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REVIEW

Alveolar Epithelial Type 2 Cell Dysfunction in Idiopathic Pulmonary Fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a progressive and irreversible pulmonary interstitial disease that seriously affects the patient's quality of life and lifespan. The pathogenesis of IPF has not been clarified, and its treatment is limited to pirfenidone and nintedanib, which only delays the decline of lung function. Alveolar epithelial type 2 (AT2) cells are indispensable in the regeneration and lung surfactant secretion of alveolar epithelial cells. Studies have shown that AT2 cell dysfunction initiates the occurrence and progression of IPF. This review expounds on the AT2 cell dysfunction in IPF, involving senescence, apoptosis, endoplasmic reticulum stress, mitochondrial damage, metabolic reprogramming, and the transitional state of AT2 cells. This article also briefly summarizes potential treatments targeting AT2 cell dysfunction.

Keywords Pulmonary fibrosis · Alveolar epithelial type 2 cell dysfunction · Metabolic reprogramming · Transitional state

Introduction

Idiopathic pulmonary fibrosis (IPF) is the most common and fatal idiopathic interstitial pneumonia. The median survival time from diagnosis is 2–4 years [1]. Because of the unclear pathogenesis, limited treatment drugs are available. Pirfenidone and nintedanib, antifibrotic medicines approved by FDA, were shown to slow down the forced vital capacity (FVC) reduction rate, but they do not improve the survival rate of patients [2]. Better understanding of the pathogenesis of IPF will lead to the development of new treatment strategies and medications.

In Europe and North America, the incidence of IPF is between 2.8 and 19 cases per 100,000 people per year, and the prevalence of IPF increases with age. Disease onset is usually at 60 years old, and the peak of disease occurs between 60 and 70 years of age [3]. IPF is an aging-related disease, and AT2 cell aging participates in the occurrence of IPF [4]. AT2 cells in IPF patients show mitochondrial stress, endoplasmic reticulum stress, and gene mutation. Recent studies have also identified metabolic reprogramming and paracrine changes in AT2 cells of IPF patients [5, 6]. Compared with AT2 cells directly transforming into mesenchymal cells, the transformation of fibroblasts into muscle fibroblasts caused by the paracrine signals of AT2 cells is more important to IPF [6]. This article summarizes the role of AT2 cells in IPF from two aspects: the dysfunction of AT2 cells in IPF and the change of signaling between AT2 cells and surrounding cells (mesenchymal cells, macrophages) during IPF (Table 1).

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AT2 Cell Senescence or Apoptosis is Dominant in IPF

Mutations in telomerase and telomere genes can lead to abnormal telomere shortening. Clinically, this molecular abnormality is manifested as telomere syndromes characterized by aging. IPF is the most common clinical manifestation of human telomere syndrome [31]. In IPF patients, AT2 cells have the shortest telomere length compared with club cells and myofibroblasts; AT2 cells also show the most

