ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Pathogenic Mechanisms Underlying Idiopathic Pulmonary Fibrosis

Benjamin J. Moss,[1] Stefan W. Ryter,[2] and Ivan O. Rosas[1]

[1]Section of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, Baylor College of Medicine, Houston, Texas 77030, USA; email: benjamin.moss@bcm.edu, ivan.rosas@bcm.edu

[2]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Weill Cornell Medicine, New York, NY 10021, USA; email: str2020@med.cornell.edu

Annu. Rev. Pathol. Mech. Dis. 2022. 17:515–46

First published as a Review in Advance on November 23, 2021

The *Annual Review of Pathology: Mechanisms of Disease* is online at pathol.annualreviews.org

https://doi.org/10.1146/annurev-pathol-042320-030240

Keywords

cellular senescence, interstitial lung disease, pulmonary fibrosis, single-cell RNA sequencing

Abstract

The pathogenesis of idiopathic pulmonary fibrosis (IPF) involves a complex interplay of cell types and signaling pathways. Recurrent alveolar epithelial cell (AEC) injury may occur in the context of predisposing factors (e.g., genetic, environmental, epigenetic, immunologic, and gerontologic), leading to metabolic dysfunction, senescence, aberrant epithelial cell activation, and dysregulated epithelial repair. The dysregulated epithelial cell interacts with mesenchymal, immune, and endothelial cells via multiple signaling mechanisms to trigger fibroblast and myofibroblast activation. Recent single-cell RNA sequencing studies of IPF lungs support the epithelial injury model. These studies have uncovered a novel type of AEC with characteristics of an aberrant basal cell, which may disrupt normal epithelial repair and propagate a profibrotic phenotype. Here, we review the pathogenesis of IPF in the context of novel bioinformatics tools as strategies to discover pathways of disease, cell-specific mechanisms, and cell-cell interactions that propagate the profibrotic niche.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease with high mortality and limited treatment options. Decades of research have revealed a complex underlying pathophysiology of IPF with alterations in many aspects of molecular and cellular physiology, including genetics, epigenetics, microRNAs (miRNAs), developmental reprogramming, cell-signaling pathways, apoptosis, metabolism, and autophagy. Similar to cancer biology and other complex natural phenomena, IPF demands a systems-level approach toward understanding its pathology. Recently, advancements in single-cell RNA sequencing (scRNA-seq) technology have provided a powerful new tool for the systems biology armament. The unbiased nature of scRNA-seq allows investigation of the full range of cell type–specific transcriptional abnormalities and predicted cell-cell cross talk. With this more comprehensive transcriptional cell atlas in hand, we are better positioned to integrate and interpret abundant data that have accumulated over the past decades.

In general, fibrosis is defined as excessive, pathologic deposition of extracellular matrix (ECM) during wound healing. Fibrogenesis is a highly orchestrated process that integrates multiple cell types and signaling mechanisms across organ systems. Different triggers, such as burns, infections, autoimmunity, operative and nonoperative wounds, foreign materials, and tumors all converge on similar fibrotic pathways. Initiation of the wound healing process by one of these triggers elicits an inflammatory response that ultimately recruits fibroblasts and activates a subset of cells, myofibroblasts, to deposit ECM in the form of collagen and other proteins. While wound healing typically resolves with apoptosis of myofibroblasts, in fibrotic disease states, there is persistence of profibrotic activators and myofibroblasts.

IPF is characterized by remodeling of the interstitium, distal airway, and alveolar spaces. Classic histopathologic findings of IPF tissue include honeycomb cysts, fibroblastic foci, and hyperplastic epithelial cells. These changes are associated with an overall bronchiolization of the distal airways, featuring ectopic mucociliary epithelium in dilated alveolar spaces. Interestingly, this pathology is predominantly found in basilar, posterior regions of the lung and typically begins in a subpleural distribution. In one model, excessive tractional forces likely occur in these subpleural, posterior basal areas, which, due to their peripheral location, are especially prone to alveolar collapse (1). Excessive tractional forces in the alveolus may promote repetitive epithelial injury (2). Thus, factors that increase the likelihood of alveolar collapse increase risk for disease, which may further impede passage of venous blood and explain both the high rates of pulmonary hypertension (PH) and the lack of pleural effusions observed in IPF (1).

The pathologic features of honeycombing, traction bronchiectasis, and fibroblastic foci are frequently observed in IPF and represent remodeling of the normal lung architecture. Once the underlying pathology has progressed to clinical and radiographic abnormalities, the prognosis for the patient is poor (3). Thus, detection of IPF at earlier stages is an important area of translational research. Radiographic appearance of interstitial lung abnormalities (ILAs) provides one potential means of identifying early-stage IPF. ILAs are correlated with histologic evidence of pulmonary fibrosis (4) and with demographic and clinical features of IPF, such as age, tobacco use, and restrictive lung defects (5). Future research into ILAs and early-stage IPF will be critical in identifying at-risk populations and designing novel therapeutic interventions to curb the high mortality associated with IPF.

EPIDEMIOLOGY

Of the idiopathic interstitial pneumonias, IPF is the most prevalent and morbid (6). IPF has a poor prognosis, with median survival only 2.5–3.5 years from the time of diagnosis (3, 7, 8). Most patients have a slow, progressive decline in lung function, ultimately leading to intractable respiratory

failure, whereas 10–15% have an unusually rapid decline within months (8). The mechanisms and key attributes of these two different phenotypes remain under investigation. Determining the exact incidence and prevalence of IPF is difficult due to diagnostic challenges, misdiagnosis, and a lack of diagnostic coding uniformity across providers. Epidemiologic studies of North American and European populations demonstrate an incidence of 3–9 cases per 100,000 people per year, with overall incidence increasing over time (9). A recent study of US Medicare recipients over age 65 showed increasing prevalence, from 202 to 495 cases per 100,000 people, from 2001 to 2011 (10). The most prominent nonmodifiable risk factors for IPF are male gender and age (11–13). In one United Kingdom cohort, 85% of patients were older than 70 years when first diagnosed with IPF (14). A study in the United States showed declining overall mortality from 2004 to 2017, which may be related to decreased prevalence of tobacco smoking, a reduction in the use of immunosuppressants, and the introduction of novel therapies (15). This conclusion is supported by registry data indicating improved survival and lung function in patients on antifibrotic medications (16, 17).

ENVIRONMENT

As the lungs and respiratory tract are in continuity with ambient air, the respiratory epithelium is constantly exposed to organic and inorganic particulates from both the external (e.g., pollutants, animal antigens, and occupational exposures) and host (e.g., microaspiration, gastroesophageal reflux, and commensal microbes) environments. As a key feature of IPF pathology is dysregulated epithelial repair, chronic injury to the alveolar epithelium through environmental exposures may be an important contributor to disease pathogenesis. Environmental exposures may provide the necessary trigger for the epigenetic modifications that have been observed in IPF. These include upregulation of histone deacetylases (18) and repression of antifibrotic chemokines by histone methylation (19). Importantly, exposure to cigarette smoke (CS) leads to genome-wide changes in DNA methylation (20). Epigenetic modification via miRNAs and long noncoding RNAs may also contribute to fibrosis (21).

Certain viral exposures have also been associated with an increased risk of IPF, including exposure to Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 7 (HHV-7), and HHV-8 (22, 23). EBV and CMV viral loads were found to be higher in IPF, and EBV antigens were detected in alveolar epithelial cells (AECs) in IPF subjects. Intriguingly, this antigen was also associated with endoplasmic reticulum (ER) stress markers, suggesting a mechanistic link between latent viral infection and development of IPF (24). However, the causal role of these chronic viral infections in the pathophysiology of IPF remains to be determined.

While it is challenging to demonstrate a causal link between environmental exposures and IPF, many correlations are well documented. Associations exist between IPF and occupational exposures, such as exposure to agricultural chemicals, livestock, wood dust, metal dust, stone, and sand, as well as with more general exposures, such as CS and air pollution (25). The microbiome represents another persistent exposure to the lung and airways. Animal models have shown an association between the microbiome and fibrosis, with an increased bacterial burden and loss of microbiome diversity in patients with IPF (26, 27). However, changes in underlying lung architecture in IPF likely alter the local flora, so drawing conclusions about causality and the microbiome is challenging.

GENETICS

IPF occurs both sporadically and in families, consistent with an underlying genetic predisposition. Familial pulmonary fibrosis (FPF), defined as having two or more family members with idiopathic interstitial pneumonia, represents about 5–20% of IPF cases (3). Importantly, up to 1 in 3 cases of

Table 1 Common (found in >5% of cases) and rare genetic variants of IPF. Common variants are set in bold

Category	Gene	Reference
Mucin production	***MUC5B***	30
	MUC2	31
Surfactant	*SFTPC*	94
	SFTPA1	234
	SFTPA2	235
	ABCA3	236
Telomeres	***TERT***	59
	TERC	59
	TINF2	237
	DKC1	238
	RTEL1	239
	PARN	240
	STN1	178
	OBFC1	31
Cell-cycle regulation	*KIF15*	34
	MAD1L1	34
	CDKN1A	241
	TP53	241
Cytokines and immune function	***IL1RN***	242
	IL8	243
	IL4	244
	TGFB1	245
	FAM13A	31
	HLA-DRB1	246
Toll-like receptor signaling	***TOLLIP***	31
	TLR3	180
	ATP11A	31
Cell adhesion and interaction	***DSP***	31
	MDGA2	178
	MAPT	31
	DPP9	31
RhoA signaling	***AKAP13***	247
mTOR signaling	*DEPTOR*	34

Abbreviations: IPF, idiopathic pulmonary fibrosis; mTOR, mechanistic target of rapamycin complex.

sporadic IPF have a family history of pulmonary fibrosis, suggesting that genetic variation is a key determinant for the development of IPF (28, 29). Beginning with observations in FPF, a host of genetic susceptibilities to lung fibrosis have been identified, with significant overlap between IPF and FPF.

There is a substantial gap in knowledge of the functional significance of most genetic associations (**Table 1**). However, discrete genetic variations have been associated with pathogenic mechanisms such as surfactant mutations, protein misfolding, ER stress, and telomere shortening associated with abnormalities in DNA repair. While specific mutations have contributed to our

understanding of IPF pathogenesis, most described genetic variations are neither necessary nor sufficient for the development of pulmonary fibrosis.

The most common genetic variant in IPF is the *MUC5B* r35705950 allele, which is present in 38% of patients with IPF and is associated with a 21-fold risk for disease in homozygous individuals (30). The pathologic mechanism of this mutation may be related to excess mucin production and impaired mucociliary clearance. The second highest region conferring risk for IPF in genome-wide association studies (GWAS) is in the desmoplakin gene (*DSP*), which is important for cell-cell adhesion (31). This *DSP* risk allele is associated with reduced *DSP* gene expression in AECs and upregulation of ECM genes (32). Disruption of the structural integrity of the alveolus by decreased *DSP* expression and reduced cell-cell adhesion may contribute to chronic alveolar injury and dysregulated epithelial repair (32).

While genetics influence the development of IPF, in a study of 14 IPF susceptibility variants in a European population, the known genetic variants explained only 12.4% of disease liability in the general population and 17.7% in people over 65 years old. Notably, *MUC5B* was accountable for three times more disease liability than all other variants combined (33). Correlations can be found between other genetic variants and lung function (34), disease progression, and treatment response (35). The introduction of genetic testing into clinical practice to assist with disease stratification and treatment response is expected in the next decade.

ANIMAL MODELS

Animal models have provided many important insights into the pathobiology of IPF (36, 37). The most commonly used model involves single-dose intratracheal instillation of bleomycin, which leads to an acute inflammatory lung injury followed by a temporary period of fibrosis. The main disadvantage to this model is the acute onset and self-resolving course of fibrosis compared to IPF, which is characterized by chronically progressive, nonresolving disease. Pathologically, fibrosis in the bleomycin model also does not fully recapitulate IPF, as it lacks basal and subpleural predominance and is characterized by more limited AEC remodeling (38–40). Recently, protocols involving repetitive instillation of bleomycin have shown promise in more faithfully recapitulating human disease at the histologic level (41). Other murine models of IPF include cytokine overexpression, genetic knockout and manipulations, and chemically induced fibrosis (e.g., using fluorescein isothiocyanate). Studying aged mice may improve the fidelity of IPF models, as the association between aging, senescence, and IPF has been an important area of recent investigation (see the section titled Aging, Cell Senescence, and Mitochondrial Dysfunction). Aged mice have an increased fibrotic response to bleomycin (42), and induction of senescence in type 2 alveolar epithelial (AT2) cells results in spontaneous lung fibrosis (43). Pulmonary fibrosis has been studied in other animals, including some that develop spontaneous fibrosis (e.g., cats and dogs), but none of these models have gained traction, likely due to inherent difficulties in working with these animals. While all these models have contributed to our understanding of the basic pathology of fibrosis, it is important to recognize that no model will ever fully capture the complexity of IPF. Thus, it will be crucial for future research not only to continue to improve on the current models but also to test and validate hypotheses across multiple model systems.

ALVEOLAR EPITHELIAL CELLS

In healthy lungs, type 1 alveolar epithelial (AT1) cells are the primary mediators of gas exchange and provide the bulk of the epithelial surface area. AT2 cells produce surfactant and serve as the primary progenitors for injured AT1 cells (44). Injury to the alveolus requires a healthy, functioning population of regenerative AT2 cells. In IPF lungs, there are higher levels of

apoptosis, senescence, abnormal differentiation and impaired renewal capacity of AT2 cells. A combination of extrinsic and intrinsic factors, including aging, ER stress, mitochondrial dysfunction, and telomere shortening, ultimately leads to an inability of the AT2 cell to contribute to effective repair of injured epithelium. Data from scRNA-seq comparing IPF and control lung tissue have provided evidence for an aberrant epithelial cell that localizes to the leading edge of the fibroblastic foci and exhibits markers of both AECs and basal cells. This profibrotic cell type expresses markers of specific genes and cellular programs known to be dysregulated in IPF, including matrix metalloproteinase 7 (MMP7), integrin αVβ6, cellular senescence, and epithelial-mesenchymal transition (EMT), and exhibits abnormal expression of developmental genes. Importantly, this cell type is not found in healthy lungs and is rarely found in other chronic lung diseases such as chronic obstructive pulmonary disease but appears in nearly every IPF lung (45, 46). Thus, aberrant epithelial cells may represent a major driver of IPF pathogenesis. Here, we discuss mechanisms of dysregulated alveolar epithelial repair, highlighting cellular senescence as a key mediator of IPF pathology (**Figure 1**).

Early studies in IPF identified a unique bronchiolar epithelial cell type characterized by abnormal cell cycle regulation within fibroblastic foci in IPF lungs but not in other interstitial lung diseases, such as acute or nonspecific interstitial pneumonias and bronchiolitis obliterans organizing pneumonia (47). Supporting this bronchiolarization, Krt5, a marker of airway basal cells normally absent from distal airways, was observed in IPF fibroblastic foci. These same cells also expressed AT1 and AT2 markers, suggesting a transitional cell with both alveolar epithelial and basal features (48). Further studies showed that treatment of AECs in vitro with an IPF cytokine cocktail led to upregulation of airway-associated genes (*TP63*, *Krt5*, and *MUC5B*) and *SOX2*, a regulator of distal airway morphogenesis (49). In murine models of alveolar injury, a population of $Krt5^+$ and $Krt8^+$ transitional cells were identified that expressed markers of cell senescence, DNA damage, and TP53 signaling (50–52). A cluster of cells identified as secretory primed basal cells was upregulated in end-stage IPF and localized to areas of high *MUC5B* expression in honeycomb cysts (46, 53). Additional scRNA-seq analyses of IPF lungs demonstrated similar development of a unique population of epithelial cells that had features of AT1, AT2, basal, and proximal airway cells (43, 45, 46). These cells expressed markers known to be highly dysregulated in IPF, such as MMP7, had upregulation of EMT gene expression pathways, had features of senescence, and localized to the epithelial layer of the fibroblastic foci.

