Role of various imbalances centered on alveolar epithelial cell/fibroblast apoptosis imbalance in the pathogenesis of idiopathic pulmonary fibrosis

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Abstract
There have been recent extensive studies and rapid advancement on the pathogenesis underlying idiopathic pulmonary fibrosis (IPF), and intricate pathogenesis of IPF has been suggested. The purpose of this study was to clarify the logical relationship between these mechanisms. An extensive search was undertook of the PubMed using the following keywords: “etiology,” “pathogenesis,” “alveolar epithelial cell (AEC),” “fibroblast,” “lymphocyte,” “macrophage,” “epigenomics,” “histone,” “acytlation,” “methylation,” “endoplasmic reticulum stress,” “mitochondrial dysfunction,” “telomerase,” “proteases,” “plasminogen,” “epithelial-mesenchymal transition,” “oxidative stress,” “inflammation,” “apoptosis,” and “idiopathic pulmonary fibrosis.” This search covered relevant research articles published up to April 30, 2020. Original articles, reviews, and other articles were searched and reviewed for content; 240 highly relevant studies were obtained after screening. IPF is likely the result of complex interactions between environmental, genetic, and epigenetic factors; environmental exposures affect epigenetic marks; epigenetic processes translate environmental exposures into the regulation of chromatin; epigenetic processes shape gene expression profiles; in turn, an individual’s genetic background determines epigenetic marks; finally, these genetic and epigenetic factors act in concert to dysregulate gene expression in IPF lung tissue. The pathogenesis of IPF involves various imbalances including endoplasmic reticulum, telomere length homeostasis, mitochondrial dysfunction, oxidant/antioxidant imbalance, Th1/Th2 imbalance, M1–M2 polarization of macrophages, protease/antiprotease imbalance, and plasminogen activation/inhibition imbalance. These affect each other, promote each other, and ultimately promote AEC/fibroblast apoptosis imbalance directly or indirectly. Excessive AEC apoptosis and impaired apoptosis of fibroblasts contribute to fibrosis. IPF is likely the result of complex interactions between environmental, genetic, and epigenetic factors. The pathogenesis of IPF involves various imbalances centered on AEC/fibroblast apoptosis imbalance.

Keywords: Alveolar epithelial cell; Apoptosis; Idiopathic pulmonary fibrosis; Fibroblast; Pathogenesis

Introduction
Idiopathic pulmonary fibrosis (IPF) is a chronic, age-related, progressive lung disease characterized by progressive lung fibrogenesis and the histological picture of usual interstitial pneumonia (UIP). It is associated with increased cough and dyspnea and impaired quality of life. The median survival of patients with IPF is 3 to 5 years from diagnosis.[1]

The paradigm about disease pathogenesis has shifted from belief that chronic inflammation is the direct cause of IPF[2,3] to the idea that oxidative stress plays an important role in the onset and progression of IPF[4,5] to more recent suggestion to the idea that oxidative stress plays an important role in the belief that chronic inflammation is the direct cause of IPF[2,3] to the idea that oxidative stress plays an important role in the onset and progression of IPF[4,5] to more recent suggestion to the idea that oxidative stress plays an important role in the belief that chronic inflammation is the direct cause of IPF[2,3] to the idea that oxidative stress plays an important role in the onset and progression of IPF[4,5] or to the idea that repetitive micro-injuries and dysfunction of the alveolar epithelial cells (AECs) injury lead to uncontrolled activation and proliferation of fibroblasts, and excessive accumulation of extracellular matrix (ECM), by generating crucial profibrotic signaling and mediators.[5,6] Correspondingly, the therapy for IPF has also undergone a transition from treatment with anti-inflammatory and immunosuppressant drugs (eg, prednisone and azathioprine) to treatment with antioxidant drugs (eg, N-acetylcysteine [NAC]) and to treatment with more recent antifibrotic drugs (nintedanib and pirfenidone). Unfortunately, it gradually became evident that anti-inflammatory and antioxidative therapy was ineffective in improving survival in patients with IPF.[7,8] Although pirfenidone and nintedanib are effective at reducing lung function decline, neither is curative for the
disease. Does the ineffectiveness of anti-inflammatory and antioxidative therapy mean that inflammation and oxidant/antioxidant imbalance are not particularly important in the pathogenesis of IPF?

Various mechanisms underlying IPF have been identified. A body of evidence indicates that IPF is related to possible triggers or environmental risk factors, genetic predisposition, and epigenetics, and these various mechanisms — such as endoplasmic reticulum (ER) stress, telomere length homeostasis, mitochondrial dysfunction, oxidant/antioxidant imbalance, $T$ helper type 1 cell (Th1/Th2) imbalance, M1–M2 polarization of macrophages, protease/antiprotease imbalance, plasminogen activation/inhibition imbalance, AEC/ fibroblast apoptosis imbalance, epithelial-mesenchymal transition (EMT), and transforming growth factor (TGF)-β — are involved in the pathogenesis of IPF. What is the relationship during environmental risk factors, genetic predisposition, and epigenetics, and what are their underlying mechanisms for initiating IPF? What relationships and interactions exist among various mechanisms mentioned above?

In the present review, we begin with a discussion on the role of possible triggers, genetic instability, and epigenetic changes in the pathogenesis of IPF and their interplay, followed by the contribution of various imbalances centered around an AEC/fibroblast apoptosis imbalance in the pathogenesis of IPF and their interaction and end with opinions on potential therapeutic targets based on the above-mentioned complex pathogenesis.

**Etiology: Environmental, Genetic, and Epigenetic Factors**

**Possible triggers/environmental risk factors**

Although the exact cause of IPF remains unclear, toxic exposures, including cigarette smoking, environmental and occupational exposures, infection, and gastroesophageal reflux, appear to be important contributing factors.

**Genetic predisposition**

Several genetic mutations have been implicated in familial IPF, which broadly are subdivided into two categories: genes related to surfactant protein processing and trafficking ($SFTPA1$, $SFTPA2$, $SFTPB$, $SFTPC$, ATP-binding cassette-type 3) and genes that maintain telomere length ($TERT$ and $TERC$).

The mutation results in an aberrant surfactant protein that cannot be correctly processed, resulting in protein misfolding, accumulation, induction of ER stress, and apoptosis of AECs. Mutations in $TERT$ or $TERC$ lead to loss of telomerase activity. Consequently, telomeres shorten successively with each cell division, and when they achieve a critical length they activate p53-dependent apoptosis or replicative senescence. Additionally, a genome-wide association study found that a single-nucleotide polymorphism in the promoter region of the mucin 5B gene and a loss-of-function polymorphism in $TOLLIP$ which is a gene encoding the inhibitor of toll-like receptor 4 (TLR4) are strongly associated with IPF. Some of these mutations have been reported not only in several familial IPF cases but also in sporadic cases of IPF, which suggests that sporadic and familial cases of IPF probably reflect a continuum of genetic risk and that sporadic IPF is also a disease with a genetic predisposition.

**Epigenetics**

Epigenetic factors contribute to the dysregulation of gene expression in IPF lung, which leads to changes in gene expression without a change in the gene-coding sequences. The most common epigenetic mechanisms include DNA methylation and histone modifications and non-coding RNA (ncRNA) regulation.

**Methylation of DNA**

Methylation of DNA is usually associated with decreased gene expression. For example, $Thy1$, a receptor that inhibits the differentiation of fibroblasts to myofibroblasts, is not expressed in fibroblastic foci in vivo. Loss of $Thy1$ occurs through epigenetic silencing caused by hypermethylation of cytosine-guanine islands in the gene promoter. As a result, such epigenetic change leads to the aggressive behavior of IPF fibroblasts.

