thickening of the alveolar walls and severe fibrosis after injury (33). This finding suggests that dysregulation of lipid composition in AECs exacerbates lung remodeling. From all these altered genes identified in IPF, only PTEN has been found decreased in small airway epithelium from patients with COPD (34).

There is likely not a unique genetic architecture for IPF susceptibility, but several gene variant combinations that result in a common problem: the progressive loss of the epithelial integrity.

Loss of epithelial integrity and lack of stable basement membrane scaffolding may change the spatial orientation for AEC permanence, spreading, and migration and may result in the failure to appropriately respond to injuries that usually affect many people without IPF outcome, such as microaspiration, viral infections, and cigarette smoke. Importantly, it may also influence the ability of the epithelium to contain the stress associated with mechanical stretch and may explain, at least partially, the characteristic basal and peripheral initiation of IPF (Figure 2). In this context, a mathematical-mechanical model describing the dynamic of the lungs during inflation suggested that the most susceptible areas to stretch overload are precisely the basal and peripheral ones where the disease initiates (35).

A Distinctive Epigenetic Reprogramming Affects Both Epithelial Cells and Fibroblasts

Epigenetic changes provoked by environmental (and endogenous) factors may contribute to the development of IPF in genetically predisposed individuals. Epigenomic mechanisms involve several reversible modifications in chromatin structure without changes in nucleotide sequence and include primarily DNA methylation, histone tail modification, and microRNA regulation. Importantly, a variety of epigenetic alterations affect cells with aging. In IPF, two studies have evaluated the global methylation patterns, although surprisingly with very little overlap, making them difficult to interpret (36, 37). Differences in the control lungs and platforms may partially explain these divergent results. An additional problem is

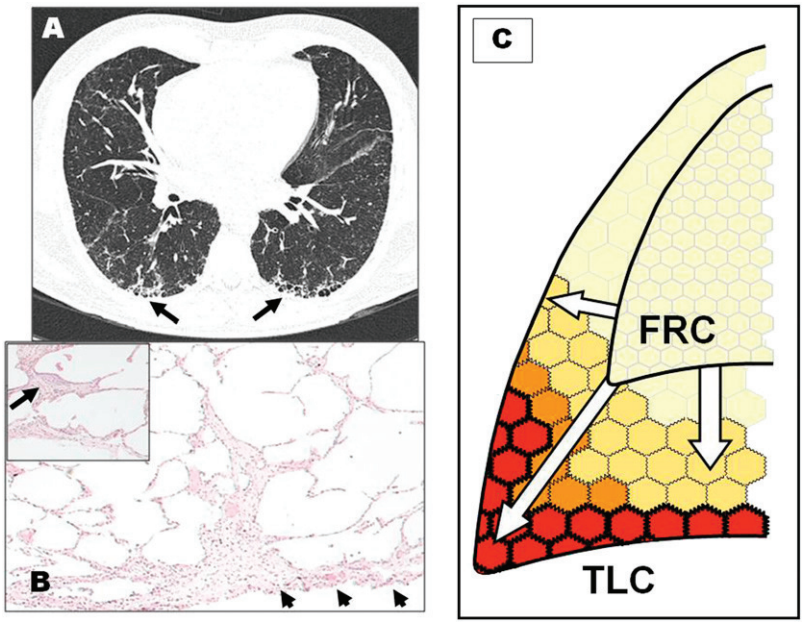

Figure 2. Characteristic initial chest tomography (*A*) and morphologic (*B*) alterations in idiopathic pulmonary fibrosis. The arrows indicate the typical bibasal, subpleural reticular and cystic images (*A*), and the subpleural fibrotic lesion in otherwise almost normal lung (*B*). The *arrow* in the inset shows a subepithelial fibroblastic focus and hyperplastic alveolar epithelial cells. (*C*) Hypothetical distribution of maximal mechanical stress obtained by a simplified mathematical model (29). In functional residual capacity (FRC) the alveoli are "semiopen." In inspiration the air is heterogeneously distributed within the lung parenchyma with the maximal mechanical stress in the peripheral (subpleural) areas of the lung that represent sites of early lesions.

that these studies evaluated whole lungs, which may mask cell-specific changes. In this context, studies approaching specific genes likely associated with an increased fibrotic response in isolated lung cells have supported that abnormal DNA methylation affects the expression of several genes likely involved in the pathogenesis of IPF (38).

