Advances in cellular senescence in idiopathic pulmonary fibrosis (Review)

SHAN HAN[1,2*], QIANGWEI LU[1*] and XIAOQIU LIU[1]

[1]Department of Respiratory and Critical Care Medicine, The Second Hospital of Jilin University, Changchun, Jilin 130000; [2]Department of Respiratory and Critical Care Medicine, Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi 712000, P.R. China

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Abstract. Idiopathic pulmonary fibrosis (IPF) is a progressive, irreversible and fatal interstitial lung disease of unknown cause, with a median survival of 2-3 years. Its pathogenesis is unclear and there is currently no effective treatment for IPF. Approximately two-thirds of patients with IPF are >60 years old, with a mean age of 66 years, suggesting a link between aging and IPF. However, the mechanism by which aging promotes development of PF remains unclear. Senescence of alveolar epithelial cells and lung fibroblasts (LFs) and their senescence-associated secretion phenotype (SASP) may be involved in the occurrence and development of IPF. The present review focus on senescence of LFs and epithelial and stem cells, as well as SASP, the activation of profibrotic signaling pathways and potential treatments for pathogenesis of IPF.

Contents

Correspondence to: Dr Xiaoqiu Liu, Department of Respiratory and Critical Care Medicine, The Second Hospital of Jilin University, 218 Zi Qiang Street, Nanguan, Changchun, Jilin 130000, P.R. China
E-mail: liuxiaoq@jlu.edu.cn

*Contributed equally

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1. Introduction

Pulmonary fibrosis (PF) is caused by factors including toxic, autoimmune, drug-induced, traumatic injury and infectious diseases. As a result, a 'reparative response' involving both fibroblasts and myofibroblasts of lung tissue may be triggered (1). In certain cases, the failure to establish normal tissue repair in damaged lung results in marked alveolar disorganization with imbalanced epithelial cell proportions, endothelial cell loss or migration to incorrect locations. Furthermore, sustained alveolar epithelial cell proliferation (AECs), repeated injury and interstitial fibroblast proliferation with concomitant deposition of collagenous extracellular matrix (ECM) result in increased fibrogenesis (1). Idiopathic PF (IPF) is a chronic progressive interstitial lung disease of unknown origin. Histologically, IPF is characterized by massive accumulation of fibroblasts, myofibroblasts, AECs and macrophages and a significant deposition of ECM (2). A previous review showed that AECs, as the main source of pro-fibrogenic cytokines in IPF, express a variety of cytokines and growth factors, which can promote the migration, proliferation and accumulation of extracellular matrix of fibroblasts; these are key events of cell dysfunction in PF, which involve abnormal wound healing and participate in the formation of patchy fibroblast myofibroblast lesions in the pathogenesis of IPF (3). AECs are damaged by pathogenic microorganisms, dust, drugs, chemicals and oxygen free radicals which, when coupled with risk factors such as aging and genetics, may decrease the ability of alveolar epithelial type II (ATII) cells and lung fibroblasts (LFs) to repair damage to the lung (4,5). LFs proliferate locally, migrate to the injury site and differentiate into myofibroblasts, which produce a large amount of ECM and exhibit contractile function. AS myofibroblasts typically vanish after successful repair; dysregulation of the normal repair process can lead to persistent myofibroblast activation (5). Therefore, decreasing fibroblast activation can limit the progression of fibrosis (5). Myofibroblast apoptosis is key for normal regression of the wound repair response and impaired myofibroblast apoptosis is associated with tissue fibrosis (6). Furthermore, depletion of myofibroblasts by apoptosis is key for normal wound healing. However, this process does not occur in the fibroblast foci of IPF (6), as patients with IPF have abnormal repair processes, including decreased mesenchymal stem cell

(MSC) proliferation, differentiation and repair capacity. These changes lead to scarring and subsequent respiratory failure (6). Accordingly, the primary clinical manifestations of IPF are progressive dyspnea, decreased lung function and respiratory failure and death. IPF mostly occurs in middle-aged and elderly people, and aging is a risk factor for IPF (6). LFs and AECs are senescent in lung tissue of patients with IPF and lung fibrosis animal models (7,8). Bone marrow (B-)MSCs are depleted, indicating that cellular senescence is associated with pathogenesis of IPF (7).

Aging is an underlying decline in age-related physiological function, leading to increased age-associated mortality and reduced reproductive capacity (9). Cellular senescence is irreversible stagnation of the cell cycle, resulting in loss of intercellular transport and communication and age-associated intrinsic cellular functions, such as cell division and replication (9,10). There are two types of cell senescence: Replicative and premature. Replicative senescence is caused by telomerase damage (10), not by telomere length, whereas premature senescence is caused by stress, oncogenes and loss of tumor suppressor factors (11). The key characteristic of aging is secretion of a large number of mediators during the stagnation of the cell cycle. These mediators are collectively known as the senescence-associated secretory phenotype (SASP) protein (12). Two primary aging signaling pathways induce cell senescence (13). The first is the cyclin-dependent kinase inhibitor (p16)/retinoblastoma (Rb) pathway, where p16 can competitively bind to CDK4/6 and inhibit its kinase activity, thereby decreasing phosphorylation of Rb to prevent activation of downstream transcription factors, thus leading to stagnation of the cell cycle. The second pathway is p53/cyclin-dependent kinase inhibitor 1 (p21). When cells are stressed, tumor suppressor p53 is activated, p21 is upregulated and the phosphorylation of Rb is inhibited, leading to cell cycle arrest (14). The mechanisms of aging associated with development of IPF are illustrated in Fig. 1.

Studies (3,5,6,15) have shown that AECs (primarily ATII cells), fibroblasts and myofibroblasts are involved in the occurrence and development of PF. Although pirfenidone and nintedanib slow the progression of IPF, the disease continues to progress and, to the best of our knowledge, there is no cure other than lung transplantation. To the best of our knowledge, while IPF is an aging-related disease, the underlying mechanism linking aging to IPF remains unclear. The present review summarizes research on the pathogenesis and treatment of IPF associated with cell aging and provides an important direction for the future treatment of PF.

2. Senescence of AECs and IPF

ATII cells play an important role in maintaining pulmonary homeostasis and their main functions include proliferation, differentiation into ATI cells, secretion of surfactants and involvement in biological activities such as pulmonary inflammatory response, immune response, regulation of ECM and damage repair (15). As AECs are key cells in the initial phase of IPF, sustained and repeated AEC injury leads to abnormal changes that promote fibrosis repair. When the alveolar epithelium is repeatedly damaged, the function and morphology of ATII cells change and the basal layer shedding caused by aging and apoptosis may reflect the initial destructive events in progression of the disease. However, to the best of our knowledge, the molecular mechanisms involved remain unclear (2). Studies (16-27) have shown that aging and apoptosis of ATII cells may be associated with endoplasmic reticulum (ER) stress and autophagy as well as telomere damage, mitochondrial dysfunction and epigenetic changes.

ER stress. In IPF, ER stress is observed in AECs (16). Viral infection and aging trigger a hyperinflammatory response due to expansion of the ER (28). In addition, susceptibility to ER stress increases during aging. ER stress initially stimulates an adaptive unfolded protein response (UPR) to promote cellular survival but, in the case of persistent chronic stress, UPR triggers the apoptotic cell death program (16). ER stress is associated with fibrosis via cell apoptosis, activation/differentiation of fibroblasts, epithelial-mesenchymal transition (EMT) and activation or polarization of inflammatory responses (16). In the aging lung, ATII cells are particularly sensitive to ER stress. ATII cells with knock out of glucose regulatory protein (GRP) 78, a key protein of ER stress, show ER stress, injury, senescence and decreased differentiation, accompanied by abnormal activation of TGF-β1/SMAD signaling (17). In addition, GRP78 is reduced in ATII cells in patients with IPF and elderly mice with IPF. These results suggest that GRP78 reduction is a potential mechanism underlying the association between ER stress of aging ATII cells and IPF (17). There is a dual role of autophagy under ER stress and crosstalk between autophagy and apoptosis is complicated. Furthermore, ER stress effectively induces autophagy activation, typically via the Bcl-2 signaling pathway (29). Prolonged or excessive ER stress induces apoptosis in epithelial cells through several UPR-dependent downstream mechanisms, including C/EBP homologous protein induction, activation of the ER-bound caspase or activation of JNK (30). However, with an increase in age, sustained ER stress decreases PTEN-induced putative kinase 1 (PINK1) expression and inhibits autophagy (30,31). In brief, autophagy decreases with aging and accelerated aging may be attributed to reduced autophagy.