In all these models, the transitional epithelial cell types have features of the proximal airway, suggesting that in times of extensive distal airway or alveolar damage, either progenitor cells are recruited from the airway or preexisting alveolar progenitor cells adopt an airway phenotype. Increased expression of *SOX9*, known to regulate airway development (54), may promote this bronchiolar airway phenotype. In IPF, the development of a unique, senescent population of AECs may hinder epithelial repair and promote a fibrotic cellular niche (43, 45). Intriguingly, a similar population of cells was identified in explants of patients with severe acute respiratory distress syndrome due to coronavirus disease 2019, which corresponded to fibrotic histopathology (55). The fact that these cells exist in both acute and chronic injury and are consistently seen in IPF suggests that they may be a common and necessary step in the perpetuation of nonresolving lung fibrosis.

Other mechanisms may also contribute to aberrant differentiation of AT2 cells. For example, IL-1β is required for normal differentiation of AT2 cells into AT1 cells, but persistent IL-1β signaling, which is upregulated in bronchoalveolar lavage (BAL) of IPF patients, inhibits this pathway (52). This may be one mechanism whereby chronic inflammation leads to fibrosis. Below, we discuss the principal contributions of AECs to IPF pathogenesis, highlighting pathways dysregulated in the aberrant epithelial cells: senescence, mitochondrial function, metabolism, autophagy, proteostasis, and developmental signaling.

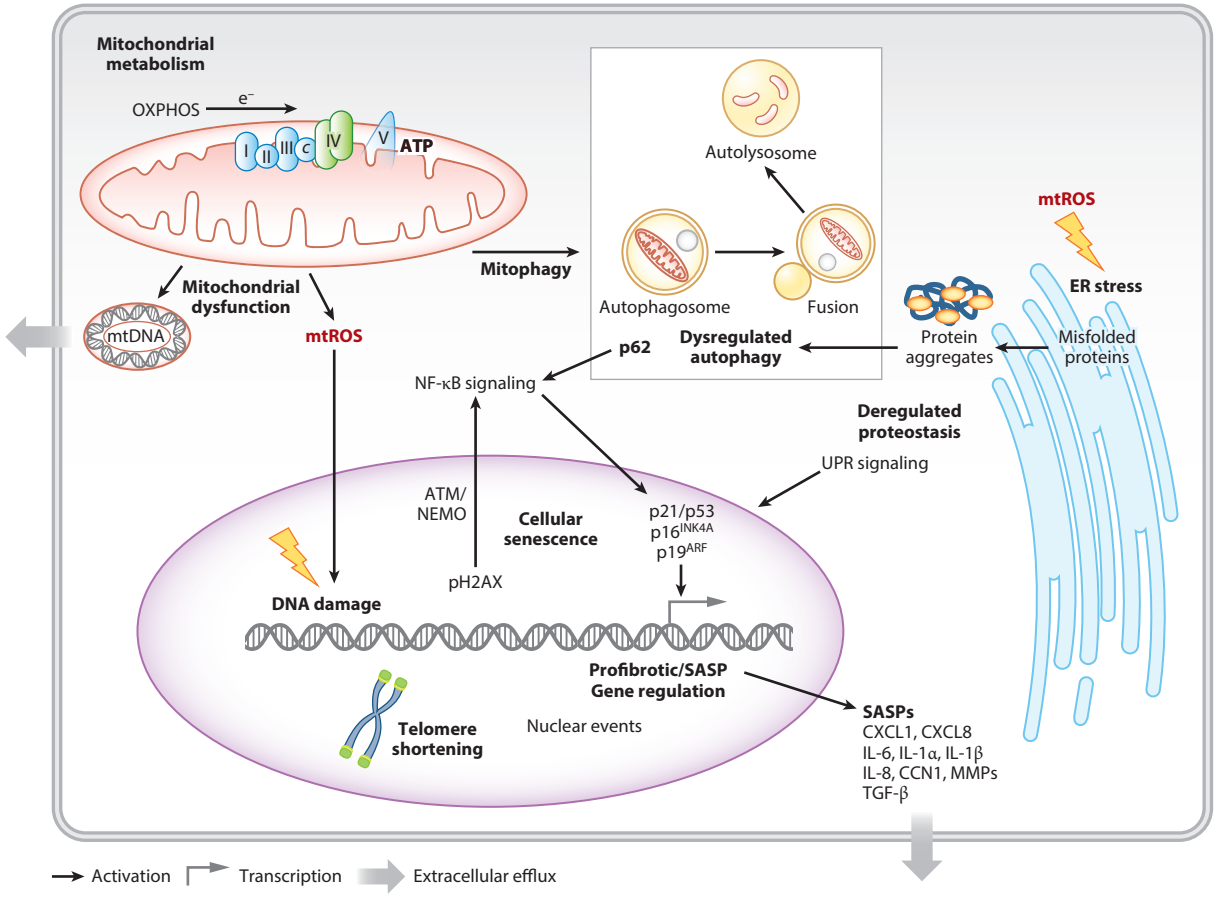

Figure 1

Dysregulated cellular pathways leading to senescence. The figure shows a hypothetical target cell in which dysregulated cellular pathways promote profibrotic and prosenescence phenotypes. Epithelial dysregulation represents a primary initiating event in IPF pathogenesis, although common elements of cellular dysregulation (e.g., autophagy, ER stress, and mitochondrial dysfunction) may also occur in other cell types, including fibroblasts, endothelial cells, and immune cells. Metabolic deregulation in epithelial cells by profibrotic stimuli may lead to aberrant bioenergetics, disrupting mitochondria-dependent pathways such as fatty acid oxidation. Additionally, mitochondrial dysfunction is associated with mitochondrial membrane depolarization and mtROS production. Loss of proteostasis due to profibrotic triggers or genetic variants (e.g., surfactant mutations) leads to accumulation of misfolded or damaged proteins, ER stress, and activation of the UPR. Autophagy allows for degradation of cellular substrates through autophagosome capture and subsequent fusion to degradative lysosomes and represents a common cellular homeostatic pathway that may be dysregulated or impaired in epithelial cells or fibroblasts during IPF pathogenesis. Loss of autophagy may trigger fibrogenesis via inadequate mitochondrial quality control and processing of protein aggregates and may also lead to aberrant NF-κB signaling via p62 accumulation. Taken together, aberrant mitochondrial function, proteostasis, and impaired autophagy may lead to pathways activating cell senescence. Both telomere attrition and mtROS-induced DNA damage may trigger fibrotic pathways via pH2AX, ATM, NEMO/NF-κB signaling, and activation of p21. Furthermore, activation of the UPR may also lead to modulation of prosenescence transcriptional regulators, including p21 and p53. Thus, initial epithelial injury translates to profibrotic effects, including epithelial cell senescence, SASP generation, and, ultimately, cell-cell interactions that promote fibrosis within the alveolar niche. Abbreviations: ATM, ataxia-telangiectasia mutated; ER, endoplasmic reticulum; IPF, idiopathic pulmonary fibrosis; MMP, matrix metalloproteinase; mtDNA, mitochondrial DNA; mtROS, mitochondrial reactive oxygen species; NEMO, NF-κB essential modifier; NF-κB, nuclear factor κB; OXPHOS, oxidative phosphorylation; pH2AX, phospho H2A histone family X; SASP; senescence-associated secretory phenotype; TGF-β, transforming growth factor-β; UPR, unfolded protein response.

Aging, Cell Senescence, and Mitochondrial Dysfunction

Aging is the most significant risk factor for IPF, such that the prevalence of IPF doubles with every decade after age 50 (13). Characteristic pathophysiologic hallmarks of aging are seen in IPF and include telomere attrition, DNA damage, epigenetic modifications, abnormal proteostasis, mitochondrial dysfunction, and dysregulated autophagy (56) (see **Figure 1**). At the cellular level, there is substantial overlap in the pathology of aging and IPF, demonstrated by increased expression of markers of cellular senescence. Senescence, characterized by arrest of cell growth and a diminished replicative potential, predisposes lungs to fibrosis by impairing regeneration of alveolar progenitor cells and fostering a profibrotic cellular environment. In IPF, AT2 cells exhibit markers of senescence, and scRNA-seq data suggest that aberrant epithelial cells, which preferentially localize to fibroblastic foci, express especially high levels of senescent protein transcripts (43, 45).

The development and maintenance of senescence involves a complex array of signaling pathways, many of which are altered in IPF. One example is telomere shortening, which is a hallmark of aging and senescence (57). GWAS studies have identified genetic mutations in several telomere-related genes in IPF (31, 58, 59) (**Table 1**). Furthermore, disruption of telomere maintenance in murine AT2 cells, but not mesenchymal cells, promotes fibrosis and accumulation of senescent epithelial cells (60). Exposure to CS, another important risk factor for IPF, also leads to telomere shortening (61). Oxidative injury, which occurs as a result of both aging and CS exposure, is increased in IPF (56, 62). Oxidative injury, aging, and ineffective telomere quality control all may converge to cause an impaired DNA damage response. Mitochondrial dysfunction and the generation of mitochondrial reactive oxygen species (mtROS) lead to DNA damage and ineffective repair, at least in part due to telomere maintenance dysfunction. Continued DNA damage activates a cascade of responses leading to senescence, including activation of ataxia-telangiectasia mutated/nuclear factor κB (NF-κB) essential modifier (ATM/NEMO), p53, plasminogen activator inhibitor-1 (PAI-1), and phosphoinositide 3-kinase (PI3K)/Akt signaling. NF-κB is also an important mediator of this response via p21, among other molecules, which may promote cellular senescence (63–65). Furthermore, NF-κB expression is increased in IPF lungs, and its expression specifically in AECs is necessary for senescence (66). Recent studies have uncovered a mechanistic association between dysregulated PI3K/Akt and downstream NF-κB activation leading to profibrotic gene expression, which may be present in both fibroblasts and epithelial cells (67).

The development of senescence, whether mediated by NF-κB or the other pathways mentioned above, is characterized by secretion of inflammatory proteins, termed the senescence-associated secretory phenotype (SASP). Individual SASP components (SASPs) contribute to fibrosis and include well-established mediators of IPF such as transforming growth factor-β (TGF-β), MMPs, IL-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) (68). While secretion of SASPs may occur in both senescent fibroblasts and AECs, only the secretion of SASPs from AECs appears to be important for progression of fibrosis (43, 68). Targeting cellular senescence may be a promising therapeutic avenue, as several studies in animal models have demonstrated attenuation of fibrosis following administration of senolytics, drugs that specifically target senescent cells (68–70).

Data from scRNA-seq revealed localized increased expression of senescent markers (CDKN1A/p21, CDKN2A/p16, TP53, MDM2, and CCND1) to AECs (43, 45, 46, 71). In particular, the aberrant population of AECs displayed upregulation of cell senescence pathways (45). AT2 and aberrant basaloid epithelial cells exhibited the most features of cellular senescence compared to other IPF cell types (43). Although senescence has also been observed in mesenchymal cells, inducing senescence in AT2 cells, but not mesenchymal cells, led to fibrosis in animal models (43, 57, 60). Furthermore, genetic induction of p53-dependent senescence in murine AT2 cells was sufficient to cause progressive fibrosis (54).

Mitochondrial homeostasis is intimately linked to the regulation of cell senescence. Mitochondria, as vital organelles, play key roles in cellular energy production and metabolic homeostasis. Consequently, mitochondrial dysfunction, as characterized by altered respiratory chain activity, membrane depolarization, and increased mtROS production, can promote senescence. Aging renders the lung more susceptible to enlarged mitochondria with increased mtROS production (72). Recent studies suggest that depletion of mitochondria attenuates senescent phenotypes (73), and disruption of mitochondrial quality maintenance mechanisms, such as mitochondrial biogenesis and mitophagy, may predispose the AT2 cell to senescence. AT2 cells are especially susceptible to mitochondrial dysfunction due to the high metabolic demands of surfactant production. Abnormally enlarged and swollen mitochondria have been observed in AT2 cells from IPF patients (74).

DNA damage may be upstream of mitochondrial dysfunction and senescence. Telomere dysfunction associated with senescence can repress mitochondrial biogenesis pathways via p53-mediated downregulation of peroxisome proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α) and PGC-1β, leading to further compromise of mitochondrial quality and elevated mtROS production (75). Telomere-associated DNA damage can induce mechanistic target of rapamycin (mTOR) and promote epithelial senescence via increased mtROS production. Accordingly, mTOR is upregulated in several chronic lung diseases, including IPF (76). Thus, various factors in the IPF AEC, including telomere dysfunction, high metabolic demand for surfactant production, and aging, promote mitochondrial dysfunction and senescence.

Specific proteins involved in senescence and mitochondrial function are dysregulated in IPF. For example, phosphatase and tensin homolog (PTEN)-induced kinase-1 (PINK1) normally facilitates mitophagy and stabilizes mitochondrial membrane integrity. Several studies have highlighted the importance of PINK1 in fibrosis, as PINK1-deficient mice are more susceptible to the development of fibrosis (74, 77). PINK1 also provides a mechanistic link between ER stress (discussed in the section titled Proteostasis) and mitochondrial dysfunction. ER stress downregulates PINK1 via activating transcription factor 3 (ATF3) and leads to an analogous program of mitochondrial unfolded protein response (UPR) via ATF4 (78). Mitochondrial dysfunction associated with PINK1 deficiency leads to fibrosis through the release of mitochondrial DNA (mtDNA), which activates Toll-like receptors (TLRs) and stimulates TGF-β (79).

Sirtuins are a group of NAD^+-dependent deacetylases tightly linked with aging, mitochondria, and cellular energy sensing. Low levels of sirtuin 1 (SIRT1), an antifibrotic deacetylase that represses p53 and promotes autophagy, have been observed in lung biopsies in IPF (80). SIRT3, which inhibits fibrosis through activation of glycogen synthase kinase 3β and helps protect against mtDNA damage through antioxidant mechanisms, is also decreased in IPF. Mice lacking SIRT3 have an increased fibrotic response to bleomycin (81). Extracellular vesicles released from fibroblasts containing specific miRNAs downregulate SIRT3, leading to increased mtROS production and cellular senescence (82). Together, these findings suggest that deficiency in sirtuins contributes to oxidative and mitochondrial dysregulation involved in fibrogenesis. Sirtuins are a prominent target in aging research, and resveratrol, an antiaging molecule that induces SIRT1 expression, can protect against bleomycin-induced fibrosis in mice.

