

Treatment of pulmonary fibrosis: From disease mechanisms to future novel therapies (Review)

SEN LU^{1*}, YUNFEI LIU^{2*}, XIAOHUA LI³ and QIPENG YAO⁴

¹Department of Critical Care Medicine, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, Sichuan 610072, P.R. China; ²Department of Anesthesiology, Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, Sichuan 610072, P.R. China; ³Department of Thoracic Surgery, Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Sichuan Clinical Research Center for Kidney Diseases, Chengdu, Sichuan 610072, P.R. China; ⁴Department of Chinese Medicine, Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, Sichuan 610072, P.R. China

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Abstract. Pulmonary fibrosis (PF) is a progressive and fatal interstitial lung disease characterized by irreversible lung scarring and frequently associated with lung cancer. Currently,

there remains a lack of effective therapies capable of significantly improving long-term outcomes or reversing the disease course. Although antifibrotic drugs are widely used and have enhanced the mechanistic understanding of PF, their efficacy is limited. This review systematically explores the core pathobiological processes and epigenetic regulatory networks involved in PF pathogenesis. Simultaneously, a critical review of the most promising emerging therapeutic strategies in recent years, including stem cell therapy, novel targeted agents, nucleic acid delivery technologies and epigenetic interventions, is provided. An in-depth analysis of the transformative role of artificial intelligence (AI) in integrating multi-omics data, predicting disease trajectories and optimizing personalized treatment plans is also presented. However, significant challenges hinder the clinical translation of these novel approaches. While AI-based models offer valuable insights, they are constrained by the complex heterogeneity of PF. Epigenetic therapies, despite their promise, face obstacles related to drug development, delivery efficiency and long-term clinical impact. Moving forward, the fundamental shift from palliative management to a disease-modifying paradigm for PF will not rely on a single technological breakthrough. Instead, it necessitates deep interdisciplinary integration. This involves the systematic convergence of the potential of regenerative medicine, the precision of gene editing, the molecular intervention of targeted therapy and the dynamic decision-making capabilities driven by AI. The goal is to construct a next-generation, individualized treatment framework capable of adapting to disease heterogeneity and evolving with the patient's condition. Despite the considerable challenges, this multimodal integrated strategy is paving a viable new path toward ultimately conquering pulmonary fibrosis.

Correspondence to: Professor Qipeng Yao, Department of Chinese Medicine, Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, 32 West Section 2, First Ring Road, Qingyang, Chengdu, Sichuan 610072, P.R. China
E-mail: yaoqipeng2024@163.com

Dr Xiaohua Li, Department of Thoracic Surgery, Sichuan Provincial People's Hospital, School of Medicine, University of Electronic Science and Technology of China, Sichuan Clinical Research Center for Kidney Diseases, 32 West Section 2, First Ring Road, Qingyang Chengdu, Sichuan 610072, P.R. China
E-mail: 1147101387@qq.com

*Contributed equally

Abbreviations: PF, pulmonary fibrosis; IPF, idiopathic PF; AI, artificial intelligence; siRNA, small interfering RNA; BSCs, airway basal stem cells; iPSCs, induced pluripotent stem cells; ECM, extracellular matrix; TGF, transforming growth factor; EMT, epithelial-mesenchymal transition; GWAS, genome-wide association studies; MUC5B, mucin 5B gene; AEC2, alveolar epithelial type II; ROS, reactive oxygen species; MMPs, matrix metalloproteinases; AT1, type I alveolar epithelial cells; SASP, senescence-associated secretory phenotype; BLM, bleomycin; HDACs, histone deacetylases; 5mC, 5-methylcytosine; m6A, N6-methylcytosine; MSCs, mesenchymal stem cells; 5-AZA, 5-azacytidine; PH, pulmonary hypertension; AAVs, adeno-associated viruses; EVs, extracellular vesicles; LNPs, lipid nanoparticles; ML, machine learning; DL, deep learning

Key words: pulmonary fibrosis, stem cell therapy, targeting agent, epigenetic therapy, nucleic acid delivery, artificial intelligence

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1. Introduction

Pulmonary fibrosis (PF) is a fatal interstitial lung disease of unknown etiology, ultimately leading to the loss of pulmonary function and respiratory failure (1). Epidemiological data show that idiopathic PF (IPF), the main form of PF, affects ~3 million individuals worldwide (2), with a higher incidence observed in North America compared to Asia and Europe, and its burden is increasing despite regional variability (3). Although current therapies (e.g., Nintedanib and Pirfenidone) can slow the progression of PF, these treatments are not curative and their efficacy remains limited. The pathophysiology for PF is complex, involving damage to alveolar epithelial cells, abnormal activation of fibroblasts and myofibroblasts and the promotion of fibrosis through multiple signaling pathways (including TGF- β , Wnt/ β -catenin and PI3K/Akt) (4). Consequently, there is a pressing rationale to explore beyond conventional antifibrotic strategies and critically evaluate the most promising emerging therapeutic paradigms.

Recent advances in regenerative and molecular medicine have opened new avenues. Stem cell therapies, particularly airway basal stem cells and induced pluripotent stem cells (iPSCs), have demonstrated that these cells can differentiate into various lung cell types in preclinical models (5-7), promoting the repair of damaged lung tissue. Furthermore, iPSC technology has made it possible to generate patient-specific stem cells, overcoming issues related to cell sourcing and transplantation compatibility (8,9). Gene editing technologies, such as CRISPR-Cas9, have shown immense potential in repairing gene mutations associated with PF and regulating the expression of fibrosis-related genes (8). Targeted drugs are considered the mainstay of PF treatment, and numerous novel targeted agents have been developed to inhibit key signaling pathways of PF. Research into epigenetics has provided new insights into PF therapy. Epigenetic modifications, including DNA methylation, histone modification and regulation by non-coding RNAs, have been verified to play crucial roles in the onset and progression of PF. Furthermore, RNA delivery technologies, such as small interfering RNA (siRNA) and mRNA, are rapidly emerging as novel therapeutic strategies that can target fibrosis-inducing genes and inhibit the fibrotic response. Finally, the potential application of AI technologies in predicting therapeutic targets for PF was discussed, particularly in optimizing personalized treatment regimens.

This review aims to analyze these interconnected advances, thereby providing a comprehensive overview of the pathobiological underpinnings of PF and a critical appraisal of the next generation of therapeutic strategies - from stem cells and targeted agents to epigenetic modulators, nucleic acid delivery and AI-driven solutions. The convergence of these disciplines holds the promise of shifting the treatment

paradigm from palliative management to true disease modification, ultimately aiming to improve patient prognosis and quality of life.

2. Methodology

This narrative review was conducted based on a comprehensive literature search performed in PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), one of the most widely used biomedical databases. This work aimed to identify relevant studies and reviews related to PF and its emerging therapeutic strategies. The following keywords and medical subject headings terms were used in various combinations: 'pulmonary fibrosis', 'lung fibrosis', 'interstitial lung disease', 'pulmonary fibrosis treatment', 'anti-fibrotic therapy', 'stem cell therapy', 'induced pluripotent stem cells', 'gene therapy', 'epigenetic modifications', 'epigenetic interventions', 'targeted therapy', 'precision medicine' and 'artificial intelligence'. Boolean operators (AND, OR) were applied to combine terms and maximize retrieval sensitivity. The search was limited to English-language publications, with no restrictions on publication date to ensure inclusion of foundational and recent advances. Articles were screened by title and abstract for relevance, followed by full-text evaluation for final inclusion. Additional references were identified through manual searching of cited references in key articles.

3. Pathogenesis of PF

PF is a chronic, irreversible disease characterized by progressive scarring of lung tissue. The core pathological change involves repeated injury to alveolar epithelial cells, leading to abnormal repair mechanisms. This in turn causes excessive activation of fibroblasts and extensive deposition of extracellular matrix (ECM), and ultimately disrupts the normal structure of the lung parenchyma and severely impairs gas exchange function. According to epidemiological data, the median survival time of patients diagnosed with PF is typically only 3-5 years (10), with a poor prognosis and a five-year survival rate of <30%, a figure lower than that of most malignant cancers. Although significant progress has been made in recent years in understanding the etiology of PF, its exact pathogenesis remains incompletely understood. Currently, it is widely accepted that PF results from a complex disease process in genetically predisposed individuals under the long-term influence of various environmental factors. This process involves multiple layers of interaction, including genetic background, epigenetic regulation, age-related changes, environmental exposures and immune system abnormalities (Fig. 1).

