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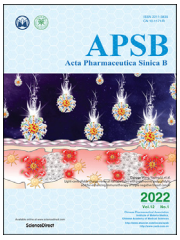

REVIEW

Targeting PI3K/AKT signaling for treatment of idiopathic pulmonary fibrosis

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Drug therapy

Abstract Idiopathic pulmonary fibrosis (IPF) is a chronic progressive fibrotic interstitial pneumonia with unknown causes. The incidence rate increases year by year and the prognosis is poor without cure. Recently, phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/AKT) signaling pathway can be considered as a master regulator for IPF. The contribution of the PI3K/AKT in fibrotic processes is increasingly prominent, with PI3K/AKT inhibitors currently under clinical evaluation in IPF. Therefore, PI3K/AKT represents a critical signaling node during fibrogenesis with potential implications for the development of novel anti-fibrotic strategies. This review epitomizes the progress that is being made in understanding the complex interpretation of the cause of IPF, and demonstrates that PI3K/AKT can directly participate to the greatest extent in the formation of IPF or cooperate with other pathways to promote the development of fibrosis. We further summarize promising PI3K/AKT inhibitors with IPF treatment benefits, including inhibitors in clinical trials and pre-clinical studies and natural products, and discuss how these inhibitors mitigate fibrotic progression to explore possible potential agents, which will help to develop effective treatment strategies for IPF in the near future.

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1. Introduction

Idiopathic pulmonary fibrosis (IPF) is one of the chronic, progressive fibrotic interstitial lung diseases without an identifiable cause. It is identified as one of the most common and severe idiopathic interstitial pneumonia[1], accompanied with difficult breathing, coughing, and worsening lung function. IPF is reported with quite a high prevalence of 58.7 per 100,000 person[2,3], as well as a high mortality rate[4]. Respiratory failure contributes the most to the death of IPF patients, other causes include coronary heart disease, pulmonary embolism and lung cancer[5]. Currently, only pirfenidone and nintedanib are approved by the US Food and Drug Administration for IPF treatment. Both of them can interfere with fibroblast proliferation and migration, and reduce fibroblasts-embedded collagen gel contraction and excess extracellular matrix (ECM) secretion[6,7]. However, gastrointestinal and skin-related adverse events (AEs) are reported in pirfenidone treatment[8], and diarrhea and increase of hepatic enzymes are common AEs for nintedanib therapy[9], preventing the widespread use of these drugs. Therefore, it is urgent to seek for other appropriate therapeutic approaches for IPF.

Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB/AKT) signaling pathway is one of the core signaling pathways in cells that regulates cell growth, proliferation, motility, metabolism and survival[10]. PI3K is a group of lipid kinases associated with the plasma membrane, and it can be divided into three categories: classes I, II, and III[11]. Class I PI3Ks are heterodimer formed by the p110 catalytic subunit and the P85 regulatory subunit, and exist in four isoforms including class IA (PI3Kα, PI3Kβ and PI3Kδ) and class IB (PI3Kγ). Among these four isoforms, which can be expressed in human lung fibroblasts[12], PI3Kα is often up-regulated or mutated in lung-related diseases[13], and PI3Kγ is usually found overexpressed in IPF lung homogenate and fibroblasts[14], while class III PI3Ks are involved in the formation of autophagosome membranes which may affect pulmonary fibrosis[15]. AKT is a serine/threonine protein kinase with three subtypes: AKT1, AKT2 and AKT3[16], can be activated in response to upstream PI3K. Since AKT3 is predominantly expressed in brain tissue, research related to pulmonary fibrosis mainly focuses on AKT1 and AKT2 subtypes. AKT1-mediated mitophages contribute to alveolar macrophage apoptosis resistance, which is required for pulmonary fibrosis development[17], and AKT2-deficient mice are protected against bleomycin (BLM)-induced pulmonary fibrosis and inflammation[18], indicating that PI3K/AKT signaling plays important roles during IPF development.