Interestingly, a recent genome-wide methylation analysis performed in small airways from patients with COPD revealed 1,120 differentially methylated genes, 97% of them hypermethylated (39). Three pathways were enriched, including G protein coupled receptor signaling, aryl hydrocarbon receptor signaling, and cAMP-mediated signaling, which are known to play a role in small airway biology. In contrast, a relatively balanced distribution of hypermethylation and hypomethylation or even a predominance of hypomethylated CpG islands were found in IPF, and the analysis of functional pathways indicated that cellular assembly and organization and cell migration and proliferation are among the critical pathways regulated by DNA methylation. In general, differentially methylated genes and the enriched pathways observed in COPD revealed marked differences with both IPF studies.

Few studies have addressed the role of histone modifications in the pathogenesis of IPF, but in one of them, reduced histone H3 and H4 acetylation, was associated with decreased COX-2 expression and PGE-2 (a strong antifibrotic mediator) production by fibroblasts (40).

Epigenetic changes may also be involved in differential pre-mRNAs splicing, which is an important mechanism for proteome diversity. Changes in alternative splicing patterns are implicated in normal development and in physiological responses, but aberrant splicing generates variants that may contribute to aging-related diseases. In this context, a recent whole transcriptome-scale study of differential splicing in IPF lungs revealed that numerous nondifferentially expressed genes displayed a switch between major and minor isoforms, suggesting that they may contribute to the IPF phenotype (41). However, this analysis was done in whole tissues, and the switch of these isoforms in fibroblasts and epithelial cells, which express a specific cell-type splicing program, is unknown.

A Distinctive microRNA Profile Affects Epithelial Cell and Fibroblast Biology in IPF Lungs

MicroRNAs (miRs) are short noncoding RNAs of ~22 nucleotides that are critical gene regulators by inhibiting posttranscriptional expression of target mRNAs.

Several studies have revealed that a number of biopathologically meaningful microRNAs are up- or down-regulated in IPF lungs. Some of them affect the epithelial cells and others the fibroblasts (31, 42–46). Let-7d, miR-200, and miR-326, which target TGF-β signaling and/or other fibrosis-related pathways, are decreased, whereas miR-21, which promotes epithelial-mesenchymal transition (EMT), is increased in AECs from IPF lungs (43, 45, 47). Other differentially expressed microRNAs affect fibroblast behavior/activation, usually targeting TGF-β canonical and noncanonical signaling pathways. For example, the expression of miR-199a-5p, which targets caveolin-1, is increased in IPF fibroblasts. Down-regulation of caveolin-1 impairs TGF-β/TGF-βR complex degradation promoting TGF-β signaling (44). Likewise, increased expression of miR-21 in IPF fibroblasts enhances TGF-β1–induced activation by targeting the inhibitory Smad7 (31).

An interesting connection of microRNA deregulation and the pathogenesis of IPF is related to the reactivation of fetal gene programs, such as Wnt/β-catenin and Sonic hedgehog signaling pathways, which may contribute to the abnormal activation of epithelial cells and fibroblasts (48). Actually, some microRNAs that have a profound effect on these developmental pathways are deregulated in IPF. For example, up-regulation of Wnt/β-catenin signaling may be associated with the decrease of miR-375 and miR-487b, which target Frizzled 8 and WNT5A, and with the increase of miR-154, which targets WNT inhibitors, causing activation of this pathway (49–51). In sharp contrast with IPF, WNT/β-catenin signaling is down-regulated in experimental emphysema, and decreased nuclear β-catenin–positive AECs are detected in COPD (52). Moreover, the analysis of gene expression in COPD compared with smokers without obstruction indicated that

Wnt receptors frizzled homolog 5 and 7 are decreased, whereas several miRNAs that target this network were identified (53). Taken together, these data indicate that COPD, in contrast to IPF, is characterized by decreased WNT/β-catenin signaling activity.