Impaired autophagy has been observed in models of fibrosis and may represent a propathogenic feature of the IPF lung. Autophagy denotes a cellular homeostatic program that governs the turnover of long-lived proteins and dysfunctional organelles (i.e., mitochondria) via sequestration in double membrane–bound autophagosomes and subsequent lysosome-dependent degradation. Inducible autophagy, a hallmark of antiaging pathways, acts as a prosurvival mechanism during cellular stress, including ER stress, hypoxia, starvation, and oxidative stress. TGF-β inhibits autophagy via activation of mTOR complex 1 (mTORC1), an inhibitor of autophagy (83).

mTORC1 is persistently activated in both aged and IPF cells, leading to reduced autophagy (84). The functional significance of impaired autophagy in IPF pathogenesis remains unclear but may be related to impaired mitochondrial quality control, impaired turnover of lipids and collagen, and impaired pathogen clearance, among other factors. Inhibition of autophagy promotes EMT by epithelial-fibroblast cross talk (85). Surfactant protein C mutation leads to a late block in autophagy and accumulation of p62 (i.e., cargo adaptor protein or sequestosome-1) in AT2 cells (86). Accumulations of p62 have been associated with aberrant downstream signaling potentially linked to fibrogenesis, including NF-κB regulation, NF-E2-related factor 2 (NRF2) regulation, or both.

Proteostasis

Proteostasis refers to the regulation of protein production, trafficking, maintenance, and degradation. Abnormalities in any one of these steps may contribute to lung disease (87, 88), but the most studied proteostatic changes in IPF are triggered by ER stress and the UPR. ER stress refers to imbalances in protein homeostasis that lead to an accumulation of unfolded proteins. Under conditions of ER stress, the UPR is activated. The UPR aids in protein synthesis, protein folding, and protein degradation. If the UPR is unable to mitigate the stress of the ER, apoptosis pathways are triggered.

ER stress and the UPR have been identified as upstream mediators of fibrosis in many disease states, including IPF. Markers for ER stress and the UPR are elevated in AT2 cells of IPF lungs (89–91). Similar factors are associated with both ER stress and IPF, including aging, cell senescence, and exposure to CS (92). AT2 cells are especially sensitive to protein homeostasis due to their high production of surfactant proteins. The importance of ER stress in IPF was first introduced by the characterization of surfactant mutations in FPF. The most common mutations in FPF are located in regions of the surfactant protein essential for folding and trafficking, leading to accumulation of unfolded proteins, ER stress, and activation of the UPR (93, 94) (**Table 1**). Markers of ER stress occur in asymptomatic relatives of patients with IPF and histologically normal regions in IPF lungs, suggesting that ER stress is important in the development of disease (24). AT2 cell–specific induction of ER stress in mice leads to lung fibrosis, epithelial cell apoptosis, and senescence (95). The increased expression of ER protein folding chaperones during ER stress (e.g., FK506-binding protein 13) has been observed in fibrotic lungs and correlates with worse lung function (96).

In addition to surfactant mutations, environmental factors can also lead to ER stress. Hypoxia induces ER stress via hypoxia-inducible factor-1 (HIF-1), which is overexpressed in AECs in biopsies of IPF lungs (97, 98). Viral infections, specifically herpesviruses, have been correlated with ER stress, and several studies have associated these viruses with development of IPF (89). CS and occupational exposures have also been linked to ER stress (99). Dysfunctional lipid synthesis and signaling have been implicated in the ER stress response. Lipid synthesis is deficient in AECs, and augmentation of lipid production attenuates fibrosis (19). A high-fat diet rich in palmitic acid results in epithelial cell apoptosis and ER stress in the murine bleomycin injury model (100). Other triggers of ER stress include telomere attrition, genomic instability, dysregulated nutrient sensing, and mitochondrial dysfunction (56), nearly all of which are also associated with aging.

ER stress is a prominent mechanism leading to AT2 cell depletion and apoptosis, a hallmark pathologic feature of IPF lungs (90, 101, 102). Increased expression of markers of apoptosis in AECs is evident in areas of fibrosis (102). Surfactant mutations cause apoptosis through ER stress, while telomere mutations cause apoptosis through DNA damage (103). Other mediators of AEC apoptosis in IPF include TGF-β (104), glutathione deficiency, and ROS (56). Recent reports have

identified necroptosis, a form of programmed cell death that can release proinflammatory and profibrotic mediators, in AECs of fibrotic areas of IPF (105).

Inability of AT2 cells to resolve ER stress leads not only to apoptosis but also to recruitment of profibrotic macrophages and cytokines (106) and EMT (107). Signaling from AECs undergoing ER stress contributes to differentiation of fibroblasts into myofibroblasts, promoting fibrosis (107, 108). In addition to stimulating release of proinflammatory cytokines and activating the nucleotide-binding oligomerization domain (NOD)-like receptor–, leucine-rich region–, and pyrin domain–containing-3 (NLRP3) inflammasome, ER stress also favors the M2, profibrotic macrophage population (109). Interestingly, ER stress upregulates MUC5B, whose ectopic expression in honeycomb cysts likely contributes to IPF pathogenesis (110). However, despite these many studies implicating ER stress in fibrosis, ER stress is not sufficient to cause fibrosis in animal models (107).

Wnt and Notch Signaling

In development, Wnt and its primary nuclear mediator, β-catenin, are required for differentiation of the bronchiolar and alveolar epithelium (111). AT2 progenitor cells are typically maintained by Wnt signaling from nearby fibroblasts; however, sustained Wnt signaling after injury inhibits AT2 cell differentiation (112). Excessive activation of canonical Wnt signaling through β-catenin is observed in fibrotic lungs (113, 114), and Wnt signaling in AT2 cells induces expression of IL-1β, which leads to fibrosis through TGF- β (115). Importantly, scRNA-seq data reveal that these changes are restricted to an aberrant population of AECs (45). Thus, while some degree of Wnt signaling is necessary for normal alveolar epithelial repair, in IPF, sustained Wnt signaling prevents normal repair.

Notch signaling, which is required for normal alveologenesis (116), is altered in AT2 cells in IPF (18, 117). Notch controls SOX2 signaling, which typically promotes a proximal airway phenotype and may contribute to the formation of honeycomb cysts (118). Dysregulation of this pathway is evident in the aberrant population of AECs, which likely drives their proximal airway phenotype (45). Together with dysregulation of other pathways in IPF, such as TGF-β, Sonic hedgehog (119), fibroblast growth factor (FGF), FOXO3, and T-box (120), dysregulation of the developmental programs of Wnt and Notch impairs alveolar differentiation and promotes fibrosis. Notably, activation of these developmental pathways resembles a similar activation during the pseudoglandular stage of lung development (120). One hypothesis, then, is that an inciting trigger activates developmental lung patterning, leading to the development of honeycomb cysts and bronchiolarization of the distal airways in IPF.

FIBROBLASTS

Once epithelial cell injury has occurred, the lung, like other organs, repairs itself through the process of wound healing. Normal wound healing involves the recruitment of fibroblasts, deposition of ECM, and differentiation of myofibroblasts, which secrete collagen and generate contractile force for wound closure. Resolution of wound repair is a complex process itself and entails deactivation of myofibroblasts, derecruitment of wound-healing fibroblasts, and resorption of ECM and cellular debris by macrophages. Fibrotic diseases are typically characterized by upregulation of TGF-β signaling, which promotes recruitment of fibrotic mesenchymal cells and inflammatory mediators, stimulating ECM deposition. TGF-β signaling has long been known to be involved in pulmonary fibrosis, and its contributions to IPF have been extensively reviewed elsewhere (104, 121) (see the sidebar titled TGF-β: A Master Regulator of Fibrosis).

TGF-β: A MASTER REGULATOR OF FIBROSIS

TGF-β is critical to both physiologic and pathologic wound healing and is one of the key signaling molecules in fibrosis. TGF-β is secreted in a latent form by different cell types, including epithelial, fibroblast, and immune cells. Once secreted, various molecules lead to TGF-β activation and binding to receptors on target cells, initiating a fibrotic signaling cascade (121). In IPF, TGF-β is expressed by AECs and alveolar macrophages, and its expression localizes to fibroblastic foci (230, 231). Data from scRNA-seq highlight the TGF-β pathway as among the most upregulated pathways in the aberrant epithelial cell population (45). Attenuation of TGF-β signaling may represent one of the mechanisms of action of the antifibrotic drug pirfenidone.

In IPF, it is difficult to distinguish recurrent, injury-driven wound repair from dysregulation of the wound repair process itself. Furthermore, when evaluating the role of mesenchymal cells in the pathogenesis of IPF, it is important to consider that the alveolar niche has undergone systemic reprogramming to generate a profibrotic environment. Although a host of mesenchymal genes and signaling pathways are dysregulated in IPF, these may occur downstream of an initial insult to the AEC.

Single-Cell Sequencing and Mesenchymal Cell Populations

Researchers have used scRNA-seq to identify populations of fibroblasts and myofibroblasts present in IPF (see the sidebar titled Single-Cell RNA Sequencing). Earlier studies relied on specific staining markers, such as α-smooth muscle actin (α-SMA), to differentiate fibroblasts from myofibroblasts and epithelial cells. However, an emerging theme from single-cell data points to a spectrum of overlapping and specialized mesenchymal cell populations that cannot be differentiated by a unique marker. Single-cell analysis of murine mesenchymal cells in response to injury demonstrated a heterogeneous population of mesenchymal cells serving distinct roles, with, for example, a specific profibrotic role for a Wnt-responsive fibroblast (122). In humans, IPF myofibroblasts exist on a continuous spectrum with normal myofibroblasts and are not derived from a different cell type (123). Importantly, multiple markers used to identify myofibroblasts, including α-SMA, are not specific markers for collagen-producing cells, as high levels are also seen in smooth muscle cells and pericytes (123). Overall, genes related to ECM were upregulated across all mesenchymal cell populations (124), suggesting that there may not be one specific IPF fibroblast responsible for the persistence of fibrosis.

SINGLE-CELL RNA SEQUENCING

Compared to bulk RNA sequencing, which measures average transcript expression from a heterogenous population of cells, scRNA-seq allows for quantification of cell-specific transcript expression and unbiased identification of novel cell types. Since its introduction in 2009, applications of scRNA-seq have become widespread. Alongside advances in microfluidic technology allowing for the processing of thousands of single cells, there has been an enormous output of computational algorithms designed for scRNA-seq data. These include tools for identifying gene regulatory networks and cell lineage reconstruction and discovering cellular trajectories along differentiation pathways. By quantifying cell-specific expression of ligands and receptors, connectomes, or detailed analyses of the interactions between cells, can be explored. Importantly, investigators are sharing their scRNA-seq data—for example, with the creation of the Human Cell Atlas. In combination with other omics technologies, such as metabolomics, lipidomics, and proteomics, further advancements in single-cell technologies will undoubtedly contribute to our understanding of the complex cellular biology of diseases such as IPF (232, 233).

When comparing mesenchymal cells from IPF and control lungs, scRNA-seq did identify a unique cluster of *Cthrc1*-expressing cells in IPF, derived from alveolar fibroblasts and expressing high levels of ECM-related genes (123). Similar to *Cthrc1*-expressing cancer cells, these cells have highly invasive and migratory potential. Thus, these pathologic fibroblasts are likely important to IPF progression. Another scRNA-seq study demonstrated that mesenchymal progenitor cell populations in IPF were more heterogenous and less differentiated compared to control mesenchymal cells (125). These progenitor cells localized to the perimeter of the fibroblastic foci and appeared to differentiate into α-SMA-positive myofibroblasts. These data suggest that in IPF, mesenchymal cells acquire a fibrogenic phenotype early in their differentiation process and contribute to disease pathogenesis.

The origin of IPF fibroblasts and myofibroblasts has been an area of controversy, with candidate progenitor cells including AECs via EMT, interstitial fibroblasts, lipofibroblasts, circulating fibrocytes, and pericytes (126). Circulating fibrocytes may contribute to the myofibroblast population in IPF and are recruited by factors released from apoptotic and necroptotic AECs that release CXCL13 and CCL2 (127). However, the exact lineage of these fibrocytes is unclear. They may instead represent a subset of monocyte-derived macrophages (128). While certain pericyte populations differentiate into lung fibroblasts in murine models of fibrosis (129, 130), to date no evidence has demonstrated this phenomenon in IPF. Furthermore, other murine lineage tracing experiments have not validated a pericyte origin for lung fibroblasts (131). Recent studies identified a population of lipofibroblasts as a potential source for activated myofibroblasts (132). Ultimately, evidence continues to mount against an epithelial origin, as suggested by scRNA-seq data, which support a paracrine signaling role for AECs through EMT.

Epithelial-Mesenchymal Transition and Myofibroblasts

EMT is an initial step in physiologic wound healing. Typically, EMT is identified histologically by the loss of epithelial cell-cell adhesion molecules, such as E-cadherin, and gain of mesenchymal markers, such as N-cadherin, vimentin, or α-SMA. This process occurs as part of development and wound repair but is dysregulated in diseases including IPF and cancer. Evidence for EMT in IPF arises mostly from colocalization of mesenchymal and epithelial markers in histopathologic stains of IPF lungs (133–135). While several early lineage tracing experiments in the murine bleomycin injury model suggested that the colocalization of these markers implied an epithelial origin for IPF mesenchymal cells (136, 137), later lineage tracing studies suggested otherwise (131). IPF lung scRNA-seq data were used to identify mesenchymal markers within the aberrant transitional epithelial cells, and these data have not shown an epithelial origin for fibroblast populations (45, 123). Thus, similar to observations in renal fibrosis (138), EMT is most likely a process whereby epithelial cells acquire mesenchymal markers that promote local fibrosis instead of transforming into mesenchymal cells.

Transition of fibroblasts to myofibroblasts is a normal event in wound healing and is classically marked by increased α-SMA expression. Myofibroblasts are necessary to generate the contractile force for wound closure and are associated with increased collagen deposition (139). In IPF, increased numbers of myofibroblasts contribute to fibrosis and disease progression. Myofibroblast differentiation and resistance to apoptosis is mediated by a host of factors, including TGF-β, integrin αVβ6, platelet-derived growth factor (PDGF), connective tissue growth factor, vascular endothelial growth factor (VEGF), overexpression of Wnt, decreased expression of bone morphogenetic protein 2 (BMP2), decreased AMP-activated protein kinase (AMPK) activation, and imbalance of oxidant-antioxidants. Many of these signaling pathways, including TGF-β, PDGF receptor, epidermal growth factor, FGF, and Wnt, specifically promote expression of

mesenchymal genes and downregulation of epithelial genes and thus activation of fibroblasts. There may also be a role for the coagulation cascade, as Factor X induces myofibroblast activation via PAR1, integrin αVβV, and TGF-β (140).

Myofibroblasts are activated and maintained by signaling pathways common to the wound healing process, including physical properties of the matrix itself. Fibroblasts use integrins to sense matrix stiffness (141). Increased matrix stiffness promotes myofibroblast differentiation and activation via TGF-β and TGF-β-independent mechanisms (141, 142). The transcription factors yes-associated protein 1 and transcriptional coactivator with PDZ-binding motif, which have elevated expression in IPF lungs, have been identified as important mediators of this process (143). Prostaglandin E2, which is associated with decreased matrix stiffness, is decreased in IPF and may contribute to the lack of dedifferentiation of myofibroblasts and resolution of wound healing (144, 145). Mechanical stretch, another mechanical property of the matrix, activates TGF-β, creating a feedforward loop of ECM activation (146).

Other activators of myofibroblast populations are currently under investigation as therapeutic agents in IPF. For example, inhibitors of Rho-associated coiled coil–forming protein kinase (ROCK), which can be activated by tissue stiffness and lead to downstream profibrotic signaling (147), are currently in Phase II clinical trials.