Existing evidence suggested that overexpression of alpha-smooth muscle actin (α-SMA) in lung fibrosis was related to the activation of PI3K/AKT[12], and the interaction between transforming growth factor-β (TGF-β) and PI3K/AKT promoted the formation of pulmonary fibrosis[19]. Besides, the activation of PI3K/AKT can participate in pulmonary fibrosis by regulating its downstreams such as mammalian target of rapamycin (mTOR), hypoxia inducible factor-1α (HIF-1α) and FOX family[14,18]. It is precisely because of the important role of PI3K/AKT in regulating receptor-mediated signal transduction, making targeting PI3K/AKT to be a new strategy for IPF treatment.

Here, we review the comprehensive pathogenesis of IPF, and systematically sort out the key role of PI3K/AKT signaling during the initial stage of epithelial cell damage, coagulation cascade, immune activation, and fibroblast accumulation. Based on the important role of PI3K/AKT signaling in IPF, we sum up the potential drugs targeting PI3K/AKT signaling which have been evaluated as therapeutic agents for IPF. Among them, GSK2126458, HEC68498 and rapamycin are currently under the clinical evaluation for the treatment of patients with IPF. Besides, substantial progresses are made in the treatment of IPF with other small molecules and natural compounds targeting PI3K/AKT signaling, broadening the treatment landscape of IPF and accelerating the advent of new promising drugs.

2. PI3K/AKT signaling in different development stage of IPF

The pathogenesis of IPF disease is largely unknown; however, singular strides have been made over the past few years. Numerous studies have suggested that some environmental and vocational exposures are related to the onset of IPF disease[20], and corresponding speculation about the potential role of genetic mutations and interplay with assumed external factors has been made[21,22]. Currently, IPF is considered to be an abnormal wound healing response caused by damage to alveolar epithelial cells (AECs)[23,24]. Routine wound healing goes through four distinct phases: the clotting/coagulation phase, the inflammatory cell migration phase, the fibroblast migration/proliferation/activation phase, and tissue remodeling and decomposition[25]. Injured AECs activate multiple inflammatory responses, repair pathways and signaling pathways, including the PI3K−AKT pathway[26], to release profibrotic mediators and disturb the balance between profibrotic and anti-fibrotic mediators[27]. This response is accompanied by abnormal epithelial−mesenchymal crosstalk[20,28], fibroblast proliferation, and fibroblast to myofibroblast transformation[29]. Additionally, myofibroblasts secrete ECM, mainly collagen, which leads to chaotic lung remodeling[30], and ultimately progressive pulmonary fibrosis and loss of function.

Pulmonary trauma repair is a complex, coordinated, and orderly process. If dysregulation or lung injury persists at any stage of the tissue repair process, fibrosis will develop, eventually leading to the development of multiple lung diseases (Fig. 1). Further investigation into the pathogenesis of IPF will provide a theoretical basis for the treatment of multiple fibrotic diseases[26,29].

2.1. *Disease initiation: Epithelial cell damage*

Numerous studies have revealed the critical role of AECs in the pathogenesis of IPF, and several pieces of evidence support the opinion that injury to the alveolar epithelium is central to disease initiation. Initially, several genetic studies showed that defects of the alveolar epithelium are the basis of disease development[31]. The variant of the mucin 5B (*MUC5B*) gene has been reported to be one of the most relevant risk factors for familial and sporadic IPF. Moreover, the primary mucin-expressing cells in microscopic honeycomb cysts of IPF are AECs, suggesting that alveolar epithelium defects are the primary contributors to IPF[32]. Mutations in lung epithelial restricted genes (*SFTPC*, *SFTPA2*, and *ABCA3*) have also been implicated in familial forms of pulmonary fibrosis. Additionally, genome-wide association studies have also verified that variants in telomerase reverse transcriptase (*TERT*) and regulator of telomere elongation helicase 1 (*RTEL1*) notably shorten telomeres and increase the risk of IPF disease[33]. Defects in telomere maintenance have been linked to epithelial cell senescence and an impaired response to epithelial injury[34]. Further, established environmental exposures such as smoking[35], inhaled