Table 1 Signaling pathways of AT2 cell dysfunction in IPF

Pathways	Objects	References
Senescence and apoptosis		
Trf2/DNA damage at telomeres/p53 pathway	Mice, primary murine AT2 cells	[7]
Bmi-1/TIME signaling/SASP	human pulmonary samples, mice, primary murine AT2 cells and fibroblasts	[8]
Sin3a/p53-p21 axis/AT2 senescence	IPF patients, mice, primary murine epithelial cells	[9]
TSPAN1/p-IκBα/NF-κB/AT2 cell apoptosis	IPF patients, mice, A549 cell line, primary murine AT2 cells	[10]
PAI-1/p53 Phosphorylation	mice, primary murine AT2 cells and fibroblasts	[11]
Endoplasmic reticulum stress and mitochondrial damage		
Grp78/ER stress/AT2 cell aging phenotype	Mice	[12]
FKBP13/UPR/ER stress	IPF patients, mice	[13]
ER stress/ATF3/PINK1/Mitophagy	Mice, A549 cell line, primary human AECIIs	[14]
ER stress/UPR/PACS2-TRPV1/ER-Mito tethering	IPF patients, MLE12 cell line, MEL188 cell line,	[15]
mitoROS/NAD+/PARP1/SIRT1/mitophagy	Mice, MLE12 cell line	[16]
SIRT3/OGG1/mitophagy/mtDNA	Mice, early-passage human lung fibroblasts	[17]
Klotho/AKT signaling/mtDNA damage	Mice, primary murine AT2 cells, A549, MLE12, and RLE-6TN cell lines	[18]
Transitional state and paracrine		
RhoGTPase Cdc42 /AT2 intermediate cell/ AT2 differentiation	IPF patients, mice, primary murine AT2 cells and stromal cells	[19]
PATS/AT2 differentiation	Mice, alveolar organoid	[20]
Nedd4-2/TGFβ- p-SMAD2/ p-SMAD3	IPF patients, mice, primary murine AT2 cells	[21]
MIR100HG/miR-29a-3p/Tab1/TIMP-1/MMP-1	Mice, MLE12 cell line	[22, 23]
EGFR-RAS-ERK/ZEB1/tPA/TGFβ-1	IPF patients, primary human parenchymal lung fibroblast, primary human AT2 cells	[24]
PDGFA / PDGFRA/AT2 differentiation	Mice, human organoids, Mouse lung organoids	[25]
SIX1/MIF/ Proliferation of fibroblasts	IPF patients, primary human AT2 cells and fibroblasts, mice, primary murine AT2 cells, MLE12 cell line	[26]
Inflammation		
Lrrk2/CCL2-CCR2 axis	Mice	[27]
MCP-1/CCR2/TGF-β axis	Mice, primary murine macrophages and AT2 cells	[28]
Shh/Gli1-OPN-JAK2/STAT3	Mice, bone marrow-derived macrophages, MLE12 cell line	[29]
MCU/Cpt1a/Bcl-2/TGFβ-1	Mice, human THP-1 monocytes, Mouse MH-S alveolar macrophage cells	[30]

serious DNA damage, which is mainly caused by telomere shortening [32]. AT2 cells in mice with telomeric repeat-binding factor 2 (*Trf2*) gene knockout exhibit decreased proliferation and differentiation ability, but apoptosis was not increased, suggesting that telomere damage in AT2 cells preferentially initiates the cell aging process and damages cell renewal and differentiation ability. AT2 cells with *Trf2* gene deletion show upregulated *p53* expression through the paracrine pathway, induce mesenchymal cell apoptosis, and hinder normal lung development in mice [7]. *Bmi-1* deficiency leads to the accumulation of ROS and cytoplasmic p16, which induces AT2 cell senescence and senescence-associated phenotype (SASP) via the TGF-β1/IL-11/MEK-ERK (TIME) pathway [8]. Sin3a gene deletion also induces cell cycle arrest and aging of AT2 cells by activating the p53-p21 axis, which promotes the occurrence of pulmonary fibrosis [9].

AT2 cell apoptosis in IPF is also a research hotspot. Studies have found that specific depletion of AT2 cells induces pulmonary fibrosis in mice. At present, there are three models of AT2 cell-specific injury: the murine surfactant protein C (SPC) promoter and the diphtheria toxin receptor gene (SPC-DTR) mouse model induced by diphtheria toxin; the tamoxifen-inducible SPC-CreER mouse model, which drives the expression of diphtheria toxin A in AT2 cells; and the ganciclovir-induced transgenic mouse model, in which the SPC promoter drives expression of mutant SR39TK herpes simplex virus-1 thymidine kinase in AT2 cells [33–35]. TSPAN1 is a member of the tetraspanins family and is downregulated in lung tissue of patients with IPF and bleomycin-induced pulmonary fibrosis mice. TSPAN1 inhibited AT2 cell apoptosis by inhibiting p-IκBα, which attenuated nuclear NF-κB translocation and activation [10]. The apoptotic sensitivity of AT2 cells increases, while the

apoptotic sensitivity of fibroblasts decreases in the lung of aged mice, which is related to the fact that plasma activator inhibitor 1 (PAI-1) can positively or negatively regulate the phosphorylation and expression of *p53* according to cell type [11].