Fibroblast Apoptosis Resistance, Autophagy, and Senescence

Similar to what occurs in epithelial cells, dysregulation of autophagy, metabolism, and cellular senescence occurs in IPF fibroblasts. In our recent studies, we have shown that deregulation of autophagy via unrestricted PI3K/Akt signaling in fibroblasts promotes profibrotic responses, including ECM gene regulation via accumulation of p62 and downstream activation of NF-κB (67).

Fibroblast lineages derived from IPF patients demonstrate features of senescence, including telomere attrition (148), expression of p21 and p16 (69), altered morphology, and proinflammatory SASPs (69). Senescence, however, may represent a normal feature of wound healing, signaling resolution of repair and thereby limiting the fibrotic repair process (149). Multiple lines of evidence show that IPF fibroblasts are resistant to apoptosis, mediated by downregulation of the cell death receptor Fas and upregulation of antiapoptotic genes (150). SIRT3, an important aging molecule, blocks fibroblast to myofibroblast differentiation and is decreased in IPF lungs (80).

Metabolic programming is an essential component of fibroblast health, as activated fibroblasts require augmented glycolysis and utilization of the serine-glycine synthesis pathway to produce sufficient collagen (151). Augmented glycolysis supports a profibrotic phenotype via secretion of lactate, which upregulates profibrotic macrophage genes via histone lactylation (152). Metabolic reprograming is mediated by TGF-β, leading to decreased activation of AMPK signaling (19, 153) and increased mTORC1 signaling, in turn, promoting apoptosis resistance and decreased autophagy in fibroblasts (84). In animal models, activation of AMPK by metformin attenuates fibrosis by stimulating autophagy and downregulating collagen production (154, 155), although human studies on metformin have so far been unsuccessful (156). Other mediators of apoptosis resistance include binding of type 1 collagen to surface receptors and activation of 3-phosphoinositide–dependent protein kinase 1/Akt (19), inhibition of ROCK (19), decreased expression of PTEN, and decreased expression of NRF2, a master regulator of the antioxidant response (157).

Metabolic reprogramming may also be critical for dedifferentiation of myofibroblasts in wound healing. During resolution of fibrosis in the murine bleomycin injury model, myofibroblasts dedifferentiate into lipofibroblasts, a process mediated by PPARγ activation of an adipocyte-like pattern of gene expression (132). In this model, active TGF-β signaling inhibits PPARγ, diminishing the

lipofibroblast dedifferentiation pathway and worsening fibrosis. Accordingly, histologic staining of IPF lungs shows a decreased amount of lipofibroblasts. Metformin, in an AMPK-independent fashion, can promote upregulation of BMP2 and phosphorylation of PPARγ (155), suggesting that this pathway may be a potential therapeutic avenue.

ENDOTHELIAL CELLS

Endothelial cells (ECs) have been implicated in IPF. Angiogenesis is a normal feature of wound repair, supplying cells and nutrients necessary for tissue healing. Early observations of fibrotic lung demonstrated vascular abnormalities, including pulmonary systemic anastomoses and neovascularization around areas of fibrosis (158). More detailed studies later clarified that while there is increased vascularity surrounding fibrotic areas, vascularity is decreased within the fibrotic foci, with large, dilated vessels in areas of honeycombing (159–161). The lack of vessels in areas of fibrosis leads to increased pulmonary vascular resistance and ultimately PH. Indeed, PH is present in 8–15% of patients with IPF and up to 60% of those with end-stage disease and is associated with increased mortality (162). It remains unclear whether these vascular abnormalities contribute to fibrosis or constitute an adaptive response to fibrosis. Several mechanisms have been proposed for abnormal vascular patterns in IPF, including consequences of hypoxic vasoconstriction, microvascular injury due to antibodies against the endothelium (163), decreased circulating endothelial progenitor cells, and imbalances in angiogenic or angiostatic factors. Consistent with the pattern of bronchiolization of the distal airways in IPF, scRNA-seq identified a population of COL15A1 peribronchial proximal airway ECs aberrantly localized to fibroblastic foci in distal airways (45). Thus, changes to the endothelium likely occur alongside more global changes to the cellular niche.

Whether or not the abnormal vasculature in IPF is a cause or result of fibrosis, there is a clear imbalance in angiogenic versus angiostatic factors, overall favoring angiogenesis. Consistent with decreased vascularity of fibroblastic foci, there is overexpression of angiostatic factors such as pigment epithelial-derived factor (PEDF) and minimal expression of angiogenic factors such as VEGF within these regions (161). In contrast, there is increased VEGF and IL-8, another angiogenic factor, within capillary ECs and AT2 cells of regions in IPF lungs spared fibrosis (160). VEGF is one of the key mediators of angiogenesis and may contribute to fibrosis by inhibiting EC apoptosis while promoting EC proliferation, migration, and differentiation. The data from patients with IPF are conflicting, as studies have shown both increases and decreases in serum VEGF compared to control populations (164, 165). In IPF lung and BAL, VEGF is reduced (160, 165), whereas overexpression of VEGF in bleomycin injury models reduces fibrosis (165). This remains controversial, as there is evidence that TGF-β and HIF-1α, which are upregulated in IPF, induce VEGF transcription (98), and one of the proposed mechanisms of action of nintedanib is via VEGF inhibition. Another important angiogenic factor is endothelin-1 (ET-1), which induces vasoconstriction and vascular smooth muscle cell growth. ET-1 expression is increased in IPF (166) and PH (167). Although antagonists are used in PH, clinical trials of endothelin receptor antagonists have not shown success in IPF patients with early-stage disease (168). Other angiogenic factors include PDGF, which is required for angiogenesis; TGF-β, which induces proliferation of vascular smooth muscle cells (169, 170); FGF-2; MMPs; hyaluronic acid; and chemokines such as IL-8 (171).

Angiostatic factors include PEDF, which inhibits VEGF and induces EC apoptosis and is elevated in fibroblastic foci and increased in BAL (160), and endostatin, which is elevated in the serum of IPF and inhibits angiogenesis (172). Elevation of angiostatic factors in IPF may be a compensatory response to increased angiogenesis. The overall imbalance in angiogenesis results

in microvascular remodeling, and there is evidence that ECs differentiate into fibroblasts through the process of endothelial-to-mesenchymal transition (EndMT), akin to EMT as discussed above. This phenomenon is more well-studied in PH, although there is evidence in the bleomycin model that EndMT is induced by TGF-β and Ras/mitogen-activated protein kinase signaling (173).

IMMUNE CELLS

IPF was initially described as a disease driven by inflammation (174). Subsequent clinical trials of immunosuppressive agents failed to show benefit and even showed harm (175). However, the inflammatory response, including all facets of both the innate and adaptive immune systems, is involved in nearly all forms of wound healing and fibrosis (176). Given the presence of fibrosis in IPF and that the immune system is central to normal wound healing, there is naturally a profibrotic inflammatory milieu in IPF. Accordingly, many studies have demonstrated a shift toward a profibrotic cellular inflammatory response in IPF with a predominance of M2 macrophages and Th2 lymphocytes. Altered inflammasome regulation may contribute to macrophage inflammatory responses. A central observation has been alteration of the alveolar macrophage (AM) population in IPF. Data from scRNA-seq have highlighted a role for monocyte-derived AMs in propagating fibrosis via release of inflammatory mediators within a dysregulated cellular niche. Furthermore, GWAS have identified mutations in genes related to innate immune function, which may predispose certain individuals to pulmonary fibrosis (177). While current investigations focus on deciphering the mechanisms underlying this response, it will be crucial to determine the degree to which the inflammatory response is causative of fibrosis and if there exist specific, targetable inflammatory mechanisms for development of therapeutics. Here we discuss the contributions of general innate immune mechanisms as well as different inflammatory cell types to IPF pathogenesis.

Damage-Associated Molecular Patterns and the Inflammasome

Substances released from damaged tissue or cells, known as damage-associated molecular patterns (DAMPs), can trigger a profibrotic inflammatory response. In IPF, DAMPs may be released from apoptotic or injured AECs. TLRs bind DAMPs and trigger downstream inflammatory responses. Mutations in TLRs, as well as regulators of TLRs, have been identified in GWAS of IPF (178). TLR4 is important for normal repair of AECs (179). A polymorphism in *TLR3* leading to defective TLR3 function is associated with mortality and lung function decline in IPF (180). Both thrombin and high mobility group box1 protein, a TLR4 agonist, are elevated in BAL of IPF patients (181, 182). Thrombin activates fibrinogen, a DAMP, which signals through TLR4 signaling (183). Furthermore, murine models of mice lacking TLR2 and TLR4 have increased fibrosis in the bleomycin injury model (179).

The innate immune system responds to both endogenous and exogenous particles via activation of inflammasomes. Inflammasomes are cytosolic multimeric protein complexes that sense potentially dangerous particles and trigger an immune response by regulating the maturation and secretion of proinflammatory cytokines (i.e., IL-1β and IL-18). The inflammasomes predominantly reside in macrophages and other immune cells, but other noninflammatory cell types may also express inflammasome components.

The most studied inflammasome in fibrosis is the NLRP3-dependent inflammasome, which recognizes pathogen-associated molecular patterns and DAMPs. Fibrotic lung injury mediated by exogenous substances, such as asbestosis, silicosis, and bleomycin, is associated with inflammasome activation (184, 185). The NLRP3 inflammasome can promote fibrosis via pathways

involving TGF-β1 and EMT (186). NLRP3 inflammasome activation in fibrosis may be exacerbated by deficits in aging and metabolism, including autophagy deficiency and increased mtROS production (187). In cancer models, the inflammasome regulates the release of SASPs and development of senescence (188). The inflammasome is also linked to metabolism via glycolysis, as dysregulated macrophage glycolysis activates the inflammasome (189, 190). The NLRP3 inflammasome localizes to both AMs and alveolar ECs in IPF and contributes to myofibroblast differentiation in vitro (184, 191). Inflammasome activation is impaired in macrophages from BAL of IPF patients (192). Pirfenidone antagonizes NLRP3 activation (193), indicating that the inflammasome may serve as a potential therapeutic drug target. The absent in melanoma-2 (AIM2) inflammasome, which binds pathogen or host double-stranded DNA, has also been implicated in IPF. Extracellular mtDNA correlates with mortality in IPF and can activate the AIM2 inflammasome, which is elevated in AMs in IPF and in the bleomycin injury model (194).

Macrophages and Monocytes

The normal lung contains distinct populations of macrophages: AMs that reside in the airways and interstitial macrophages in the tissue. In general, these lung-resident macrophages are capable of self-renewal (195, 196). In response to inflammation and injury, a pool of resident AMs persists, while a monocyte-derived population is recruited to assist in repair. As injury resolves, this monocyte-derived population typically declines and undergoes apoptosis (197). Lineage-tracing experiments in a murine model of fibrosis showed expansion of monocyte-derived AM pools expressing profibrotic genes. Deletion of this line of macrophages attenuated fibrosis, suggesting that they play a causal role in disease (198, 199). Furthermore, scRNA-seq data from a model of asbestos-induced lung fibrosis demonstrated that monocyte-derived AMs expressed profibrotic genes and localized to areas of fibrosis adjacent to fibroblasts and injured epithelial cells (200). The principal monocyte chemokine CCL2 is elevated in IPF (201), and elevated monocyte counts in IPF have been associated with worse outcomes (202). Together, these data support the idea that a monocyte-derived AM, which normally promotes wound repair, persists in IPF and contributes to the fibrotic niche.

During normal wound healing, macrophages release various cytokines [e.g., IL-1, IL-6, TNF-α, TGF-β, MMPs, and insulin-like growth factor 1 (IGF-1)] that regulate EC proliferation, fibroblast activation, angiogenesis, and ECM deposition to facilitate scar tissue formation (176). Macrophages are also involved in limiting fibrosis by secreting tissue inhibitors of MMPs (176). Previous work has distinguished two primary macrophage phenotypes. The M1 phenotype (classical activation) is triggered by lipopolysaccharide, TNF-α, and interferon-γ (IFN-γ) and is associated with inflammation. The M2 phenotype (alternative activation) is activated by IL-4, IL-10, granulocyte-macrophage colony-stimulating factor, and IL-13 and is generally profibrotic but is also involved in resolving inflammation. Monocytes are capable of differentiating into either phenotype based on the cytokine milieu. The profibrotic cytokine milieu of IPF may favor M2 populations, which secrete factors implicated in IPF, such as TGF-β, FGF, PDGFα, IGF-1, and VEGF, and can directly contribute to ECM growth (203).

Although there is evidence supporting the polarization of macrophages into M1 and M2 phenotypes, more recent work suggests that macrophages exist along a spectrum instead of in binary subtypes (128, 204). For example, in the bleomycin injury model, a granulocyte/monocyte hybrid subtype, rather than a specific M1 or M2 macrophage, was critical for fibrosis (204). However, several lines of evidence point to predominance of a profibrotic population in IPF. Mechanistically, profibrotic macrophages contribute to fibrosis by stimulating fibroblasts through release of

soluble factors (205, 206) and ECM remodeling (207). In BAL and serum of IPF and acute exacerbation of IPF, CCL18, a chemokine typically associated with the M2 phenotype, is upregulated. CCL18 stimulates fibroblasts in culture models (45, 128, 208), and levels of CCL18 correlate with mortality (209, 210). CCL18 recruits T lymphocytes and perpetuates a positive feedback loop with pulmonary fibroblasts to increase collagen production (211). Another indicator of M2 activation is elevation of chitinase-3 like-1 in IPF BAL, which is associated with worse prognosis (128). Macrophages also promote fibrosis through phagocytosis of apoptotic cells, termed efferocytosis, which activates TGF-β. Thus, targeting macrophages may represent one strategy to mitigate the worsening of fibrosis in IPF (212).

Results from scRNA-seq demonstrate an increase in secreted phosphoprotein-1 (SPP1)-expressing macrophages that localize to the fibroblastic foci (45, 71, 213). SPP1 has previously been implicated in organ fibrosis and may support macrophage proliferation (71, 213). Another population of airway macrophages lacking transferrin receptor and with increased expression of genes associated with fibrosis was identified in IPF (214). In the murine model, bleomycin injury leads to an increase in interstitial macrophages enriched in IL-1β expression. IL-1β maintains impaired generation of AT1 cells from the transitional progenitor epithelial cell (52), suggesting a link between profibrotic macrophages and dysregulated alveolar epithelial repair.

T Cells

The immune system uses distinct populations of T cells to respond to inflammation and fibrosis in health and disease. There are fewer immunomodulatory cells, such as T cells and B cells, overall in fibrotic compared to normal lung (215). However, there remains evidence for their involvement in IPF. In general, T helper 2 (Th2) cytokines promote fibrosis, whereas Th1 cytokines (e.g., IFN-γ and IL-12) promote inflammation. Th2 cytokines include IL-4, IL-5, IL-9, and IL-13 and are important for fibrosis across multiple disease states. Epithelial cells recruit T cells by secreting cytokines such as TGF-β, IL-1β, CXC, and CC, which induce migration of adaptive immune cells (216). Not surprisingly, most T cells found in IPF lungs tend toward the Th2 axis (215). Accordingly, early studies attempted to improve fibrosis by promoting the less fibrotic Th1 response. IL-12 may attenuate bleomycin-induced fibrosis in mice by stimulating IFN-γ (217); however, clinical trials of IFN-γ failed to show benefit (218). Profibrotic chemokines from Th2 cells (i.e., IL-13 and IL-4) promote differentiation toward myofibroblasts (219). A population of PD-1$^+$ CD4$^+$ cells express relatively higher levels of TGF-β and IL-17A in IPF and may promote fibrosis via signal transducer and activator of transcription 3 signaling (220). Other T cells, such as T regulatory cells, Th9, Th22, and γδT cells, have been associated with both anti- and profibrotic effects depending on the stage of inflammation and fibrosis (221).