**Histone modifications**

In quiescent cells, genomic DNA is wrapped around histones to form nucleosomes, restricting transcriptional access to the DNA. Methylation, acetylation, phosphorylation, and ubiquitylation of histone tails occur at specific residues and control gene expression by regulating DNA accessibility to RNA polymerase II and transcription factors. For example, acetylation of histones results in the relaxation of chromatin, facilitating gene transcription. Histone acetylation and deacetylation on lysine residues by histone acetyltransferases and histone deacetylases are closely associated with active and repressive chromatin states and increased and decreased transcription factor binding to specific gene promoters, respectively. Prostaglandin (PG)E2, a cyclooxygenase (COX)-dependent arachidonic acid metabolite in the lung, exerts an important antifibrotic effect by promoting the survival of AECs while increasing the sensitivity of fibroblasts/myofibroblasts to apoptosis, whereas fibroblasts from IPF patients are unable to up-regulate the COX-2 enzyme and are thereby deficient in PG2E2 production. Defective histone acetylation is responsible for the diminished expression of COX-2. One study found aberrant expression and activity of histone deacetylases in sporadic IPF and observed that compared with control lungs, protein levels of HDACs are significantly elevated in IPF lungs, and apoptosis resistance in IPF fibroblasts is mediated by enhanced activity of HDAC enzymes. Pan-HDAC inhibition by LBH589 may present a novel therapeutic option for patients with IPF. Huang et al. found that increased histone deacetylase expression was partially responsible for the down-regulation of the factor-associated suicide (Fas) death receptor in fibroblast fibroblasts, another reason for apoptosis resistance in fibroblasts.
ncRNA regulation

ncRNAs are often considered a part of the epigenome. ncRNAs are functional RNA molecules that are not translated into proteins, including transfer RNAs, ribosomal RNAs, small nucleolar RNAs, microRNAs (miRNAs), and long non-coding RNAs.[54] miRNAs are the most extensively studied family of small ncRNAs. miRNAs are short (∼22 nt) single-stranded ribonucleic acids functioning as post-transcriptional regulators of gene expression that play important roles by binding to specific sequences, blocking translation, or causing degradation of the target mRNA, which results in gene silencing. Through various mechanisms, some miRNAs (eg, miR-21,[55,56] miR-31,[57] miR-145,[58] miR-154,[59] and miR-199a[60]) play a role in promoting fibrosis during the pathogenesis of IPF, and their expression levels are often elevated, while others (such as let-7d,[56,61] miR-9,[62] miR-18a,[63] miR-26a,[56] miR-27b,[64] miR-29,[65] miR-30a,[56,66] miR-155,[67] miR-200,[68] miR-221,[69] miR-323a,[70] miR-326,[71] miR-338,[72] miR-375,[73] and miR-486[74]) prevent fibrosis, and their expression levels are reduced. One recent study demonstrated that the long-intervening non-coding RNAs (lincRNAs) LINC00960 and LINC01140 can regulate fibroblast proliferation and inflammation, while changes in LINC01140 expression may mediate a reduced inflammatory response in IPF fibroblasts.[75] While the mechanisms by which these lincRNAs regulate these responses are unknown, it is speculated that the biological actions of lincRNAs may be mediated through domains containing conserved sequences, which interact with proteins and/or base pairs with RNA/DNA.[76]

Interactions between environmental, genetic, and epigenetic factors

IPF is likely the result of complex interactions between environmental, genetic, and epigenetic factors.

First, environmental exposures strongly affect epigenetic marks.[77] Epigenetic processes translate environmental exposures into the regulation of chromatin. Cigarette smoke, the main environmental risk factor for IPF, influences the methylation of specific promoters in genes involved in the pathogenesis of IPF, such as WNT7A.[78] One study revealed how cigarette smoke influences histone modifications and chromatin accessibility.[79] Additionally, epigenetic processes shape the gene-expression profiles involved in disease pathophysiology. In turn, an individual’s genetic background determines epigenetic marks in two ways: by direct inheritance[80] and by genetic variants. Genome-wide studies demonstrate a strong genetic component to inter-individual variation in methylation and histone modification profiles.[81] Finally, genetic and epigenetic factors act in concert to dysregulate gene expression in IPF lung [Figure 1].

![Figure 1: The etiology and pathogenesis of IPF: various imbalances centered on AECs/fibroblasts apoptosis imbalance, AECs: Alveolar epithelial cells; EMT: Epithelial-mesenchymal transition; ER: Endoplasmic reticulum; IPF: Idiopathic pulmonary fibrosis; TGF-β: Transforming growth factor-β; Th: T helper.](image-url)
Various Imbalances Centered on an Apoptosis Imbalance in AECs and Fibroblasts

ER stress
Accumulation of mutant and misfolded proteins in the ER has been shown to induce severe ER stress. Reduced clearance of misfolded proteins (e.g., proteasomal dysfunction, impaired autophagy), disturbances in redox homeostasis, nutrient deprivation, and environmental insults (e.g., viral infection) can bring about the aggregation of unfolded or misfolded proteins, exacerbating ER stress, which triggers the activation of the unfolded protein response (UPR) to alleviate ER stress and sustain the cellular homeostasis.[86] However, when stress is overloaded or prolonged, the UPR becomes maladaptive and triggers apoptotic cell death through activating cell death pathways such as caspase-4, c-Jun NH2 terminal kinase, and C/EBP homologous protein.[83-85] ER stress may cause fibrosis through AEC apoptosis, EMT, myofibroblast differentiation, and M2 macrophage polarization.[14-16]

Telomere length homeostasis
A telomere is a region of tandem repeats of short DNA sequences at the ends of chromosomes, which are important for their stability and allow the complete replication of the ends. Telomere length homeostasis is essential for proper cellular function.[86] Telomeres shorten with every cell division owing to incomplete replication of telomere caps on the ends of chromosomes. Telomerase, a specialized RNA-directed DNA polymerase, elongates telomere sequences at the termini of chromosome DNA, preventing progressive telomere loss and maintaining chromosome stabilization. Mutations in the essential telomerase genes, found in both sporadic and familial IPF cases, result in telomerase loss of function and reduced telomerase activity, thereby accelerating the telomere shortening.[40] In addition, other factors such as smoking,[87] chronic inflammation, and cumulative oxidative stress can cause telomere shortening.[88]

When telomeres shorten to a critical length, they can be sensed as double-stranded DNA breaks, activating the DNA damage sensor and checkpoint inhibitor p53, which leads to apoptosis or replicative senescence,[17] impairing mitochondrial biogenesis by repressing expression of a key mediator of mitochondrial biogenesis, Peroxisome proliferator-activated receptor gamma (PPARγ) coactivator-1α, resulting in inefficient oxidative phosphorylation and increased ROS production.[89] Second, phosphatase and tensin homolog-induced putative kinase 1 (PINK1) labels the dysfunctional mitochondrion for trafficking to the autophagosome. ER stress then causes downregulation of PINK1 expression, resulting in an accumulation of dysmorphic mitochondria, deficient mitophagy, reduced ETC activity, and increased ROS production.[90] As a consequence, ROS produced by dysfunctional mitochondria can cause further ER stress, thus forming a vicious circle.[91] Third, oxidative stress can cause mitochondrial dysfunction. Excessive ROS promote mitochondrial DNA (mtDNA) damage,[92] but additionally, that mtDNA damage can augment further increases in ROS production.[18,93] leading to a vicious feed-forward cycle that worsens mitochondrial dysfunction. In addition, a profibrotic environment promotes mitochondrial dysfunction in pulmonary epithelial cells. One study found that TGF-β down-regulates mitochondrial ETC function, leading to increased ROS production.[94] Increased ROS can further activate latent TGF-β, thus creating a vicious feed-forward cycle with the potential to recruit fibroblasts and promote fibrogenesis.[95] Mitochondrial dysfunction can also activate TGF-β through metabolic reprogramming: the metabolic shift from the highly efficient method of ATP production to the less efficient method of glycolysis due to mitochondrial dysfunction — and correspondingly, fatty acid oxidation increases in response to the shift to glycolysis in fibroblasts or alveolar macrophages in fibrotic lung tissue — resembles the cancer-associated Warburg effect.[96-100] Glycolytic flux increases lactate production and lowers the local tissue pH, causing increased activation of TGF-β, which induces differentiation of fibroblasts to myofibroblasts.[96]

The consequences of mitochondrial dysfunction
To summarize, telomere shortening, ER stress, oxidative stress, and a profibrotic environment (TGF-β) promote mitochondrial dysfunction in pulmonary epithelial cells; as a consequence, mitochondrial dysfunction can further stimulate ER stress, oxidative stress, and TGF-β, creating a vicious feed-forward cycle. In addition, mitochondrial dysfunction promotes AEC apoptosis and senescence.[18]

Mitochondria-regulated homeostasis

Contents of mitochondrial dysfunction
Mitochondrial dysfunction, which means mitochondria-regulated homeostasis is broken, in IPF includes reduced efficiency of electron transport chain (ETC) function along with excessive production of reactive oxygen species (ROS), decreased mitochondrial biogenesis, and impaired mitophagy — a key pathway for mitochondria turnover and the removal of dysfunctional mitochondria.