Decreased autophagy. As a cellular protective mechanism, autophagy plays an important role in cell homeostasis and removal of harmful substances (18). Autophagy can be triggered by stress factors, such as reactive oxygen species (ROS), ER stress and hypoxia. Moreover, insufficient autophagy may accelerate senescence in epithelial cells and potentiate both EMT and myofibroblast differentiation. Additionally, inhibition of autophagy in epithelial cells leads to enhanced EMT and fibrosis (19). Expression of multifunctional protein p62 and ubiquitinated protein in the lung of patients with IPF is increased, indicating insufficient autophagy in lung tissue (18,20). Beclin-1, a key autophagic protein, is downregulated in fibroblasts isolated from patients with IPF (32). Conditional knockout of the tuberous sclerosis complex 1 gene in mouse epithelial cells renders bleomycin (BLM)-induced mice more prone to PF; this is reversed by activation of autophagy by rapamycin (33). Another study (34) has revealed that aging mice with loss of autophagic proteins [light chain 3β(LC3B)-/- and autophagy-related 4B cysteine peptidase (ATG4B)-/-] are more susceptible to BLM-induced lung

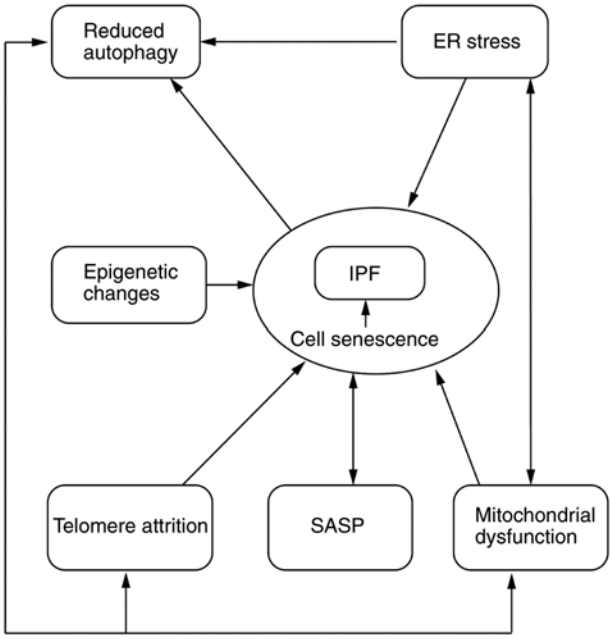

Figure 1. Mechanisms of aging involved in the development of IPF. Hallmarks of cellular senescence are decreased autophagy, telomere attrition, epigenetic changes, mitochondrial dysfunction, ER stress and SASP, which contribute to the occurrence and development of IPF. Mechanisms in cell senescence interact with each other and form a complex network. IPF, idiopathic pulmonary fibrosis; ER, endoplasmic reticulum; SASP, senescence-associated secretion phenotype.

fibrosis and linked cathepsin A, a binding partner to LC3B. In addition, ER stress increased apoptosis of epithelial cells in aging mice with loss of autophagic proteins (LC3B-/- and ATG4B-/-) (34).

Studies (18-20,32-34) confirm that autophagy is decreased in AECs of patients with IPF. Autophagy deficiency leads to epithelial cell dysfunction and promotes PF, while autophagy activation enhances the repair ability of epithelial cells and inhibits PF. However, the molecular mechanism underlying regulation of autophagy and its effect on epithelial cell function remains unclear and warrants further investigation.

Telomere attrition. Telomeres are composed of DNA repetitive sequences and binding proteins that maintain structural integrity of chromosomes. The binding proteins consist of the protein components telomeric repeat-binding factor (TRF) 1, TRF2, Ras-related protein Rap1, TRF1-interacting nuclear factor 2, telomere protective protein 1 (TPP1) and protection of telomeres 1 gene (35). Telomeric DNA typically contains clusters of three or four guanines (for example, 5'-TTGGGG-3' in tetrahymena and 5'-TTAGGG-3' in humans) (35). These telomeric repeats are added by the enzyme telomerase. Telomerase comprises three parts: Human telomerase RNA (hTR); telomerase synergistic protein 1 and hT reverse transcriptase(hTRT). Telomerase performs catalytic reverse transcription (21). Typically, normal aging is accompanied by telomere shortening. However, mutations in the telomerase complex genes hTRT and hTR are found in 8-15% of familial patients with IPF and 1-3% of sporadic cases; these mutations accelerate telomere shortening and cause replicative aging of AECs (21-23). V144M, R865C and R865H mutants of hTRT are key because these mutants can explain how the hereditary hTERT mutation causes telomere shortening in IPF patients, so as to further understand the role of naturally occurring telomerase mutations in the pathophysiology of some age-related disease states; *in vitro* experiments have determined that V144 and R865 in hTRT are key residues required for the normal function of cell telomerase (36,37). Moreover, 98 G→A, 37A→G, 108C→U and 325G→U hTR substitution are often noted in familial patients with IPF. These mutations are predicted to impair base pairing in a helix in the key pseudoknot domain of hTR (21,38). The knockout of TRF1 in ATII cells causes severe telomere dysfunction in the lung of mice and induces PF by inducing DNA damage and upregulating cell cycle suppressor protein p21/p53 (39). Similarly, specific knockout of TRF2 in mouse ATII cells is characterized by telomere dysfunction, resulting in increased p53 and p21 expression. As the ability of ATII cells to self-renew and differentiate is limited, these cells are less able to repair BLM-induced lung damage (40). A recent study (41) suggested that a key role in alveolar stem cell dysfunction is played by telomere shortening or uncapping, bridging the gap in telomere abnormality and fibrotic lung pathology. Failure to regenerate alveoli due to alveolar stem cell dysfunction may expose lung cells to elevated mechanical tension, which may activate the TGF-β signaling loop to promote the fibrotic process (41). In addition, short telomeres signal and activate p53, which suppresses phosphatidylglycerol phospholipase C (PGC)-1α and PGC-1β promoters, leading to mitochondrial dysfunction and cell aging (42). These findings indicate that telomere shortening serves a key role in the occurrence of IPF.

Mitochondrial dysfunction. Mitochondrial dysfunction drives cell senescence. In pathogenesis of IPF, mitochondrial dysfunction primarily involves an imbalance in mitochondrial ROS levels, mitochondrial DNA changes, mitochondria-mediated reduced autophagy and electron transport chain imbalance. Mitochondrial dysfunction is associated with telomere attrition and ER stress (24,25,31). Epithelial cell damage is associated with increased mitochondrial ROS in lung tissue of patients with IPF (24). With aging, mitochondrial function is impaired, leading to increased ROS production, which in turn causes deterioration of mitochondrial function (24). Changes in mitochondrial DNA metabolism and lack of phagocytosis often occur in patients with IPF, leading to increased sensitivity to apoptosis (24). Kim *et al* (25) showed that Klotho protein plays a role in protecting AEC mitochondrial DNA integrity. Knockout of PINK1 in ATII cells of mouse lung tissue leads to mitochondrial swelling and dysfunction, impaired mitochondrial autophagy and increased susceptibility to PF (31). Furthermore, there is evidence of a role played by PINK1/parkin RING-in-between-RING (RBR) E3 ubiquitin protein ligase-mediated mitochondrial autophagy in IPF. Furthermore, there is growing evidence supporting the role played by PINK1-PARK2-mediated mitochondrial autophagy in IPF (43).