Innate Lymphoid Cells

A recently identified class of profibrotic immune cells, the innate lymphoid cells (ILCs), are cells of lymphoid lineage that do not express T or B cell receptors (222, 223). Like other immune cells, there appear to be both anti- and profibrotic subtypes, with the ILC2 subtype secreting typical profibrotic mediators, including IL-4, IL-5, IL-9, and IL-13. Elevated ILC2 populations have been observed in IPF lungs (223). In a parasitic mouse model of pulmonary fibrosis, IL-25, which is elevated in lungs of patients with IPF, stimulated production of IL-13-expressing ILC2 cells (223). IL-33, another profibrotic cytokine released by macrophages and ECs (224) that is highly expressed in IPF, induces polarization and activation of ILC2 cells (and M2 macrophages), enhancing IL-13 and TGF-β production (225).

Neutrophils

Although neutrophils are rarely observed histologically in IPF lungs, several lines of evidence point to the involvement of neutrophils in IPF through release of profibrotic cytokines and ECM remodeling. For example, the presence of neutrophils in BAL of IPF patients predicts early mortality (226), and chemotactic factors for neutrophils (i.e., IL-8/CXCL8) are increased in IPF (227). Neutrophils secrete neutrophil elastase, which is increased in BAL, and deletion of this enzyme in mice attenuates fibrosis (221, 228). Neutrophils also secrete various MMPs, playing a role in ECM deposition and maintenance (221). Finally, neutrophils release neutrophil extracellular traps when responding to injury, which are associated with lung fibrosis and stimulate fibroblasts in an autophagy-dependent manner in vitro (229).

CONCLUSIONS AND FUTURE DIRECTIONS

Much progress has been made over the past two decades in defining the biochemical, cellular, and genetic mechanisms underlying the pathogenesis of IPF and related fibrotic disorders. While a dominant hypothesis has emerged suggesting that epithelial cell injury drives the disease process, leading to epithelial cell dysfunction and transformation in the alveolar niche, it is increasingly clear that diverse cell types may also contribute to IPF pathogenesis via paracrine signaling events and intracellular cross talk. The biochemical events leading to cellular dysfunction in individual cell types are complex and involve multiple elements. Mitochondrial dysfunction involving mtROS generation and leakage of mtDNA into the cytoplasmic and extracellular compartments has emerged as a key event in epithelial cell dysfunction. These events may be associated with perturbations in mitochondrial control mechanisms, such as mitochondrial dynamics, mitophagy, and mitochondrial biogenesis. Furthermore, ER stress and proteostasis disequilibrium may play cardinal roles in cellular injury, leading to the accumulation of toxic protein aggregates. Autophagy, which is dysregulated or impaired in IPF, serves a vital function in mitigating cellular damage by scavenging dysfunctional mitochondria and protein aggregates. Emerging concepts include a key role for epithelial, fibroblast, and immune cell senescence in the pathogenic process and as a final regulatory target for cellular dysregulation in mitochondrial and proteostatic pathways. Enhanced DNA damage via mtROS, telomere instability, and ER stress are potential contributors to initiating senescence pathways.

In summary, much has been learned about IPF pathogenesis from reductionist cell biology approaches and in vivo modeling. Despite these advances, a holistic picture of the complex systemic interactions by which multiple cell types interact to promote IPF pathogenesis remains lacking. The emerging bioinformatics and systems biology revolution has yielded powerful new tools to fuel insights into the complex array of genetic perturbations that may be associated with disease progression across multiple cell types. Among these, scRNA-seq approaches have provided a novel approach to survey transcriptomics profiles in multiple cell types at once, generating a comparative overview of transcriptomics changes that may be associated with disease and thus providing signatures of disease progression. Connectivity analyses of scRNA-seq data provide a further methodological approach to uncover, to a degree not previously possible with conventional model systems, novel cell-cell interactions between different cell types that may converge to drive disease pathogenesis (**Figure 2**). The data presented in **Figure 2** are just one example of the types of analyses and discovery of intracellular interactions that are becoming possible with novel analytical methods. It should be noted that scRNA-seq and connectome analyses can be useful for hypothesis generation and identification of novel cell-cell interactions, but such data ultimately require validation using gene and protein expression measurements and

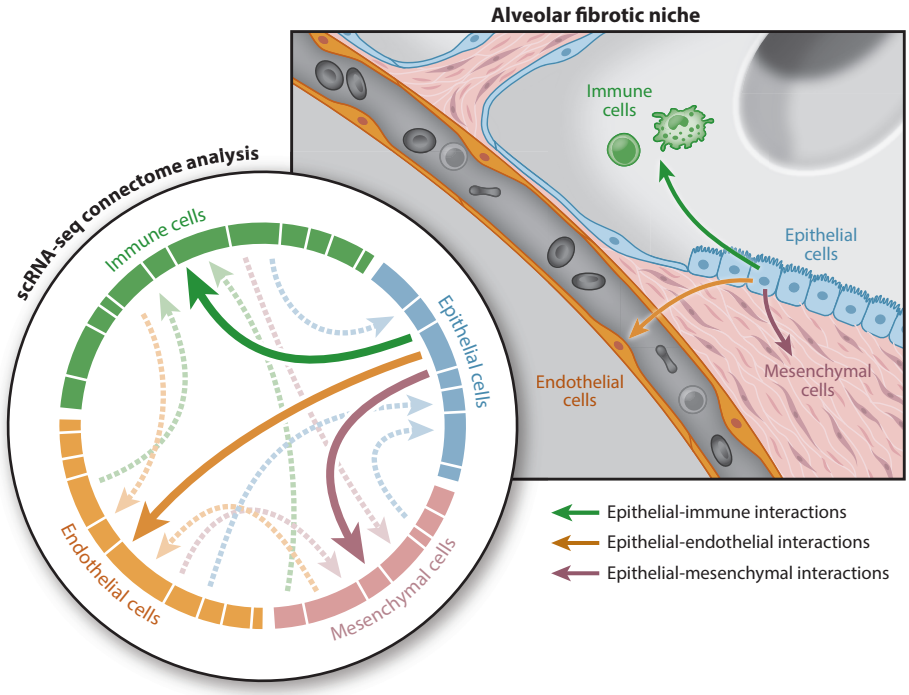

Figure 2

Connectome analyses from scRNA-seq can be used to discover novel cell-cell regulatory interactions associated with fibrogenesis in the alveolar niche. A representative connectome map of the fibrotic niche depicts pairing between effector and target (ligand and receptor) gene regulation. For illustrative purposes, hypothetical epithelial cell interactions with other cell types are featured in the niche diagram and highlighted (*bold arrows*) in the connectome map, although interactions between other cell types may also be uncovered by these types of analyses (*dotted arrows*). These data are provided for exemplary purposes; scRNA-seq data of this nature are useful for hypothesis building, but further validation of the findings is required. Such validation may include RT-PCR and proteomics confirmation of gene and protein expression as well as biochemical and cellular assays to confirm the observed cellular interactions. Currently, epithelial-mesenchymal (including fibroblasts) interactions represent the most common type of cell-cell interaction proposed in fibrogenesis. Epithelial cell dysfunction may also affect other target cell types, including endothelial and immune cells. Dysregulated immune cells, such as macrophages, may engage in cross talk with alveolar epithelial cells, mesenchymal cells, and endothelial cells. Thus, scRNA-seq data are able to inform cell-cell interactions implicated in IPF pathogenesis to uncover novel paracrine effects and specific ligand-receptor interactions. Abbreviations: IPF, idiopathic pulmonary fibrosis; RT-PCR, reverse transcription polymerase chain reaction; scRNA-seq, single-cell RNA sequencing.

cell-based assays. Further progress in this area will likely inform novel therapeutic approaches in IPF.

FUTURE ISSUES

1. Additional rare genetic variants need to be identified, and the functional significance of already identified gene variants should be fully defined.

2. The cellular interactions and cross talk within the fibrotic alveolar niche need to be better defined.
3. Researchers should develop a systems-level approach by integrating large data sets, including single-cell technologies, epigenomics, proteomics, metabolomics, and lipidomics.
4. In silico drug discovery methods, such as connectivity map analysis, should be used to rapidly screen for novel therapeutics.
5. The use of novel idiopathic pulmonary fibrosis (IPF) models, such as organoids and ex vivo lung slices, needs to be expanded to elucidate IPF biology.
6. Researchers need to identify the underlying susceptibilities for high-risk populations and develop means of prevention and monitoring for disease progression.

DISCLOSURE STATEMENT

I.O.R. is a consultant for Boehringer Ingelheim, Roche/Genentech, Immunomet, and the Three Lakes Foundation.

LITERATURE CITED

1. Leslie KO. 2012. Idiopathic pulmonary fibrosis may be a disease of recurrent, tractional injury to the periphery of the aging lung: a unifying hypothesis regarding etiology and pathogenesis. *Arch. Pathol. Lab. Med.* 136:591–600
2. Wu H, Yu Y, Huang H, Hu Y, Fu S, et al. 2020. Progressive pulmonary fibrosis is caused by elevated mechanical tension on alveolar stem cells. *Cell* 180:107–21.e17
3. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, et al. 2011. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am. J. Respir. Crit. Care Med.* 183:788–824
4. Miller ER, Putman RK, Vivero M, Hung Y, Araki T, et al. 2018. Histopathology of interstitial lung abnormalities in the context of lung nodule resections. *Am. J. Respir. Crit. Care Med.* 197:955–58
5. Putman RK, Rosas IO, Hunninghake GM. 2014. Genetics and early detection in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 189:770–78
6. Travis WD, Costabel U, Hansell DM, King TE Jr., Lynch DA, et al. 2013. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am. J. Respir. Crit. Care Med.* 188:733–48
7. Strongman H, Kausar I, Maher TM. 2018. Incidence, prevalence, and survival of patients with idiopathic pulmonary fibrosis in the UK. *Adv. Ther.* 35:724–36
8. Ley B, Collard HR, King TE Jr. 2011. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 183:431–40
9. Hutchinson J, Fogarty A, Hubbard R, McKeever T. 2015. Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. *Eur. Respir. J.* 46:795–806
10. Raghu G, Chen SY, Yeh WS, Maroni B, Li Q, et al. 2014. Idiopathic pulmonary fibrosis in US Medicare beneficiaries aged 65 years and older: incidence, prevalence, and survival, 2001–11. *Lancet Respir. Med.* 2:566–72
11. King TE Jr., Tooze JA, Schwarz MI, Brown KR, Cherniack RM. 2001. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am. J. Respir. Crit. Care Med.* 164:1171–81
12. Mannino DM, Etzel RA, Parrish RG. 1996. Pulmonary fibrosis deaths in the United States, 1979–1991. An analysis of multiple-cause mortality data. *Am. J. Respir. Crit. Care Med.* 153:1548–52

13. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. 2006. Incidence and prevalence of idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 174:810–16
14. Br. Lung Found. 2021. Lung disease in the UK. *British Lung Foundation.* **https://statistics.blf.org.uk/**
15. Jeganathan N, Smith RA, Sathananthan M. 2021. Mortality trends of idiopathic pulmonary fibrosis in the United States from 2004 through 2017. *Chest* 159(1):228–38
16. Guenther A, Krauss E, Tello S, Wagner J, Paul B, et al. 2018. The European IPF registry (eurIPFreg): baseline characteristics and survival of patients with idiopathic pulmonary fibrosis. *Respir. Res.* 19:141
17. Zurkova M, Kriegova E, Kolek V, Lostakova V, Sterclova M, et al. 2019. Effect of pirfenidone on lung function decline and survival: 5-yr experience from a real-life IPF cohort from the Czech EMPIRE registry. *Respir. Res.* 20:16
18. Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, et al. 2017. Idiopathic pulmonary fibrosis. *Nat. Rev. Dis. Primers* 3:17074
19. Sgalla G, Kulkarni T, Antin-Ozerkis D, Thannickal VJ, Richeldi L. 2019. Update in pulmonary fibrosis 2018. *Am. J. Respir. Crit. Care Med.* 200:292–300
20. Vaz M, Hwang SY, Kagiampakis I, Phallen J, Patil A, et al. 2017. Chronic cigarette smoke-induced epigenomic changes precede sensitization of bronchial epithelial cells to single-step transformation by *KRAS* mutations. *Cancer Cell* 32:360–76.e6
21. Tzouvelekis A, Kaminski N. 2015. Epigenetics in idiopathic pulmonary fibrosis. *Biochem. Cell. Biol.* 93:159–70
22. Moore BB, Moore TA. 2015. Viruses in idiopathic pulmonary fibrosis: etiology and exacerbation. *Ann. Am. Thorac. Soc.* 12(Suppl. 2):S186–92
23. Sheng G, Chen P, Wei Y, Yue H, Chu J, et al. 2020. Viral infection increases the risk of idiopathic pulmonary fibrosis: a meta-analysis. *Chest* 157:1175–87
24. Kropski JA, Pritchett JM, Zoz DF, Crossno PF, Markin C, et al. 2015. Extensive phenotyping of individuals at risk for familial interstitial pneumonia reveals clues to the pathogenesis of interstitial lung disease. *Am. J. Respir. Crit. Care Med.* 191:417–26
25. Sack C, Raghu G. 2019. Idiopathic pulmonary fibrosis: unmasking cryptogenic environmental factors. *Eur. Respir. J.* 53(2):1801699
26. Lipinski JH, Moore BB, O'Dwyer DN. 2020. The evolving role of the lung microbiome in pulmonary fibrosis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 319:L675–82
27. O'Dwyer DN, Garantziotis S. 2021. The lung microbiome in health, hypersensitivity pneumonitis, and idiopathic pulmonary fibrosis: a heavy bacterial burden to bear. *Am. J. Respir. Crit. Care Med.* 203(3):281–83
28. Loyd JE. 2003. Pulmonary fibrosis in families. *Am. J. Respir. Cell Mol. Biol.* 29:S47–50
29. Fernandez BA, Fox G, Bhatia R, Sala E, Noble B, et al. 2012. A Newfoundland cohort of familial and sporadic idiopathic pulmonary fibrosis patients: clinical and genetic features. *Respir. Res.* 13:64

30. Identification of the *MUC5B* polymorphism, the most common genetic variant in IPF.

30. Seibold MA, Wise AL, Speer MC, Steele MP, Brown KK, et al. 2011. A common *MUC5B* promoter polymorphism and pulmonary fibrosis. *N. Engl. J. Med.* 364:1503–12

31. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, et al. 2013. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. *Nat. Genet.* 45:613–20
32. Hao Y, Bates S, Mou H, Yun JH, Pham B, et al. 2020. Genome-wide association study: functional variant rs2076295 regulates desmoplakin expression in airway epithelial cells. *Am. J. Respir. Crit. Care Med.* 202:1225–36
33. Leavy OC, Ma SF, Molyneaux PL, Maher TM, Oldham JM, et al. 2021. Proportion of idiopathic pulmonary fibrosis risk explained by known common genetic loci in European populations. *Am. J. Respir. Crit. Care Med.* 203(6):775–78
34. Allen RJ, Guillen-Guio B, Oldham JM, Ma SF, Dressen A, et al. 2020. Genome-wide association study of susceptibility to idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 201:564–74
35. Oldham JM, Ma SF, Martinez FJ, Anstrom KJ, Raghu G, et al. 2015. *TOLLIP*, *MUC5B*, and the response to *N*-acetylcysteine among individuals with idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 192:1475–82