Mechanisms of mitochondrial dysfunction
First, telomere shortening stimulates the DNA damage sensor and checkpoint inhibitor p53; activated p53 then further impairs mitochondrial biogenesis by repressing expression of a key mediator of mitochondrial biogenesis, Peroxisome proliferator-activated receptor gamma (PPARγ) coactivator-1α, resulting in inefficient oxidative phosphorylation and increased ROS production.[89] Second, phosphatase and tensin homolog-induced putative kinase 1 (PINK1) labels the dysfunctional mitochondrion for trafficking to the autophagosome. ER stress then causes downregulation of PINK1 expression, resulting in an accumulation of dysmorphic mitochondria, deficient mitophagy, reduced ETC activity, and increased ROS production.[90] As a consequence, ROS produced by dysfunctional mitochondria can cause further ER stress, thus forming a vicious circle.[91] Third, oxidative stress can cause mitochondrial dysfunction. Excessive ROS promote mitochondrial DNA (mtDNA) damage,[92] but additionally, that mtDNA damage can augment further increases in ROS production.[18,93] leading to a vicious feed-forward cycle that worsens mitochondrial dysfunction. In addition, a profibrotic environment promotes mitochondrial dysfunction in pulmonary epithelial cells. One study found that TGF-β down-regulates mitochondrial ETC function, leading to increased ROS production.[94] Increased ROS can further activate latent TGF-β, thus creating a vicious feed-forward cycle with the potential to recruit fibroblasts and promote fibrogenesis.[95] Mitochondrial dysfunction can also activate TGF-β through metabolic reprogramming: the metabolic shift from the highly efficient method of ATP production to the less efficient method of glycolysis due to mitochondrial dysfunction — and correspondingly, fatty acid oxidation increases in response to the shift to glycolysis in fibroblasts or alveolar macrophages in fibrotic lung tissue — resembles the cancer-associated Warburg effect.[96-100] Glycolytic flux increases lactate production and lowers the local tissue pH, causing increased activation of TGF-β, which induces differentiation of fibroblasts to myofibroblasts.[96]

Oxidant/antioxidant imbalance
Both endogenous and exogenous sources included in the oxidative stress and generation of ROS
One theory regarding the pathogenesis of IPF is that an oxidant/antioxidant imbalance, known as oxidative stress, exists in the alveolar regions of affected lungs. In lungs from IPF patients, ROS are produced by inflammatory cells (lymphocytes, macrophages, and neutrophils) and parenchymal cells (e.g., myofibroblasts and fibroblasts) in response to cytokines, growth factors, and exogenous oxidizing agents such as air pollutants and cigarette smoke.[101,102] Some factors, such as TGF-β, inflammation, and mitochondrial dysfunction, can induce the sustained production of ROS.[18,93]
Effects of oxidant/antioxidant imbalance

ROS further amplify the profibrotic TGF-β downstream signal and promote inflammation. Therefore, reciprocal promotion of TGF-β and ROS, of inflammation and ROS, and of mitochondrial dysfunction and ROS results in a perverse and vicious cycle for fibrosis. ROS also cause direct damages to AECs, predisposing individuals to lung fibrosis. Importantly, ROS may contribute to a protease/antiprotease imbalance because they can directly induce matrix metalloproteinase (MMP) transcription, and inactivate protease inhibitors. In addition, mitochondrial ROS promote AEC cell apoptosis and senescence, while fibroblasts are resistant to apoptosis. One reason for this is that ROS activate the persistent platelet-derived growth factor (PDGF) receptor production that is implicated in fibroblast differentiation and proliferation in fibrosis. Considered together, ROS result in pathophysiological consequences, including TGF-β activation, inflammation, protease/antiprotease imbalance, AEC damage, apoptosis and senescence, and fibroblast differentiation; all of these are implicated in driving lung fibrosis.

Inflammation and immunity

Th1/Th2 imbalance

The toll-like receptor (TLR) signaling pathway and T-cell differentiation mediated by fate-specifying cytokines

Although the role of inflammation in the pathogenesis of IPF remains controversial, evidence supports the fact that immune responses play an important role in the initiation or progression of IPF. Pathogen-associated molecular patterns and damage-associated molecular patterns are recognized by TLRs expressed in monocytes, macrophages, dendritic cells (DCs), mast cells, neutrophils, eosinophils, basophils, and epithelial cells, thereby initiating an innate immune response, which activates nuclear factor-kB (NF-κB) to produce proinflammation cytokines or activates interferon (IFN) regulatory factors to produce type I IFNs. Cytokines produced by these cells prime the differentiation of naïve T cells into Th1, Th2, Th17, or regulatory T cell (Treg) phenotypes according to the antigenic stimulation involved [Figure 2].

The existence and causes of Th1/Th2 imbalance in IPF

T-cell activation in IPF patients is likely skewed toward a Th2 response. Studies have shown that Th1 cells and secretory cytokine (IFNg) levels decrease in the bronchoalveolar lavage or circulation of patients with IPF, whereas Th2 cells and associated cytokines (interleukin [IL]-4, IL-5, and IL-13) increase in the lungs or circulation of patients with IPF. Th1/Th2 imbalance toward a Th2 phenotype is induced by damaged tissues or mediators that promote Th2 differentiation. One recent study showed an elevated level of galectin-1 in bronchoalveolar lavage of patients with IPF, which contributed to a Th1/Th2 imbalance toward a Th2 phenotype by selectively inducing apoptosis on Th1 and Th17 cells, but not on naïve, Th2, or regulatory FoxP3+ T cells. PGE2 can direct Th2 differentiation by inhibiting IL-12 production by monocytes cultured in the presence of IL-4 and granulocyte-macrophage colony-stimulating factor. In addition, a decrease in a suppressor of cytokine signaling (SOCS)-1 expression is involved in IPF, because SOCS-1 may inhibit Th2 differentiation as an inhibitor of profibrotic cytokines, such as IL-4.

Th1 and Th2 cells take on opposite roles in fibrogenesis

Th1 cells and their secretory cytokines (IFNg) are thought of as being antifibrotic. For instance, one study showed that IL-12 exerted an antifibrotic effect by promoting the
production of IFNγ in a bleomycin-induced animal model of IPF. Inhibiting Th1 differentiation by targeting of the transcription factor T-bet increased bleomycin-induced lung injury. By contrast, Th2 and associated cytokines (IL-4, IL-5, and IL-13) exhibit a fibrogenic property by stimulating fibroblast proliferation, fibroblast differentiation into myofibroblasts, and collagen production. Therefore, the imbalance between Th1 and Th2 is actually an imbalance between profibrosis and antiﬁbrosis.

**M1–M2 polarization of macrophages**

Macrophages can be broadly classiﬁed as M1 and M2 according to their phenotype and role. The M1–M2 polarization of macrophages and the Th1–Th2 polarization of T cells are well-correlated processes with positive feedback between each other. A high amount of IFN-γ produced by Th1 cells is the major inducing factor in the activation of M1 macrophages, whereas high amounts of IL-4 and IL-13 produced by Th2 cells can activate M2 macrophages, which suppress inﬂammation and confer a tissue repair function. An overzealous or prolonged M2 polarization results in excessive amounts of proﬁbrotic mediators (TGF-β1, PDGF, and tissue inhibitors of metal proteinase 1 [TIMP1], and CCL18), which promote ﬁbroblast accumulation and the differentiation of ﬁbroblasts into myoﬁbroblasts, and collagen production and deposition. Thus, M2 macrophages act as the key effector arm of Th2-driven type 2 responses in ﬁbrosis. ER stress has been reported to regulate skew toward M2 polarization.

**Protease/antiprotease imbalance**

MMPs are a family of endopeptidases with 23 members in humans. These enzymes are responsible for ECM degradation and for cleaving membrane receptors and various bioactive mediators (such as cytokines, growth factors, and chemokines). It is well known that an imbalance of MMPs and their inhibitors, the TIMPs, mainly due to overexpression of MMPs, is implicated in the pathogenesis of IPF. Most MMPs, such as MMP1, MMP3, MMP7, MMP9, MMP12, and MMP28, are overexpressed in IPF lungs compared with controls and play a profibrotic role, contributing to ﬁbroblast growth factor (FGF) expression levels decrease (eg, FGF-10) and FGF plays a protective role in IPF.