Remarkably, the available information indicates that the IPF microRNA profile is very different from COPD. Evaluation of lung tissues from smokers with COPD and smokers without lung disease revealed 71 miRNAs differentially expressed (53). Impressively, most of them are usually unchanged in IPF lungs. From the few deregulated miRNAs shared by COPD and IPF, the most down-regulated in both diseases was miR-487b, which is silenced by epigenetic mechanisms associated with cigarette smoke (51). Evaluation of individual microRNAs in COPD also shows marked differences with IPF. For instance, mir-21, which is increased in IPF, is significantly decreased in exhaled breath condensates of patients with COPD (31, 54). Likewise, miR-17-92, which among other mRNAs target several components of the TGF-β signaling pathway, is reduced in IPF lungs and increased in COPD tissues (42).

Another interesting likely miRNA-associated difference between IPF and COPD is related to COX expression. Fibroblasts from COPD display increased COX expression and PGE-2 production, which is mediated, at least partially, by decreased expression of miR-146a (55). This microRNA is not modified in IPF, and, as mentioned, IPF fibroblasts exhibit decreased COX-2 expression likely related to histone hypoacetylation (40).

Genetic Architecture and Epigenetic Reprogramming Result in the Aberrant Activation of Epithelial Cells

Reestablishing epithelial integrity is critical to orchestrate a correct regenerative response. In IPF, AEC damage and apoptosis result in an aberrant lung reepithelialization. Therefore, epithelial cells exhibiting severe endoplasmic reticulum stress (a process that has also been associated with aging) and apoptosis (56) coexist with others showing premature senescence. Additionally, there are epithelial cells that are proliferating while others display a partial EMT-like process,

Table 1: Mediators Overexpressed by Epithelial Cells and Their Likely Functions in the Pathogenesis/Progression of Idiopathic Pulmonary Fibrosis

Mediator	Some Putative Profibrotic Roles
Growth factors and related molecules	
Transforming growth factor-beta (TGF-β) (74)	Likely the strongest profibrotic factor. The primary inductor of fibroblast to myofibroblast differentiation and of epithelial to mesenchymal transition
Platelet-derived growth factor (PDGF) (75)	Induces migration and proliferation of fibroblasts
Connective-tissue growth factor (CTGF) (76)	Induced by TGF-β and appears to be a mediator of some of its profibrotic effects. It provokes transcriptional activation of Col1α2
Tumor necrosis factor-alpha (TNF-α) (77)	Induces loss of fibroblast Thy-1 surface expression which is associated with Thy-1 shedding, smad phosphorylation, and myofibroblast differentiation.
Osteopontin (78)	Induces migration and proliferation of fibroblasts and epithelial cells. In fibroblasts, up-regulates TIMP-1 and type I collagen and down-regulates MMP-1 expression. In epithelial cells causes up-regulation and activation of MMP-7.
Insulin-like growth factor-I (IGF-I) (79)	Stimulates collagen production
Insulin-like growth factor binding proteins 3 and 5 (80)	Induces collagen and fibronectin production by lung fibroblasts
Angiotensinogen (81)	Enhances fibroblast migration and proliferation and ECM synthesis. It induces epithelial cell apoptosis.
Fibroblast growth factor 9 (82)	Unknown
Neuregulin NRG1α (83)	May contribute to bronchiolization of the distal lung as seen in IPF
Endothelin-1, and endothelin-converting enzyme (84)	Induce epithelial-to-mesenchymal transition. Differentiation of fibroblasts to myofibroblasts. They may contribute to pulmonary arterial hypertension
Matrix metalloproteinases (MMP) and tissue inhibitors of MMP	
MMP-1 (85)	Induces alveolar epithelial cell migration and proliferation, protects from apoptosis, and represses mitochondrial oxygen consumption. Induces fibroblast migration
MMP-7 (86)	Cleaves E-cadherin and may induce epithelial cell migration. Cleaves osteopontin, potentiating its effect
MMP-2 (87, 88)	Basement membrane degradation
Membrane type-1 and 2 MMP (88)	Unknown. They activate proMMP-2 and might be involved in cell growth and migration
TIMP-4 (89)	Unknown, it might inhibit MMPs
Chemokines	
CCL17/thymus and activation-regulated chemokine (TARC) (90)	Associated with a Th2 (profibrotic) profile
CCL2/monocyte chemotactic protein-1 (91)	Involved in the profibrotic effects of thrombin. It stimulates fibroblast collagen expression and endogenous up-regulation of TGF-β.
CXCL12 (92)	Acts as a potent chemotactic factor for fibrocytes through the CXCL12/CXCR4 axis