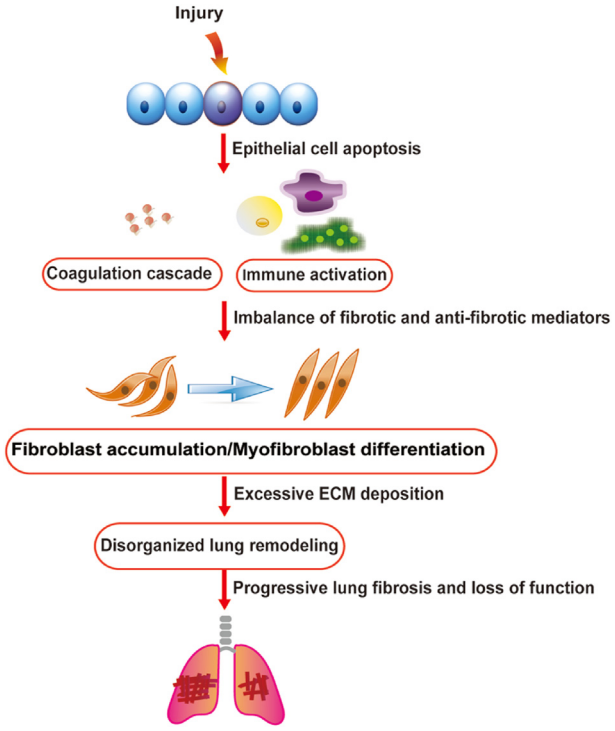

Figure 1 The pathogenesis of IPF. Injured AECs can activate multiple inflammatory responses, coagulation cascade and repair programs, releasing quite a few profibrotic mediators and breaking the balance between profibrotic and anti-fibrotic mediators. Then fibroblasts will accumulate in large numbers and transdifferentiate into myofibroblasts. Myofibroblasts secrete excess extracellular matrix, causing chaotic lung remodeling, which eventually generates progressive pulmonary fibrosis and loses functions.

particulates due to occupational factors (*e.g.*, sawdust and metal dust), microbial (viral, bacterial, and fungal) infections[36,37], and gastroesophageal reflux disease risk factors for pulmonary fibrosis may act as sources of recurrent damage to the alveolar epithelium. Together, these studies imply that AECs play prominent roles in driving early IPF disease pathogenesis.

It is common to observe abnormal epithelial cells, such as bronchial epithelial cells and proliferative type II AECs, lined with honeycomb fibrotic areas in IPF lung biopsies, and studies have shown that AECs damage is sufficient to cause pulmonary fibrosis[38]. Obvious AECs apoptosis in areas of positive remodeling and regions with high myofibroblast activity have also been found in IPF lung biopsies, suggesting that AECs apoptosis is associated with the onset of IPF[39,40]. Moreover, AECs produce key fibrogenic mediators, including connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), TGF-β[41–43].

The interaction of predisposing risk factors, including genetic susceptibility and environmental exposure, plays a vital role in repeated micro-damage of epithelial cells followed by epithelial–mesenchymal transition (EMT), senescence, and apoptosis, which is considered to be the initial cause of IPF responses. The exact mechanisms of epithelial cell damage are complex and have not yet been elucidated. However, it is clear that EMT, senescence, and apoptosis of vulnerable alveolar epithelium are central to this process, among which, the PI3K/AKT signaling pathway is widely involved.

2.1.1. *EMT*

EMT is indispensable to the pathogenesis of IPF in that it allows epithelial cells to obtain a mesenchymal phenotype through disassembly of epithelial cell–cell contacts, resulting in the loss of cell polarity[44]. The role of EMT in fibrosis is pernicious, and activation of EMT in the lung has been advocated as one relevant mechanism leading to alveolar cell loss, myofibroblast accumulation, and lung fibrosis in both human and experimental studies[45]. Several studies have shown that the EMT was disrupted when PI3K/AKT is inhibited, and the use of AKT inhibitors can partially reverse EMT[46]. TGF-β is the most important EMT inducer in fibrosis and cancer. In renal fibrosis, EMT can be promoted by enhancing the expression of PI3K subunit p110δ induced by TGF-β and the phosphorylation of AKT. Activated AKT activates HIF-1α, which promotes the conversion of AECs to fibroblasts and mediates EMT to participate in pulmonary fibrosis[47]. Additionally, PI3K P85 was highly expressed in silica-induced lung fibrosis, HBE cells, and A549 cells. Indeed, siRNA mediated knockout of PI3K P85 in HBE and A549 cells reduced the severity of pulmonary fibrosis by weakening the process of EMT[48]. Recent research confirmed that the non-SMAD signaling pathway of PI3K/AKT plays a key role in BLM-induced EMT[49]. These findings demonstrated that PI3K/AKT promotes EMT and contributes to the pathogenesis of fibrosis.