**Plasminogen activation/inhibition imbalance**

Plasmin plays an antifibrotic role in the pathogenesis of IPF by promoting ﬁbrinolysis as well as degradation of ECM. Plasmin has been shown previously to induce COX-2, PGE2, and hepatocyte growth-promoting factor (HGF) synthesis in AECs and ﬁbroblasts, which are important effectors of antiﬁbrotic actions. By contrast, plasminogen activator inhibitor 1 (PAI-1) plays a profibrotic role in the pathogenesis of IPF as a primary inhibitor of urokinase-type and tissue-type plasminogen activators, respectively, which convert plasminogen into plasmin. One mechanism whereby increased PAI-1 promotes lung ﬁbrosis involves increasing the sensitivity of ﬁbroblasts to TGF-β. PAI-1 expression is increased relative to the plasminogen activator, contributing to plasminogen activation/inhibition imbalance. AEC damage causes PAI-1 overexpression in AECs and lung macrophages. Evidence shows that there is a positive feedback loop between PAI-1 and TGF-β. Moreover, ROS, IL-1β, and tumor necrosis factor (TNF) promote expression of PAI-1.

**AEC/fibroblast apoptosis imbalance**

**Homeostasis of AECs and ﬁbroblasts**

In the normal repair of AECs, activated ﬁbroblasts produce ECM and provide a provisional scaffold for AEC migration, proliferation, and re-epithelialization. After the AEC damage has been repaired, ﬁbroblasts undergo apoptosis in order to restore normal cellular homeostasis and maintain tissue architecture and organ function. Fibroblast apoptosis is essential in normal wound healing but is dysregulated in IPF.

**Imbalance of AEC and ﬁbroblast apoptosis in IPF**

In IPF, the phenotypes of ﬁbroblasts and AECs change. Considering AECs as an example, aberrantly activated AECs, which are characterized by cells undergoing apoptosis, senescence, or damage, secrete cell-type-speciﬁc proﬁbrotic and proinﬂammatory cytokines. As a result, the dysregulated crosstalk and abnormal mediators between them lead to AEC apoptosis, ﬁbroblast anti-apoptosis, and abnormal ECM.

**Down-regulated factors promoting the survival of AECs and inducing ﬁbroblast apoptosis**

For instance, in IPF, PGE2 produced by AECs and ﬁbroblasts, and HGF and keratinocyte growth factor produced by ﬁbroblasts, reduce. Studies showed that both miR-30a and miR-29 in AECs can suppress AEC apoptosis and promote apoptosis of lung ﬁbroblasts in an IPF animal model. Their expression decreases in AECs during IPF development. Interestingly, the conclusions of the research on ﬁbroblast growth factor (FGF) expressed by both ﬁbroblasts and AECs in the lung are controversial. Some studies report that FGF expression levels decrease (eg, FGF-10) and FGF plays a pathological role (ﬁbroblast invasion) in IPF, while other studies report that FGF expression levels increase (eg, FGF-1, basic FGF, FGF-9 and FGF-18) and FGF-23 and FGF plays a protective role (AEC survival and ﬁbroblast apoptosis). A possible explanation for these ﬁndings is that, on the one hand, FGF may have a potential dual nature in the lung, while, on the other hand, this heterogeneity may originate from different specimen types and different microenvironments (in vivo or ex vivo).
Overexpressed factors inducing the apoptosis of AECs and promoting proliferation or anti-apoptosis of fibroblasts are overexpressed

TGF-β and ROS overexpressed by fibroblasts promote AEC apoptosis while increasing resistance to apoptosis in fibroblasts. AECs can also excessively secrete profibrotic mediators, including TGF-β, connective tissue growth factor (CTGF), PDGF, and some Th2 cytokines (eg, IL-17E) [Figure 3]. These mediators not only promote AEC apoptosis via an autocrine mechanism but also incite overactivation and proliferation of fibroblasts.

Other overexpressed factors inducing apoptosis of AECs

In addition to the factors mentioned above (TGF-β, ROS, CTGF, PDGF, and IL-17E), the following further elaborate on other factors that can promote AEC apoptosis. Angiotensin-II increased in AECs of IPF is indirectly induce apoptosis of adjacent AECs. The lincRNA maternally expressed gene 3 increased in AECs of IPF is known to induce apoptosis by activating pro-apoptotic protein P53. TNF-related apoptosis-inducing ligand, a member of the TNF superfamily, which is upregulated in AECs of IPF lung tissue samples, has been shown to induce apoptosis via DR4 and DR5 receptor binding. Numerously studies prove that telomeret attrition, uncheckered ER stress, and mitophagy deficiency can induce AEC apoptosis in lungs with IPF [Figure 3].

Other factors promoting proliferation or anti-apoptosis of fibroblasts

In addition to the factors mentioned above (TGF-β, ROS, CTGF, PDGF, and IL-17E), the following further elaborate on other factors that can promote the proliferation or anti-apoptosis of fibroblasts. Increased expression of inhibitors of apoptosis in fibroblasts, such as surviving, the Fas death receptor, cellular FLICE-like inhibitory protein, secreted protein acidic and rich in cysteine, and X-linked inhibitor of apoptosis (XIAP), protects lung fibroblasts from apoptosis. Studies demonstrate decreased Fas expression in fibroblasts during histone modifications and ECM metalloproteinase inducer overexpression in fibroblasts from IPF can induce an apoptosis-resistant phenotype of fibroblasts in IPF. Apoptosis resistance in fibroblasts is partially mediated by TLR4 activation, which results in the transcription of pro-survival signaling factors via NF-κB and PI3K-Akt activation. Of note, injured or activated AECs produce virtually mediators contributing to apoptosis-resistant phenotypes of fibroblasts, such as TNF-α, endothelin 1, CXCL12, IL-25, and IL-17BR (IL-25’s receptor). The underlying mechanism is as follows: increased levels of SPARC, TGF-β, and endothelin-1 in IPF lung promote fibroblast resistance to apoptosis by activating the Akt signal pathway. Both TGF-β and endothelin-1 induce XIAP expression to inhibit Fas-mediated apoptosis in fibroblasts, CXCL12, a potent chemoattractant for local fibroblasts, is essential for EMT and fibroblast-to-myofibroblast differentiation. One study demonstrated that the mRNA and protein levels of IL-25 and IL-17BR (IL-25’s receptor) are significantly higher in IPF patients and that IL-25 potentiates the expression of CTGF in AECs and the recruitment and proliferation of lung fibroblasts.

AEC/fibroblasts apoptosis imbalance is the core of IPF

In summary, the pathogenesis of IPF involves various imbalances including ER, telomere length homeostasis, mitochondrial dysfunction, oxidant/antioxidant imbalance, Th1/Th2 imbalance, M1–M2 polarization of macrophages, protease/antiprotease imbalance, plasminogen activation/inhibition imbalance, and AEC/fibroblast apoptosis imbalance. As can be seen from the above description and Figure 1, among them, AEC/fibroblast apoptosis imbalance is the core of IPF, because although other imbalances affect each other and promote each other, they ultimately promote AEC/fibroblast apoptosis imbalance directly or indirectly.

Excess AEC apoptosis induces alveolar basement membrane destruction, leading to inefficient re-epithelialization and the recruitment of fibroblasts to the site of damage to
promote excess ECM deposition and pulmonary architecture destruction. An alternative mechanism is that the apoptotic AECs can directly trigger progressive fibrosis by inducing a response in neighboring cells. Studies have revealed that uptake of apoptotic AECs by macrophages contributes to fibrosis through the increased expression of TGFβ, a growth factor with both anti-inflammatory and profibrotic activities.

Fibroblast apoptosis is the primary mechanism by which fibroblasts are removed, whereas fibroblasts differentiate into myofibroblasts in profibrotic microenvironments owing to impaired apoptosis of fibroblasts, resulting in the aggregation of activated myofibroblasts. Myofibroblasts are the major actors in the fibrogenic process and are characterized by expression of α-smooth muscle actin and synthesis of varying amounts of ECM, including collagens, glycoproteins, and proteoglycans, which will ultimately lead to lung architecture destruction and fibrosis.

As the research progresses, we will find that inflammation and immune response is still important in the pathogenesis of IPF, but it is complicated and needs to be further studied.

**Views on Therapeutic Approaches Based on Complex Pathogenesis**

**Therapy targeting inflammation and immune response**

The following evidence could help explain why anti-inflammatory drugs are mostly ineffective in IPF: First, B cells are the antibody-producing cells of the adaptive immune system and B cell abnormalities are involved in the pathogenesis of IPF, but lung diseases involving humoral immunity mediated by B cells are usually insufficiently responsive or refractory to glucocorticosteroid therapy.

Second, studies show that glucocorticosteroid treatment is not sufficient to inhibit the activity of alveolar macrophages, instead, it activates a class of M2 macrophages and causes M1–M2 polarization of macrophages. In addition, a recent study demonstrates that activated and non-proliferating lymphocytes and mature DCs are involved in the pathogenesis of IPF, and glucocorticosteroid-based therapy has a poor activity on differentiated lymphocytes and especially mature DCs.