(Continued)

Table 1: (*Continued*)

Mediator	Some Putative Profibrotic Roles
Coagulation factors	
TF/FVIIa/FX ternary complex (93)	FXa is a potent inducer of the myofibroblast differentiation.
Plasminogen activator inhibitor-1 (94)	Promotes proliferation, activation, and collagen synthesis, and inhibits apoptosis of lung fibroblasts. It promotes AEC2 apoptosis. A positive feedback loop between PAI-1 and TGF-β1 has been described.
Protease-activated receptor-1 and -2 (91, 95)	Activation of PAR1 may lead to increased local CCL2 release. The PAR-2/TF/FVIIa axis may contribute to the coagulation cascade. PAR-1 is the main receptor responsible for mediating thrombin's effects on fibroblast function.
Developmental pathways	
Wnt-pathway components (96)	It induces epithelial cell proliferation and differentiation, epithelial-to-mesenchymal transition, fibroblast migration and myofibroblast differentiation. It induces IL-1β expression.
Sonic hedgehog (97, 98)	It increases proliferation, migration, extracellular matrix production, and survival of fibroblasts. Smoothened, the obligatory signal transducer of the pathway is required for TGF-β1–induced myofibroblastic differentiation.
Other mediators	
Pigment epithelium-derived factor (99)	It has angiostatic and neurotrophic activities. Colocalizes with TGF-β1, prominently within the epithelium overlying the fibroblastic focus
Dimethylarginine dimethylaminohydrolase (DDAH) (100)	Metabolize asymmetric dimethylarginine which is an endogenous inhibitor of nitric oxide synthase. Increases collagen synthesis.
Autotaxin (101)	Hydrolysis of lysophosphatidylcholine by the phospholipase D activity resulting in the production of lysophosphatidic acid which promotes fibroblast migration and resistance to apoptosis
Hypoxia-inducible factor-1α (102)	It plays a role in hypoxia-induced TGF-β1 and vascular endothelial growth factor up-regulation.
Sphingosine-1-phosphate (103)	A pleiotropic bioactive lipid mediator. Stimulates extracellular matrix synthesis and increase the expression of profibrotic mediators such as connective tissue growth factor
Heparan sulfate (HS) 6-O-endosulfatase 2 (104)	Unknown. It regulates TGF-β1 signaling?

Definition of abbreviations: ECM = extracellular matrix; IPF = idiopathic pulmonary fibrosis; TGF = transforming growth factor.

likely linked to a migratory program and a transition to a wound-healing phenotype (57). Thus, increasing numbers of phenotypically diverse epithelial cells repopulate the IPF lungs. Migration and proliferation of bronchiolar basal cells and type 2 AECs, and the (yet unproven) presence of a plastic stem cell–like population may participate in this reepithelialization. However, the source of the epithelial cells populating the IPF lungs is uncertain.

Directional and coordinated cell migration, proliferation, and differentiation are required for proper reepithelialization. Impaired cell function or loss of the basement membrane integrity (as occurs in IPF) has dramatic consequences on the ability of epithelial cells to interpret directionality signals.

Remarkably, epithelial cells in IPF lungs are not only increased in numbers and showing abnormal phenotypes but also extremely active, and strong evidence demonstrates that they synthesize virtually all the mediators that participate in the formation of the fibroblastic foci and in the progressive tissue remodeling (1) (Table 1). In addition, it has been found that after lung injury in mice, activated epithelial cells produce type I collagen and actually behave similar to activated fibroblasts expressing several mesenchymal genes and leading to progressive fibrosis (58). However, whether this process also occurs in IPF is presently unknown.