Genetic susceptibility plays a foundational role in PF pathogenesis. In terms of genetic factors, genome-wide association studies (GWAS) have been performed to identify several susceptibility loci for PF. Notably, a single nucleotide polymorphism, rs35705950, located in the promoter region of the mucin 5B gene (MUC5B), was confirmed as the strongest genetic risk factor, with this variant increasing the risk of developing PF by 4-7 times, as validated in multiple independent cohorts (11-14). MUC5B, a key component of airway mucus, may lead to abnormal mucus secretion due to mutations

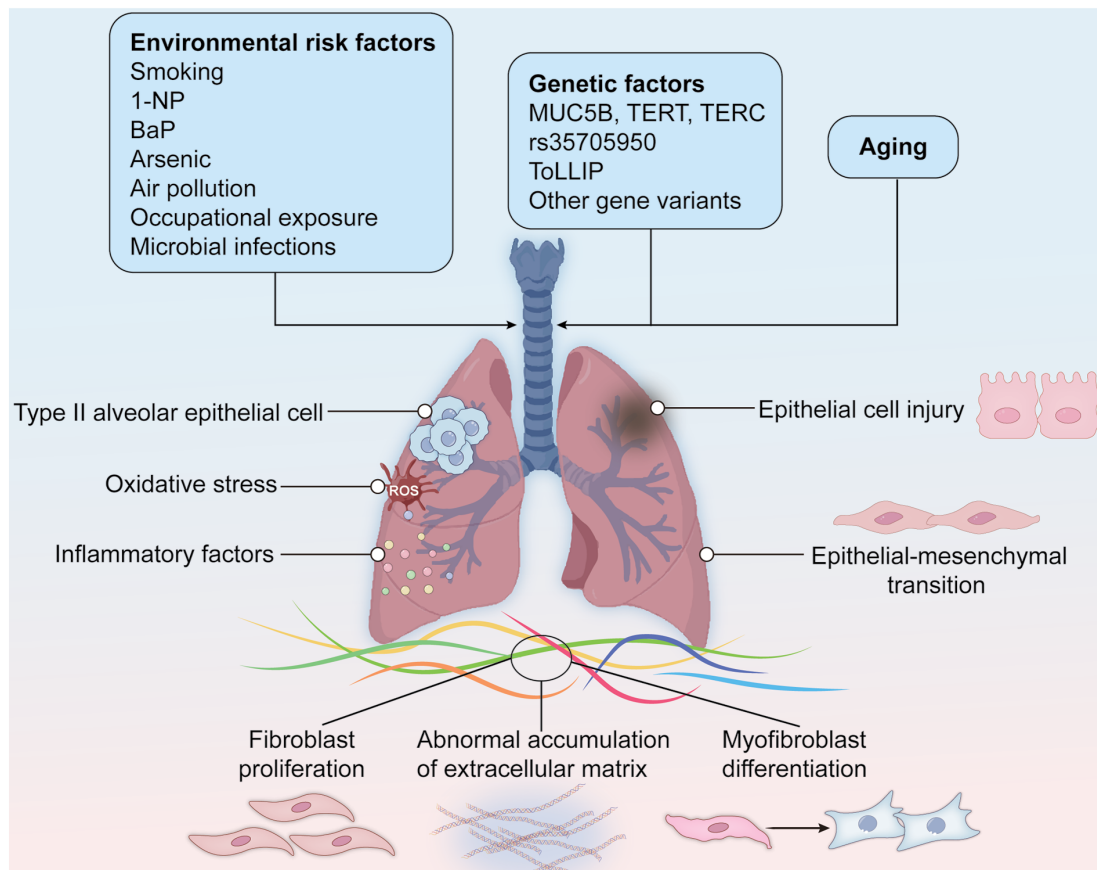


Figure 1. Overview of pathogenesis and pathophysiological progression in pulmonary fibrosis. The schematic illustrates the multi-layered interactions-including genetic predisposition, environmental exposures, age-related decline and immune dysregulation - that collectively drive disease initiation. The interplay of these factors triggers a sequential pathogenic cascade beginning with alveolar epithelial cell injury, followed by dysregulated inflammation and immune activation, leading to fibroblast recruitment and myofibroblast differentiation and culminating in excessive extracellular matrix deposition and tissue remodeling. ROS, reactive oxygen species; 1-NP, 1-nitropyrene; BaP, benzo[a]pyrene; MUC5B, mucin 5B, oligomeric mucus/gel-forming; TERT, telomerase reverse transcriptase; TERC, telomerase RNA component; rs35705950, a single nucleotide polymorphism in the MUC5B promoter region; TOLLIP, toll interacting protein.

in its promoter region, thereby affecting the homeostasis of the alveolar microenvironment (15). In addition to MUC5B, mutations in telomerase-associated genes, such as telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC), are closely associated with familial PF. These mutations cause telomere shortening, accelerating the aging process of alveolar epithelial cells (16-18). Polymorphisms in the Toll-interacting protein gene may influence the intensity of the inflammatory response of lung tissue to injury by regulating the Toll-like receptor signaling pathway (19,20). Notably, these genetic risk factors often exhibit a pronounced age-dependent penetrance, among which aging can lead to a decline in the repair capacity of alveolar epithelial cells, telomere shortening and mitochondrial dysfunction. This helps explain why PF primarily affects middle-aged and elderly populations (21).

Aging is a key biological driver of PF. Aging is one of the core biological processes in the occurrence and development of PF. It has been reported that pulmonary fibroblasts isolated from patients with PF often exhibit accelerated aging phenotypes, including increased β -galactosidase activity, elevated expression of p21, p16, p53 and cytokines related to the senescence-associated secretory phenotype (SASP), as well as reduced proliferative capacity (22). Abnormal transcription factors related to endothelial cell senescence have

been identified as the contributors for the sustained activation of fibroblasts responsible for collagen production during PF progression (23,24). Raslan *et al* (25) performed single-cell RNA sequencing on the lungs of young and aged mice with bleomycin-induced lung injury and then demonstrated that endothelial activation resolved in the lungs of young mice but persisted in the lungs of aged mice. The abnormal state of activated pulmonary endothelial cells *in vivo* may lead to impaired lung injury repair and sustained fibrosis. Additionally, the decline in tissue stem cell function due to aging is a key factor in the development of PF. Specifically, the decreased self-renewal and differentiation capacity of alveolar epithelial type II (AEC2) cells, which serve as progenitor cells for alveolar epithelium, directly impacts the repair capacity of lung tissue. Lu *et al* (26) designed a gene-editing nanoparticle platform, incorporating a CRISPR-Cas9 system, to effectively clear reactive oxygen species (ROS) while reducing SASP factors by knocking down the senescence-promoting gene lysine acetyltransferase 7, thereby providing robust antioxidant and anti-aging effects for alveolar epithelial type II (AT2) cells.

Environmental exposure: A trigger in PF pathogenesis. Air pollutants, particularly fine particulate matter (PM2.5) and inhalable particles (PM10), have been confirmed to be highly correlated with the incidence and mortality of PF (27,28).

These particles promote fibrosis development through various mechanisms, such as inducing oxidative stress, mitochondrial dysfunction, telomere attrition, initiating chronic inflammation and directly damaging alveolar epithelium (29). There is a close link between metabolic products and IPF. Air pollution may induce changes in metabolites. For instance, Wang *et al.* (30) found that each pollutant-associated metabolic feature was positively correlated with the risk of IPF. At the molecular level, PM10 exposure upregulates the expression of matrix metalloproteinases (MMPs) and disrupts the integrity of the basement membrane (25,27-29,31). PM2.5 exposure increases the expression of TGF- β 1, α -smooth muscle actin and type I collagen in mouse lungs, while activating the TGF- β /Smad signaling pathway and promoting the transformation of fibroblasts into myofibroblasts (32). Gaseous pollutants, such as nitrogen dioxide, primarily contribute to PF pathogenesis by inducing respiratory inflammation and increasing vascular permeability (33). Tobacco smoke is another major environmental risk factor, and active and passive smoking increase the risk of PF by 2-3 times (34). Thousands of chemicals in cigarettes (e.g., benzopyrene, ROS) can cause DNA damage and abnormal apoptosis in alveolar epithelial cells, while activating pulmonary fibroblasts (35,36). It is worth noting that the widespread use of e-cigarettes has caused new public health concerns. It was found that heavy metal particles (e.g., lead, cadmium) and volatile organic compounds (e.g., formaldehyde) in e-cigarette vapor may promote PF development through inducing oxidative stress and inflammatory responses (37). These occupational exposures (such as silica dust, asbestos and coal dust) have been proven to be the direct causes of certain types of PF, such as pneumoconiosis. These occupational dusts often accumulate in the lungs over many years. After inhalation, silica particles are phagocytosed by macrophages but resist degradation, triggering excessive ROS production and inflammatory signaling pathways, continuously stimulating local inflammation and fibrosis (38,39). Histone deacetylase 10 has become a key regulator of oxidative stress and inflammation in silicosis. Furthermore, certain chemicals, such as herbicides and chemotherapy drugs, are related to the onset of PF, potentially through direct cytotoxic or immune-modulating mechanisms. Importantly, environmental exposure can induce changes in epigenetic patterns, thereby altering gene expression profiles and contributing to the development and progression of PF.