The association between mitochondrial dysfunction and low PINK1 expression indicates that PINK1 may serve an important role in maintaining the morphology and function of mitochondria and selectively degrades damaged mitochondria through autophagy (31). Mitochondrial dysfunction occurs

with and further promotes aging, leading to an imbalance in the electron transport chain. NAD^+ is an electron acceptor and oxidant, as well as a cofactor in numerous metabolic and signaling pathways. The ratio of NAD^+ to NADH is key and NAD^+ improves mitochondrial function and longevity in aging mice. One mechanism by which NAD^+ affects PF is the regulation of cellular function by regulating the activity of sirtuins (SIRTs). Deacetylase SIRT is an NAD^+-dependent histone deacetylase (HDAC) and serves an important role in transcription, cell cycle regulation and subsequent translation and modification (44-46). Aging decreases expression of SIRT3, leading to an increase in acetylation and thereby increasing the levels of mitochondrial ROS and DNA damage (44). SIRT3 inhibits TGF-β1 signaling and controls myofibroblast transformation (47). Increased expression levels of NADPH oxidase 4 (NOX4) have been reported in the lung of patients with IPF (48). NOX4 is considered to be a mediator of mitochondrial dysfunction (49). NOX4 enzyme interacts with mitochondria and affects mitochondrial function and production of mitochondrial ROS and SIRT, jointly promoting epithelial injury and lung fibrosis (50). NOX4 also regulates protein and collagen concentrations of α-smooth muscle actin (α-SMA) by controlling Smad2/3 and regulating platelet-derived growth factor (PDGF) to induce fibroblast migration (48). Telomere shortening is a feature of premature aging, partly due to increased mitochondrial ROS (51). There is an interaction between mitochondria and ER. ER stress can downregulate PINK1 to regulate mitochondrial function and increase apoptotic response. Meanwhile, during ER stress, the transfer of calcium ions from ER to mitochondria leads to mitochondrial swelling (31).

Epigenetic changes. Epigenetic marks are cell-type specific. A previous study revealed that microRNA (miRNA or miR)-34a, miR-34b and miR-34c, members of the aging-associated miR-34 family, are highly expressed in AECs from patients with IPF (26). The deletion of miR-34a effectively improves BLM-induced epithelial cell senescence in animal models (27,52). The mechanism of miR-34 in IPF may involve p53 activation and downregulation of the transcription factor early 2 factor to inhibit cell proliferation (26). The activation of p53 induces p21, leading to cell cycle arrest by inhibiting the expression of cyclin E and CDK2. Cui *et al* (27) showed that miR-34a is increased in AECs but not in LFs. This suggests that miR-34a expression may be regulated differently in different types of lung cell. The role of AECs and SASP in IPF is displayed in Fig. 2.

3. Senescence of fibroblasts and IPF

Fibroblasts serve a central role in the fibrotic process. Deposition of fibroblasts in the pulmonary interstitium and generation of ECM serve a key role in the development of IPF (53). Primary LFs isolated from the lung tissue of patients with IPF exhibit more senescence compared with age-matched controls. These senescent fibroblasts increase levels of senescence-associated β-galactosidase, P16, P21, P53 and SASP (5). The pathogenesis of fibroblast senescence in IPF remains unclear but mitochondrial dysfunction, telomere shortening, epigenetic changes and decreased autophagy are involved in IPF. To the best of our knowledge, however, most current research has focused on epigenetics and autophagy (5,54).

Epigenetic alterations. Epigenetic changes mainly involve DNA methylation, histone post-translational modifications and miR regulation (55). Human DNA contains cytosine-phosphate-guanine(CpG)sites and the methylation of the CpG island via DNA methyltransferases (DNMTs) prevents RNA polymerase complex binding to the promoter region and therefore suppresses gene expression. Additionally, the majority of human CpG sites are methylated (56). Abnormal DNA methylation either silences or activates expression of genes that drive fibrosis and alter the mRNA expression of several genes. For example, Neveu *et al* (57) found that thymocyte differentiation antigen 1 (Thy-1) expression is epigenetically modified. Treatment with DNMT inhibitor 5-azacytidine attenuates TGF-β1-induced collagen type I α 1 chain gene and protein expression and α-SMA gene expression in LFs. Furthermore, inhibiting DNMT1 attenuates TGF-β1-induced DNMT activity, downstream suppression of Thy-1 expression and decreases Thy-1 promoter methylation. In addition, DNA methylation induces differentiation of fibroblasts into myofibroblasts and deposition of collagen matrix (58). O6-alkylguanine DNA alkyltransferase (MGMT) is a key DNA repair enzyme; however, MGMT is hypomethylated with overexpression in IPF fibroblasts (59). These findings suggest that inhibition of DNMT might prevent lung fibrosis (57).

At present, a few histone modifications have been described. The best-elucidated modifications (60) include acetylation by histone acetyltransferases (HATs) (61), deacetylation by HDAC (62), methylation by histone methyltransferase (HMT), demethylation, phosphorylation, ubiquitination, sumoylation and poly ADP-ribosylation (63). Currently, acetylation is hypothesized to activate cell transcription by decompression of the chromatin. IPF is characterized by increases in 'restrictive' histone post-translational modifications that result in decreased anti-fibrotic and pro-apoptotic gene expression (63). A previous study has shown that decreased acetylation of histone H3 and H4 in patients with IPF decreases expression of cyclooxygenase 2 (COX-2) in fibroblasts, thereby decreasing synthesis of prostaglandin E2 and effectively inhibiting activation of fibroblasts (64). Moreover, Fas expression is decreased in IPF and linked to elevated H3K9 trimethylation and decreased H3 pan-acetylation at the Fas promoter (63). Taken together, the aforementioned studies indicate an imbalance in both histone acetylation and methylation in IPF; this imbalance prevents transcriptional activation of anti-fibrotic and pro-apoptotic genes.

Numerous miRNAs are up- or downregulated in patients with IPF. For example, increased expression of miR-21 in IPF fibroblasts inhibits Smad7 activation by TGFβ1, thereby aggravating PF (65). Additionally, miR-21 is hypothesized to induce EMT and promote fibrotic processes via inhibition of Smad7 (65). Furthermore, miR-144-3p is a miRNA that is upregulated >70-fold in IPF fibroblasts; it can increase α-SMA levels and its mimic has been shown to downregulate relaxin/insulin-like family peptide receptor 1in IPF LFs (66).

Decreased autophagy. Transformation of PF fibroblasts into pulmonary myofibroblasts is a key part of PF. Inhibition of autophagy leads to increased differentiation of myofibroblasts