36. Jenkins PG, Moore BB, Chambers RC, Eickelberg O, Königshoff M, et al. 2017. An official American Thoracic Society workshop report: use of animal models for the preclinical assessment of potential therapies for pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 56(5):667–79
37. Carrington R, Jordan S, Pitchford SC, Page CP. 2018. Use of animal models in IPF research. *Pulm. Pharmacol. Ther.* 51:73–78
38. Moore BB, Hogaboam CM. 2008. Murine models of pulmonary fibrosis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 294(2):L152–60
39. Baron RM, Choi AJS, Owen CA, Choi AMK. 2012. Genetically manipulated mouse models of lung disease: potential and pitfalls. *Am. J. Physiol. Lung Cell Mol. Physiol.* 302(6):L485–97
40. Tashiro J, Rubio GA, Limper AH, Williams K, Elliot SJ, et al. 2017. Exploring animal models that resemble idiopathic pulmonary fibrosis. *Front. Med.* 4:118
41. Redente EF, Black BP, Backos DS, Bahadur AN, Humphries SM, et al. 2021. Persistent, progressive pulmonary fibrosis and epithelial remodeling in mice. *Am. J. Respir. Cell Mol. Biol.* 64(6):669–76
42. Sueblinvong V, Neujahr DC, Mills ST, Roser-Page S, Ritzenthaler JD, et al. 2012. Predisposition for disrepair in the aged lung. *Am. J. Med. Sci.* 344(1):41–51
43. Yao C, Guan X, Carraro G, Parimon T, Liu X, et al. 2021. Senescence of alveolar type 2 cells drives progressive pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 203(6):707–17
44. Barkauskas CE, Cronce MJ, Rackley CR, Bowie EJ, Keene DR, et al. 2013. Type 2 alveolar cells are stem cells in adult lung. *J. Clin. Investig.* 123:3025–36
45. Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, et al. 2020. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci. Adv.* 6(28):eaba1983
46. Habermann AC, Gutierrez AJ, Bui LT, Yahn SL, Winters NI, et al. 2020. Single-cell RNA sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. *Sci. Adv.* 6(28):eaba1972
47. Chilosi M, Poletti V, Murer B, Lestani M, Cancellieri A, et al. 2002. Abnormal re-epithelialization and lung remodeling in idiopathic pulmonary fibrosis: the role of ΔN-p63. *Lab. Investig.* 82:1335–45
48. Smirnova NF, Schamberger AC, Nayakanti S, Hatz R, Behr J, Eickelberg O. 2016. Detection and quantification of epithelial progenitor cell populations in human healthy and IPF lungs. *Respir. Res.* 17:83
49. Schruf E, Schroeder V, Le HQ, Schönberger T, Raedel D, et al. 2020. Recapitulating idiopathic pulmonary fibrosis related alveolar epithelial dysfunction in a human iPSC-derived air-liquid interface model. *FASEB J.* 34:7825–46
50. Kobayashi Y, Tata A, Konkimalla A, Katsura H, Lee RF, et al. 2020. Persistence of a regeneration-associated, transitional alveolar epithelial cell state in pulmonary fibrosis. *Nat. Cell Biol.* 22:934–46
51. Strunz M, Simon LM, Ansari M, Kathiriya JJ, Angelidis I, et al. 2020. Alveolar regeneration through a Krt8+ transitional stem cell state that persists in human lung fibrosis. *Nat. Commun.* 11:3559
52. Choi J, Park JE, Tsagkogeorga G, Yanagita M, Koo BK, et al. 2020. Inflammatory signals induce AT2 cell-derived damage-associated transient progenitors that mediate alveolar regeneration. *Cell Stem Cell* 27:366–82.e7
53. Carraro G, Mulay A, Yao C, Mizuno T, Konda B, et al. 2020. Single cell reconstruction of human basal cell diversity in normal and IPF lung. *Am. J. Respir. Crit. Care Med.* 202(11):1540–50
54. Danopoulos S, Alonso I, Thornton ME, Grubbs BH, Bellusci S, et al. 2018. Human lung branching morphogenesis is orchestrated by the spatiotemporal distribution of ACTA2, SOX2, and SOX9. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 314:L144–49
55. Bharat A, Querrey M, Markov NS, Kim S, Kurihara C, et al. 2020. Lung transplantation for patients with severe COVID-19. *Sci. Transl. Med.* 12(574):eabe4282
56. Korfei M, MacKenzie B, Meiners S. 2020. The ageing lung under stress. *Eur. Respir. Rev.* 29(156):200126
57. Alder JK, Barkauskas CE, Limjunyawong N, Stanley SE, Kembou F, et al. 2015. Telomere dysfunction causes alveolar stem cell failure. *PNAS* 112:5099–104
58. Mushiroda T, Wattanapokayakit S, Takahashi A, Nukiwa T, Kudoh S, et al. 2008. A genome-wide association study identifies an association of a common variant in TERT with susceptibility to idiopathic pulmonary fibrosis. *J. Med. Genet.* 45:654–56

45. The largest single-cell atlas of IPF, characterizing unique cell populations, including aberrant AECs.

59. Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, et al. 2007. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 356:1317–26

59. First identification of the role of telomere mutations in fibrotic lung disease pathogenesis.

60. Naikawadi RP, Disayabutr S, Mallavia B, Donne ML, Green G, et al. 2016. Telomere dysfunction in alveolar epithelial cells causes lung remodeling and fibrosis. *JCI Insight* 1:e86704
61. Morla M, Busquets X, Pons J, Sauleda J, MacNee W, Agusti AG. 2006. Telomere shortening in smokers with and without COPD. *Eur. Respir. J.* 27:525–28
62. Fois AG, Paliogiannis P, Sotgia S, Mangoni AA, Zinellu E, et al. 2018. Evaluation of oxidative stress biomarkers in idiopathic pulmonary fibrosis and therapeutic applications: a systematic review. *Respir. Res.* 19:51
63. Tilstra JS, Robinson AR, Wang J, Gregg SQ, Clauson CL, et al. 2012. NF-κB inhibition delays DNA damage–induced senescence and aging in mice. *J. Clin. Investig.* 122:2601–12
64. Zhao J, Zhang L, Lu A, Han Y, Colangelo D, et al. 2020. ATM is a key driver of NF-κB-dependent DNA-damage-induced senescence, stem cell dysfunction and aging. *Aging* 12:4688–710
65. Nicolae CM, O'Connor MJ, Constantin D, Moldovan GL. 2018. NFκB regulates p21 expression and controls DNA damage-induced leukemic differentiation. *Oncogene* 37:3647–56
66. Tian Y, Li H, Qiu T, Dai J, Zhang Y, et al. 2019. Loss of PTEN induces lung fibrosis via alveolar epithelial cell senescence depending on NF-κB activation. *Aging Cell* 18:e12858
67. Tsoyi K, Liang X, De Rossi G, Ryter SW, Xiong K, et al. 2021. CD148 deficiency in fibroblasts promotes the development of pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 204(3):312–25
68. Lehmann M, Korfei M, Mutze K, Klee S, Skronska-Wasek W, et al. 2017. Senolytic drugs target alveolar epithelial cell function and attenuate experimental lung fibrosis ex vivo. *Eur. Respir. J.* 50(2):1602367
69. Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, et al. 2017. Cellular senescence mediates fibrotic pulmonary disease. *Nat. Commun.* 8:14532
70. Hohmann MS, Habiel DM, Coelho AL, Verri WA Jr., Hogaboam CM. 2019. Quercetin enhances ligand-induced apoptosis in senescent idiopathic pulmonary fibrosis fibroblasts and reduces lung fibrosis in vivo. *Am. J. Respir. Cell Mol. Biol.* 60:28–40
71. Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, et al. 2019. Single-cell transcriptomic analysis of human lung provides insights into the pathobiology of pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 199:1517–36
72. Mora AL, Bueno M, Rojas M. 2017. Mitochondria in the spotlight of aging and idiopathic pulmonary fibrosis. *J. Clin. Investig.* 127:405–14
73. Correia-Melo C, Marques FD, Anderson R, Hewitt G, Hewitt R, et al. 2016. Mitochondria are required for pro-ageing features of the senescent phenotype. *EMBO J.* 35:724–42

74. Key demonstration of the importance of age-related mitochondrial dysfunction and defective mitophagy in IPF pathogenesis.

74. Bueno M, Lai YC, Romero Y, Brands J, St Croix CM, et al. 2015. PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. *J. Clin. Investig.* 125:521–38

75. Sahin E, Colla S, Liesa M, Moslehi J, Muller FL, et al. 2011. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature* 470:359–65
76. Summer R, Shaghaghi H, Schriner D, Roque W, Sales D, et al. 2019. Activation of the mTORC1/PGC-1 axis promotes mitochondrial biogenesis and induces cellular senescence in the lung epithelium. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 316:L1049–60
77. Patel AS, Song JW, Chu SG, Mizumura K, Osorio JC, et al. 2015. Epithelial cell mitochondrial dysfunction and PINK1 are induced by transforming growth factor-beta1 in pulmonary fibrosis. *PLOS ONE* 10:e0121246
78. Jiang D, Cui H, Xie N, Banerjee S, Liu RM, et al. 2020. ATF4 mediates mitochondrial unfolded protein response in alveolar epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 63:478–89
79. Bueno M, Zank D, Buendia-Roldán I, Fiedler K, Mays BG, et al. 2019. PINK1 attenuates mtDNA release in alveolar epithelial cells and TLR9 mediated profibrotic responses. *PLOS ONE* 14:e0218003
80. Mazumder S, Barman M, Bandyopadhyay U, Bindu S. 2020. Sirtuins as endogenous regulators of lung fibrosis: a current perspective. *Life Sci.* 258:118201
81. Kim SJ, Cheresh P, Williams D, Cheng Y, Ridge K, et al. 2014. Mitochondria-targeted Ogg1 and aconitase-2 prevent oxidant-induced mitochondrial DNA damage in alveolar epithelial cells. *J. Biol. Chem.* 289:6165–76

82. Kadota T, Yoshioka Y, Fujita Y, Araya J, Minagawa S, et al. 2020. Extracellular vesicles from fibroblasts induce epithelial cell senescence in pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 63(5):623–36
83. Patel AS, Lin L, Geyer A, Haspel JA, An CH, et al. 2012. Autophagy in idiopathic pulmonary fibrosis. *PLOS ONE* 7:e41394
84. Romero Y, Bueno M, Ramirez R, Alvarez D, Sembrat JC, et al. 2016. mTORC1 activation decreases autophagy in aging and idiopathic pulmonary fibrosis and contributes to apoptosis resistance in IPF fibroblasts. *Aging Cell* 15:1103–12
85. Hill C, Li J, Liu D, Conforti F, Brereton CJ, et al. 2019. Autophagy inhibition-mediated epithelial–mesenchymal transition augments local myofibroblast differentiation in pulmonary fibrosis. *Cell Death Dis.* 10:591
86. Nureki SI, Tomer Y, Venosa A, Katzen J, Russo SJ, et al. 2018. Expression of mutant *Sftpc* in murine alveolar epithelia drives spontaneous lung fibrosis. *J. Clin. Investig.* 128:4008–24
87. Balch WE, Sznajder JI, Budinger S, Finley D, Laposky AD, et al. 2014. Malfolded protein structure and proteostasis in lung diseases. *Am. J. Respir. Crit. Care Med.* 189:96–103
88. Romero F, Summer R. 2017. Protein folding and the challenges of maintaining endoplasmic reticulum proteostasis in idiopathic pulmonary fibrosis. *Ann. Am. Thorac. Soc.* 14:S410–13
89. Lawson WE, Crossno PF, Polosukhin VV, Roldan J, Cheng DS, et al. 2008. Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 294:L1119–26
90. Korfei M, Ruppert C, Mahavadi P, Henneke I, Markart P, et al. 2008. Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 178:838–46
91. Korfei M, von der Beck D, Henneke I, Markart P, Ruppert C, et al. 2013. Comparative proteome analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP) and organ donors. *J. Proteom.* 85:109–28
92. Jorgensen E, Stinson A, Shan L, Yang J, Gietl D, Albino AP. 2008. Cigarette smoke induces endoplasmic reticulum stress and the unfolded protein response in normal and malignant human lung cells. *BMC Cancer* 8:229
93. Kabore AF, Wang WJ, Russo SJ, Beers MF. 2001. Biosynthesis of surfactant protein C: characterization of aggresome formation by EGFP chimeras containing propeptide mutants lacking conserved cysteine residues. *J. Cell Sci.* 114:293–302
94. Thomas AQ, Lane K, Phillips J III, Prince M, Markin C, et al. 2002. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. *Am. J. Respir. Crit. Care Med.* 165:1322–28
95. Borok Z, Horie M, Flodby P, Wang H, Liu Y, et al. 2020. *Grp78* loss in epithelial progenitors reveals an age-linked role for endoplasmic reticulum stress in pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 201:198–211
96. Tat V, Ayaub EA, Ayoub A, Vierhout M, Naiel S, et al. 2020. FK506-binding protein 13 expression is upregulated in interstitial lung disease and correlated with clinical severity: a potentially protective role. *Am. J. Respir. Cell Mol. Biol.* 64(2):235–46
97. Delbrel E, Soumare A, Naguez A, Label R, Bernard O, et al. 2018. HIF-1α triggers ER stress and CHOP-mediated apoptosis in alveolar epithelial cells, a key event in pulmonary fibrosis. *Sci. Rep.* 8:17939
98. Tzouvelekis A, Harokopos V, Paparountas T, Oikonomou N, Chatziioannou A, et al. 2007. Comparative expression profiling in pulmonary fibrosis suggests a role of hypoxia-inducible factor-1α in disease pathogenesis. *Am. J. Respir. Crit. Care Med.* 176:1108–19
99. Kropski JA, Blackwell TS. 2018. Endoplasmic reticulum stress in the pathogenesis of fibrotic disease. *J. Clin. Investig.* 128:64–73
100. Chu SG, Villalba JA, Liang X, Xiong K, Tsoyi K, et al. 2019. Palmitic acid–rich high-fat diet exacerbates experimental pulmonary fibrosis by modulating endoplasmic reticulum stress. *Am. J. Respir. Cell Mol. Biol.* 61:737–46
101. Uhal BD, Joshi I, Hughes WF, Ramos C, Pardo A, Selman M. 1998. Alveolar epithelial cell death adjacent to underlying myofibroblasts in advanced fibrotic human lung. *Am. J. Physiol.* 275:L1192–99
102. Plataki M, Koutsopoulos AV, Darivianaki K, Delides G, Siafakas NM, Bouros D. 2005. Expression of apoptotic and antiapoptotic markers in epithelial cells in idiopathic pulmonary fibrosis. *Chest* 127:266–74