Role of microbial infections. In recent years, the relationship between microbial infections and PF has garnered increasing attention. Compared to control groups, several bacterial species, such as *Haemophilus influenzae*, *Streptococcus* and *Moraxella catarrhalis*, were found to be more prevalent in the lung tissue of patients with PF (40,41). These bacteria may directly cause damage to airway epithelial cells by inducing host immune responses or through the activation of chronic low-level antigen stimulation, which triggers a wound-healing cascade. Additionally, they may indirectly promote fibrosis by inducing persistent inflammation (40). Viral infections, particularly the PF caused by severe acute respiratory syndrome-coronavirus (SARS-CoV)-2 infection, have become a clinical focus. SARS-CoV-2 infection can trigger a cytokine storm, leading to pro-fibrotic responses and resulting

in significant fibrotic changes in the lungs, which are associated with respiratory failure and high mortality rates (42,43). Furthermore, various viral infections, including those caused by human T-cell leukemia virus, cytomegalovirus, human immunodeficiency virus and Epstein-Barr virus, are associated with the development of PF. These viral infections induce immune-mediated damage resulting in the accumulation of macrophages, neutrophils, eosinophils and type 2 T-helper (Th2) cells at the site of injury, thereby releasing large amounts of pro-inflammatory and profibrotic cytokines (44).

In summary, the pathogenesis of PF is not caused by a single factor but results from the combined effects of genetic predisposition, aging and various environmental exposures (including air pollutants, smoking and microbial infections). These factors lay the foundation or provide the initial 'hit' for sustained alveolar epithelial injury and abnormal repair, disrupting pulmonary homeostasis and thereby initiating a complex pathophysiological process. The core features of this process include abnormal cellular behavior and molecular signaling activation under the interaction of genetic and environmental factors, ultimately leading to irreversible fibrosis.

4. Pathophysiological process of PF

Alveolar epithelial cell injury and abnormal activation. The pathophysiology of PF is initiated by alveolar epithelial cell injury, particularly involving alveolar epithelial type I (AT1) and AT2 cells (45). Repeated lung injury damages AT2 cells, which subsequently become hyperactivated, leading to the overactivation of Wnt/ β -catenin and SHH signaling pathways and the secretion of TGF- β . Furthermore, injured AT2 cells secrete SASP factors, leukotrienes and prostaglandins, adopting a senescent phenotype that promotes the proliferation of myofibroblasts.

Role of inflammation and immune cells. After epithelial injury, abnormal inflammatory and immune responses are rapidly activated. Injured epithelial cells release pro-inflammatory cytokines (e.g., TNF- α , IL-1 β) and chemokines (e.g., C-C motif chemokine ligand 2, C-X-C motif chemokine ligand 12), recruiting neutrophils, monocytes and lymphocytes to the site of damage. In PF, this inflammatory response is often chronic and dysregulated. Macrophages play a dual role in PF progression: Initially, they are predominantly pro-inflammatory M1-type, secreting cytokines such as IL-1 β and IL-6; later, they gradually polarize toward an anti-inflammatory and pro-fibrotic M2-type, secreting cytokines like TGF- β and IL-10 (46,47). Other immune cells, such as Th2 cells, promote fibroblast activation and ECM production through the secretion of IL-4 and IL-13, while the regulatory function of regulatory T cells may become imbalanced, collectively contributing to the formation of a fibrotic microenvironment (48).

Activation of fibroblasts/myofibroblasts and ECM deposition. The combined effects of the aforementioned injury and inflammatory microenvironment drive the core execution phase of fibrosis. Under continuous stimulation by injury signals and inflammatory factors (especially TGF- β), fibroblasts in the lung interstitium are activated and differentiate into myofibroblasts. These cells highly express α -smooth muscle actin

(α -SMA), possess strong contractile ability, and exhibit a high capacity for ECM secretion. Myofibroblasts originate from diverse sources, including the activation of local fibroblasts, recruitment of bone marrow-derived fibrocytes, epithelial-mesenchymal transition (EMT), endothelial-mesenchymal transition and pericyte transdifferentiation.

TGF- β has been verified to be the most central pro-fibrotic factor (49). It promotes fibroblast proliferation and differentiation into myofibroblasts via both Smad-dependent (e.g., Smad2/3 phosphorylation) and non-Smad-dependent (e.g., MAPK, PI3K/Akt) signaling pathways. Besides, it stimulates the synthesis of large amounts of ECM components (primarily type I and III collagen), while suppressing the activity of MMPs and increasing the expression of their tissue inhibitors, resulting in reduced ECM degradation and net increased deposition.

ECM remodeling and mechanosignaling feedback. As the disease progresses, excessive ECM abnormally accumulates in the lung interstitium, leading to alveolar wall thickening, structural destruction and the formation of a 'honeycomb lung.' This structural remodeling not only impairs gas exchange but also alters the mechanical properties of the lung tissue (e.g., increased stiffness). The altered mechanical environment itself can further activate mechanosensitive signaling pathways in fibroblasts, such as Yes-associated protein (YAP)/transcriptional co-activator with PDZ-binding motif (TAZ), thereby continuously driving their activation and pro-fibrotic gene expression, forming a self-reinforcing vicious cycle (50,51). Myofibroblasts also secrete various ECM-modifying enzymes (e.g., MMP1, MMP3, MMP7, MMP9) and components [e.g., collagen type III α 1 chain (COL3A1), COL6A1], exacerbating the pathological remodeling of the ECM (52).

In summary, the pathophysiology of PF is a dynamic, multi-stage network process. It begins with epithelial injury, progresses through complex intercellular communication and abnormal activation of signaling pathways (centered on TGF- β and integrating multiple pathways such as Wnt, Hedgehog and YAP/TAZ (53), and ultimately leads to irreversible destruction of lung structure characterized by myofibroblast aggregation and excessive ECM deposition. Importantly, these complex molecular and cellular events are largely subject to precise regulation at the epigenetic level.

5. Epigenetic changes in PF

Epigenetics is the study of gene expression and functional changes that do not involve changes to the DNA sequence but are regulated by other molecular mechanisms.

Epigenetic changes include abnormalities in DNA methylation, dysregulation of histone modifications and disturbances in non-coding RNA expression. DNA methylation changes typically occur in CpG dinucleotide clusters within the gene promoter regions (also known as 'CpG islands'), and these changes are closely related to the transcriptional silencing of the affected genes. By contrast, DNA demethylation leads to the reactivation and expression of genes, a process that is of great significance in epigenetics. Studies have shown that half of the loci in GWAS gene regions exhibit methylation changes

in patients with IPF, and DNA methylation plays a crucial role in the expression of specific genes in PF lungs (54-56). McErlean *et al* (57) compared the DNA methylation characteristics of primary airway macrophages (Ams) obtained from patients with IPF and healthy donors and found that the changes in the DNA methylome were related to the differentiation and phenotype of Ams in the process of PF. They suggested that the metabolic functions of AMs are involved in the pathogenesis of PF. The epigenetic changes in lipid and glucose metabolism-related genes were associated with the clinical severity of PF. Wang *et al* (50) showed that in patients with IPF and bleomycin (BLM)-induced PF mouse models, overexpression of methyl-CpG-binding domain 2 protein in myofibroblasts inhibited the expression of the erythroid differentiation regulator 1 promoter. These epigenetic changes promoted the differentiation of fibroblasts into myofibroblasts, thereby exacerbating the progression of PF.

Histone modification is another important mechanism in epigenetics, where histones undergo modifications including acetylation, methylation and phosphorylation, altering chromatin structure and thereby affecting gene expression. Histone deacetylases (HDACs) are key enzymes in regulating chromatin remodeling and gene transcription, and increasing evidence suggests that the HDAC family is closely associated with the progression of chronic fibrotic diseases (58). Hua *et al* (59) found that the HDAC2/SIN3 transcription regulator family member A (Sin3A)/methyl-CpG-binding protein (MeCP) 2 complex acts as an endogenous inhibitor of connective tissue growth factor in lung fibroblasts. Jeong *et al* (60) discovered that HDAC3 promotes alveolar EMT and fibroblast migration under hypoxic conditions. RNA epigenetic modifications play a crucial role in PF, particularly the roles of N6-methyladenosine (m6A) and 5-methylcytosine (m5C) - two prevalent RNA modifications that regulate gene expression - in the fibrosis process. YTH domain-containing protein 1 (YTHDC1), which is primarily expressed in AECII cells, exhibits significantly reduced expression in these cells during PF. This downregulation of YTHDC1 contributes to disease progression, as YTHDC1 has been shown to counteract stress-induced pulmonary senescence and fibrosis through a non-canonical mechanism independent of its m6A-binding ability. Mechanistically, YTHDC1 promotes the interaction between TopBP1 and MRE11, thereby activating ATR and facilitating DNA damage repair (61-63). Additionally, m5C modification has been shown to play a critical role in fibroblast activation and inflammatory pathways, and acts as a key factor in the development of PF (64,65).