As the research progresses, we will find that inflammation and immune response is still important in the pathogenesis of IPF, but it is complicated and needs to be further studied.

**Therapy targeting oxidant/antioxidant imbalance**

As shown in Figure 4, oxidant mainly refers to the following ROS: \( \cdot O_2^-, \cdot O_2, \cdot HO_2, HO, \) and \( H_2O_2. \) Endogenous ROS are mainly produced through the ETC in mitochondria and by chemical reactions catalyzed by NADPH oxidase (NOX). The antioxidant defense system includes small-molecular-weight antioxidants (eg, vitamin E/C, glutathione [GSH]), superoxide dismutase, and enzymes that catalyze \( H_2O_2 \) metabolism (GSH peroxidase, GSH reductase, and catalase).

NAC is an antioxidant that not only can be metabolized into GSH precursor, cysteine, to supplement intracellular GSH. Clinical trials or meta-analysis indicate NAC offers no benefit for the preservation of forced vital capacity compared with placebo in IPF. This by no means implies that oxidant/antioxidant imbalance is no longer important in the pathogenesis of IPF. As can be seen from Figure 4, GSH is just one branch of the body’s antioxidant defense system.

One study found that mitochondrial catalase–overexpressed transgenic mice are protected against lung fibrosis in part via the prevention of AEC mtDNA damage. Evidence has also demonstrated that NOX-4 is a significant enzyme that catalyzes the generation of ROS in the pathogenesis of IPF. Directly reducing ROS
adjustment of AEC/treatment plan for IPF should be mainly based on the imbalance is at the core of the pathogenesis of IPF, so the apoptosis of regulation of factors leading to apoptosis of AECs and anti-(cIAP), has been shown to restore apoptotic sensitivity of fibroblasts from IPF; animal studies have shown AT-406 to be of benefit in treating IPF. The resistance to apoptosis of fibroblasts could be caused by downregulating the expression of miR-29c as well as Fas receptor by TGF-β. Therefore, the upregulation of miR-29 expression in IPF lungs could restore the sensitivity to apoptosis of lung fibroblasts and inhibit ECM production. Additionally, FasL is up-regulated in lung tissues of IPF; therefore, specific inhibition of the Fas-FasL pathway in AECs may prevent the development of pulmonary fibrosis. Up-regulation of factors reducing apoptosis of AECs and restoring the sensitivity to apoptosis of fibroblasts is another potential treatment. For example, miR-30a expression decreases in AECs, and the upregulation of miR-30a can suppress AEC apoptosis.

As mentioned above, fibrosis may be heterogeneous and multifactorial in etiology and pathogenesis. Therefore, attempts to prevent or counteract a single upstream pathway may not be enough to inhibit the progression of fibrosis. Future research should focus on treatment methods that target multiple mechanisms, with adjustment of AEC/fibroblast apoptosis imbalance as the central focus and adjustment of various other imbalances as the secondary points of focus.

Conclusions
IPF is likely the result of complex interactions between environmental, genetic, and epigenetic factors.

First, environmental exposures strongly affect epigenetic marks, and epigenetic processes translate environmental exposures into the regulation of chromatin. Second, epigenetic processes shape the gene expression profiles involved in disease pathophysiology. In turn, an individual’s genetic background determines epigenetic marks in two ways: by direct inheritance and by genetic variants. Finally, genetic and epigenetic factors act in concert to dysregulate gene expression in IPF lung. The pathogenesis of IPF involves various imbalances centered on AEC/fibroblast apoptosis imbalance, including ER, telomere length homeostasis, mitochondrial dysfunction, oxidant/antioxidant imbalance, Th1/Th2 imbalance, M1–M2 polarization of macrophages, protease/antiprotease imbalance, and plasminogen activation/inhibition imbalance. Among them, AEC/fibroblast apoptosis imbalance is the core of IPF, because although other imbalances affect each other and promote each other, they eventually lead to dysregulated crosstalk between AECs and fibroblasts. As a result, the dysregulated crosstalk and abnormal mediators between them result in AEC apoptosis, fibroblast anti-apoptosis, and abnormal ECM. It is worth noting that the ineffectiveness of anti-inflammatory and antioxidative therapies for IPF patients in no way means that inflammation and oxidant/antioxidant imbalances are no longer important in the pathogenesis of IPF. Fibrosis may be heterogeneous and multifactorial in etiology and pathogenesis. Therefore, attempts to prevent or counteract a single upstream pathway may not be enough to inhibit the progression of fibrosis. Future research should focus on treatment methods that target multiple mechanisms, with adjustment of AEC/fibroblast apoptosis imbalance as the central focus and adjustment of various other imbalances as the secondary points of focus.

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Conflicts of interest
None.

References
1. Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M, et al. Idiopathic pulmonary fibrosis. Nat Rev Dis Primers 2017;3:17077. doi: 10.1038/nrdp.2017.74.
2. Keogh BA, Crystal RG. Alveolitis: the key to the interstitial lung disorders. Thorax 1982;37:1–10. doi: 10.1136/thx.37.1.1.
3. Gross TJ, Hunninghake GW. Idiopathic pulmonary fibrosis. N Engl J Med 2001;345:517–525. doi: 10.1056/NEJMra003200.
4. Kinnula VL, Fattman CL, Tan RJ, Ouy TD. Oxidative stress in pulmonary fibrosis: a possible role for redox modulatory therapy. Am J Respir Crit Care Med 2005;172:417–422. doi: 10.1164/rccm.200310-017PP.
5. Fernandez IE, Eickelberg O. New cellular and molecular mechanisms of lung injury and fibrosis in idiopathic pulmonary fibrosis. Lancet 2012;380:680–688. doi: 10.1016/S0140-6736(12)61144-1.
6. Selman M, King TE, Pardo A. American Thoracic Society, European Respiratory Society, American College of Chest Physicians. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med 2001;134:136–151. doi: 10.7326/0003-4819-134-2-200101160-00015.
7. Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ. Idiopathic Pulmonary Fibrosis Clinical Research Network. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med 2012;366:1968–1977. doi: 10.1056/NEJMoa1113354.
8. Martinez FJ, de Andrade JA, Anstrom KJ, King TE Jr, Raghu G. Idiopathic Pulmonary Fibrosis Clinical Research Network. Randomized trial of acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med 2014;370:2093–2101. doi: 10.1056/NEJMoa1401739.
9. Skandamis A, Kani C, Markantonis SL, Souliotis K. Systematic review and network meta-analyses of approved medicines for the treatment of idiopathic pulmonary fibrosis. J Drug Assess 2019;8:55–61. doi: 10.1080/21405660.2019.1397726.
10. Fleetwood K, McCoil R, Glenville J, Edwards SC, Gsteiger S, Daigl M, et al. Systematic review and network meta-analysis of idiopathic pulmonary fibrosis treatments. J Manag Care Spec Pharm 2017;23(3 Suppl):S5–S16. doi: 10.18553/jmcp.2017.23.3-b5-s.

11. Spagnoli P, Sverzutti N, Rossi G, Cavazza A, Tsouvelakis A, Cestanti B, et al. Idiopathic pulmonary fibrosis: an update. Ann Med 2015;47:15–27. doi: 10.1007/s00284-014-9821-5.

12. Macneal K, Schwartz DA. The genetic and environmental causes of pulmonary fibrosis. Proc Am Thorac Soc 2012;9:120–125. doi: 10.1513/pats.201112-055AW.

13. Lee JS, Song JW, Wolters PJ, Elicker BM, King TE Jr, Kim DS, et al. Genetic variants associated with usual interstitial pneumonitis and cellular pulmonary fibrosis trials: lessons from cancer. Eur Respir J 2013;41:262–269. doi: 10.1183/09031936.0011512.

14. Tanjore H, Lawson WE, Blackwell TS. Endoplasmic reticulum stress as a pro-fibrotic stimulus. Biochem Biophys Acta 2013;1832:940–947. doi: 10.1016/j.bbabio.2012.11.011.

15. Tanjore H, Blackwell TS, Lawson WE. Emerging evidence for type alveolar epithelial cells patients with idiopathic pulmonary fibrosis. J Exp Med 2012;216:2724–2735. doi: 10.1084/jem.20122351.