Endoplasmic Reticulum Stress of AT2 Cells Promotes IPF

GRP78 is one of the important modifiers of controlling protein quality by activating the unfolded protein response (UPR) in the endoplasmic reticulum. Mice with Grp78 gene knockout develop spontaneous pulmonary fibrosis, and AT2 cells with *Grp78* gene knockout are characterized by endoplasmic reticulum stress, apoptosis, senescence, and impaired stem cell ability [12]. Endoplasmic reticulum stress inhibitors decrease the expression of markers of cell aging, apoptosis, and mesenchymal cells [12]. The UPR maintains protein homeostasis by enhancing the ability of the ER to refold proteins and reducing the translation of abnormal proteins [36]. *Fkbp13* (13-kD FK506-binding protein) level positively correlated with the UPR marker GRP78 and total XBP1. *Fkbp13* knockout mice were more sensitive to bleomycin and showed increased early inflammatory cells (macrophages, neutrophils, lymphocytes) and proinflammatory factors (IL-6, TGFβ-1) [13]. In mice harboring L188Q mutation in *SFTPC*, which encodes SPC, ER stress is induced but the mice do not show spontaneous fibrosis; upon exposure to bleomycin, much greater lung fibrosis was observed compared with fibrosis in WT mice [37]. In the *SFTPC* BRICHOS mouse model with C121G mutation in *SFTPC*, mice show high levels of ER stress in AT2 cells and spontaneous pulmonary fibrosis [38]. These models indicate ER stress as a key driver of lung fibrosis.

Excessive ER stress damages the signal network with mitochondria and causes mitochondrial dysfunction (Fig. 1). ER stress of AT2 cells impairs mitophagy by activating transcription factor 3 (ATF3) and repressing transcription of the PTEN-induced putative kinase 1 (PINK1) gene [14]. Mitophagy dysfunction promotes AT2 cell aging and IPF [39]. When UPR cannot be compensated due to persistent ER stress, ER-mitochondrial tethering will decrease, and the expression of transient receptor potential cation channel subfamily V member 1 (TRPV1) and phosphofulin acid cluster sorting protein 2 (PACS2) are downregulated. The deletion