6. Urgent need for new potential treatment options for PF

In recent years, an in-depth understanding of the pathophysiological mechanisms and epigenetic regulation of PF has pointed the way toward developing novel therapies that go beyond traditional anti-fibrotic drugs. However, current clinical treatment options remain limited. Currently, the standard treatment involves medication, with pirfenidone and nintedanib being the two approved drugs for PF. These medications can slow the progression of the disease and are selected for palliative treatment in the later stages of PF. The two antifibrotic drugs have similar efficacy: Pirfenidone primarily works by inhibiting

fibroblast proliferation and collagen synthesis, mainly through the regulation of TGF β . Its side effects include gastrointestinal reactions and skin sensitivity or allergies. Nintedanib is a tyrosine kinase inhibitor targeting platelet-derived growth factor (PDGF), fibroblast growth factor and VEGF receptors, which interferes with active processes in fibrosis, such as fibroblast proliferation, migration, differentiation and ECM secretion (66). However, its side effects are more prominent, particularly diarrhea, nausea and vomiting. The treatment effectiveness of both drugs generally depends on their tolerance by patients, with discontinuation rates ranging from 10 to 20% (67). Although these drugs have demonstrated some clinical efficacy, their primary role is to slow disease progression rather than reverse the fibrotic process or existing fibrotic damage, and their impact on improving lung function is limited.

Other treatment options may include oxygen therapy, pulmonary rehabilitation and lung transplantation in advanced stages, but these treatments are not suitable for all patients and have specific limitations. New drugs targeting novel pathways are currently under development, such as NADPH oxidase 1/4 inhibitors (68), pirfenidone analogs (69), translation initiation factor 3A modulators (70), antifibrotic agents (71) and senolytics (72). However, their efficacy and clinical applications remain to be validated.

At present, although the use of antifibrotic drugs has slowed the decline in lung function in patients with IPF, neither drug has shown effectiveness in relieving symptoms, and few patients experience safety and tolerability issues, mainly gastrointestinal. Importantly, comorbidities and complications, such as acute exacerbations, pulmonary hypertension (PH), cardiovascular diseases, gastroesophageal reflux disease and lung cancer, further exacerbate the disease burden and contribute to the high mortality rate of IPF (73). When all drug treatments fail, lung transplantation becomes the only treatment option. However, given the limited availability of lung donors and the clinical issues associated with post-transplant immune rejection, current treatment options for PF are highly limited. Therefore, there is an urgent need to develop new treatment strategies for PF.

7. Emergence of novel treatment approaches

As our understanding of the pathophysiological mechanisms of PF has deepened, treatment strategies have also been continuously innovated. Traditional therapeutic methods have gradually shown their limitations. Therefore, an increasing number of emerging treatment approaches are being explored and applied. These new therapies emphasize precision medicine and focus on individual patient differences, driving the treatment of PF toward a multidisciplinary and personalized approach. In addition to seeking drugs that effectively inhibit disease progression, there is a growing demand to alleviate treatment side effects and improve the cost-effectiveness of therapies. The rise of cutting-edge technologies, such as stem cell therapy, nucleic acid delivery techniques, targeted drugs and epigenetic interventions, has brought new hope for patients with PF and provided diversified options and prospects for clinical treatment. To systematically elaborate on the core characteristics and clinical prospects of these emerging

strategies, this article summarizes their mechanisms of action, advantages and challenges (Fig. 2 and Table I).

Stem cell therapy. Stem cell therapy for IPF is an important component of translational medicine, offering emerging regenerative medical treatments for this disease (74,75). Stem cells used in the treatment of IPF include pulmonary-derived stem cells, mesenchymal stem cells (MSCs) derived from bone marrow (BMSCs), adipose tissue and placenta, iPSCs and embryonic stem cells (75). The therapeutic mechanisms of these stem cells mainly focus on their ability to exert immune modulation, alleviating pulmonary inflammation (76,77). These processes are essential for inhibiting the progression and worsening of PF. Additionally, stem cells can repair lung tissue by secreting anti-fibrotic factors and angiogenesis factors that promote tissue healing (78), while differentiating into various cell types to replace dysfunctional cells, thus recovering lung function (79).

Another promising approach is using natural or synthetic scaffolds to generate bioengineered functional lung tissue for medical purposes. To date, various decellularization techniques have been applied to lung tissue. For instance, decellularized 3D hydrogel-based organoids can be used to assess the impact of the ECM microenvironment on fibroblast phenotypes, tissue homeostasis and disease function, and can potentially be transplanted into damaged lung tissue (80). However, the effectiveness of these methods still requires further validation.

Pulmonary-derived stem cells mainly include basal cells from the nasal epithelium (5), proximal trachea and bronchial cells, and AT2 cells from the alveolar region (81). AT2 cells are considered the progenitor cells of AT1 cells. In a BLM-induced PF rat model, transplantation of AT2 cells reduced PF, decreased the lung scar area, accelerated body weight recovery and lowered the hydroxyproline content (82). Furthermore, AT2 cell transplantation effectively alleviated pulmonary edema, collagen deposition and immune cell infiltration, all of which contribute to the progression of PF. Numerous studies have also shown that basal stem cell transplantation has a certain therapeutic effect on PF (83,84).

Although autologous lung stem cells have therapeutic potential, there are issues such as limited donor availability, reduced self-renewal capacity due to increased donor age, and interference with genetic and epigenetic memory. These issues have, to a certain extent, limited their clinical application (85,86). Autologous lung stem cells are difficult to expand and their biological characteristics gradually change with successive passages. To date, no method has been established that allows for widespread *ex vivo* proliferation of lung stem cells while maintaining their self-renewal and differentiation potential. Considering the inherent challenges in obtaining and expanding primary lung stem cells, current studies focus on establishing functional induced lung stem cells *in vitro*.

MSCs can be expanded *in vitro* and recruited to injured areas after allogeneic transplantation, promoting epithelial tissue repair and exhibiting immune-regulatory functions (87). MSC-based therapies include both systemic and local administration (88). However, challenges such as low cell survival rates, dependency on dosage and frequency for therapeutic efficacy, and issues like graft rejection and tumorigenicity, have limited their widespread use (89-91). In recent years,

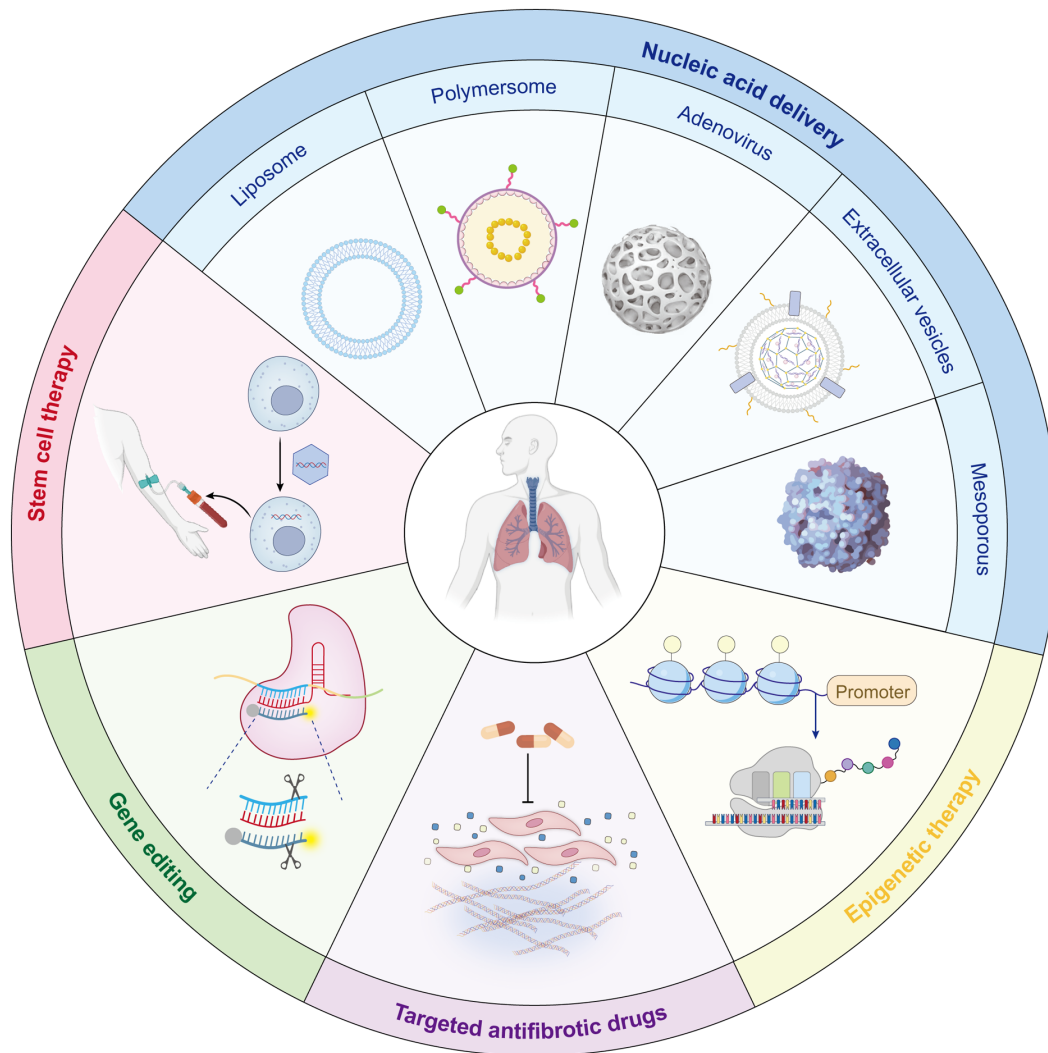


Figure 2. PF treatment strategy. Various modalities to treat PF (including stem cell therapy, nucleic acid delivery, epigenetic therapy, targeted drug therapy, gene editing). PF, pulmonary fibrosis.