16. Nathan N, Giraud V, Picard C, Nunes H, Dastot-Le Moal F, Copin B, et al. Germ-line SFTPA1 mutation in familial idiopathic pulmonary hypertension and lung cancer. Hum Mol Genet 2016;25:1457–1467. doi: 10.1093/hmg/ddw014.

17. Tsuchiya S, Liu J, Wang J, Yqiao M, Sun Y, Zou Y, et al. Genetic defects in surfactant protein A2 are associated with usual interstitial pneumonitis and cellular pulmonary fibrosis. Am J Hum Genet 2009;84:52–59. doi: 10.1016/j.ajhg.2008.11.010.

18. Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, et al. Genetic defects in surfactant protein A2 are associated with usual interstitial pneumonitis and cellular pulmonary fibrosis. Am J Hum Genet 2009;84:52–59. doi: 10.1016/j.ajhg.2008.11.010.

19. Zhang X, Jiang J, Chen WJ, Xu LX, Xie LC. Genetic characterization of the Chinese family with familial pulmonary fibrosis. Chin Med J (Engl) 2012;125:1945–1951.

20. Kim DS, Song JW, Wolters PJ, Elicker BM, King TE Jr, Song JS et al. Genetic variants associated with usual interstitial pneumonitis and cellular pulmonary fibrosis. J Clin Invest 2013;127:405–414. doi: 10.1172/JCI78440.

21. Chambarger AC, Schiller HB, Fernandez IE, Sterclova M, Heinzennel K, Hennen E, et al. Glutathione peroxode 3 localizes to the epithelial lining fluid and the extracellular matrix in interstitial lung disease. Sci Rep 2016;6:29992. doi: 10.1038/ srep29992.

22. Kazazumi M, Sonoko N, Masanori K, Takateru I, Akira O. Expression of bcl-2 protein and APO-1 (Fas antigen) in the lung tissue from patients with idiopathic pulmonary fibrosis. Microsc Res Tech 1997;38:480–487. doi: 10.1002/sct.10970092-0029.

23. Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008;214:219–220. doi: 10.1002/path.2277.

24. Hams E, Armstrong ME, Barlow JL, Saunders SP, Schwartz C, Gong Q, et al. Endoplasmic reticulum stress, a new wrestler, in the pathogenesis of idiopathic pulmonary fibrosis. Am J Transl Res 2017;9:722–733.

25. Vannella KM, Wynn TA. Mechanisms of organ injury and repair by macrophages. Annu Rev Physiol 2017;79:593–617. doi: 10.1146/annurev-physiol-022516-034356.

26. Yang J, Velikoff M, Canalis E, Horowitz JC, Kim KK. Activated alveolar epithelial cells mutate fibroblasts through autocrine and paracrine secretion of a connective tissue growth factor. Am J Pathol Lung Cell Mol Physiol 2014;306:L786–L796. doi: 10.1152/ajplung.00243.2013.

27. Gokey JJ, Snowball J, Sridharan A, Speth JP, Black KE, Hariri LP, et al. MEG3 is increased in idiopathic pulmonary fibrosis and regulates epithelial cell differentiation. JCI Insight 2018;3:e122490. doi: 10.1172/jci.insight.122490.

28. Xu X, Luo S, Li B, Dai H, Zhang J. Feature article: IL-25 contributes to lung fibrosis by directly acting on alveolar epithelial cells and fibroblasts. Exp Biol Med (Maywood) 2019;244:770–780. doi: 10.1177/1931670719843827.

29. King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet 2011;378:1949–1961. doi: 10.1016/S0140-6736(11)60024-4.
58. Yang S, Cui H, Xie N, Icyuz M, Banerjee S, Antony VB, et al. MicroRNA-326 regulates pro-inflammatory response and macrophage activation in bronchoalveolar lavage fluid from idiopathic pulmonary fibrosis patients. Mol Med Rep 2018;18:5799–5806. doi: 10.3829/mmr.2018.9565.

59. Liang H, Gu Y, Li T, Zhang Y, Huangfu L, Hu M, Shi Y, Gochuico BR, Yu G, Tang X, Osorio JC, Fernandez IE, Mao C, Zhang J, Lin S, Jing L, Xiang J, Wang M, et al. miR-27b inhibits fibroblast proliferation via targeting TGFβ receptor II. J Cell Mol Med 2014;18:2404–2416. doi: 10.1111/jcmm.12420.

60. Chi L, Xiao Y, Zhu L, Zhang M, Xu B, Xia H, et al. microRNA-155 attenuates profibrotic effects of transforming growth factor-beta on human lung fibroblasts. Am J Regul Homeost Agents 2019;33:1415–1424. doi: 10.23812/19-41A.

61. Mosmas S, Salton F, Kosmider B, Ring N, Volpe MC, Bahmed K, et al. miR-200 family members reduce senescence and restore idiopathic pulmonary fibrosis type II alveolar epithelial cell transdifferentiation. ERJ Open Res 2019;5:00138–02019. doi: 10.1183/23120341.00138-2019.

62. Wang YC, Liu JS, Tang HK, Nie J, Zhu JX, Wen LL, et al. miR-221 targets HMG2 to inhibit blomycin-induced pulmonary fibrosis by regulating TGF-β1/Smad3-induced EMT. Int J Mol Med 2016;38:1208–1216. doi: 10.3892/ijmm.2016.2705.

63. Ge L, Habiel DM, Hamsbro PM, Kim RY, Gharib SA, Edelman JD, et al. miR-32a-3p regulates lung fibrosis by targeting multiple profibrotic pathways. JCI Insight 2016;1:e90301. doi: 10.1172/jci.insight.90301.

64. Das S, Kumar M, Negi V, Patnaik B, Prakash YS, Agrawal A, et al. MicroRNA-526 regulates profibrotic functions of transforming growth factor-β in pulmonary fibrosis. Am J Respir Cell Mol Biol 2014;50:882–892. doi: 10.1165/rcmb.2013-0195OC.

65. Zhang Q, Ye H, Xiang F, Song LJ, Zhou LL, Cai PC, et al. The evolution of lncRNA repertoires and expression patterns in tetrapods. Nature 2014;505:633–640. doi: 10.1038/nature12943.

66. Saito S, et al. Defective histone acetylation is responsible for the diminished contribution to epithelial-mesenchymal transition in pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2013;305:L433–L441. doi: 10.1152/ajplung.00177-2012.

67. Kasowski M, Kyriazopoulou S, Grubert F, et al. Extensive variation in chromatin states across humans. Science 2013;342:750–754. doi: 10.1126/science.1238491.

68. Ji X, Wu B, Fan J, Han L, Ruan C, Wang T, et al. The anti-fibrotic effects and mechanisms of microRNA-486-5p in pulmonary fibrosis. Sci Rep 2017;7:14131. doi: 10.1038/s41598-017-02624.

69. Hadijarahalambour MJ, Roux BT, Coeomer E, Feghali-Bostwick CA, Murray LA, Clarke DL, et al. Long intergenic non-coding RNAs regulate human lung fibroblast function: Implications for idiopathic pulmonary fibrosis. Sci Rep 2019;9:6020. doi: 10.1038/s41598-019-42922-w.

70. Necsulea A, Soumillon M, Warnefors M, Liechti A, Dash T, Zeller U, et al. The evolution of lncRNA repertoires and expression patterns in tetrapods. Nature 2014;505:633–640. doi: 10.1038/nature12943.

71. Jirtle RL, Skinner MK. Environmental epigenetics and disease susceptibility. Nat Rev Genet 2007;8:253–262. doi: 10.1038/nrg2045.

72. Frakes AE, Dillin A. The UPRER: sensor and coordinator of organismal homeostasis. Mol Cell 2017;66:761–771. doi: 10.1016/j.molcel.2017.05.031.

73. Hetz C, Saxena S. ER stress and the unfolded protein response in neurodegeneration. Nat Rev Neurol 2017;13:477–491. doi: 10.1038/nrneurol.2017.99.

74. Bhanasi J, Khatt S, Dhawan V. Terminalia Arjuna bark extract impedes foam cell formation and promotes apoptosis in ox-LDL-stimulated macrophages by enhancing UPR-CHOP pathway. Lipids Health Dis 2019;18:195. doi: 10.1186/s12944-019-1119-z.

75. Li JS, Miralles Fusté J, Simavonar T, Bortocci C, Tsi T, Karlseder J, et al. TAZAP: a telomere-associated protein involved in telomere length control. Science 2017;355:638–641. doi: 10.1126/science.aah6752.
103. Nelson KK, Melendez JA. Mitochondrial redox control of matrix metalloproteases. Free Radic Biol Med 2004;37:768–784. doi: 10.1016/j.freeradbiomed.2004.06.008.