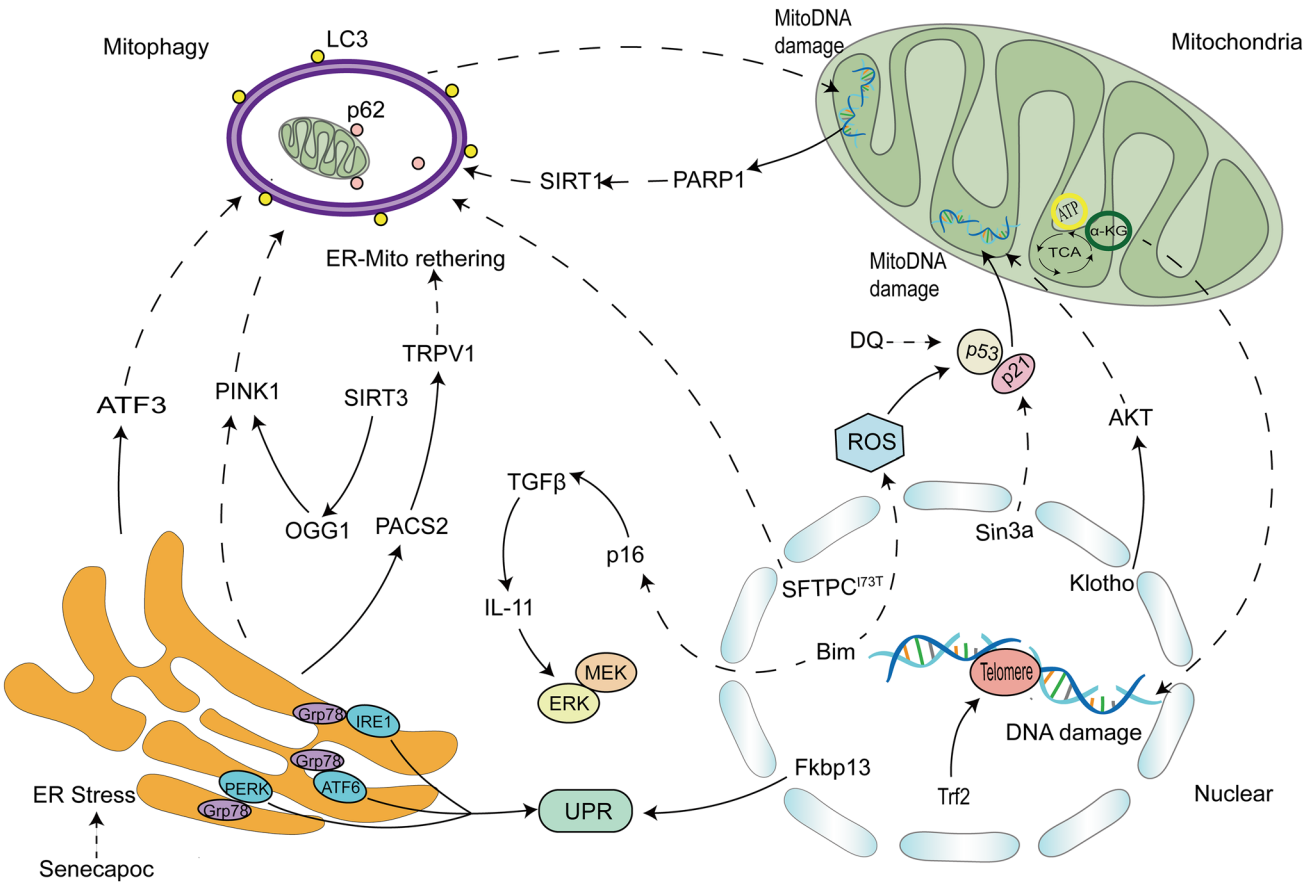

Fig. 1 The mechanism of AT2 cell senescence and apoptosis. The solid line indicates promotion and the dotted line indicates inhibition

of *Pacs2* has been proven to induce the cleavage of BAP31 to lead to mitochondrial-dependent apoptosis. In vitro and ex vivo experiments showed that ER-mitochondrial tethering induced by ER stress was related to the PACS2-TRPV1 axis [40]. The TRPV1-modulating drug capsaicin (CPS) inhibited the degradation of TRPV1, increased the level of PACS2 protein, and reduced the apoptosis of alveolar epithelial cells and collagen expression [15].

Mitochondrial Damage and Metabolism Reprogramming of AT2 Cells Participate in IPF

Mitochondrial DNA (mtDNA) and mitophagy damage are critical factors in the process of IPF (Fig. 1). Cigarette smoke (CS) may cause mitochondrial reactive oxygen species (mitoROS), mitoROS-induced DNA damage activates poly ADP-ribose polymerase (PARP1), which competitively depletes nicotinamide adenine dinucleotide (NAD+) with SIRT1. Suppressed SIRT1 leads to a lack of regulation of mitophagy, resulting in DNA damage and AT2 cell senescence. Mitophagy damage in turn leads to mitoROS, resulting in a positive feedback between mitophagy and SIRT1 [16]. SIRT1 activator and mitoROS scavenger can inhibit CS-induced AT2 cell senescence and pulmonary fibrosis [16].

SIRT3 also inhibits ROS-induced damage to mitochondrial DNA by preventing acetylation of 8-oxoguanine DNA glycosylase (OGG1). Deacetylation of OGG1 is essential for maintaining DNA integrity by preventing mitophagy from being damaged [17]. OGG1 can prevent mitophagy dysfunction caused by PINK1 deficiency to avoid AT2 cell aging and alleviate IPF [39]. SIRT7 participates in the mitochondrial UPR, which is important for AT2 cell homeostasis [41]. *Klotho* is an anti-aging gene and Klotho indirectly prevents lung fibrosis through lessening mtDNA damage and apoptosis of AT2 cells [18, 42, 43]. Klotho promotes FGFR1 binding to FGF23 and actives AKT signaling to prevent apoptosis of AT2 cells caused by mtDNA damage [44, 45].