MSC-derived exosomes (MSC-EVs) have attracted attention as a promising alternative treatment. MSC-EVs carry bioactive molecules such as microRNAs (miRNAs), proteins and lipids, which can specifically regulate the pathological processes of IPF while avoiding the risks associated with traditional cell transplantation. Zhou *et al* (92) demonstrated that BMSC-EVs could delay the progression of IPF in a mouse model by delivering miR-186. Wan *et al* (93) demonstrated that EVs derived from BMSCs overexpressing miR-29b-5p can alleviate IPF via the frizzled 6 pathway. Nevertheless, the clinical translation of MSC EVs still faces challenges, including the effective separation, modification and delivery of exosomes, which require further optimization.

As a breakthrough technology, iPSCs transform somatic cells into PSCs by genetic reprogramming (94), which can avoid the ethical problems and immune rejection caused by embryonic stem cells, and thus hold immense application potential. This gives iPSCs great potential for therapeutic applications. iPSCs can serve as effective cellular substitutes in IPF models and improve the pathological manifestations of PF. However, iPSCs still face several technical challenges in clinical treatment, including low differentiation efficiency

and cellular heterogeneity during differentiation (95,96). In response, researchers have proposed various optimization strategies. For example, Soh *et al* (6) improved the efficiency of iPSC-derived lung progenitor cells through a two-step differentiation protocol. In 2014, Huang *et al* (7) developed an efficient method to direct human pluripotent stem cells (hPSCs) to differentiate into lung and airway epithelial cells, including basal cells, goblet cells, Clara cells, ciliated cells and both AT. Later, Yamamoto *et al* (97) reported a long-term expansion method for alveolar organoids containing iPSC-derived alveolar stem cells (SFTPC+), showing that the differentiation process and cellular heterogeneity of SFTPC+ cells and their progenitors closely resemble those of AT2 cells. In 2025, Pezet *et al* (98) successfully converted hPSCs into expandable spheres, named induced respiratory progenitor cells, achieving a 95% purity of induced AT1 cells.

Furthermore, the use of CRISPR/Cas9 technology, zinc finger nucleases for homology-directed repair, small/short DNA fragments and sequence-specific transcription activator-like effector nucleases for gene editing enables the precise correction of mutations associated with genetic diseases such as cystic fibrosis (8,99). In summary, researchers use induced

Table I. Comparison of emerging therapeutic strategies for pulmonary fibrosis.

Therapeutic category	Representative agent/approach	Development stage	Key advantages	Major challenges	(Refs.)
Stem cell therapy	1. Lung-derived stem cells (e.g., AT2 cells, basal cells) 2. MSCs (from bone marrow, adipose tissue, etc.) 3. iPSCs	Preclinical (some phase I trials ongoing)	Multimodal repair, low immunogenicity (EVs), disease modeling	Tumorigenicity risk (iPSCs), scalable production, delivery efficiency high (especially for MSC-EVs)	(74-99)
Targeted drug therapy	1. Pathway Inhibitors: TGF- β (e.g., ALK5 inhibitors), Wnt, PI3K/Akt (e.g., Omipalisib), Hippo-YAP pathway inhibitors 2. Inflammatory factor antagonists: IL-13, CCL2, LIGHT/TL1A monoclonal antibodies, etc.	Phase I/II clinical trials	High specificity, combinable with existing drugs	Patient stratification needed, resistance possible	(100-123)
Epigenetic therapy	1. DNA methylation modulation: DNMT inhibitors (e.g., 5-AZA) 2. Histone modification modulation: HDAC inhibitors 3. Non-coding RNA regulation: miRNA agonists/antagonists 4. Epigenome editing: CRISPR/dCas9 systems	Preclinical to early clinical	Reverses pathogenic memory, synergistic potential	Off-target effects, toxicity (5-AZA)	(128-146)
Nucleic acid delivery therapy	1. Delivery Systems: LNPs, polymeric nanoparticles, AAV, EVs 2. Therapeutic molecules: siRNA, mRNA, miRNA, CRISPR components	Rapidly developing (siRNA drugs already approved)	Precision at the genetic level, modular and flexible platform, suitability for local lung delivery	Efficient, cell-specific delivery to the lungs; stability and immunogenicity of nucleic acid drugs; long-term safety of delivery vehicles	(92,93,149-158)
AI	AI-driven platforms & tools: e.g., AlphaFold (structure prediction), deep learning models (drug design, image analysis)	Early development (target discovery phase)	Accelerates R&D, predicts novel targets	Validation <i>in vivo</i> required, data dependency	(159-164)

AT2, alveolar epithelial type II cell; MSC, mesenchymal stem cell; iPSC, induced pluripotent stem cell; EV, extracellular vesicle; DNMT, DNA methyltransferase; HDAC, histone deacetylase; CRISPR, clustered regularly interspaced short palindromic repeats; LNPs, lipid nanoparticles; AAV, adeno-associated virus; AI, artificial intelligence.

or gene-edited iPSCs to create disease models that simulate human disease pathology. By investigating these models, researchers can gain a deeper understanding of the underlying mechanisms of the disease and identify potential drug targets, providing new directions for personalized treatment of PF.

Targeted drug therapy. Scholars have identified several important signaling pathways in the progression of PF, including the TGF- β , Wnt, PI3K-Akt and Hippo-YAP pathways. TGF- β is considered one of the most important molecules in the fibrosis process. TGF- β regulates cell proliferation, fibroblast

activation, ECM deposition and myofibroblast differentiation through both its classic Smad and non-Smad signaling pathways. In particular, TGF- β 1 plays a central role in PF by promoting the transformation of fibroblasts into myofibroblasts, thereby advancing fibrosis progression (49). To inhibit TGF- β 1 activation, several strategies have been developed in clinical and experimental studies, such as using antisense oligonucleotides to block TGF- β synthesis, targeting TGF- β ligands with proteoglycans or soluble TGF- β receptors, or inhibiting TGF- β receptor activity via activin receptor-like kinase 5 inhibitors (100). Studies have also found that the

Sloan-Kettering Institute proto-oncogene, a negative regulator of the TGF- β signaling pathway, can effectively modulate TGF- β 1/Smad signaling, slowing fibroblast proliferation and EMT, providing a new strategy for treating PF (101-103). It is well known that integrins promote TGF- β activation, and α v β 6 integrin is one of the most extensively studied potential therapeutic targets in IPF. However, the phase II clinical trial of the anti-integrin drug BG00001 showed no significant change in forced vital capacity between the treatment and placebo groups, and certain patients even experienced acute exacerbations (104). Pentoxifylline-2 (PTX-2) inhibits the production of TGF- β , possibly by suppressing the differentiation of monocytes into macrophages, which in turn reduces TGF- β levels. Zinc pentoxifylline α is a recombinant form of human PTX-2 (rhPTX-2). In phase II trials (NCT04552899, NCT02550873), rhPTX-2 showed significant efficacy in restoring lung function (104,105). However, in the 52-week phase III randomized controlled trial STARSCAPE, no significant differences were observed between rhPTX-2 and the placebo in patients with IPF (104). Galectin-3 (Gal-3), a lectin that binds to β -galactosides, is significantly elevated in various fibrotic diseases. This β -galactoside-binding lectin promotes fibrosis progression by modulating the expression of TGF- β receptors. A phase I/IIa clinical trial showed that individuals receiving the Gal-3 inhibitor TD139 exhibited reduced expression of Gal-3 compared to control subjects. The results suggested that inhibition of Gal-3 expression in the lungs correlates with a reduction in plasma biomarkers. This is closely related to the pathological biology of IPF (PDGF-BB, plasminogen activator inhibitor-1, Gal-3, CCL18 and chitinase-3-like protein 1) (106).