Another important but unsolved question is related to the spatial and temporal patterns of secretion of the numerous mediators synthesized by epithelial cells in the course of IPF. The behavior/response of these cells may depend on their origin, location, and the conditions of the intercellular contacts. For example, there is evidence that epithelial cells of contact-deprived regions are susceptible to TGF-β reprogramming and EMT, whereas the intact epithelium is not (59). If this compartmentalized response occurs in IPF, it may clarify, at least partially, the focal nature of the fibrotic reaction whereby clusters of fibroblast/myofibroblastic foci are interspersed with normal lung areas.

Also, which of the diverse sources/phenotypes of epithelial cells are responsible for the release of what mediators is uncertain. It is tempting to speculate that senescent epithelial cells, reprogrammed to a secretory phenotype and usually covering active fibrosing lesions, are the cells that are producing the diverse growth factors for fibroblasts. Likewise, epithelial cells from lesions evolving to honeycombing may produce some MMPs that may contribute to the formation of the cysts. Identifying which epithelial cells are producing which mediators will be critical to better

understand the complex pathogenic mechanisms of the disease.

Myofibroblasts Come into Action

Epithelial activation is followed by a dynamic and complex process characterized by the migration and proliferation of fibroblasts and their differentiation to myofibroblasts, a critical process for the development of fibrosis through exaggerated extracellular matrix (ECM) deposition.

The source of the myofibroblasts in IPF is unclear, and several cell types have been proposed as the putative precursor, including tissue-resident mesenchymal cells, epithelial cells (via EMT), pleural mesothelial cells (via mesothelial-to-mesenchymal transition) and bone marrow–derived cells (fibrocytes) (1). More recently, it has drawn attention to the pericyte as a major myofibroblast precursor after lung injury in mice (60). Interestingly, increased numbers of pericytes are found in IPF lungs, but most of them are negative for αSMA, indicating that they do not differentiate to myofibroblasts *in vivo* (61). Overall, one can assume that a heterogeneous population of mesenchymal cells contributes to the formation of the fibroblastic/myofibroblastic foci, although the magnitude of the individual contributions is unknown. Of note, activated epithelial cells produce virtually all the growth factors that contribute to the expansion and activation of the fibroblasts in the IPF lungs (e.g., platelet-derived growth factor [PDGF], a potent chemoattractant for local fibroblasts; CXCL12, the main chemokine that attract circulatory fibrocytes and perhaps vascular pericytes and pleural mesothelial cells through the CXCR4/CXCL12 axis; and TGF-β, which is essential for EMT and for fibroblast-to-myofibroblast differentiation) (1).

Once the fibroblast/myofibroblast focus is formed, complex epithelial–mesenchymal interactions and cross-talking through direct contacts and/or soluble mediators occur in the microenvironment that contribute to disease progression. For example, myofibroblasts lose the expression of Thy-1 producing MMP-9 after TGF-β stimulation, which may provoke the disruption of the epithelial basement membrane and the activation of latent TGF-β (62). Also, IPF myofibroblasts produce mediators able to induce epithelial apoptosis, increasing the alveolar damage/activation (1). Thus, AECs are provoking the formation of the fibroblastic foci, whereas the myofibroblasts from these foci are contributing to the destabilization of the epithelium. Myofibroblasts in the foci secrete excessive amounts of ECM components, primarily fibrillar collagens, and are finally a key component of the aberrant tissue remodeling.

Factors That Influence the Speed and the Irreversible Progression of IPF: The Dark Side of the Moon?

IPF is clinically heterogeneous, exhibiting different rates of disease progression. Thus, although most patients follow a slowly progressive course, others develop an accelerated progression. However, the mechanisms that influence these clinical courses are poorly understood.

Stiffness of the IPF matrix, which is significantly higher than in normal lungs, affects fibroblast phenotype enhancing fibroblast-to-myofibroblast differentiation and may be a critical factor driving the progression and perpetuation of established fibrosis (63). Interestingly, a recent study indicates that a major driver of the inexorably progressive characteristic of IPF is the diseased ECM and not the diseased fibroblasts/myofibroblasts. A positive feedback loop of progression regulated by miRNA-29, in which the IPF matrix modulates genes, mainly at the level of translation, provokes the adjacent spreading of the fibrotic response (64).Tissue stiffness may also affect mechano-signaling pathways that regulate epithelial plasticity, although studies in IPF are scanty.