83. One of the first studies demonstrating impaired autophagy in IPF.

103. Günther A, Korfei M, Mahavadi P, von der Beck D, Ruppert C, Markart P. 2012. Unravelling the progressive pathophysiology of idiopathic pulmonary fibrosis. *Eur. Respir. Rev.* 21:152–60
104. Fernandez IE, Eickelberg O. 2012. The impact of TGF-β on lung fibrosis: from targeting to biomarkers. *Proc. Am. Thorac. Soc.* 9:111–16
105. Lee JM, Yoshida M, Kim MS, Lee JH, Baek AR, et al. 2018. Involvement of alveolar epithelial cell necroptosis in idiopathic pulmonary fibrosis pathogenesis. *Am. J. Respir. Cell Mol. Biol.* 59:215–24
106. Maguire JA, Mulugeta S, Beers MF. 2011. Endoplasmic reticulum stress induced by surfactant protein C BRICHOS mutants promotes proinflammatory signaling by epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 44:404–14
107. Zhong Q, Zhou B, Ann DK, Minoo P, Liu Y, et al. 2011. Role of endoplasmic reticulum stress in epithelial–mesenchymal transition of alveolar epithelial cells: effects of misfolded surfactant protein. *Am. J. Respir. Cell Mol. Biol.* 45:498–509
108. Tanjore H, Cheng DS, Degryse AL, Zoz DF, Abdolrasulnia R, et al. 2011. Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress. *J. Biol. Chem.* 286:30972–80
109. Yao Y, Wang Y, Zhang Z, He L, Zhu J, et al. 2016. Chop deficiency protects mice against bleomycin-induced pulmonary fibrosis by attenuating M2 macrophage production. *Mol. Ther.* 24:915–25
110. Chen G, Ribeiro CMP, Sun L, Okuda K, Kato T, et al. 2019. XBP1S regulates MUC5B in a promoter variant–dependent pathway in idiopathic pulmonary fibrosis airway epithelia. *Am. J. Respir. Crit. Care Med.* 200:220–34
111. Mucenski ML, Nation JM, Thitoff AR, Besnard V, Xu Y, et al. 2005. β-Catenin regulates differentiation of respiratory epithelial cells in vivo. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 289:L971–79
112. Nabhan AN, Brownfield DG, Harbury PB, Krasnow MA, Desai TJ. 2018. Single-cell Wnt signaling niches maintain stemness of alveolar type 2 cells. *Science* 359:1118–23
113. Königshoff M, Balsara N, Pfaff EM, Kramer M, Chrobak I, et al. 2008. Functional Wnt signaling is increased in idiopathic pulmonary fibrosis. *PLOS ONE* 3:e2142
114. Königshoff M, Kramer M, Balsara N, Wilhelm J, Amarie OV, et al. 2009. WNT1-inducible signaling protein-1 mediates pulmonary fibrosis in mice and is upregulated in humans with idiopathic pulmonary fibrosis. *J. Clin. Investig.* 119:772–87
115. Aumiller V, Balsara N, Wilhelm J, Günther A, Königshoff M. 2013. WNT/β-catenin signaling induces IL-1β expression by alveolar epithelial cells in pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 49:96–104
116. Xu K, Nieuwenhuis E, Cohen BL, Wang W, Canty AJ, et al. 2010. Lunatic Fringe-mediated Notch signaling is required for lung alveogenesis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 298:L45–56
117. Parimon T, Yao C, Stripp BR, Noble PW, Chen P. 2020. Alveolar epithelial type II cells as drivers of lung fibrosis in idiopathic pulmonary fibrosis. *Int. J. Mol. Sci.* 21(7):2269
118. Vaughan AE, Brumwell AN, Xi Y, Gotts JE, Brownfield DG, et al. 2015. Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature* 517:621–25
119. Hu B, Liu J, Wu Z, Liu T, Ullenbruch MR, et al. 2015. Reemergence of hedgehog mediates epithelial–mesenchymal crosstalk in pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 52:418–28
120. Froidure A, Marchal-Duval E, Homps-Legrand MG, Justed A, Crestani B, Mailleux A. 2020. Chaotic activation of developmental signalling pathways drives idiopathic pulmonary fibrosis. *Eur. Respir. Rev.* 29:190140
121. Frangogiannis N. 2020. Transforming growth factor-β in tissue fibrosis. *J. Exp. Med.* 217:e20190103
122. Zepp JA, Zacharias WJ, Frank DB, Cavanaugh CA, Zhou S, et al. 2017. Distinct mesenchymal lineages and niches promote epithelial self-renewal and myofibrogenesis in the lung. *Cell* 170:1134–48.e10
123. Tsukui T, Sun KH, Wetter JB, Wilson-Kanamori JR, Hazelwood LA, et al. 2020. Collagen-producing lung cell atlas identifies multiple subsets with distinct localization and relevance to fibrosis. *Nat. Commun.* 11:1920
124. Liu X, Rowan SC, Liang J, Yao C, Huang G, et al. 2020. Definition and signatures of lung fibroblast populations in development and fibrosis in mice and men. bioRxiv 2020.07.15.203141
125. Beisang DJ, Smith K, Yang L, Benyumov A, Gilbertsen A, et al. 2020. Single-cell RNA sequencing reveals that lung mesenchymal progenitor cells in IPF exhibit pathological features early in their differentiation trajectory. *Sci. Rep.* 10:11162

126. Habiel DM, Hogaboam CM. 2017. Heterogeneity of fibroblasts and myofibroblasts in pulmonary fibrosis. *Curr. Pathobiol. Rep.* 5:101–10
127. Wolters PJ, Collard HR, Jones KD. 2014. Pathogenesis of idiopathic pulmonary fibrosis. *Annu. Rev. Pathol.* 9:157–79
128. Byrne AJ, Maher TM, Lloyd CM. 2016. Pulmonary macrophages: a new therapeutic pathway in fibrosing lung disease? *Trends Mol. Med.* 22:303–16
129. Hung C, Linn G, Chow YH, Kobayashi A, Mittelsteadt K, et al. 2013. Role of lung pericytes and resident fibroblasts in the pathogenesis of pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 188:820–30
130. Marriott S, Baskir RS, Gaskill C, Menon S, Carrier EJ, et al. 2014. ABCG2^{pos} lung mesenchymal stem cells are a novel pericyte subpopulation that contributes to fibrotic remodeling. *Am. J. Physiol. Cell Physiol.* 307:C684–98
131. Rock JR, Barkauskas CE, Cronce MJ, Xue Y, Harris JR, et al. 2011. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *PNAS* 108:E1475–83
132. El Agha E, Moiseenko A, Kheirollahi V, De Langhe S, Crnkovic S, et al. 2017. Two-way conversion between lipogenic and myogenic fibroblastic phenotypes marks the progression and resolution of lung fibrosis. *Cell Stem Cell* 20(2):261–73.e3
133. Chilosi M, Caliò A, Rossi A, Gilioli E, Pedica F, et al. 2017. Epithelial to mesenchymal transition-related proteins ZEB1, β-catenin, and β-tubulin-III in idiopathic pulmonary fibrosis. *Mod. Pathol.* 30:26–38
134. Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, et al. 2005. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-β1: potential role in idiopathic pulmonary fibrosis. *Am. J. Pathol.* 166:1321–32
135. Marmai C, Sutherland RE, Kim KK, Dolganov GM, Fang X, et al. 2011. Alveolar epithelial cells express mesenchymal proteins in patients with idiopathic pulmonary fibrosis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 301:L71–78
136. Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, et al. 2006. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *PNAS* 103:13180–85
137. Tanjore H, Xu XC, Polosukhin VV, Degryse AL, Li B, et al. 2009. Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. *Am. J. Respir. Crit. Care Med.* 180:657–65
138. Fintha A, Gasparics A, Rosivall L, Sebe A. 2019. Therapeutic targeting of fibrotic epithelial-mesenchymal transition–an outstanding challenge. *Front. Pharmacol.* 10:388
139. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. 2002. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* 3:349–63
140. Maher TM, Oballa E, Simpson JK, Porte J, Habgood A, et al. 2017. An epithelial biomarker signature for idiopathic pulmonary fibrosis: an analysis from the multicentre PROFILE cohort study. *Lancet Respir. Med.* 5:946–55
141. Haak AJ, Tan Q, Tschumperlin DJ. 2018. Matrix biomechanics and dynamics in pulmonary fibrosis. *Matrix Biol.* 73:64–76
142. Huang X, Yang N, Fiore VF, Barker TH, Sun Y, et al. 2012. Matrix stiffness–induced myofibroblast differentiation is mediated by intrinsic mechanotransduction. *Am. J. Respir. Cell Mol. Biol.* 47:340–48
143. Liu F, Lagares D, Choi KM, Stopfer L, Marinkovic A, et al. 2015. Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 308:L344–57
144. Wettlaufer SH, Scott JP, McEachin RC, Peters-Golden M, Huang SK. 2016. Reversal of the transcriptome by prostaglandin E2 during myofibroblast dedifferentiation. *Am. J. Respir. Cell Mol. Biol.* 54:114–27
145. Berhan A, Harris T, Jaffar J, Jativa F, Langenbach S, et al. 2020. Cellular microenvironment stiffness regulates eicosanoid production and signaling pathways. *Am. J. Respir. Cell Mol. Biol.* 63(6):819–30
146. Froese AR, Shimbori C, Bellaye PS, Inman M, Obex S, et al. 2016. Stretch-induced activation of transforming growth factor-β_1 in pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 194:84–96
147. Knipe RS, Tager AM, Liao JK. 2015. The Rho kinases: critical mediators of multiple profibrotic processes and rational targets for new therapies for pulmonary fibrosis. *Pharmacol. Rev.* 67:103–17
148. Alvarez D, Cardenes N, Sellares J, Bueno M, Corey C, et al. 2017. IPF lung fibroblasts have a senescent phenotype. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 313:L1164–73

149. Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, et al. 2008. Senescence of activated stellate cells limits liver fibrosis. *Cell* 134:657–67
150. Redente EF, Chakraborty S, Sajuthi S, Black BP, Edelman BL, et al. 2020. Loss of Fas signaling in fibroblasts impairs homeostatic fibrosis resolution and promotes persistent pulmonary fibrosis. *JCI Insight* 6(1):e141618
151. O'Leary EM, Tian Y, Nigdelioglu R, Witt LJ, Cetin-Atalay R, et al. 2020. TGF-β promotes metabolic reprogramming in lung fibroblasts via mTORC1-dependent ATF4 activation. *Am. J. Respir. Cell Mol. Biol.* 63(5):601–12
152. Cui H, Xie N, Banerjee S, Ge J, Jiang D, et al. 2021. Lung myofibroblast promote macrophage profibrotic activity through lactate-induced histone lactylation. *Am. J. Respir. Cell Mol. Biol.* 64(1):115–25
153. Atabai K, Yang CD, Podolsky MJ. 2020. You say you want a resolution (of fibrosis). *Am. J. Respir. Cell Mol. Biol.* 63(4):424–35
154. Rangarajan S, Bone NB, Zmijewska AA, Jiang S, Park DW, et al. 2018. Metformin reverses established lung fibrosis in a bleomycin model. *Nat. Med.* 24:1121–27
155. Kheirollahi V, Wasnick RM, Biasin V, Vazquez-Armendariz AI, Chu X, et al. 2019. Metformin induces lipogenic differentiation in myofibroblasts to reverse lung fibrosis. *Nat. Commun.* 10:2987
156. Spagnolo P, Kreuter M, Maher TM, Wuyts W, Bonella F, et al. 2018. Metformin does not affect clinically relevant outcomes in patients with idiopathic pulmonary fibrosis. *Respiration* 96:314–22
157. Artaud-Macari E, Goven D, Brayer S, Hamimi A, Besnard V, et al. 2013. Nuclear factor erythroid 2-related factor 2 nuclear translocation induces myofibroblastic dedifferentiation in idiopathic pulmonary fibrosis. *Antioxid. Redox Signal.* 18:66–79
158. Turner-Warwick M. 1963. Precapillary systemic-pulmonary anastomoses. *Thorax* 18:225–37
159. Gracey DR, Divertie MB, Brown AL Jr. 1968. Alveolar-capillary membrane in idiopathic interstitial pulmonary fibrosis: electron microscopic study of 14 cases. *Am. Rev. Respir. Dis.* 98:16–21
160. Cosgrove GP, Brown KK, Schiemann WP, Serls AE, Parr JE, et al. 2004. Pigment epithelium-derived factor in idiopathic pulmonary fibrosis: a role in aberrant angiogenesis. *Am. J. Respir. Crit. Care Med.* 170:242–51
161. Ebina M, Shimizukawa M, Shibata N, Kimura Y, Suzuki T, et al. 2004. Heterogeneous increase in CD34-positive alveolar capillaries in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 169:1203–8
162. Hamada K, Nagai S, Tanaka S, Handa T, Shigematsu M, et al. 2007. Significance of pulmonary arterial pressure and diffusion capacity of the lung as prognosticator in patients with idiopathic pulmonary fibrosis. *Chest* 131:650–56
163. Magro CM, Waldman WJ, Knight DA, Allen JN, Nadasdy T, et al. 2006. Idiopathic pulmonary fibrosis related to endothelial injury and antiendothelial cell antibodies. *Hum. Immunol.* 67:284–97
164. Ando M, Miyazaki E, Ito T, Hiroshige S, Nureki SI, et al. 2010. Significance of serum vascular endothelial growth factor level in patients with idiopathic pulmonary fibrosis. *Lung* 188:247–52
165. Murray LA, Habiel DM, Hohmann M, Camelo A, Shang H, et al. 2017. Antifibrotic role of vascular endothelial growth factor in pulmonary fibrosis. *JCI Insight* 2(16):e92192
166. Giaid A, Michel RP, Stewart DJ, Sheppard M, Corrin B, Hamid Q. 1993. Expression of endothelin-1 in lungs of patients with cryptogenic fibrosing alveolitis. *Lancet* 341:1550–54
167. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, et al. 1993. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N. Engl. J. Med.* 328:1732–39
168. Raghu G, Behr J, Brown KK, Egan JJ, Kawut SM, et al. 2013. Treatment of idiopathic pulmonary fibrosis with ambrisentan: a parallel, randomized trial. *Ann. Intern. Med.* 158:641–49
169. Farkas L, Gauldie J, Voelkel NF, Kolb M. 2011. Pulmonary hypertension and idiopathic pulmonary fibrosis: a tale of angiogenesis, apoptosis, and growth factors. *Am. J. Respir. Cell Mol. Biol.* 45:1–15
170. Collum SD, Amione-Guerra J, Cruz-Solbes AS, DiFrancesco A, Hernandez AM, et al. 2017. Pulmonary hypertension associated with idiopathic pulmonary fibrosis: current and future perspectives. *Can. Respir. J.* 2017:1430350
171. Hanumegowda C, Farkas L, Kolb M. 2012. Angiogenesis in pulmonary fibrosis: too much or not enough? *Chest* 142:200–7
172. Sumi M, Satoh H, Kagohashi K, Ishikawa H, Sekizawa K. 2005. Increased serum levels of endostatin in patients with idiopathic pulmonary fibrosis. *J. Clin. Lab. Anal.* 19:146–49