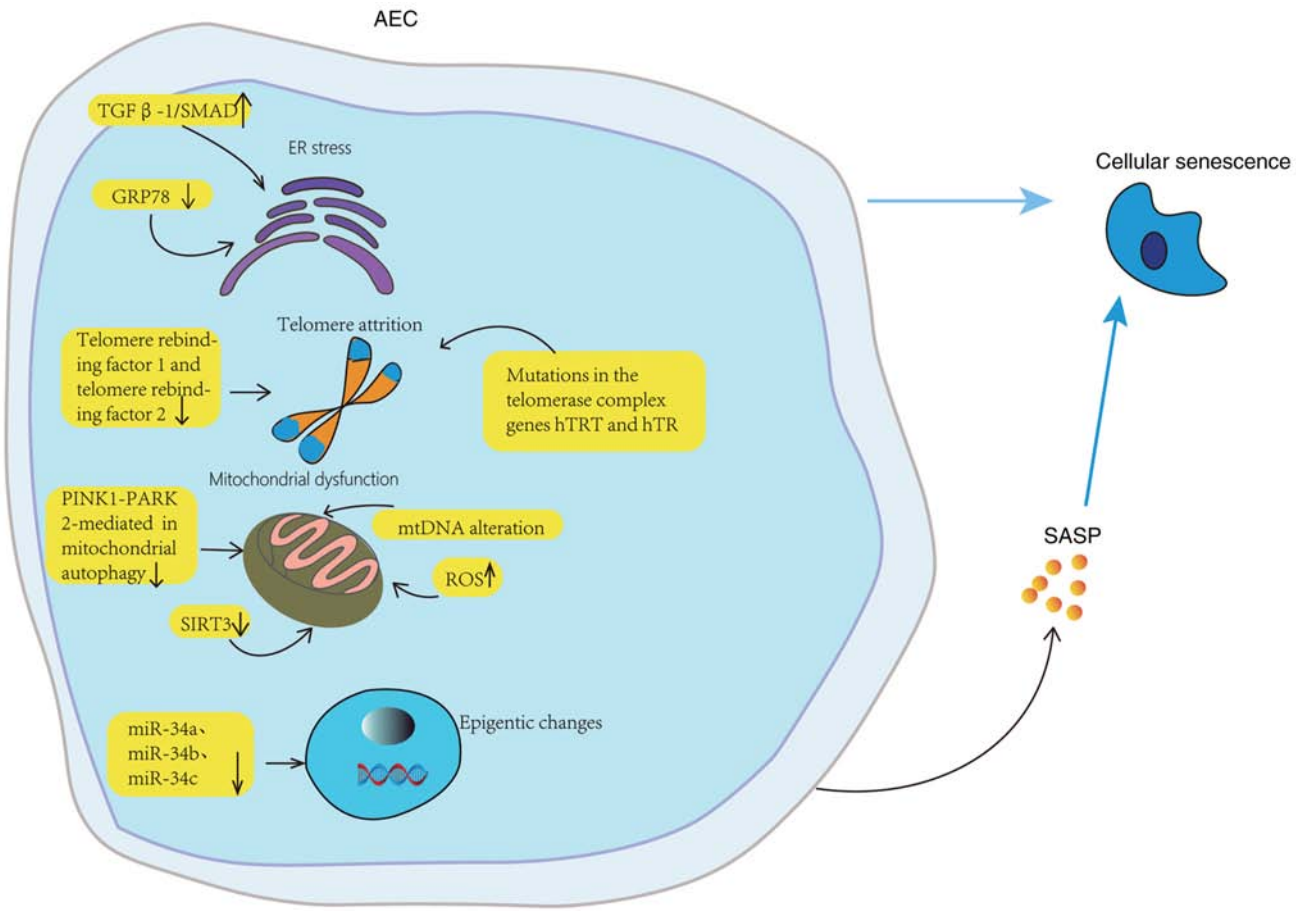

Figure 2. Aging mechanism of AECs. The senescence and apoptosis of AECs may be related to ER stress, autophagy, telomere damage, mitochondrial dysfunction, and epigenetic changes. In aging lungs, AECs are particularly sensitive to ER stress. GRP78 knockout decreases ER stress, injury, aging and differentiation, and TGF-β 1/Abnormal activation of SMAD signaling. Normal aging is accompanied by telomere shortening, while mutations in hTRT and hTR of telomerase complex gene accelerate telomere shortening and lead to replicative aging of AECs. In the pathogenesis of IPF, mitochondrial dysfunction involves the imbalance of mitochondrial ROS level, the change of mitochondrial DNA, the downregulation of mitochondrial autophagy mediated by PINK1-PARK2, and the downregulation of SIRT3 expression. The high expression of miR-34a, miR-34b and miR-34c can lead to the aging of AECs. AEC, alveolar epithelial cell; GRP78, glucose regulatory protein 78; hTRT, human telomerase reverse transcriptase; hTR, human telomerase RNA; mtDNA, mitochondrial DNA; PINK1, PTEN-induced putative kinase 1; PARK2, parkin RBR E3 ubiquitin protein ligase; ROS, reactive oxygen species; SASP, senescence-associated secretion phenotype; SIRT3, NAD-dependent deacetylase sirtuin-3; miR, microRNA.

and enhanced expression of smooth actin. A lack of autophagy may cause phenotypical transformation of fibroblasts into myofibroblasts (67). Beclin-1, a key autophagic protein, in a complex with class III phosphatidylinositol 3-kinase and ATG14 serves as a major positive regulator of autophagy (68). Genetic deletion of autophagy protein LC3 or Beclin-1 enhances expression of fibronectin, myofibroblast differentiation marker and SMA in LFs (69). TGF-β1 is a multifunctional peptide growth factor with a range of potential effects on cell proliferation and differentiation and ECM protein production. TGF-β1 also mediates the downregulation of Caveolin-1 (Cav-1) in fibroblasts via the MAPK signaling pathway and Cav-1 loss provides fibroblasts with anti-apoptotic properties (70). Another pathway by which autophagy regulates the pathological process of IPF is the mammalian target of rapamycin (mTOR) pathway. This pathway consists of upstream molecules (PI3K and AKT), tuberous sclerosis complex 1/2 and downstream molecules (eukaryotic cell translation initiation factor 4E binding protein 1 and ribosomal protein S6 kinase 1) (71). The mTOR pathway inhibits autophagy, which is characterized by increased apoptotic effector protein beclin-1 and LC3 levels. Activation of this pathway promotes differentiation of myofibroblasts and leads to the formation of PF (72). Similarly, Nho and Hergert (73) showed that deletion of PTEN in activated human chromosome 10 PTEN/AKT/mTOR signaling pathway induces autophagy in myofibroblasts for collagen synthesis. In LFs of IPF, the PTEN/AKT axis decreases the expression of FOXO3a. The decreased FOXO3a expression suppresses LC3B transcription and leads to loss of autophagy on collagen in IPF fibroblasts. Vimentin intermediate fills and Janus kinase 2/signal transduction activator 3 signaling pathways lead to increased production of the anti-apoptotic Bcl-2 family protein to inhibit autophagy, thus causing fibroblast-to-myofibroblast transformation and progression of PF (74).

A study reported the presence of telomere shortening in LFs of patients with IPF (5). This is consistent with telomere length maintained in the epithelial cells of patients with IPF (67). However, when expression of telomerase transcriptase in human LF is induced, the telomere length does not change in BLM-induced lung tissue (75,76). This suggests that the association between telomere shortening and cellular senescence occurs only in ATII cells and not in LFs, showing