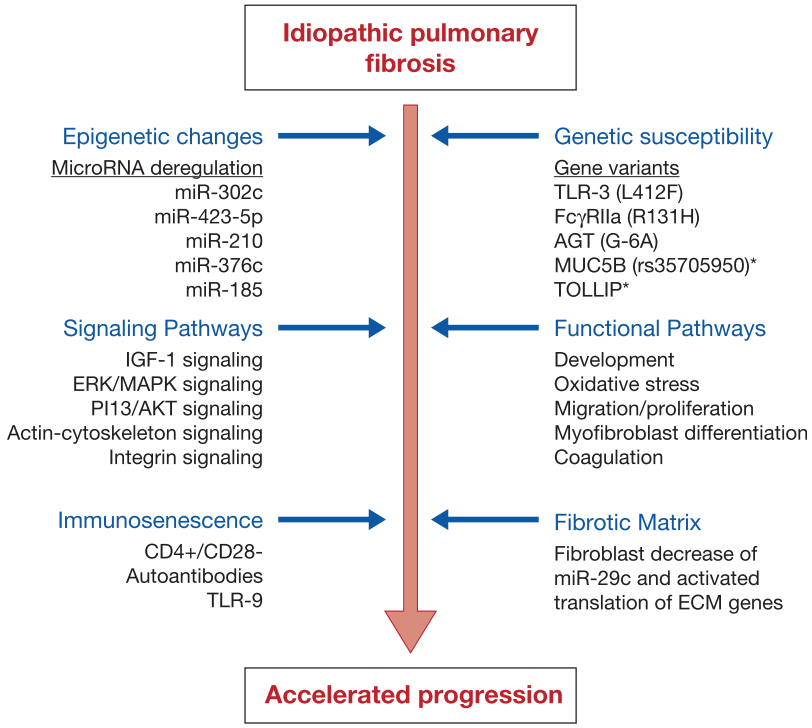

Figure 3. Mechanisms associated with idiopathic pulmonary fibrosis (IPF) progression rate. Some gene polymorphisms and epigenetic changes may contribute to an accelerated progression rate in IPF. Likewise, transcriptional signatures from lungs obtained from patients with rapid and slowly progressive IPF have revealed significant differences in several signaling and functional pathways. Some aging-associated abnormalities (e.g., immunosenescence) may also contribute to the development of a more aggressive clinical course. Fibrotic extracellular matrix (ECM) may induce a positive feedback loop provoking the decrease of antifibrotic microRNAs (e.g., miR-29c), which in turn activate the translation of ECM genes and ECM accumulation. *Variants of these genes are associated with increased risk to develop IPF but with improved survival. Individuals with the susceptibility MUC5B rs35705950_T allele who develop IPF have decreased mortality. Likewise, the major TOLLIP rs5743890_A allele increased susceptibility to IPF but confers survival advantage.

Many factors may accelerate the rate of IPF progression (Figure 3), including accelerated immunosenescence (e.g., marked down-regulation of $CD4^+/CD28^-$ T cells and/or antigen-specific autoimmunity [14, 15]) and increased expression of toll-like receptor-9 (65). Some gene variants, such as toll-like receptor 3 L412F, FcγRIIa R131H, or angiotensinogen gene G-6A, among others, may contribute to rapid progression, whereas others (MUC5B promoter polymorphism, TOLLIP variants) are associated with improved survival (24, 66–69).

Interestingly, the increase of some miRNA (e.g., miR-302c, miR-423–5p, miR-210, miR-376c, and miR-185) and the decrease of Argonaute (AGO)-1 and AGO2 (indispensable components of the miRNA processing RISC complex) have been revealed in IPF lungs from patients with rapid progression, which may partially explain the distinct lung molecular signatures in patients with relatively stable/slow and rapidly progressive IPF (70–72).

Therefore, additional complex interactions in the lung microenvironment influence the progression speed of scarring formation and remodeling. However, these are likely contributory mechanisms for the progression rate but not for the disease predisposition, initiation, and development.