2.1.2. *Senescence*

Interestingly, in the fibroblastic lesions and honeycomb areas of the IPF lung, increased aging markers were found mainly in epithelial cells[50,51]. Aging epithelial cells secrete several mediators in senescence-associated secretory phenotype (SASP), which directly affects the surrounding microenvironment, thereby triggering IPF; thus, epithelial cell senescence can be considered a pathological feature of IPF. Gene mutations related to telomere abrasion also promote the development of IPF by affecting the senescence of AECs[52]. Studies have found that in chronic obstructive pulmonary disease, oxidative stress dependent microRNA-34a can reduce the expression of anti-senescence-related sirtuin-1 and sirtuin-6 in epithelial cells through PI3Kα[53]. AKT signaling has also been reported to play a central role in controlling the proliferation and survival of fibroblasts[54]. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a key negative regulator of the PI3K pathway that can dephosphorylate PIP3 to PIP2 and prevent the activation of downstream kinases such as AKT[55]. Research shows that AEC senescence induced by the loss of PTEN occurs in an AKT-dependent manner, which may help determine potential targets for anti-aging treatment of IPF[56,57]. Based on the above findings, inhibition of PI3K and AKT are postulated to be able to delay aging and reduce lung fibrosis.

2.1.3. *Apoptosis*

A growing body of evidence suggests that apoptosis of AECs plays an important role in the pathogenesis of lung diseases[50]. Many apoptosis incentives have been identified, and the most relevant to the pathobiology of IPF include autophagy, oxidative stress and endoplasmic reticulum (ER) stress[58]. Autophagy ameliorated BLM-induced pulmonary fibrosis by inhibiting the apoptosis of lung epithelial cells[59]. Insufficient autophagy was found in IPF, mainly in the AECs of the IPF lung, and further affects fibroblast differentiation[60,61]. Studies have indicated that the PI3K/AKT signaling pathway is up-regulated and autophagy regulator mTOR activation is increased to inhibit autophagy and

exacerbate apoptosis of AECs to promote pulmonary fibrosis[62]. Oxidative stress is also important in the development of pulmonary fibrosis, and it has been proposed that the imbalance between the antioxidative and pro-oxidative state may promote apoptosis of epithelial cells and activation of fibrotic pathways[63]. Model studies of BLM-induced pulmonary fibrosis have also shown that the over-activated PI3K/AKT/HIF-1α pathway regulates abnormal cell proliferation and apoptosis through oxidative stress, thereby further affecting the normal repair of AECs, leading to the production of type III collagen and the formation of pulmonary fibrosis[64]. Studies have reported that markers (BIP, EDEM, and XBP-1) of ER stress and unfolded protein response activation were primarily elevated in the hyperplastic type II AECs overlying fibroblastic foci of patients with IPF[65]. The mechanisms by which ER stress regulates AEC apoptosis are not fully understood, and recent research has confirmed that ER stress and oxidative stress affect the apoptosis of type II AECs *via* activating PI3K/AKT pathway, leading to silicon dioxide nanoparticles-induced pulmonary fibrosis[66]. Taken together, these studies indicated that the increase in the apoptosis of AECs in IPF is closely related to the activation of PI3K/AKT.