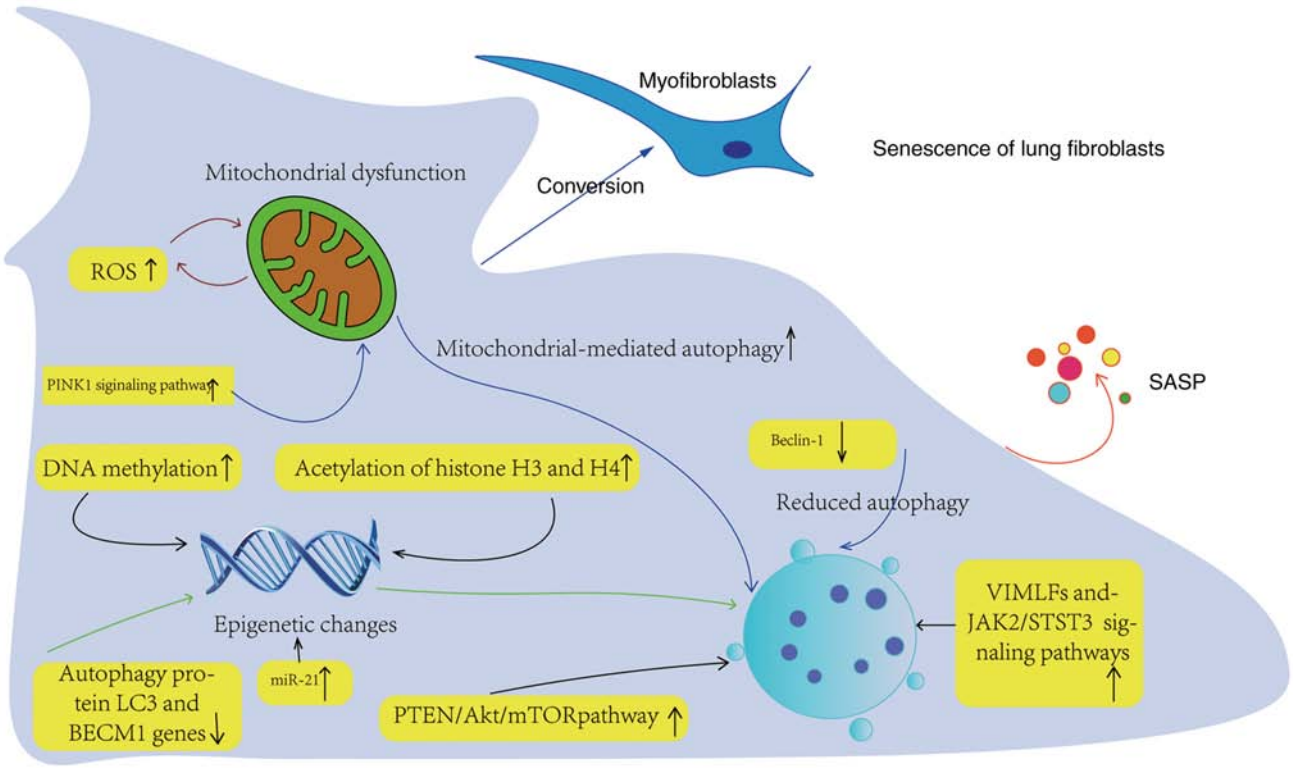

Figure 3. Aging mechanism of lung fibroblasts. Epigenetic changes mainly involve DNA methylation, histone post-translational modification and miR regulation. Abnormal DNA methylation can inhibit or activate the gene expression leading to fibrosis and change the mRNA expression of genes. The up regulation of histone H3 and H4 acetylation can promote pulmonary fibroblast fibrosis. The transformation of PF fibroblasts into pulmonary muscle fibroblasts is a key part of PF. Inhibition of autophagy may lead to the transformation of fibroblast phenotype into myofibroblast, and Beclin-1 is a key autophagic protein and a major positive regulator of autophagy. Gene deletion of autophagic protein LC3 or Beclin-1 enhanced the expression of fibronectin, myofibroblast differentiation marker and SMA in LFs. The upregulation of PTEN in PTEN/AKT/mTOR signal pathway can downregulate autophagy of myofibroblasts. Upregulation of ROS and PINK1 signaling pathway leads to mitochondrial dysfunction related to autophagy. PINK1, PTEN-induced putative kinase 1; ROS, reactive oxygen species; SASP, senescence-associated secretion phenotype; VIMFL, vimentin intermediate fill; JAK2, Janus kinase 2; STST3, signal transduction activator 3; miR, microRNA.

cell type specificity. In terms of mitochondrial dysfunction, TGF-β is a pro-fibrosis factor and TGF-β stimulation of LFs decreases PINK1 levels and promotes mitochondria-mediated phagocytosis as well as a low levels of myofibroblast differentiation (44). In addition, mitochondrial dysfunction of aging fibroblasts results in mTOR complex 1 (mTORC1) activation, which changes mitochondrial homeostasis and produces a large amount of ROS. ROS promotes DNA damage and enhances aging of LFs (77). The role of LFs and SASP in IPF is illustrated in Fig. 3.

4. Stem cell senescence and IPF

Stem cell dysfunction occurs in several aging-associated diseases, including IPF. MSCs from patients with IPF are in a transitional state of senescence and induce senescence paracrine, which can induce senescence in normal fibroblasts. A study demonstrated that adult stem cells prevent PF progression, repair lung injury and remodel lung tissue in animal models of PF (78,79). As MSCs are pluripotent, they can differentiate into a variety of cell types, including ATII cells, in response to specific stimuli (80).

Resident stem cells in the adult respiratory system have been identified, including Clara cell secreted protein (CCSP) and surfactant protein C-positive bronchioalveolar stem cells, p63-, Krt5- and/or Krt14-positive basal cells, secretoglobin family 1A member 1-positive Clara cells, tyrosine-protein kinase Kit-positive lung stem cells, E-cadherin/leucine-rich repeat containing G protein-coupled receptor 6-positive putative stem cells and ATII cells that initiate a pro-fibrotic cascade when the lung is damaged by persistent or repeated noxious stimuli, such as smoke, viruses, pollutants and genetic factors, and induces an abnormal environment to promote terminal differentiation of lung stem/progenitor cells from favoring local alveolar tissue regeneration to effector or fibroblast/myofibroblast development in IPF, ultimately promoting fibrosis progression (81).

ATII cells function as alveolar progenitors and long-term stem cells in the adult lung (82). Adult stem cells undergo dynamic changes after tissue injury. ATII cells are adult alveolar stem cells that can differentiate into ATI cells during alveolar homeostasis and post-injury repair and are involved in the process of lung repair (83). A previous study found that alveolar stem cell differentiation involves a transitional state (84) and a pre-ATI cell transitional state was identified in lung tissue regeneration. This unique state is associated with enrichment of cellular senescence and defective alveolar regeneration pathways, as well as the prolonged senescence and stress mediated pathway leading to pathological processes such as fibrosis. Furthermore, there

is a transition between ATI and ATII cells. These transition states are associated with abnormal epithelial cells, which show DNA damage response and express aging related genes on the way to ATI cells and are related to the defects of human pulmonary fibrosis (84). Metabolic lesions are associated with progressive PF (84).

Aging affects all cells, including MSCs. B-MSCs share many features with lung resident progenitor cells and have anti-inflammatory and immunomodulatory properties (85). A study demonstrated the ability of MSCs to suppress inflammation, decrease fibrosis and prolong survival in preclinical PF models (79). Tracking of radiolabeled cells revealed that when administered intravenously, MSCs primarily localize to the lung, followed by the liver and other organs (86). IPF is characterized by interstitial inflammation and epithelial cell damage, followed by fibroblast proliferation and collagen deposition. Preferentially located at sites of inflammation, MSCs inhibit ongoing damage and contribute to tissue repair (87). It has been suggested that several pathways are altered in aging MSCs and lungs, which may increase the risk of IPF (88). Scientists have observed IPF-associated extrapulmonary effects in B-MSCs. Compared with B-MSCs from age-matched controls, B-MSCs from patients with IPF exhibit increased cell size, morphological changes, DNA damage and telomere shortening with replicative senescence (89). In addition, B-MSCs from patients with IPF exhibit mitochondrial dysfunction and impaired recovery under *in vitro* and *in vivo* stimulation (89). Senescent B-MSCs have decreased paracrine capacity by senescent fibroblasts, suggesting a potential link between senescent B-MSCs and later onset of the IPF (89). In addition, systemic transfer of MSCs effectively decreases BLM-induced lung injury and fibrosis by downregulating nitric oxide metabolites, pro-inflammatory factors and angiogenic cytokines (90).

Elucidating the association between stem cell aging and PF may provide novel potential treatment options for PF.

5. SASP

There is an association between senescent AECs and fibroblasts. Senescent AECs promote activation of LFs by increasing expression of SASP. SASP includes pro-inflammatory cytokines (such as IL-6 and IL-8), growth factors [such as TGF-β and granulocyte-macrophage (GM) colony-stimulating factor (CSF)], chemokines [such as C-X-C motif chemokine ligand (CXCL) 1, CXCL3 and CXCL10] and matrix remodeling enzymes (such as metalloproteinases) (91). SASP has potent autocrine and paracrine effects and regulates the tissue microenvironment through biological processes, including cell proliferation, migration, inflammation, fibrosis, ECM degradation, neovascularization, tissue repair and regeneration, senescence clearance and EMT (92). SASP creates an inflammatory microenvironment for clearance of senescent cells and promotes senescence of surrounding cells in a paracrine manner. The SASP factor varies in different cell types and senescence-induced stimuli (92). Fibroblasts and myofibroblasts exhibit stress and senile phenotypes while secreting a range of cytokines, including pro-inflammatory cytokines (such as TNF-α, TGF-β, IL-1β, IL-6, IL-8, IL-10 and IL-18), chemokines (such as CXCL1 and monocyte chemoattractant protein-1), growth regulators [such as fibroblast growth factors, connective tissue growth factor (CTGF), GM-CSF and macrophage-CSF], matrix metalloproteinases (such as MMP-2, MMP-3, MMP-9, MMP-10 and MMP-12) and leukotrienes (LTs; such as LTA4, LTB4, LTC4, LTD4) (93,94). These cytokines serve a key role in regulation of PF and inflammation (95). IL-18 promotes senescence of LFs and expression of SASP in lung tissues by downregulating Klotho expression. Furthermore, neutralizing IL-18 by IL-18 binding protein partially inhibits aging of LFs (94). TGFβ-1 is a key component of SASP. By upregulating the cell cycle inhibitors p21, p27 and p15, TGFβ induces the senescence of adjacent cells in a paracrine manner through the SMAD signaling pathway (96).