The Wnt signaling pathway plays a crucial role in various fibrotic diseases, particularly in regulating the proliferation and differentiation of fibroblasts. Wnt signaling promotes fibroblast activation and accelerates fibrosis progression by activating the downstream β -catenin-dependent pathway (107). A study showed that betulonic acid (BA), a pentacyclic triterpenoid, can alleviate the effects of BLM-induced PF in mice, particularly in reducing collagen deposition and improving lung function. BA effectively reduces the expression of fibrotic markers, including fibronectin, collagen I and α -SMA. Further research indicates that BA can inhibit Wnt3a-induced fibroblast activation and subsequent Wnt/ β -catenin pathway activation, resulting in reduced nuclear accumulation of β -catenin and phosphorylation of key signaling proteins such as low-density lipoprotein receptor-related protein 6 and Dishevelled 2 (108). Another study explored the use of SBC-115076 as a potential treatment for pPH associated with PF (109). The results demonstrated that proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition reduced pulmonary artery thickening and right ventricular remodeling in a BLM-induced PF animal model. More importantly, SBC-115076 treatment also diminished the activation of the Wnt/ β -catenin pathway, a core driver of fibrosis and vascular remodeling. These findings suggest that PCSK9 inhibitors serve as promising therapeutic agents for PF and PH by modulating key fibrotic and vascular remodeling pathways. These studies provide a theoretical basis for targeting the Wnt signaling pathway as a potential therapeutic target.

The PI3K-Akt pathway plays a pivotal role in the pathogenesis of PF (110). PI3K is a group of membrane-associated lipid

kinases classified into three classes based on their molecular structure. Class I PI3Ks are the most widely studied in various diseases. PI3K α is commonly upregulated or mutated in lung-related diseases (111), PI3K γ is often overexpressed in IPF lungs and fibroblasts (112), and class III PI3K is involved in the formation of autophagosome membranes, potentially affecting PF (113). AKT is a serine/threonine protein kinase with three subtypes, and research on PF has primarily focused on AKT1 and AKT2. AKT1-mediated mitotic cells help alveolar macrophages resist apoptosis (114). This is essential for the development of PF, while AKT2 regulates PF by inducing macrophages to produce TGF- β 1 and IL-13. AKT2-deficient mice are protected from BLM-induced PF and inflammation (115). Evidence suggests that the PI3K/Akt signaling pathway can promote PF by activating multiple key factors involved in cell proliferation, survival, and ECM deposition (116,117). Inhibitors targeting the PI3K/Akt pathway, such as omipalisib and rapamycin, have shown potential efficacy for PF in clinical trials. These studies indicate that the PI3K/Akt pathway is an important therapeutic target for PF.

Hippo-YAP signaling pathway also plays an essential role in the pathogenesis of fibrosis. Liu *et al* (53) demonstrated that YAP, a homolog of *Drosophila* Yki, and TAZ, transcriptional co-activators, regulate fibroblast activation and matrix synthesis. Inhibition of the Hippo signaling pathway can reduce BLM-induced PF (118). Qing *et al* (119) used a G protein-coupled receptor (GPCR) ligand screening system to identify a dopamine receptor D2 antagonist that selectively blocks YAP in macrophages. By targeting GPCRs, drugs can modulate the Hippo-YAP signaling pathway, achieving anti-fibrotic effects. Zeyada *et al* (120) found that trigonelline (Trig), a natural plant alkaloid with multiple pharmacological effects, can attenuate the sphingosine kinase 1/sphingosine-1-phosphate axis in the lungs and its downstream Hippo targets YAP-1 and TAZ. After Trig administration, EMT in BLM-induced mouse lung tissue was reversed. These strategies provide new insights for developing novel targeted therapeutic drugs.

In addition to the signaling pathways, inflammation plays a critical role in the onset and progression of PF. Particularly during acute exacerbations and in certain subtypes of PF, inflammatory responses exacerbate the fibrotic process. Studies have shown that cytokines (such as IL-13, IL-6), chemokines (such as CCL2), and growth factors (such as TGF- β) are key factors driving fibroblast activation and ECM deposition (121,122), making them important targets for anti-inflammatory therapies. Recent research has indicated that inhibition of TNF superfamily (TNFSF) members, such as lymphotoxin-like, exhibits inducible expression, and competes with HSV glycoprotein D for HVEM (LIGHT/TNFSF14) and TNF-like ligand 1A (TNFSF15), can significantly reverse fibrosis in a preclinical model providing a new direction for inflammation-targeted therapies (123).

However, the widespread use of corticosteroids and immunosuppressants for anti-inflammatory treatment often yields limited effectiveness and may even worsen the condition, highlighting the need for more precise, mechanism-based therapeutic approaches. Although clinical applications of combined anti-inflammatory and anti-fibrotic treatments face several challenges, including patient selection, drug side

effects and ongoing immune modulation requirements, these recent findings lay a solid foundation for the future development of personalized, inflammation-targeted therapies for PF.

Epigenetic therapy. Epigenetic therapy has gained significant attention as a novel treatment strategy, particularly for complex diseases such as cancer, genetic disorders, cardiovascular diseases and chronic diseases like fibrosis (124-127). DNA methylation regulates gene expression by directly affecting the transcriptional activity of genes. Numerous studies have indicated that DNA methylation plays a pivotal role in the progression of PF (54,55). Currently, treatment strategies for DNA methylation focus primarily on DNA demethylating agents. DNA methyltransferases (DNMTs) are key catalytic enzymes in the process of DNA methylation and studies have shown that pharmacological inhibitors targeting DNMTs can effectively suppress the expression of fibrotic genes. For example, TGF- β 1 significantly induced the methylation of the Thy-1 promoter in lung fibroblasts, while DNMT inhibitors such as 5-azacytidine (5-AZA) could downregulate the expression of fibrotic-related genes, including α -SMA and collagen type I, significantly inhibiting collagen deposition (128). Wei *et al* (129) showed that 5-AZA and glycyrrhizic acid alleviated peroxisome proliferator-activated receptor γ (PPAR γ)-mediated fibrosis suppression through mechanisms that increased sensitivity to DNMTs, suggesting that targeting the DNMT/PPAR γ axis could benefit patients with PF. The DNA methylation inhibitors 5-AZA (Vidaza[®]) and its deoxy derivative 5-aza-2'-deoxycytidine (Dacogen[®]) are widely used in cancer treatment, particularly for hematological malignancies, where they have been proven effective in reactivating and upregulating tumor suppressor genes (130,131). However, the use of 5-AZA and similar drugs requires careful attention to their potential toxic effects. Some reports indicate that DNMT inhibitors may cause hepatotoxicity, with certain patients experiencing adverse effects such as interstitial lung fibrosis, pneumonia and acute lung injury (132,133). Therefore, it is of great significance to develop DNA methylation inhibitors with lower toxicity to ensure the safety and efficacy of these treatments in clinical applications.

In recent years, the widespread use of CRISPR/dCas9 technology has introduced a new approach for treating PF through epigenome editing. Wang *et al* (134) employed CRISPR/Cas9-mediated homology-directed repair to target the important epigenetic biomarker O6-methylguanine-DNA methyltransferase for *de novo* methylation, resulting in stable upregulation of DNMTs in edited HeLa cells. Qu *et al* (135) used CRISPR/dCas9-Dnmt3A-mediated epigenome editing to effectively reverse matrix stiffness-induced overexpression of desmoplakin. Wu *et al* (136) investigated its impact on ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) expression and DNA methylation patterns in human bronchial epithelial cells. After using CRISPR/dCas9-zeste homolog 2 to epigenetically downregulate UCHL1, they observed a reduction in mRNA expression of COL1A1 and fibronectin. These studies demonstrate the significant potential of CRISPR/Cas9-directed epigenetic silencing in functional and therapeutic research. However, off-target effects remain a major hurdle in the clinical application of CRISPR/Cas9 technology. Compared to other gene delivery platforms, such as recombinant adeno-associated

viruses (AAVs) (137), CRISPR/Cas9 has conceptual advantages, but targeting specific cell types remains a challenge.

In the study of PF, the increased activity of HDACs is considered a significant factor in promoting fibrosis (138). HDACs inhibit gene transcription by removing acetyl groups from histones, leading to a more compact chromatin structure. To date, the US Food and Drug Administration (FDA) has approved four HDAC inhibitors for cancer treatment (139), but none have been approved for fibrotic diseases. Emerging *in vitro* and *in vivo* preclinical evidence increasingly suggests that HDACs play a beneficial role in preventing or reversing fibrosis (138,140). Rubio *et al* (141) found that EP300 reduced nuclear HDAC activity and interfered with the ribonucleoprotein complex function in IPF, and inhibiting EP300 significantly reduced fibrosis markers, providing a basis for more effective treatments targeting the etiology of IPF. For patients with IPF, HDAC3 expression has been found to significantly increase and to be closely correlated with the progression of fibrosis (142). Inhibiting HDAC3 activity was shown to restore the expression of fibrosis-related genes and reduce the degree of PF. Yu *et al* (143) designed and synthesized 24 novel HDAC6, HDAC8 or dual HDAC6/8 inhibitors, identifying five HDAC inhibitors that can alleviate TGF- β -induced PF. Future HDAC inhibitors could be combined with other drugs or therapies as potential strategies for treating PF.