104. Parks WC, Wilson CL, López-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 2004;4:617–629. doi: 10.1038/nri1418.

105. Wu SM, Pizzo SV. Mechanism of hypochlorite-mediated inactivation of proteinase inhibition by alpha 2-macroglobulin. Biochemistry 1999;38:1393–13990. doi: 10.1021/bi991436r.

106. Kim SJ, Cheresh P, Jablonski RP, Williams DR, Kamp DW. The role of mitochondrial DNA in mediating alveolar epithelial cell apoptosis and pulmonary fibrosis. Int J Mol Sci 2015;16:21486–21519. doi: 10.3390/ijms160921486.

107. Kropiack JA, Blackwell TS. Endoplasmic reticulum stress in the pathogenesis of fibrotic disease. J Clin Invest 2018;128:64–73. doi: 10.1172/JCIR3560.

108. Lei H, Kazlauskas A. A reactive oxygen species-mediated, self-perpetuating loop promotes a cytochrome c release/anti-mitochondrial factor receptor a. Mol Cell Biol 2014;34:110–122. doi: 10.1128/ MCB.00839-13.

109. Amati L, Pepe M, Passeri ME, Mastronardi ML, Jirillo E, Covelli V. Toll-like receptor signaling mechanisms involved in dendritic cell activation: potential therapeutic control of T cell polarization. Curr Pharm Des 2006;12:4247–4254. doi: 10.2174/ 13816120677843583.

110. Peng SC, Hu X, Wei LQ, Li ZH. The correlation of helper T lymphocyte 1 helper lymphocyte 2 with clinical and image features in patients with idiopathic pulmonary fibrosis. Zhonghua Nei Ke Za Zhi 2013;52:489–493.

111. Passalacqua G, Minicarini M, Colombo D, Troisi G, Ferrari M, Bagnasco D, et al. IL-13 and idiopathic pulmonary fibrosis: Possible links and new therapeutic strategies. Pulm Pharmacol Ther 2017;45:95–100. doi: 10.1016/j.pupt.2017.05.007.

112. Corapi E, Carrizzo G, Compagno D, Laderach D. Endogenous galectin-1 in T lymphocytes regulates anti-prostate cancer immunity. Front Immunol 2019;10:2190. doi: 10.3389/ fimmu.2019.02190.

113. Kalinski P, Hilkens CM, Smidlers A, Smidewiet FG, Kapsenberg ML. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 1 cytokine production in maturing human naive T helper cells. J Immunol 1997;159:28–35.

114. Bao Z, Zhang Q, Wan H, He P, Zhou X, Zhou M. Expression of galectin-1 in T cells: past, present and future. Clin Immunol 2012;142:107–116. doi: 10.1016/j.clim.2011.09.011.

115. Corapi E, Carrizzo G, Compagno D, Laderach D. Endogenous galectin-1 in T lymphocytes regulates anti-prostate cancer immunity. Front Immunol 2019;10:2190. doi: 10.3389/ fimmu.2019.02190.

116. Luzina IG, Todd NW, Iacono AT, Atamas SP. Roles of T lymphocytes in pulmonary fibrosis. J Leukoc Biol 2008;83:237–244. doi: 10.1189/jlb.0707504.

117. Xu J, Mora AL, LaVoy J, Brigham KL, Rojas M. Increased bleomycin-induced lung injury in mice deficient in the transcription factor T-bet. Am J Physiol Lung Cell Mol Physiol 2006;291:L658–667. doi: 10.1152/ajplung.0073718.

118. Wynn TA. Type 2 cytokines: mechanisms and therapeutic strategies. Nat Rev Immunol 2015;15:271–282. doi: 10.1038/ nrri3831.

119. Hume DA. The many alternative faces of macrophage activation. Front Immunol 2015;6:370. doi: 10.3389/fimmu.2015.00370.

120. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature 2013;496:444–455. doi: 10.1038/nature12211.

121. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goedert S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 2014;41:14–20. doi: 10.1016/j.immuni.2014.06.008.

122. Lawrence T, Natoli G. Transcriptional regulation of macrophage polarization: enabling diversity with identity. Nat Rev Immunol 2011;11:750–761. doi: 10.1038/nri3088.

123. Wang N, Liang H, Zen K. Molecular mechanisms that influence the macrophage re-polarization balance. Front Immunol 2014;5:614. doi: 10.3389/fimmu.2014.00614.

124. Yao Y, Wang Y, Zhang Z, He L, Zhou J, Zhang M, et al. Chop deficiency protects mice against bleomycin-induced pulmonary
fibrosis by attenuating M2 macrophage production. Mol Ther 2016;24:941–925. doi: 10.1038/mt.2016.36.

126. Wang Y, Zhu J, Zhang L, Zhang Z, He L, Mou Y, et al. Role of C/EBP homologous protein and endoplasmic reticulum stress in asthma exacerbation by regulating the IL-4/signal transducer and activator of transcription 6 transcription factor ECIL-4 receptor or positive feedback loop in M2 macrophages. J Allergy Clin Immunol 2017;140:1530–1541. doi: 10.1016/j.jaci.2017.01.024.

127. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 2014;15:786–801. doi: 10.1038/nrm3904.

128. Menou A, Duitman J, Crestani B. The impaired proteases and antiproteases balance in idiopathic pulmonary fibrosis. Matrix Biol 2018;66–67:1129–1137. doi: 10.1016/j.matbio.2018.03.001.

129. Guiot J, Moermans C, Henket M, Corhay JL, Louis R. Blood biomarkers in idiopathic pulmonary fibrosis. Lung 2017;195:273–280. doi: 10.1007/s00604-017-9993-5.

130. Craig NJ, Zhang L, Haggard JS, Owen CA. Matrix metalloproteinases as therapeutic targets for idiopathic pulmonary fibrosis. Am J Respir Cell Mol Biol 2015;53:585–600. doi: 10.1165/rcmb.2015-0201TR.

131. Todd JL, Vinisko R, Liu Y, Neeley ML, Overton R, Flaherty KR, et al. IFP-PRO registry investigators. Circulating matrix metalloproteinases and tissue metalloproteinase inhibitors in patients with idiopathic pulmonary fibrosis in the multicenter IFP-PRO Registry cohort. BMC Pulm Med 2020;20:27. doi: 10.1186/s12890-020-1103-4.

132. Xiong Y, Zhang J, Shi L, Ning Y, Zhu Y, Chen S, et al. MicroRNA-29c regulates apoptosis in idiopathic pulmonary fibrosis. Sci Rep 2016;6:37445. doi: 10.1038/srep37445.

133. Gupte VV, Ramasamy SK, Reddy R, Lee J, Weinreb PH, Violette SM, et al. Overexpression of fibroblast growth factor-10 during both inflammatory and fibrotic phases attenuates bleomycin-induced pulmonary fibrosis in mice. Am J Respir Crit Care Med 2009;180:424–436. doi: 10.1164/rccm.200811-1794OC.

134. MacKenzie B, Korlet M, Heneke I, Sibnska Z, Tan X, Heuzel S, et al. Increased feedback FGF1–FGFR expression in idiopathic pulmonary fibrosis. Respir Res 2015;16:83. doi: 10.1186/s12931-015-0242-2.

135. Epstein Stouet G, Brook E, Eyal O, Edelstein E, Shitrit D. Epidermal growth factor receptor paracrine upregulation in idiopathic pulmonary fibrosis fibroblasts promotes survival and migration and inhibit myofibroblast differentiation of human lung fibroblasts in vitro. Am J Physiol Lung Cell Mol Physiol 2019;310:L615–L629. doi: 10.1152/ajlup.00185.2015.

136. Barnes JW, Duncan D, Helton S, Hutcheson S, Kurundkar D, Logudon NJ, et al. Role of fibroblast growth factor 23 and klotho cross talk in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2019;317:L141–L154. doi: 10.1152/ajlup.00246.2018.

137. Marchand-Adam S, Marchal J, Cohen M, Soler P, Gerard B, et al. Plasminogen activator inhibitor-1 and transforming growth factor-beta1 formation. Oncotarget 2017;8:71024–71037. doi: 10.18632/oncotarget.20297.

138. Xu T, Liang J, Geng Y, Liu N, Kurckyan A, Kular V, et al. MicroRNA-29c prevents pulmonary fibrosis in idiopathic pulmonary fibrosis fibroblasts. Am J Pathol 2019;186:752–762. doi: 10.1165/ajpath.201202-0302OC.