Aging ATII cells express SASPs such as PDGF, TNF, endothelin-1, CTGF, osteopontin, CXCL12 and plasminogen activator inhibitor 1 (PAI-1), inducing massive proliferation and activation of IPF LFs and myofibroblasts (97). One salient component is PAI-1 which is highly expressed in ATII cells. Overexpression of PAI-1 promotes accumulation of extracellular stroma and acts as a strong inducer of cell senescence, especially in ATII cells. TGF-β1 increases PAI-1 expression through a variety of signaling pathways (98), such as TGF-β1, induce ATII-cell senescence, and that its prosenescent effects are mediated by PAI-1 (99), TGF-β1 induces PAI-1 production in alveolar macrophages through activin receptor-like kinase 5 activation and Smad3 phosphorylation (100).

Extracellular vesicles (EVs) have been recognized as a type of SASP. Derived from plasma membranes, EVs have diameters ranging from 30 nm to 5 μm and are composed of a phospholipid bilayer. EVs effectively promote cell signaling and differentiation by transferring bioactive substances, including miRNA, mRNA and lipids. IPF LF-derived EVs transfer miR-23b-3p and miR-494-3p to lung epithelial cells (101,102). These miRNAs inhibit production of mitochondrial ROS, causing mitochondrial dysfunction, DNA damage response and accelerated cell senescence (101). The expression of miRNAs in LF-derived EV was correlated with lung function of the donor. The development of fibrosis is largely regulated by SASP. Targeted inhibition of specific miRNA species or blocking secretion of specific EVs from LF may provide new therapeutic options (101).

SASP is involved in lung fibrosis by recruiting a large number of inflammatory cells into tissue and organs. In addition, SASP promotes the synthesis of ECM proteins by stimulating proliferation and transformation of fibroblasts into myofibroblasts (101).

The senescence of AECs is controlled by the PTEN/NF-κB pathway; this is a feature of PF. NF-κB is a key target of the PTEN/PI3K/Akt pathway, a signal transduction pathway that triggers cytokine release in senescent cells, thereby contributing to the adjacent cellular microenvironment (103). Other studies have showed that NF-κB and p38 MAPK pathways are involved in regulation of SASP (104,105).

In conclusion, SASP directly or indirectly promotes cell senescence or lung fibrosis via various signaling pathways, such as blocking the Klotho, SMAD and PTEN/NF-κB pathways. Further study of this mechanism is key for the treatment of IPF.

6. Interaction between senescent ATII, LF and other cell types in IPF

Senescent cells have two effects: They can be physiologically beneficial for tissue repair but they can also be pathologically harmful in age-associated diseases such as lung fibrosis (91). One of the key pathogenic mechanisms of PF is the inability of ATII cells to repair damaged epithelial cells due to cell death, ineffective proliferation, migration and differentiation, leading to interstitial scarring. Apoptosis of ATII cells directly encourages the progression of PF. Abnormal aging and apoptosis in ATII cells during acute lung damage are initiators of fibrosis. AECs during acute lung damage activate fibroblasts to differentiate into myofibroblasts, which form fibroblast foci and secrete large amounts of ECM (91). Aging AECs promote activation of LFs by increasing expression of SASP. One hypothesis on the origin of myofibroblasts is EMT (91). Cells lose the properties of epithelial cells and acquire properties of mesenchymal cells (106). The Wnt pathway is potentially involved in EMT. In ATII cells with overexpressed Wnt-1-induced signaling proteins, secretions of certain pro-fibrosis markers, such as PAI-1, are increased. These markers induce EMT in adjacent epithelial cells. In turn, abnormally aging ATII cells activate fibroblasts by secreting chemokines. Myofibroblasts produce mediators that induce epithelial cell apoptosis, thereby destabilizing epithelial cells and increasing alveolar injury. Moreover, apoptosis of AECs leads to formation of gaps between alveoli (107). Myofibroblasts are the activated form of fibroblasts that migrate into the interstitial space and activate further fibroblast proliferation, leading to increased apoptosis in ATII cells (108), further promoting lung fibrosis.

Kadota *et al* (109) demonstrated accelerated epithelial cell mitochondrial damage and senescence during IPF. LFs from patients with IPF were found to induce cellular senescence via EV-mediated transfer of pathogenic cargo to lung epithelial cells. The transfer of miR-23b-3p and miR-494-3p via IPF LF-derived EVs also induced mitochondrial damage and senescence in lung epithelial cells. In addition, levels of miR-23b-3p and miR-494-3p from IPF LF-derived EVs correlated positively with disease severity of IPF. Furthermore, LFs from patients with IPF were found to induce cellular senescence via EV-mediated transfer of pathogenic substances to AECs. IPF LF-derived EVs increased mitochondrial ROS and associated mitochondrial damage in lung epithelial cells, leading to activation of the DNA damage response and subsequent epithelial senescence. In addition, miR-23b-3p and miR-494-3p, released from fibroblasts as EV cargo, were transferred to lung epithelial cells, where they induced mitochondrial damage and cellular senescence in HBECs. Scientists have identified a novel EMT interaction mediated by LF-derived EVs (110). This interaction induces mitochondrial damage and senescence in epithelial cells during the pathogenesis of IPF (110). Aging in ATII cells also contributes to PF. E3 ubiquitin ligase FBW7 binds TPP1, promotes TPP1 multisite ubiquitination, accelerates degradation, triggers telomere unpacking and DNA damage responses, as well as leads to stress-associated telomere dysfunction, which ultimately inhibits ATII stem cell proliferation and mediates stress-induced ATII stem cell senescence and PF (111). The proportion of ATII in total AECs is significantly decreased in patients with IPF (112).

7. Senotherapeutics

Currently, the only FDA-approved medications for treatment of IPF are pirfenidone and nintedanib (113). While both drugs slow progression of fibrosis in certain patients with IPF, they do not halt or reverse progressive fibrosis. Experiments are ongoing and numerous clinical trials have been conducted (114-116). A better understanding of cellular senescence lung fibrosis may provide novel therapeutic strategies for the prevention and treatment of aging-related PF.

Experiments have focused on the characteristics and signaling pathways of aging, but the current results are limited. Most research studies are in their early stages. There is also an increasing focus on developing anti-aging treatments known as senotherapy. In principle, aging treatment strategies are broadly divided into two categories: Anti-aging strategies that selectively remove senescent cells and homogeneous strategies that block the aging phenotype by inhibiting SASP without damaging cells (117-119). These potential therapeutics are summarized in Table I.

Senolytics

Therapeutic interventions for autophagy. Rapamycin attenuates TGF-β-induced differentiation of myofibroblasts and simultaneously activates autophagy by inhibiting mTORC1 (77). Berberine improves BLM-induced IPF by antagonizing the TGF-β1-mediated SMAD and the FAK-dependent PIK3/Akt-mTOR signaling pathways (120). Sphingosine 1-phosphate inhibits activation of mTORC, increases autophagy and decreases progression of PF (121).

Therapeutic interventions for ER stress. Chronic ER stress-mediated apoptosis in ATII cells and macrophages is a key pathogenic mechanism of IPF (122,123). Perera *et al* (124) showed that BLM increases ER stress and apoptosis in ATII cells and lung macrophages. Moreover, this effect is significantly ameliorated by *in vivo* blockade of the calcium-activated potassium channel KCa3.1 with senicapoc, a KCa3.1 ion channel blocker. Blocking KCA3.1 ion channels decreased ER stress and apoptosis in ATII cells and macrophages, which may help to reduce IPF pathology and improve lung function.

Decreasing ER stress and apoptosis in ATII cells reverse fibrosis. Liu *et al* (125) showed that 4-phenylbutyrate, a chemical ER chaperone, ameliorates ER stress, interstitial damage, collagen deposition and apoptosis in unilateral ureteral obstruction rat kidney. Research (126) has demonstrated reduced ER mitochondrial tethering in *in vitro* experimental ER stress and *in vitro* IPF ATII experiments in the presence of decreased expression of phospholipase acidic cluster classification protein 2 (PACS2). The levels of PACS2 are affected by its interaction with transient receptor potential cation channel subfamily V member 1 (TRPV1) and can be experimentally modified using capsaicin (CPS) and recovered following CPS treatment. Thus, therapeutic targeting of the PACS2/TRPV1 axis represents a novel approach to epithelial protection in IPF (126). Some ER stress inhibitors target lung cells and most experiments are *in vitro* (127,128). Therefore, to the best of our knowledge, the side effects of inhibiting ER stress in treatment of fibrosis are not mentioned in the literature. This requires further study on the side effects of ER stress inhibitors on normal cells.