Conclusions

IPF is a progressive and therapeutically frustrating disease that represents an enormous challenge for patients, clinicians, and researchers. Clarifying the pathogenic mechanisms that underlie the disease is crucial, and in this context, the longstanding notion that IPF was an inflammatory-driven fibrosis has been challenged by a growing body of evidence describing a key role of epithelial cells.

Maintaining the epithelial integrity to preserve the lung health in the face of the numerous and incessant environmental insults and the rigors of aging is a formidable task, but genome evolution has resulted in the construction of a sophisticated lung structure able to confront these challenges. Here we propose an integrative model to construct a framework that may explain the sequence of pathogenic events. First, there is a genetic architecture of IPF susceptibility that results in loss of alveolar epithelial integrity, which, in the presence of a distinctive epigenetic reprogramming, provokes an aberrant activation of the epithelial cells that produce virtually all the mediators responsible for the

Table 2: Idiopathic Pulmonary Fibrosis and Chronic Obstructive Pulmonary Disease: Common and Distinct Mechanisms

Common Features of IPF and COPD	Distinct Features	
	IPF	**COPD**
Telomere attrition (11, 16)	**Initial/primary target**	
	Alveolar epithelial cells (1)	Small airways epithelial cells (105)
Oxidative stress (13,17)	**Genetic architecture of susceptibility**	
	TERT (5p15), MUC5B (11p15), the 3q26 region near TERC, FAM13A (4q22), DSP (6p24), OBFC1 (10q24), ATP11A (13q34), DPP9 (19p13), and chromosomal regions (7q22 and 15q14-15) (23)	HHIP (4q31), CHRNA 3/5 (15q25), EGLN2 (19q13), and FAM13A (4q22) (26, 27)
Alveolar epithelial cell senescence (12,16)	**DNA methylation**	
	A relatively balanced distribution of hyper- and hypomethylation changes or even a predominance of hypomethylated CpG islands (36, 37)	97% of differentially methylated are hypermethylated (39)
Immune senescence. Increased $CD4^+/CD28^-$ T cells. Circulating autoantibodies (14, 15, 21, 22)	**MicroRNA dysregulation**	
	Up-regulated: miR-21, miR-199a-5p, miR-145, miR-154 family, miR-34a (44, 46, 47, 50)	Up-regulated: miR-223, miR-1274a, miR-144, miR-374a, miR-664, miR-17-92, miR-576-3p, miR-513a-5p, miR-25, miR-99b, miR-125b-1, miR-24 (53, 54)
	Down-regulated: let-7d, miR-200, miR-210, miR-326, miR-17-92, miR-29, miR-30 (42, 43, 45, 64)	Down-regulated: miR-21, miR-923, miR-937, miR-422a (53, 54)
Endocrine senescence. Abnormal decrease of dehydroepiandrosterone (DHEA) (106, 107)	**Wnt signaling pathway**	
	Up-regulated (48)	Down-regulated (52)
Loss of heterozygosity, microsatellite instability (10, 18)	**Autophagy**	
	Decreased (12)	Increased (108)

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; IPF = idiopathic pulmonary fibrosis.

formation/activation of the myofibroblastic foci. Activated myofibroblasts produce exaggerated amounts of ECM proteins that result in the abnormal remodeling of the lung structure. Certainly, aging-associated processes, such as telomere attrition, mitochondrial dysfunction, cellular senescence, and stem cell exhaustion, among others, play a pivotal role in the development of IPF, whereas several subsequent alterations (e.g., matrix stiffness, inflamm-aging, and infections) may contribute to disease rate of progression. Importantly, many aging-related alterations also occur in COPD, but a number of biopathological processes, including a distinctive genetic architecture and a divergent epigenetic dysregulation affecting the expression of a network of specific key target genes, differentiate both diseases (Table 2).

Our challenge for the future is to decipher the complex interplay between the genetic architecture, aging-associated processes, environmental factors, (e.g., tobacco exposure), and the genome–environment interaction (epigenetic) that impacts gene expression that finally results in the sequence of pathogenic mechanisms leading to the development of IPF. ■

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