Studies have shown that MLE12 exhibits mitochondrial respiratory inhibition after bleomycin induction for 3 h, specifically manifested as the decline in oxidative phosphorylation. Mitochondrial respiratory inhibition is accompanied by DNA damage (γ- H2AX is upregulated). The glycolysis of cells induced by bleomycin is also inhibited, which involves the significant decrease of the expression of glucose uptake and transport 1 (GLUT1), the decrease of the exchange rate of intermediate products of the tricarboxylic acid cycle, and the decrease of extracellular acidification rate (ECAR) [46]. Another study showed that iAEC2s with $SFTPC^{I73T}$ gene mutation exhibit damaged oxidative phosphorylation and higher glycolysis [5].

In addition, inhibiting glutamine metabolism can inhibit the proliferation and differentiation of AT2 cells. The specific mechanism may be that the inhibition of glutamine metabolism reduces α-KG production, which is the most important intermediate product in the tricarboxylic acid cycle. The impaired TCA cycle and limited ATP are not conducive to the regeneration of damaged cells [47]. Glutamine can maintain various intermediates of the TCA cycle and pentose phosphate pathway, glycolytic intermediates, and almost all amino acids. Bleomycin-damaged MLE12 can restore cell mitochondrial respiration and ECRA after supplementing glutamine [46]. Glutamine can also reduce the cytotoxicity of bleomycin, because glutamine and α-KG play important roles in nucleotide synthesis and DNA repair, respectively [48, 49].

Transitional State of AT2 Cells in IPF

A previous study identified the presence of aberrant basaloid cells, which is a previously unidentified epithelial cell population, that coexpress basal epithelial markers, mesenchymal markers, aging markers, developmental transcription factors, and known IPF markers [50]. AT2 cells in the IPF lung can also differentiate into KRT5+ basal cells in response to fibrotic signaling. TGFβ-1 and anti-bone morphogenic protein (anti-BMP) promote this transdifferentiation [51]. In models of progressive lung fibrosis and human IPF patients, AT2 cells can differentiate into Krt8+ alveolar differentiation intermediate (ADI), which can reprogram into AT1 cells [52]. RhoGTPase Cdc42 depletion leads to an accumulation of cells in the AT2 intermediate cell state, which prevents AT2 cells from differentiating into AT1 cells. This impaired regenerative ability causes AT2 cells to be exposed to persistently elevated mechanical tension, activates the TGF-β signal in AT2 cells, and promotes periphery-to-center progression of lung fibrosis [19]. Another study showed that there was accumulation of pre-alveolar type-1 transitional cell state (PATS), an intermediate cell state during the differentiation of AT2 cells into AT1 cells, in the pulmonary fibrosis area of IPF patients. PATS cells in human lungs exhibit enrichment of genes associated with cellular senescence, TP53 signaling, and TGF-β-regulated genes. Moreover, PATS cells are vulnerable to DNA damage during the process of differentiation from AT2 cells to AT1 cells (Fig. 2). Long-term aging and stress regulatory signals in transitional cells will promote the occurrence of fibrosis [20].

AT2 cells without Nedd4-2 can also lead to epithelial remodeling of the peripheral airway, mainly manifested as decreased club cells, increased ciliated cells and goblet cells in local and terminal airways. The expression of MUC5B in proximal and distal airways is also increased. Deletion of Nedd4-2 in AT2 cells increases the activity of ENaC,