Table I. Experimental potential senolytic drugs that target senescent alveolar epithelial cells and lung fibroblasts.

First author/s, year	Target	Potential therapeutic drugs	Mechanism	(Refs.)
Le Saux *et al*, 2013;	Telomere	Raloxifene	Induces telomerase activity	(129)
Calado *et al*, 2009;			and maintains telomere length	(130)
Arish *et al*, 2019;		Androgens		(131)
Townsley *et al*, 2016		GRN510	The small molecule telomerase activator GRN510 attenuates fibrosis in mice with bleomycin-induced PF	(132)
		Danazol	Danazol, a synthetic androgen, increases telomere length and stabilizes diffusing capacity for carbon monoxide and forced vital capacity	
Sanders *et al*, 2014;	Epigenetics	Vorinostat	Inhibits ubiquitin-histone	(139)
Korfei *et al*, 2018;			deacetylase and induces	(140)
Mora *et al*, 2017;			apoptosis of fibroblasts	(141)
Coward *et al*, 2014;		5'-azacytidine	Decreases DNA methylation	(142)
Korfei *et al*, 2015			and fibrosis	(138)
		BIX-01294 and 3-deazaneplanocin	Inhibitors of euchromatic histone-lysine N-methyltransferase 2 and enhancer of zeste homolog 2 histone methyltransferases; increase cyclooxygenase -2 expression in IPF fibroblasts, which may enhance production of anti-fibrotic prostanoid PGE2	
		LBH589 and SAHA	LBH589 downregulates mRNA expression of ACTA2 and ECM genes COL1A1, COL3A1 and FN in primary IPF fibroblasts and interferes with fibroblast-to-myofibroblast differentiation. SAHA induces apoptosis of IPF myofibroblasts, an effect that is mediated, at least in part, by upregulation of pro-apoptotic gene Bcl-2 antagonist/killer 1 and downregulation of anti-apoptotic gene Bcl-xL	
		Panobinostat	The pan-histone deacetylase-inhibitor panobinostat decreases profibrotic phenotypes, as well as inducing cell cycle arrest and apoptosis in IPF fibroblasts	
Sosulski *et al*, 2017;	Mitochondria	Hexafluoro	Increases the expression of	(47)
Sato *et al*, 2016			NAD-dependent deacetylase sirtuin-3	(145)
		Metformin	Prevents PF with NADPH oxidase 4 inhibitors	

Table I. Continued.

First author/s, year	Target	Potential therapeutic drugs	Mechanism	(Refs.)
Liu *et al*, 2016	ER	4-phenylbutyrate	A chemical ER chaperone that ameliorates ER stress, interstitial damage, collagen deposition and apoptosis in unilateral ureteral obstruction rat kidney	(125)
Romero *et al*, 2016; Lavieu *et al*, 2006;	Autophagy	Rebamycin	Inhibits mTOR complex 1 and activates autophagy	(72) (121)
Lawson *et al*, 2008		Sphingosine 1-phosphate	Inhibits activation of mTOR complex and increases autophagy	(122)
		Berberine	Antagonizes TGF-β1-mediated PIK3/Akt/mTOR signaling and increases autophagy	
Lehmann *et al*, 2017; Feng *et al*, 2019	SASP	Dasatinib and quercitin	Inhibit tyrosine kinases, induce clearance of senescent cells, decrease fibrosis and SASP production.	(149) (151)
		Citrus basic extract	Downregulates expression of SASP factors in etoposide-induced fibroblasts by activating cyclooxygenase 2	
Glassberg *et al*, 2017; Zhao *et al*, 2021	Stem cell transplantation	MSC intravenous injection	Following *in vivo* transplantation, MSCs home in on injured lung tissue, release paracrine factors and extracellular vesicles, regulate function of immune cells, decrease the local inflammatory response, inhibit fibrous proliferation and promote endogenous lung injury resistance. MSCs decrease bleomycin-induced lung tissue inflammatory response, cell infiltration and cytokine expression, extracellular matrix production and collagen deposition and improve Ashcroft score	(159) (156)

IPF, idiopathic pulmonary fibrosis; SAHA, suberoylanilide hydroxamic acid; ER, endoplasmic reticulum; MSC, mesenchymal stem cell; SASP, senescence-Associated Secretory Phenotype.

Therapeutic interventions for telomerase function. Drugs that target telomeres have therapeutic potential for lung fibrosis. Le Saux *et al* (129) demonstrated that activation of telomerase with the small molecule telomerase activator GRN510 resulted in attenuation of lung fibrosis in BLM-induced fibrosis mice, showing decreased collagen deposition and loss of lung function and protecting lung epithelial cells from senescence. The estrogen receptor modulator raloxifene and androgens have been used to induce telomerase activity and increase telomere length (130). In a prospective study, danazol, a synthetic androgen, was shown to increase telomere length (131). Danazol was also found to increase telomere length and stabilize forced vital capacity and diffusing capacity of carbon monoxide in a small-scale clinical trial (132). However, hepatotoxicity and worsening PF associated with long-term use of danazol has been reported following danazol initiation and withdrawal (133). Therefore, more studies need to be conducted for effective interventions concerning telomerase function in lung fibrosis.

Therapeutic interventions for epigenetic changes. In lung remodeling and repair, druggable targets include enzymes that catalyze DNA methylation and demethylation, as well as enzymes that catalyze post-translational modifications of histones and non-coding RNAs (miRNAs and long non-coding RNAs) (134). Investigating the role of epigenetic factors in development of IPF may provide novel therapeutic options.

DNMT inhibitor 5-aza-2'-deoxycytidine (decitabine) enhances miR 17-92 expression and decreases expression of profibrotic genes including collagen 1A1 and CTGF (134). Its effects are independent of DNA methylation that enhances apoptosis. Therefore, more selective inhibitors of DNMTs need to be studied to determine if altered DNA methylation is involved in the pathogenesis of IPF.

Histone post-translation modifications, such as acetylation and methylation, are key regulators of transcription and comprise crucial components of the 'histone code' (135). HATs and HDAC regulate histone acetylation. Histone lysine or arginine methyltransferase and histone demethylase regulate histone methylation. HDAC alters differentiation of lung myofibroblasts (136). Moreover, treatment of fibroblasts with HDAC inhibitors increases histone H3 and histone H4 acetylation close to the promoter of the Fas gene, influences Fas expression and recovers sensitivity to Fas-mediated apoptosis (63,137). In fibroblasts from patients with IPF, application of HDAC inhibitors LBH589 and suberoylanilide hydroxamic acid (SAHA) increases expression of COX-2 to levels similar to those observed in controls (64,138). The upregulation of the pro-apoptotic gene Bcl-2 antagonist/killer 1 and down-regulation of the anti-apoptotic gene Bcl-xL partially mediate SAHA-induced apoptosis of IPF myofibroblasts, indicating that HDAC inhibitors may provide a novel therapeutic strategy in IPF by regulating myofibroblast susceptibility to apoptosis (139). A few studies have demonstrated that HDAC inhibitors inhibit activation and proliferation of cultured fibroblasts and attenuate fibrosis in multiple organs in *in vivo* animal models (137,139,140). The pan-HDAC-inhibitor panobinostat decreases profibrotic phenotypes and induces cell cycle arrest and apoptosis in IPF fibroblasts, thus indicating more efficiency than pirfenidone in inactivating IPF fibroblasts (140). The aforementioned study showed that HDAC inhibitors promote apoptosis of LF, while other treatments of IPF inhibit apoptosis of epithelial cells. Vorinostat, a pan-HDAC inhibitor, decreases lung fibrosis by promoting apoptosis of myofibroblasts, further improving lung function in mouse model of lung fibrosis (141). Some preclinical evidence showed that HDAC inhibitors have beneficial effects on IPF, for example, two inhibitors of the euchromatic histone-lysine N-methyltransferase 2 and enhancer of zeste homolog 2 HMTs (BIX-01294 and 3-deazaneplanocin) increase COX-2 expression in IPF fibroblasts, which may enhance production of PGE2, an anti-fibrotic prostanoid (142).