MiRNAs regulate mRNA to alter the expression of target genes and dynamically regulate DNA methylation and other non-coding RNAs, including themselves. These regulatory mechanisms lead to changes in miRNAs, triggering chain reactions in the TGF- β 1/Smad, MAPK and PI3K/AKT pathways, ultimately resulting in the expression of a fibrotic phenotype. Animal studies have shown that miRNA-29 can target various fibrosis-related genes, including COL1A1, COL3A1 and TGF- β 1, and inhibiting these genes can slow down the occurrence of fibrosis (144). Designing miRNAs at the genetic level represents a promising therapeutic strategy and miRNA-based drugs integrated into *in vivo* and *in vitro* applications have the potential to yield the most direct clinical outcomes. Furthermore, the use of extracellular vesicles (EVs), particularly those derived from MSCs, as delivery systems for miRNAs has shown promising therapeutic effects in the treatment of PF (92,93). However, a significant challenge for targeting miRNAs in IPF is the potential difference in gene regulation by individual miRNAs between mice and humans. Therefore, future research should focus on the continuous improvement of existing models and emphasize the testing and validation of hypotheses across multiple model systems.

Long non-coding RNAs (lncRNAs) represent a key aspect of epigenetic therapy. lncRNAs play crucial roles in regulating gene expression, chromatin modifications and cell fate determination. Savary *et al* (145) found that lncRNA dynamin 3 opposite strand was one of the most strongly induced lncRNAs in a human lung fibroblast cell line (MRC-5) stimulated with TGF- β 1, through RNA sequencing and small RNA sequencing. Xia *et al* (146) showed that lncRNA SYISL promotes fibroblast-to-myofibroblast transformation via miR-23a-mediated regulation of TRIO and F-actin binding protein. *In vivo* delivery of SYISL-targeted short hairpin RNA significantly reduced collagen deposition, hydroxyproline content and fibrosis marker expression in BLM-induced mice.

These studies further demonstrate the potential of targeting lncRNAs as a therapeutic strategy for IPF.

One important feature of epigenetic therapy is its reversibility, implying that by modulating epigenetic markers, it is possible to partially restore the normal expression of genes. This offers new hope for treating numerous incurable diseases. Although epigenetic therapy has achieved some positive results in basic research, it still faces numerous challenges in clinical applications, such as selecting appropriate treatment targets, developing delivery systems and ensuring the long-term effectiveness of treatments. In the future, as research in epigenetics advances, these therapeutic approaches are expected to gradually move toward clinical use, potentially bringing new breakthroughs in the treatment of diseases like fibrosis.

Nucleic acid delivery therapy. Nucleic acid delivery therapies have fundamentally transformed the treatment of a wide range of diseases, including genetic disorders, infectious diseases and malignant tumors. By delivering nucleic acid molecules such as plasmid DNA, small interfering siRNA, miRNA and circular RNA, it is possible to regulate key signaling pathways associated with specific diseases at the molecular level. This can directly or indirectly influence gene expression, gene silencing or gene deletion, thereby inhibiting or repairing the production of abnormal proteins. Compared to traditional drug treatments, nucleic acid delivery offers more precise gene modification capabilities, enabling targeted therapy that effectively avoids systemic side effects.

The unique physiological structure of the lungs makes them an ideal route for drug delivery, facilitating both systemic drug therapy and localized treatment of lung diseases. The lung's distinct structure allows it to serve as an ideal target for drug delivery, capable of delivering both systemic therapies and local treatments for pulmonary diseases. Recent advancements have been made in the development of delivery systems targeting pulmonary capillary endothelial cells and specific lung cells, particularly the inhalation method, which has proven to be an effective approach for treating lung diseases. The outbreak of COVID-19 significantly accelerated the development and commercialization of mRNA-based vaccines. This in turn boosted the application of nucleic acid drugs in pulmonary delivery, opening new prospects for vaccine development and gene therapy (147,148).

However, the unique characteristics of the lungs present challenges. Due to continuous exposure to external air, the lungs are susceptible to infections and inflammation, which necessitates a heightened focus on the safety of nucleic acid delivery carriers. There are two primary routes for nucleic acid delivery to the lungs: Systemic intravenous injection and localized inhalation. Both routes face distinct challenges, especially when nucleic acids must cross the cell membrane. Due to their large molecular structure and negative charge, nucleic acids typically struggle to penetrate the cell membrane directly, and RNA is prone to degradation, which limits the delivery effectiveness. Therefore, enhancing nucleic acid stability, improving intracellular expression and ensuring the stability of aerosolization have become key research areas in lung nucleic acid delivery.

To overcome these challenges, researchers have developed various innovative carrier systems, such as liposomes, polymer

NPs and LNPs. These carriers effectively enhance the stability of RNA, preventing degradation in external environments and by nucleases, while improving delivery efficiency. As delivery technologies continue to advance, the prospects for pulmonary nucleic acid drug delivery are increasingly promising, offering new hope for the treatment of pulmonary diseases.

Applications of liposomes and polymer NPs. As an effective delivery vehicle, liposomes have been extensively explored and applied for nucleic acid drug delivery. In 2018, the US FDA approved the first siRNA drug formulated with liposomes. Liposomes are used for siRNA delivery and can decorate collagen-binding peptides and collagenases, target fibrotic lung tissue and aid in the restoration of normal lung structure (149,150). Zhao *et al* (151) developed a non-inflammatory LNP exhibited a 40-fold enhancement in pulmonary protein expression without inducing significant inflammatory responses compared to traditional LNP formulations. Furthermore, they developed ursolic acid-incorporated phosphoramidate-based LNPs encapsulating mRNA encoding nuclear receptor subfamily 1 group D member 1. These non-inflammatory LNPs effectively combat pulmonary fibrosis by reducing inflammation and oxidative stress, promoting fibroblast conversion, and enhancing angiogenesis. Polymer NPs also serve as an effective drug delivery system, improving drug loading efficiency and solubility. Polysaccharide-based natural products, such as chitosan, are of particular interest due to their excellent biocompatibility and biodegradability, and have been widely studied for gene drug delivery. Vlasova *et al* (152) proposed a method for efficiently synthesizing cationic poly(ethyleneimine) derivatives, using a fission-Ugi reaction to synthesize ionizable polymers for lung delivery of various sizes of RNA and gene editing tools. Compared to *in vivo* polyethylenimine, lipid-polymer-lipid hybrid NPs showed a 300-fold increase in efficiency for systemic mRNA delivery to the lungs. These NPs were also able to deliver complex CRISPR-Cas9 gene RNA to the lungs, achieving ~6% gene editing in lung tissue. Bai *et al* (153) developed an inhalable and mucosal-penetrating NP system that was formulated from PLGA-PEG and G0-C14 (termed PPGC), which enabled efficient mucosal delivery of siIL11. In a mouse model, siIL11@PPGC NPs significantly reduced fibrosis development and improved lung function without inducing systemic toxicity.

Application of AAVs. AAV, a small single-stranded DNA virus, has been studied for pulmonary nucleic acid drug delivery. AAV exhibits low immunogenicity and has been used in clinical trials to treat Leber congenital amaurosis and spinal muscular atrophy (154). Studies show that AAV can bind to glycosaminoglycan receptors in bronchial mucus and effectively penetrate the mucus layer for pulmonary gene delivery. Additionally, AAV6 has been used to deliver miRNA-21-5p in a hyperoxic acute lung injury rat model to prevent apoptosis of AT2 cells (155). Wang *et al* (156) found that AAV6-mediated knockdown of nestin inhibited TGF- β signaling and significantly alleviated PF in mouse models and patients with IPF. Although AAV application faces challenges in safety and immune response, it still shows great potential as a gene therapy tool for lung diseases, particularly for fibrosis treatment.

Exosome delivery. EVs, composed of phospholipid bilayers, possess excellent biocompatibility and targeting

capabilities, making them potential carriers for nucleic acid delivery (92,93). EVs can maintain lung health by modulating immune responses, inducing tissue repair and maintaining pulmonary homeostasis. They can be detected in lung tissue and biological fluids such as bronchoalveolar lavage fluid and blood, providing information about disease progression and serving as biomarkers for diseases (157). The efficacy of exosomes derived from umbilical MSCs (hUCMSC-EVs) has been validated in clinical trials (158) (MR-46-22-004531, ChiCTR2300075466). Patients who received nebulized hUCMSC-EVs as an adjunct therapy showed degenerative changes in lung fibrosis on consecutive CT scans, compared to those who only received standard treatment. Due to their natural membrane protection and targeting abilities, EVs have broad application potential in gene therapy and drug delivery.