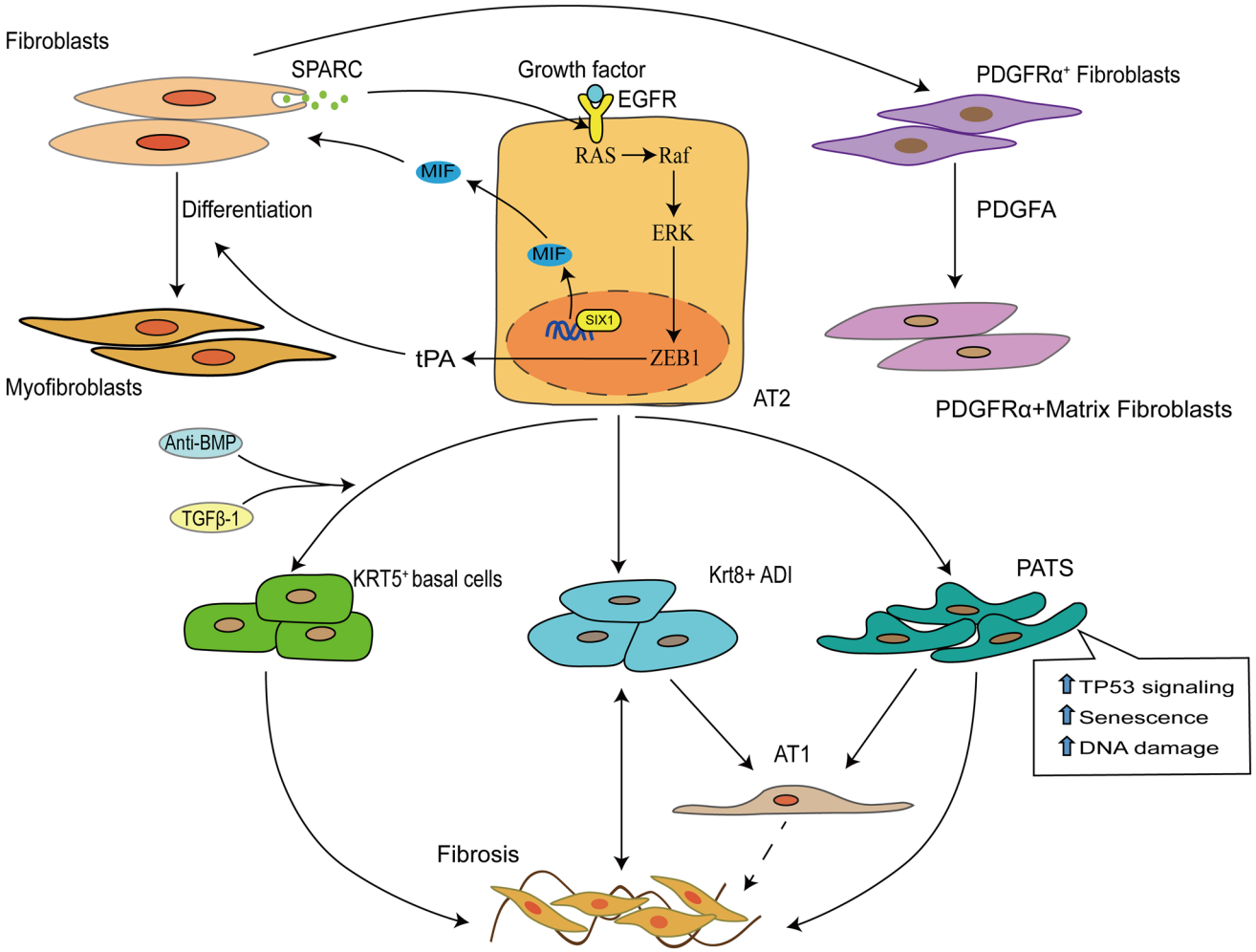

Fig. 2 The mechanism of transitional state and paracrine signals of AT2 cells. The solid line indicates promotion and the dotted line indicates inhibition

which leads to the consumption of airway surface fluid and decreases mucociliary clearance [21]. The lncRNA mir-100-let-7a-2-mir-125b-1 cluster host gene (MIR100HG) modulates TGF-β1-induced fibrotic changes in AT2 cells. It can increase the expression of TGF-β-activated kinase 1/MAP3K7 binding protein 1 (Tab1) by inhibiting microRNA-29a-3p (mir-29a-3p), promote the production of TIMP-1, and inhibit the degradation of collagen by MMP-1 [22, 23]. Mir-29 can also activate the Wnt/β-catenin pathway to promote TGF-β1-induced extracellular matrix synthesis [53].