139. Ban C, Wang T, Zhang S, Xin P, Liang L, Wang C, et al. Fibromytic system related to pulmonary artery pressure and lung function in patients with idiopathic pulmonary fibrosis. Clin Respir J 2017;11:640–647. doi: 10.1111/crj.12397.

140. Seo JY, Park J, Yu MR, Kim YS, Ha H, Lee HB. Positive feedback loop between plasminogen activator inhibitor-1 and transforming growth factor-beta1 during renal fibrosis in diabetes. Am J Nephrol 2009;30:481–490. doi: 10.1159/000242477.

141. Liu RM. Oxidative stress, plasminogen activator inhibitor 1, and lung fibrosis. Antioxid Redox Signal 2008;10:303–319. doi: 10.1089/ars.2007.1903.

142. Keerthsingam CB, Jenkins RG, Harrison NK, Hernandez-Rodriguez NA, Booth H, Laurent GJ, et al. Cyclooxygenase-2 deficiency results in a loss of the anti-proliferative response to transforming growth factor-beta in human fibrotic lung fibroblasts and promotes bleomycin-induced pulmonary fibrosis in mice. Am J Pathol 2001;158:1411–1422. doi: 10.1006/ajph.2001.1490.

143. Marchal-Adam S, Marchal J, Cohen M, Soler P, Gerard B, Castre Y, et al. Defect of hepatocyte growth factor secretion by fibroblasts in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2005;171:1368–1379. doi: 10.1164/rcrm.200212-1514OC.

144. Xie T, Lian G, Geng Y, Liu N, Kurckyan A, Kular V, et al. MicroRNA-29c: prevents pulmonary fibrosis by regulating epithelial cell renewal and apoptosis. Am J Respir Cell Mol Biol 2017;57:721–732. doi: 10.1165/rcmb.2017-0133OC.

145. Matsuushima S, Iihara S, McCelland M, Kimura H, Williams S, et al. Matrix metalloproteinase-19 is a key regulator of lung fibrosis in mice. Am J Physiol Lung Cell Mol Physiol 2019;316:L1025–L1034. doi: 10.1152/ajlup.00185.2015.

146. Barnes JW, Duncan D, Helton S, Hutcheson S, Kurundkar D, Logudon NJ, et al. Role of fibroblast growth factor 23 and klotho cross-talk in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2019;317:L141–L154. doi: 10.1152/ajlup.00246.2018.

147. Barnes JW, Duncan D, Helton S, Hutcheson S, Kurundkar D, Logudon NJ, et al. Role of fibroblast growth factor 23 and klotho cross-talk in idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2019;317:L141–L154. doi: 10.1152/ajlup.00246.2018.

148. Akram KM, Lomajs NJ, Forsyth NR, Spiteri MA. Alveolar epithelial cells in idiopathic pulmonary fibrosis: a role in fibrosis and repair? Front Physiol 2017;8:128. doi: 10.3389/fphys.2017.00117.

149. Liu RM. Oxidative stress, plasminogen activator inhibitor 1, and lung fibrosis. Antioxid Redox Signal 2008;10:303–319. doi: 10.1089/ars.2007.1903.

150. Akram KM, Lomajs NJ, Forsyth NR, Spiteri MA. Alveolar epithelial cells in idiopathic pulmonary fibrosis display upregulation of TRAIL, DR4 and DR5 expression with simultaneous preferential over-expression of pro-apoptotic factor p53. Int J Clin Exp Pathol 2014;7:552–564.

151. Arsh N, Petukhov D, Wallach-Dayan SB. The role of telomerase and telomeres in interstitial lung diseases: from molecules to clinical implications. Int J Mol Sci 2019;20:2996. doi: 10.3390/ijms2012996.

152. Sisson TH, Maher TM, Asayi IO, King JE, Higgins PD, Booth AJ, et al. Increased survivin expression contributes to apoptosis resistance in IPF fibroblasts. Adv Biol Sci Biotechnol 2012;3:657–664. doi: 10.4236/abb.2012.36008.

153. Golan-Gerstl R, Wallach-Dayan SB, Zisman P, Cardoso WV, Goldenstein RH, Breuer R. Cellular FLICE-like inhibitory protein deviates myofibroblast fas-induced apoptosis toward proliferation during lung fibrosis. Am J Respir Cell Mol Biol 2012;47:271–279. doi: 10.1165/rcmb.2010-0284RC.

154. Huang WT, Akhter H, Jiang C, MacEwen M, Ding Q, Antony V, et al. Plasminogen activator inhibitor 1, fibroblast growth factor-10, fibroblast growth factor, and activator of transcription 6/transformer EBP homologous protein and endoplasmic reticulum stress in mice with idiopathic pulmonary fibrosis. Sci Rep 2016;6:37445. doi: 10.1038/srep37445.
160. Hasaneen NA, Cao J, Pulko-Gross A, Zucker S, Foda HD. Extracellular matrix metalloproteinase inducer (EMMPRIN) promotes lung fibroblast proliferation, survival and differentiation to myofibroblasts. Respir Res 2016;17. doi: 10.1186/s12931-016-0334-7.

161. He Z, Yao Y, Deng Y, Li W, Chen Y, Xing S, et al. Lipopolysaccharide induces lung fibroblast proliferation through Toll-like receptor 4 signaling and the phosphoinositide3-kinase-Akt pathway. PLoS One 2012;7:e35926. doi: 10.1371/journal.pone.035926.

162. Hanson KM, Hernady EB, Reed KC, Johnston CJ, Groves AM, Finkelstein JN. Apoptosis resistance in fibroblasts precedes progressive scarring in pulmonary fibrosis and is partially mediated by toll-like receptor 4 activation. Toxicol Sci 2019;170:489–498. doi: 10.1093/toxsci/kfr103.

163. Selman M, Pardo A. Role of epithelial cells in idiopathic pulmonary fibrosis: from innocent targets to serial killers. Proc Am Thorac Soc 2006;3:364–372. doi: 10.1513/pats.200601-003TK.

164. Du Z, Lin Z, Wang Z, Liu D, Tian D, Xia L. SPOCK1 overexpression induced by platelet-derived growth factor-BB promotes hepatic stellate cell activation and liver fibrosis through the integrin α5β1/PI3K/Akt signaling pathway. Lab Invest 2020;10:1042–1056. doi: 10.1038/s41747-020-0425-4.

165. Ajayi IO, Sisson TH, Higgins PD, Booth AJ, Sagana RL, Huang SK, et al. X-linked inhibitor of apoptosis regulates lung fibroblast resistance to Fas-mediated apoptosis. Am J Respir Cell Mol Biol 2013;49:86–95. doi: 10.1165/rcmb.2012-0224OC.

166. Fattman CL. Apoptosis in pulmonary fibrosis: too much or not enough? Antioxid Redox Signal 2008;10:379–385. doi: 10.1089/ars.2007.1901.

167. Rogliani P, Calzetta L, Cavalli F, Matare MG, Cazzola M. Pirfenidone, nintedanib and N-acetylcysteine for the treatment of idiopathic pulmonary fibrosis: a systematic review and meta-analysis. Pulm Pharmacol Ther 2016;40:95–103. doi: 10.1016/j.pupth.2016.07.009.

168. Behr J, Bendstrup E, Crestani B, Günther A, Olschewski H, Skold CM, et al. Safety and tolerability of acetylcyesteine and pirfenidone combination therapy in idiopathic pulmonary fibrosis: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Respir Med 2016;4:445–453. doi: 10.1016/S2213-2600(16)30044-3.

169. Derrick L, Vitalt R, Jones T, Jagirdar R, Luckhardt TR, Horowitz JC, et al. NADPH oxidase-4 mediates myofibroblast activation and fibrogenic responses to lung injury. Nat Med 2009;15:1077–1081. doi: 10.1038/nm.2005.

170. Amara N, Goven D, Prost F, Muloway R, Crestani B, Boczkowski J. NOX4/NAPDH oxidase expression is increased in pulmonary fibroblasts from patients with idiopathic pulmonary fibrosis and mediates TGFbeta1-induced fibroblast differentiation into myofibroblasts. Thorax 2010;65:738–742. doi: 10.1136/thx.2009.113536.

171. Ashley SL, Sisson TH, Wheaton AK, Kim KK, Wilke CA, Ajayi IO, et al. Targeting inhibitor of apoptosis proteins protects from bleomycin-induced lung fibrosis. Am J Respir Cell Mol Biol 2016;54:482–492. doi: 10.1165/rcmb.2015-0148OC.

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