173. Hashimoto N, Phan SH, Imaizumi K, Matsuo M, Nakashima H, et al. 2010. Endothelial-mesenchymal transition in bleomycin-induced pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 43:161–72
174. Am. Thorac. Soc. 2000. Idiopathic pulmonary fibrosis: diagnosis and treatment: international consensus statement. *Am. J. Respir. Crit. Care Med.* 161:646–64
175. Idiopathic Pulm. Fibros. Clin. Res. Netw. 2012. Prednisone, azathioprine, and *N*-acetylcysteine for pulmonary fibrosis. *N. Engl. J. Med.* 366:1968–77
176. Wick G, Grundtman C, Mayerl C, Wimpissinger TF, Feichtinger J, et al. 2013. The immunology of fibrosis. *Annu. Rev. Immunol.* 31:107–35
177. Michalski JE, Schwartz DA. 2020. Genetic risk factors for idiopathic pulmonary fibrosis: insights into immunopathogenesis. *J. Inflamm. Res.* 13:1305–18
178. Noth I, Zhang Y, Ma SF, Flores C, Barber M, et al. 2013. Genetic variants associated with idiopathic pulmonary fibrosis susceptibility and mortality: a genome-wide association study. *Lancet Respir. Med.* 1:309–17
179. Liang J, Zhang Y, Xie T, Liu N, Chen H, et al. 2016. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. *Nat. Med.* 22:1285–93
180. O'Dwyer DN, Armstrong ME, Trujillo G, Cooke G, Keane MP, et al. 2013. The Toll-like receptor 3 L412F polymorphism and disease progression in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 188:1442–50
181. Kimura M, Tani K, Miyata J, Sato K, Hayashi A, et al. 2005. The significance of cathepsins, thrombin and aminopeptidase in diffuse interstitial lung diseases. *J. Med. Investig.* 52:93–100
182. Hamada N, Maeyama T, Kawaguchi T, Yoshimi M, Fukumoto J, et al. 2008. The role of high mobility group box1 in pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 39:440–47
183. Millien VO, Lu W, Shaw J, Yuan X, Mak G, et al. 2013. Cleavage of fibrinogen by proteinases elicits allergic responses through Toll-like receptor 4. *Science* 341:792–96
184. Gasse P, Mary C, Guenon I, Noulin N, Charron S, et al. 2007. IL-1R1/MyD88 signaling and the inflammasome are essential in pulmonary inflammation and fibrosis in mice. *J. Clin. Investig.* 117:3786–99
185. Dostert C, Petrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. 2008. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320:674–77
186. Tian R, Zhu Y, Yao J, Meng X, Wang J, et al. 2017. NLRP3 participates in the regulation of EMT in bleomycin-induced pulmonary fibrosis. *Exp. Cell Res.* 357:328–34
187. Stout-Delgado HW, Cho SJ, Chu SG, Mitzel DN, Villalba J, et al. 2016. Age-dependent susceptibility to pulmonary fibrosis is associated with NLRP3 inflammasome activation. *Am. J. Respir. Cell Mol. Biol.* 55:252–63
188. Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, et al. 2013. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat. Cell Biol.* 15:978–90
189. Cho SJ, Moon JS, Nikahira K, Yun HS, Harris R, et al. 2020. GLUT1-dependent glycolysis regulates exacerbation of fibrosis via AIM2 inflammasome activation. *Thorax* 75:227–36
190. Moon JS, Hisata S, Park MA, DeNicola GM, Ryter SW, et al. 2015. mTORC1-induced HK1-dependent glycolysis regulates NLRP3 inflammasome activation. *Cell Rep.* 12:102–15
191. Ji J, Hou J, Xia Y, Xiang Z, Han X. 2021. NLRP3 inflammasome activation in alveolar epithelial cells promotes myofibroblast differentiation of lung-resident mesenchymal stem cells during pulmonary fibrogenesis. *Biochim. Biophys. Acta Mol. Basis Dis.* 1867:166077
192. Lasithiotaki I, Giannarakis I, Tsitoura E, Samara KD, Margaritopoulos GA, et al. 2016. NLRP3 inflammasome expression in idiopathic pulmonary fibrosis and rheumatoid lung. *Eur. Respir. J.* 47:910–18
193. Li Y, Li H, Liu S, Pan P, Su X, et al. 2018. Pirfenidone ameliorates lipopolysaccharide-induced pulmonary inflammation and fibrosis by blocking NLRP3 inflammasome activation. *Mol. Immunol.* 99:134–44
194. Cho SJ, Hong KS, Jeong JH, Lee M, Choi AMK, et al. 2019. DROSHA-dependent AIM2 inflammasome activation contributes to lung inflammation during idiopathic pulmonary fibrosis. *Cells* 8(8):938
195. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, et al. 2013. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. *Immunity* 38:792–804

196. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, et al. 2013. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. *J. Exp. Med.* 210:1977–92
197. Janssen WJ, Barthel L, Muldrow A, Oberley-Deegan RE, Kearns MT, et al. 2011. Fas determines differential fates of resident and recruited macrophages during resolution of acute lung injury. *Am. J. Respir. Crit. Care Med.* 184:547–60
198. Misharin AV, Morales-Nebreda L, Reyfman PA, Cuda CM, Walter JM, et al. 2017. Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span. *J. Exp. Med.* 214:2387–404
199. McCubbrey AL, Barthel L, Mohning MP, Redente EF, Mould KJ, et al. 2018. Deletion of c-FLIP from CD11b^{hi} macrophages prevents development of bleomycin-induced lung fibrosis. *Am. J. Respir. Cell Mol. Biol.* 58:66–78
200. Joshi N, Watanabe S, Verma R, Jablonski RP, Chen CI, et al. 2020. A spatially restricted fibrotic niche in pulmonary fibrosis is sustained by M-CSF/M-CSFR signalling in monocyte-derived alveolar macrophages. *Eur. Respir. J.* 55(1):1900646
201. Iyonaga K, Takeya M, Saita N, Sakamoto O, Yoshimura T, et al. 1994. Monocyte chemoattractant protein-1 in idiopathic pulmonary fibrosis and other interstitial lung diseases. *Hum. Pathol.* 25:455–63
202. Kreuter M, Bradley SJ, Lee JS, Tzouvelekis A, Oldham JM, et al. 2021. Monocyte count as a prognostic biomarker in patients with idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 204(1):74–81
203. Heukels P, Moor CC, von der Thusen JH, Wijsenbeek MS, Kool M. 2019. Inflammation and immunity in IPF pathogenesis and treatment. *Respir. Med.* 147:79–91
204. Satoh T, Nakagawa K, Sugihara F, Kuwahara R, Ashihara M, et al. 2017. Identification of an atypical monocyte and committed progenitor involved in fibrosis. *Nature* 541:96–101
205. Bitterman PB, Wewers MD, Rennard SI, Adelberg S, Crystal RG. 1986. Modulation of alveolar macrophage-driven fibroblast proliferation by alternative macrophage mediators. *J. Clin. Investig.* 77:700–8
206. Zhou Y, Peng H, Sun H, Peng X, Tang C, et al. 2014. Chitinase 3–like 1 suppresses injury and promotes fibroproliferative responses in mammalian lung fibrosis. *Sci. Transl. Med.* 6:240ra76
207. Madsen SJ, Gach HM, Hong SJ, Uzal FA, Peng Q, Hirschberg H. 2013. Increased nanoparticle-loaded exogenous macrophage migration into the brain following PDT-induced blood–brain barrier disruption. *Lasers Surg. Med.* 45:524–32
208. Schupp JC, Binder H, Jager B, Cillis G, Zissel G, et al. 2015. Macrophage activation in acute exacerbation of idiopathic pulmonary fibrosis. *PLOS ONE* 10:e0116775
209. Cai M, Bonella F, He X, Sixt SU, Sarria R, et al. 2013. CCL18 in serum, BAL fluid and alveolar macrophage culture supernatant in interstitial lung diseases. *Respir. Med.* 107:1444–52
210. Prasse A, Probst C, Bargagli E, Zissel G, Toews GB, et al. 2009. Serum CC chemokine ligand-18 concentration predicts outcome in idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 179:717–23
211. Prasse A, Pechkovsky DV, Toews GB, Jungraithmayr W, Kollert F, et al. 2006. A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18. *Am. J. Respir. Crit. Care Med.* 173:781–92
212. Raghu G, van den Blink B, Hamblin MJ, Brown AW, Golden JA, et al. 2018. Effect of recombinant human pentraxin 2 vs placebo on change in forced vital capacity in patients with idiopathic pulmonary fibrosis: a randomized clinical trial. *JAMA* 319:2299–307
213. Morse C, Tabib T, Sembrat J, Buschur KL, Bittar HT, et al. 2019. Proliferating SPP1/MERTK-expressing macrophages in idiopathic pulmonary fibrosis. *Eur. Respir. J.* 54(2):1802441
214. Allden SJ, Ogger PP, Ghai P, McErlean P, Hewitt R, et al. 2019. The transferrin receptor CD71 delineates functionally distinct airway macrophage subsets during idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 200:209–19
215. Nuovo GJ, Hagood JS, Magro CM, Chin N, Kapil R, et al. 2012. The distribution of immunomodulatory cells in the lungs of patients with idiopathic pulmonary fibrosis. *Mod. Pathol.* 25:416–33
216. Distler JHW, Györfi AH, Ramanujam M, Whitfield ML, Königshoff M, Lafyatis R. 2019. Shared and distinct mechanisms of fibrosis. *Nat. Rev. Rheumatol.* 15:705–30

217. Keane MP, Belperio JA, Burdick MD, Strieter RM. 2001. IL-12 attenuates bleomycin-induced pulmonary fibrosis. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 281:L92–97
218. King TE Jr., Albera C, Bradford WZ, Costabel U, Hormel P, et al. 2009. Effect of interferon gamma-1b on survival in patients with idiopathic pulmonary fibrosis (INSPIRE): a multicentre, randomised, placebo-controlled trial. *Lancet* 374:222–28
219. Hashimoto S, Gon Y, Takeshita I, Maruoka S, Horie T. 2001. IL-4 and IL-13 induce myofibroblastic phenotype of human lung fibroblasts through c-Jun NH_2-terminal kinase–dependent pathway. *J. Allergy Clin. Immunol.* 107:1001–8
220. Celada LJ, Kropski JA, Herazo-Maya JD, Luo W, Creecy A, et al. 2018. PD-1 up-regulation on $CD4^+$ T cells promotes pulmonary fibrosis through STAT3-mediated IL-17A and TGF-β1 production. *Sci. Transl. Med.* 10(460):eaar8356
221. Kolahian S, Fernandez IE, Eickelberg O, Hartl D. 2016. Immune mechanisms in pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 55:309–22
222. Horsburgh S, Todryk S, Ramming A, Distler JHW, O'Reilly S. 2018. Innate lymphoid cells and fibrotic regulation. *Immunol. Lett.* 195:38–44
223. Hams E, Armstrong ME, Barlow JL, Saunders SP, Schwartz C, et al. 2014. IL-25 and type 2 innate lymphoid cells induce pulmonary fibrosis. *PNAS* 111:367–72
224. Luzina IG, Kopach P, Lockatell V, Kang PH, Nagarsekar A, et al. 2013. Interleukin-33 potentiates bleomycin-induced lung injury. *Am. J. Respir. Cell Mol. Biol.* 49:999–1008
225. Li D, Guabiraba R, Besnard AG, Komai-Koma M, Jabir MS, et al. 2014. IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. *J. Allergy Clin. Immunol.* 134:1422–32.e11
226. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE Jr. 2008. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. *Chest* 133:226–32
227. Xaubet A, Agusti C, Luburich P, Barbera JA, Carrion M, et al. 1998. Interleukin-8 expression in bronchoalveolar lavage cells in the evaluation of alveolitis in idiopathic pulmonary fibrosis. *Respir. Med.* 92:338–44
228. Chua F, Dunsmore SE, Clingen PH, Mutsaers SE, Shapiro SD, et al. 2007. Mice lacking neutrophil elastase are resistant to bleomycin-induced pulmonary fibrosis. *Am. J. Pathol.* 170:65–74
229. Chrysanthopoulou A, Mitroulis I, Apostolidou E, Arelaki S, Mikroulis D, et al. 2014. Neutrophil extracellular traps promote differentiation and function of fibroblasts. *J. Pathol.* 233:294–307
230. Broekelmann TJ, Limper AH, Colby TV, McDonald JA. 1991. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. *PNAS* 88:6642–46
231. Khalil N, O'Connor RN, Unruh HW, Warren PW, Flanders KC, et al. 1991. Increased production and immunohistochemical localization of transforming growth factor-α in idiopathic pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* 5:155–62
232. Kashima Y, Sakamoto Y, Kaneko K, Seki M, Suzuki Y, Suzuki A. 2020. Single-cell sequencing techniques from individual to multiomics analyses. *Exp. Mol. Med.* 52:1419–27
233. Hwang B, Lee JH, Bang D. 2018. Single-cell RNA sequencing technologies and bioinformatics pipelines. *Exp. Mol. Med.* 50:1–14
234. Takezaki A, Tsukumo SI, Setoguchi Y, Ledford JG, Goto H, et al. 2019. A homozygous SFTPA1 mutation drives necroptosis of type II alveolar epithelial cells in patients with idiopathic pulmonary fibrosis. *J. Exp. Med.* 216:2724–35
235. Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, et al. 2009. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am. J. Hum. Genet.* 84:52–59
236. Campo I, Zorzetto M, Mariani F, Kadija Z, Morbini P, et al. 2014. A large kindred of pulmonary fibrosis associated with a novel ABCA3 gene variant. *Respir. Res.* 15:43
237. Alder JK, Stanley SE, Wagner CL, Hamilton M, Hanumanthu VS, Armanios M. 2015. Exome sequencing identifies mutant *TINF2* in a family with pulmonary fibrosis. *Chest* 147:1361–68
238. Kropski JA, Mitchell DB, Markin C, Polosukhin VV, Choi L, et al. 2014. A novel dyskerin (*DKC1*) mutation is associated with familial interstitial pneumonia. *Chest* 146:e1–7
239. Cogan JD, Kropski JA, Zhao M, Mitchell DB, Rives L, et al. 2015. Rare variants in *RTEL1* are associated with familial interstitial pneumonia. *Am. J. Respir. Crit. Care Med.* 191:646–55

240. Stuart BD, Choi J, Zaidi S, Xing C, Holohan B, et al. 2015. Exome sequencing links mutations in *PARN* and *RTEL1* with familial pulmonary fibrosis and telomere shortening. *Nat. Genet.* 47:512–17
241. Korthagen NM, van Moorsel CHM, Barlo NP, Kazemier KM, Ruven HJT, Grutters JC. 2012. Association between variations in cell cycle genes and idiopathic pulmonary fibrosis. *PLOS ONE* 7:e30442
242. Korthagen NM, van Moorsel CHM, Kazemier KM, Ruven HJT, Grutters JC. 2012. IL1RN genetic variations and risk of IPF: a meta-analysis and mRNA expression study. *Immunogenetics* 64:371–77
243. Ahn MH, Park BL, Lee SH, Park SW, Park JS, et al. 2011. A promoter SNP rs4073T>A in the common allele of the interleukin 8 gene is associated with the development of idiopathic pulmonary fibrosis via the IL-8 protein enhancing mode. *Respir. Res.* 12:73
244. Kishore A, Zizkova V, Kocourkova L, Petrkova J, Bouros E, et al. 2016. Association study for 26 candidate loci in idiopathic pulmonary fibrosis patients from four European populations. *Front. Immunol.* 7:274
245. Xin L, Jiang M, Su G, Xie M, Chen H, et al. 2018. The association between transforming growth factor beta1 polymorphism and susceptibility to pulmonary fibrosis: a meta-analysis (MOOSE compliant). *Medicine* 97:e11876
246. Xue J, Gochuico BR, Alawad AS, Feghali-Bostwick CA, Noth I, et al. 2011. The HLA class II allele DRB1*1501 is over-represented in patients with idiopathic pulmonary fibrosis. *PLOS ONE* 6:e14715
247. Allen RJ, Porte J, Braybrooke R, Flores C, Fingerlin TE, et al. 2017. Genetic variants associated with susceptibility to idiopathic pulmonary fibrosis in people of European ancestry: a genome-wide association study. *Lancet Respir. Med.* 5:869–80