Paracrine Signals in AT2 Cells Contribute to IPF

Some studies have pointed out that only a small part of fibroblasts in fibrotic lesions come from AT2 cells [6]. Compared with the direct transformation of AT2 cells into mesenchymal cells, the transformation from fibroblasts to myofibroblasts caused by the imbalance of paracrine signals is more important to EMT (Fig. 2). EGFR-RAS-ERK signaling in AT2 cells upregulates transcription regulator zinc finger E-box-binding homeobox 1 (ZEB1) and promotes AT2 cells to secrete tissue plasminogen activator (tPA), which can enhance TGF-β-induced myofibroblast differentiation [24]. AT2 cells can secrete TGF-β2 after injury, which highlights the role of paracrine signals between AT2 cells and fibroblasts in mediating EMT [24]. Fibroblasts activated by TGF-β can secrete secreted protein acidic and rich in cysteine (SPARC), which can activate EGFR-RAS signal transduction in AT2 cells. Thus, the bidirectional epithelial–mesenchymal crosstalk promotes the progression of fibrosis [54]. Studies have shown that AT2 cells in IPF patients have not completely lost the ability to differentiate into AT1 cells. PDGFA ligands activate beneficial feedback of the epithelial–mesenchymal crosstalk by promoting

PDGFRα+ fibroblast differentiation into beneficial PDGFRA+ matrix fibroblasts. PDGFRA+ matrix fibroblasts promote the differentiation of AT2 cells into AT1 cells [25]. A specific balance between the activation of the PDGFA ligand and inhibition of the PDGF-B ligand may enhance alveolar repair [25]. These findings suggest that the changes in signal connections between AT2 cells themselves or between AT2 cells and mesenchymal cells are involved in the occurrence of pulmonary fibrosis.

The developmental transcription factor Sine oculis homeobox homolog 1 (SIX1) plays an important role in lung development [55]. The mRNA and protein levels of Six1 and its transcriptional coactivators (EYA1 and EYA2) were increased in AT2 cells of bleomycin-induced pulmonary fibrosis model mice and telomere dysfunction pulmonary fibrosis model mice [26, 56]. The mechanism of Six1 in promoting the progression of pulmonary fibrosis may be related to the downstream macrophage migration inhibitory factor (MIF). SIX1 directly binds to the 5′-TCAGG-3′ consensus sequence of the MIF promoter, and MIF then promotes the proliferation of fibroblasts and the expression of α-SMA and COL1A1 [26]. The treatment of pulmonary fibrosis targeting MIF, mainly in the form of anti-MIF antibody therapy and MIF antagonist, is still under study [57, 58].

Inflammation activated by AT2 cells aggravates IPF

iACE2s with *SFTPC*^{I73T} mutation activates the NF-κB pathway and promote the secretion of inflammatory mediators (GM-CSF, CXCL5, and MMP-1) [5]. Activated WNT/β-catenin signal in AT2 cells also induces downstream IL-1β and IL-6, which activates TGF-β or STAT3 signal transduction, respectively [59]. Macrophage colony-stimulating factor (M-CSF) stimulates monocyte macrophages to produce CC chemokine ligand 2 (CCL2), which is involved in the occurrence of IPF [60]. The number of macrophages and neutrophils in alveolar lavage fluid and the secretion of IL-6 and monocyte chemoattractant protein 1 (MCP-1) are increased in pulmonary fibrosis mice induced by GRP78 knockout [12].