As highlighted by a recent review, studies discovered that non-coding RNAs may be a potential treatment for IPF (60,65). Many miRNAs(miR-21, miR-133a, miR-106b-5p) associated with lung fibrosis regulate TGF-β1 expression or function, inflammation, actin expression or cell signaling (65,143,144). Moreover, blocking upregulated lung 'fibro-miRs' or restoring downregulated 'anti-fibro-miRs' attenuates the lung fibrotic response (60).

Epigenetic marks are cell-type specific, yet, to the best of our knowledge, most research in this area has involved whole-lung tissue because isolation of sufficient material for specific cell types is often not feasible in human subjects. Another challenge with epigenomics is to integrate the epigenetic mechanisms that affect transcription and translation.

Therapeutic interventions for mitochondrial dysfunction. Metformin prevents PF via NOX4 inhibition (145). Metformin-mediated AMPK activation inhibits TGF-β-induced NOX4 expression. Rangarajan *et al* (146) found that metformin, as well as other AMPK activators, reverse lung fibrosis by promoting inactivation and apoptosis of myofibroblasts due to a lack of AMPK activation. The antifibrotic effects of normal SIRT3 expression levels in AECs and fibroblasts have potential therapeutic applications by decreasing formation of destructive cellular ROS and may be used in treatment of IPF. Furthermore, hexafluoro (a novel fluorinated synthetic honokiol analogue) maintains SIRT3 levels in LFs treated with TGF-β1 cytokines. This compound partly decreases TGF-ß-induced mitochondrial oxidative stress and activation of fibroblasts via SIRT3 stimulation. In addition, hexafluoro decreases levels of profibrotic factors such as α-SMA and fibronectin, which promote EMT in lung fibrosis (147).

Senomorphics

Therapeutic interventions for SASP. Removal of senescent cells decreases expression of SASP factors in regulating fibrotic pathogenesis of IPF. Targeting SASP components may be a feasible strategy to block detrimental functions of senescent cells of IPF. SASP, which is composed of cytokines, modulates the tissue microenvironment through various biological processes, including cell proliferation and migration, inflammation, fibrosis, ECM degradation, neovascularization and paracrine and autocrine pathways, as well as EMT (103). To investigate if SASP regulators decrease lung fibrosis, Justice *et al* (148) conducted a two-center, open-label study of dasatinib (D) + quercetin (Q), a senolytic, in patients with IPF to evaluate the feasibility of senolytic intervention. D is a tyrosine kinase inhibitor, while Q is a natural product that targets Bcl-2, insulin/insulin-like growth factor 1 and hypoxia-inducible factor 1α SCAP network components. It selectively eliminates senescent fibroblasts in BLM-induced fibrosis mouse models (95,148). A previous study reported that D + Q inhibits tyrosine kinase, induces clearance of senescent cells, decreases fibrosis and inhibits production of SASP (149). Furthermore, D + Q eliminates the anti-apoptosis effect of fibrotic fibroblasts induced by Fas ligand or TNF-associated apoptosis-inducing ligand, further reversing PF induced by BLM and decreasing mortality rate, making D + Q a potential therapeutic target for IPF (150). Citrus basic extract downregulates expression of SASP factors in etoposide-induced fibroblasts by activating COX-2 (151). Shentu *et al* (152) used human MSC-derived EVs (mEVs) to inhibit TGF-β1 and stimulate both normal and IPF myofibroblast differentiation. Their findings demonstrated anti-myofibroblastic effects of Thy-1-mediated mEV uptake and anti-myofibroblast in IPF fibroblasts and revealed that mEV-enriched miRs may mediate these effects.

mEVs represent a promising cell-free therapeutic approach for an array of disorders. Human bronchial epithelial cell (HBEC)-derived EVs contain a variety of miRNAs, including miR-16, miR-26a, miR-26b, miR-141, miR-148a and miR-200a (153). By attenuating Wnt signaling, these miRNAs inhibit TGF-β1-mediated induction of both myofibroblast differentiation and lung epithelial cellular senescence. However, this effect

in HBEC EVs is more pronounced than the effects observed in mEVs (153). Kadota *et al* (153) demonstrated that intratracheal administration of HBEC EVs attenuates BLM-induced lung fibrosis. To the best of our knowledge, however, there is no appropriate *in vitro* ATII culture system to prepare sufficient quantities of ATII EVs for injection therapy.

Stem cell transplantation. Cell therapy for lung diseases is developing rapidly. After tissue damage, MSCs are activated and recruited to the injured site. MSCs secrete bioactive molecules, regulate local immune response and establish a microenvironment to promote regeneration (154). Phase 1b non-randomized non-placebo clinical trials involving stem cells and a clinical study (155,156) have been performed using lung stem/progenitor ATII cells (155,157-159). Data from these trials support the safety of transplantation of MSCs or ATII cells in patients with IPF of different severity levels. Furthermore, Zhao *et al* (156) systematically reviewed 36 preclinical studies that used MSCs to treat BLM-induced acute lung injury and PF in rodent models. They found that MSCs significantly improve BLM-induced PF and suggested that MSCs could be a potential treatment for both IPF and virus-induced PF. In addition, intravenous injection of MSCs is feasible and safe for the treatment of patients with moderate to severe IPF (160). It is challenging to evaluate parameters such as tissue origin, cell type and delivery method in large-scale human studies because these parameters determine the effect of cell therapy. Poggio *et al* (161) compared intervention strategies of MSCs administered once and twice weekly and reported that repeated administration has more potent immunosuppressive effects on T cells (primarily $CD8^+$ T cells). In a murine model of BLM-induced PF, repeated administration of MSCs (three times/3 days) had comparable antifibrotic effects to the continuous administration of pirfenidone (161).

8. Conclusion

IPF is a progressive and fatal diffuse interstitial lung disease that has a poor prognosis and its primary mechanism remains unclear. Currently, there is no effective treatment for IPF other than lung transplantation.

IPF is an aging-associated lung disease in which LF and AEC senescence play a complex role in pathogenesis. Numerous studies (16-20,24-28,31-34,41) have revealed that ATII cell senescence and apoptosis are associated with ER stress and autophagy, telomere damage, mitochondrial dysfunction and epigenetic changes, leading to development of PF. The activation of LF and deposition of ECM proteins are key steps in the development of IPF. Epigenetic changes and reduced activation of autophagy promote myofibroblast differentiation, ultimately leading to PF. Aging AECs promote LF activation by increasing expression of SASP, thereby increasing occurrence and development of PF. In short, cell senescence is an important mechanism of IPF pathogenesis (5,9,54).

AECs (primarily ATII cells) and LF have therapeutic pathways and the same pathogenic pathways in the process of mediating PF. These pathways may act as potential new therapeutic targets for PF in future. More efforts on clinical testing need to be performed to validate promising therapeutic compounds.

Over the past decades, scientists have explored the mechanisms that lead to aging, damage and fibrosis of lung cells and have yielded useful insights (3,5,6,9,12,15,19,53,95). However, the exact mechanism of cellular senescence remains unclear and requires further research. Aging affects not only different types of cell in the lungs but also cells in other organs. The treatment of lung cell senescence is being actively explored, but whether the molecular mechanism involved in lung cell senescence therapy adversely affects normal and aging cells of extrapulmonary organs remains unclear. It is difficult to treat a specific pathogenic target accurately, because the expectation of this treatment is to promote the apoptosis of lung senescent cells and the proliferation of normal lung cells. It is worth exploring whether targeted therapy drugs can be developed that accurately target disease-causing senescent cells in the lung. Stem cell transplantation has been a hot research topic in recent years. Existing research has advanced in mouse models of BLM-induced PF and it may provide new ideas for the treatment of IPF. Novel therapies targeting cellular senescence may provide new treatment strategies for IPF and improve survival and quality of life for patients with IPF.

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Authors' contributions

SH and QL both wrote the manuscript. SH, QL and XL revised the manuscript. SH and QL contributed equally to this work and should be considered co-first authors. Data authentication is not applicable. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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