In summary, research on nucleic acid delivery therapies and their carrier systems is continuously advancing towards improving delivery efficiency, overcoming physiological barriers and enhancing therapeutic efficacy. By using various carrier systems such as liposomes, polymer NPs, adeno-associated viruses and exosomes, the effective delivery of nucleic acid drugs can be achieved, showing great potential in the treatment of both local and systemic diseases.

AI rise. The development of new drugs is a multi-step, lengthy and costly process. Although traditional drug discovery methods are valuable, they face numerous challenges, including low success rates and inefficient predictions of drug properties, toxicity and drug-target interactions. In recent years, advancements in AI have provided rapid and effective strategies to gradually overcome these obstacles, significantly reshaping the landscape of drug discovery and development. AI has already achieved remarkable results in various therapeutic fields, including oncology, infectious diseases, neurology and rare diseases. By utilizing machine learning (ML), deep learning (DL) and natural language processing, various stages of the drug development process, including target identification, drug screening, drug design, drug-target interactions, drug repurposing, prediction of physicochemical properties, toxicity evaluation and pharmacokinetic forecasting, have been significantly enhanced. Through the analysis of large datasets, AI can identify potential drug molecules and accurately predict their biological activity, thereby accelerating the drug development process.

With the continuous progress of AI technologies, scientists can identify potential therapeutic targets for diseases such as PF more quickly and design effective drugs targeting these specific targets. AI can mine key biomarkers of diseases from large biomedical datasets and discover numerous potential molecular mechanisms, providing strong support for the development of new drugs. Various AI models and AI-driven tools have now been developed to screen, design, discover and optimize drugs, such as AlphaFold, Chemistry42, Clinico and ProTox-II. Utilizing AI-supported platforms, Pun *et al* (159) identified therapeutic targets for amyotrophic lateral sclerosis. Xu *et al* (160) successfully identified Traf2 and Nck-interacting kinase (TNIK) as key regulators in the pathology of IPF. Furthermore, AI can optimize clinical trials by analyzing patient data and recruitment patterns. Xu *et al* (160) reported the first clinical trial of TNIK-targeting

inhibitors (NCT05938920), where they used AI to streamline preclinical candidate nomination to just 18 months and reduced the completion time of Phase 0/I clinical trials by at least 30 months from target discovery. This is the first reported case where an AI platform successfully identified disease-related targets and compounds, marking a revolutionary shift toward streamlining drug discovery.

DL plays a multifaceted role in drug discovery, with applications ranging from predicting compound activity, generating new chemical structures and predicting chemical reactions, to calculating ligand-protein interactions and analyzing biomedical imaging. Witten *et al* (161) created a dataset containing >9,000 LNP activity measurements and used it to train a directed information transfer neural network for predicting nucleic acid delivery with various lipid structures. They evaluated 1.6 million lipids *in silico* and identified two structures, FO-32 and FO-35, that exhibited localized mRNA delivery to mouse muscle and nasal mucosa. Gerckens *et al* (162) developed an end-to-end deep learning model by analyzing thousands of immunofluorescence-stained ECM images obtained through automated high-throughput microscopy. This model screened a small molecule drug repurposing library to inhibit ECM deposition, with AI-driven fiber pattern detection identifying Tranilast as an effective anti-fibrotic inhibitor.

ML, a branch of AI, involves deriving models or rule sets from an initial training set. These models are then used to assess new datasets. ML has made significant contributions to the analysis of high-resolution X-ray computed tomography scans for predicting the progression of IPF (163). Muratov *et al* (164) explored the application of ML in identifying transcriptomic changes related to lung diseases caused by titanium dioxide (TiO₂)-NPs. This approach has markedly contributed to understanding the potential effects of TiO₂-NP inhalation, advancing the field of nanotoxicology, and supporting the development of safer nanotechnology applications. As omics platforms increasingly integrate with machine learning, one major obstacle remains the cost-effectiveness of using these expensive assays and complex machine learning techniques for diagnostics. However, as these technologies become more mainstream and affordable, this may no longer pose a significant issue. Proteomics and machine learning may uncover new relationships that could revolutionize the diagnosis and management of future patients.

Despite the enormous potential AI holds for drug development, there are still several challenges to address. Issues such as data accessibility, the integration of diverse datasets and the interpretability of AI models remain pressing concerns. The 'black box' nature of AI makes it difficult for researchers to understand the decision-making process of AI models, which could impact transparency and trust in drug development. As technology continues to evolve, improvements in algorithms, interdisciplinary data integration and stronger collaboration between laboratories and AI technologies will further drive the application of AI in treating diseases like PF.

Furthermore, AI has made it possible to explore vast chemical spaces, optimize clinical trials and identify new therapeutic targets, thus paving the way for the development of precision medicine. However, challenges such as limited data availability, integration of diverse datasets, AI model interpretability and ethical concerns remain key obstacles.

Overcoming these limitations through improved algorithms, standardized databases and interdisciplinary collaboration is crucial. Overall, AI is continuously reshaping drug discovery by shortening timelines, increasing success rates and driving the development of innovative and accessible therapies to address unmet medical needs.

8. Conclusion and outlook

Significant progress has been made in the treatment of PF over the past few decades, yet its complex pathological mechanisms and treatment challenges continue to pose a substantial clinical hurdle. Although existing treatments such as anti-fibrotic drugs and lung transplantation have helped slow disease progression to a certain extent, their effectiveness remains limited and they cannot cure the disease. With the development of cutting-edge technologies, including stem cell therapy, targeted drugs, epigenetic therapy, nucleic acid delivery and AI, the treatment of PF is poised to enter a new era.

Stem cell therapy, as a potential regenerative medicine approach, has demonstrated promise in experimental studies for repairing lung tissue and reversing the fibrotic process. However, improving stem cell survival, targeting and long-term effects remains a significant challenge for clinical applications. Targeted drug therapies, which precisely inhibit key fibrosis-related molecules such as TGF- β and PDGF, have shown some clinical success. However, the effectiveness of single-target treatments is often hindered by resistance and pathological remodeling, necessitating the combination with other therapeutic strategies for more effective combination therapies.

Epigenetic therapy offers a novel treatment approach by regulating gene expression and repairing epigenetic changes caused by genetic damage or environmental factors. While the development of epigenetic drugs is still in its early stages, their potential to intervene in the fibrotic process and restore normal gene function should not be underestimated. Nucleic acid delivery technologies, utilizing small RNA molecules and gene editing techniques, enable direct regulation of gene expression and the repair of disease-causing genes, providing a more precise and personalized treatment option for PF. Despite challenges such as efficient delivery, targeting and side effects, advancements in delivery technology hold the promise of breakthroughs in this field. The application of AI in PF treatment has also become an emerging research focus. AI can leverage big data analysis, machine learning and other technologies to precisely screen drugs, predict disease progression and optimize individualized treatment plans, markedly enhancing treatment efficiency. Nevertheless, the future of PF treatment remains fraught with challenges, particularly in effectively integrating these advanced technologies into clinical practice.

Given the significant heterogeneity of PF and the possible variation in the disease mechanisms across different patients, future treatment strategies should focus more on individualized and precision therapies. Additionally, how to effectively combine various treatment approaches to overcome the limitations of single therapies remains a critical issue. Overall, the future treatment of PF will rely on interdisciplinary collaboration, combining stem cells, gene therapy, targeted drugs, epigenetic repair and AI, offering new hope for the treatment

of this disease. However, further clinical validation and technological breakthroughs are needed before these innovations can be widely applied.

9. Limitations

Although this review aims to systematically elaborate on the progress and prospects of emerging therapeutic strategies for PF, it is subject to several limitations. First, most of the advanced therapies discussed (e.g., iPSC-based cell therapy, epigenetic editing and novel nucleic acid delivery systems) are currently primarily in the preclinical or early clinical trial stages. Their long-term efficacy and safety in human patients have not yet been fully validated. Second, existing studies exhibit heterogeneity across different model systems (e.g., various animal models, cell lines) and patient populations, which limits the extrapolation and generalizability of some conclusions. Additionally, the discussion was performed largely based on published literature, which may not cover all the latest or unpublished clinical trial data, and the literature selection may have been influenced by the authors' search strategy and database scope. Furthermore, although this review emphasizes the importance of multi-target and combination therapies, understanding of the optimal combination regimens, timing and underlying interaction mechanisms remains incomplete. PF itself is highly heterogeneous (e.g., different etiologies, disease stages), yet most current research on novel therapies still tends to treat IPF as a homogeneous entity; the efficacy for specific subtypes requires further exploration. Finally, achieving truly individualized and precise treatment depends on a robust biomarker system, and research in this area is still in its early stages. Acknowledging these limitations helps in viewing the current research findings more objectively and points the way toward key breakthroughs needed in the future. Before these innovations can be widely applied, further clinical validation and technological breakthroughs are required.

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Authors' contributions

SL and YL performed data acquisition and data analysis and wrote the manuscript. XL and QY conceived and supervised the work. 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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