The interaction between AT2 cells and immune cells such as pulmonary macrophages is also involved in pulmonary fibrosis. *Lrrk2* deletion can not only damage autophagy through ERK and JNK signaling pathways of AT2 cells, but also leads to activation of the CCL2/CCR2 axis and recruitment of monocyte-derived macrophages [27]. Fibrosis induced by the MCP-1/CCR2/TGF-β axis was also present between injured AT2 cells and macrophages [28]. AT2 cells can also secrete Sonic hedgehog (Shh), initiate the Shh/Gli signal cascade, induce macrophages to secrete osteopontin, activate the JAK2/STAT3 pathway, mediate M2 polarization of macrophages, and promote pulmonary fibrosis [29]. Apoptosis resistance of pulmonary macrophages promotes the occurrence of pulmonary fibrosis. The mitochondrial calcium uniporter induces metabolic reprogramming to fatty acid β-oxidation and promotes the binding of carnitine palmitoyltransferase 1a (CPT1A) in mitochondria to the BH3 domain of mitochondrial B-cell lymphoma-2 (Bcl-2), decreases the proapoptotic proteins (Puma and Noxa), and inhibits the apoptosis of pulmonary macrophages. The interaction between CPT1A and Bcl-2 increases TGF-β1 in pulmonary macrophages, reduces antifibrosis protein (TNF-α), and promotes pulmonary fibrosis [30].

Potential Therapy Targeting AT2 Cells

Senescence and Regeneration

Dasatinib plus quercetin (DQ) can reduce aging markers in lung tissue of Sin3a-deficient mice and alleviate pulmonary fibrosis [9]. The clinical trial of DQ in the treatment of IPF shows that this drug can improve the physical function of patients [61]. Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme in the production of NAD+, and abnormal metabolism of NAMPT occurs in some aging-related diseases [62]. Research has found that MSCs inhibit AT2 cell aging by inhibiting lysosome-mediated degradation of NAMPT [63]. Human clinical trials have shown that a high-dose of allogeneic MSCs can delay the progress of IPF patients [64]. Recent studies propose that serum-free cultured MSCs (SF-MSCs) are easier to implant into mouse lungs after intravenous administration than serum cultured MSCs (S-MSCs). The antifibrosis effect of SF-MSCs is more pronounced compared with S-MSCs [65]. Lung niche mesenchymal cells promote the repair and regeneration of AT2 cells by secreting growth hormones, Wnt5A and chemokines. Ghr-enriched EVs promote the expression of Ghr in AT2 cells and the regeneration of AT2 cells [66].

ER Stress

Tauroursodeoxycholic acid (TUDCA) reduces ER stress markers (GRP94 and CHOP) as well as mesenchymal markers [12]. TUDCA inhibits BLM-induced CHOP mRNA expression in a dose-dependent manner and presents protective effects against BLM-induced pulmonary fibrosis in mice [67]. Calcium and calmodulin-dependent kinase II (CaMKII) inhibition blocks ER stress and apoptosis induced by bleomycin in MLE12 cells. A transgenic mouse model of CaMKII inhibition in type II pneumocytes exhibited a lower degree of pulmonary fibrosis than WT mice [68].

Mitochondrial Damage

MitoROS is involved in CS-induced senescence of AT2 cells. Mitochondria-targeted antioxidant mitoquinone (MitoQ) protects mice from CS-induced pulmonary fibrosis. SIRT1 activator and supplementation of NAD with its precursors can restore SIRT1 activity, prevent AT2 cell senescence, and inhibit CS-induced pulmonary fibrosis [16]. Thyroid hormone restores mitochondrial respiration in bleomycin-induced AT2 cells and limits the severity of pulmonary fibrosis in mice [69].

Conclusion and Future Insights

The pathogenesis of IPF mainly involves cell aging and apoptosis, mitochondria and endoplasmic reticulum dysfunction, abnormality of AT2 cell transitional state, paracrine signals in AT2 cells and inflammatory response. Mitochondria and endoplasmic reticulum oxidative stress, which can damage mitochondrial DNA, mitochondrial autophagy and the UPR, promote AT2 cell aging and apoptosis. The transformation of fibroblasts into myofibroblasts through the paracrine pathway of AT2 cells and AT2 cell transitional state also participate in IPF. The study on the pathogenesis of IPF should not be limited to AT2 cells themselves, and the paracrine effect on surrounding cells should also be studied. More potential therapies targeting AT2 cells including DQ, TUDCA, MitoQ, and Ghr-enriched EVs need to be further explored.

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Declarations

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

Ethical Approval Not applicable.

Consent to Participate Not applicable.

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