2.2. *Disease progression: Coagulation cascade*

The coagulation cascade is responsible for fibrin formation at sites of damaged blood vessels, and functions to prevent blood loss[67]. In the early stages of wound healing, endothelial and epithelial damage can activate the coagulation cascade, resulting in the production of thrombin, followed by thrombin-mediated conversion of serum-derived fibrinogen to fibrin to form a pre-matrix[68]. Accumulating evidence suggests that the physiological function of the coagulation cascade is not limited to coagulation, and that this cascade also plays a key role in influencing inflammatory and tissue damage repair responses[69]. Therefore, uncontrolled coagulation contributes to the pathophysiology of various diseases, including acute and chronic lung injury[70]. Previous studies have shown that several zymogens of both the exogenous coagulation cascade and plasminergic systems, including factor X, thrombin, and plasminogen, are locally produced and activated in IPF fibrotic foci[71], and that this cascade is closely related to fibrin deposition in the lungs of patients with IPF. Furthermore, increased pro-coagulant activity has been observed in the bronchoalveolar lavage fluids of patients with IPF[72], and there is sufficient evidence to show that the balance of pro coagulation is increased in patients with IPF[73–75]. Under these coagulation promoting conditions, ECM degradation decreases, leading to fibrosis-promoting effects, and fibroblast differentiation into myofibroblasts is induced by protease-activated receptor[25,76]. It well recognized that activation of the coagulation cascade and PI3K/AKT may affect the pathogenesis of pulmonary fibrosis (Fig. 2).

The alveolar blood vessels are affected by damage to the alveolar structure and removal of AECs in the basement membrane, which leads to increased vascular permeability. Extravasation of coagulation factors into the tissue leads to extravascular coagulation[77]. Subsequently, endothelial cells and endothelial progenitor cells will proliferate to form new blood vessels. Studies have shown that the quantity of endothelial progenitor cells in patients with IPF is significantly reduced, which to a large extent leads to the failure of reendothelialization, and may further lead to dysfunction of the alveolar-capillary barrier, profibrotic response, and increase in vascular endothelial growth factor (VEGF)[78,79]. VEGF combines with the receptor KDR on vascular endothelial cells to activate the PI3K/AKT signaling pathway, thereby promoting the growth and migration of vascular endothelial cells and the formation of new blood vessels[80]. Strong expression of VEGF

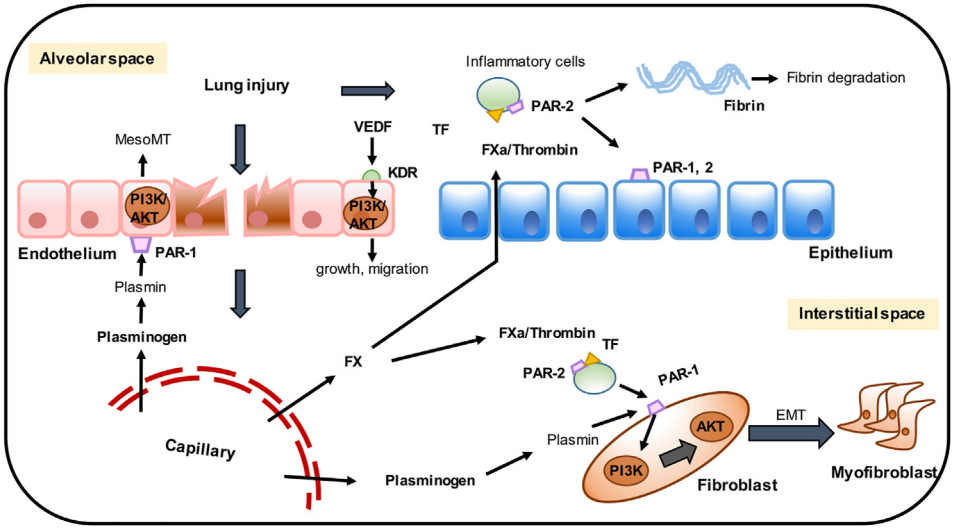

Figure 2 The involvement of PI3K/AKT in coagulation cascade of IPF. Injured lung will cause the damage of the endothelium and epithelium, resulting in the activation of the coagulation cascade. The integrity of the alveolar-capillary barrier is disrupted, allowing factor X (FX), thrombin and plasminogen to enter the alveolar and interstitial spaces. Meanwhile, reactively increased VEGF combines with the receptor KDR in vascular endothelial cells to activate PI3K/AKT signaling pathway to advocate the growth and migration of endothelium. Endogenous tissue factor (TF) facilitates zymogen protease activation, causing fibrin deposition. Plasmin contributes to the activation of PI3K/AKT, which promotes the mesothelial−mesenchymal transition (MesoMT) and EMT *via* protease activated receptor 1 (PAR-1).

can be detected in the lavage fluid and serum of patients with IPF, and pulmonary fibrosis can be reduced by inhibiting the expression of VEGF through the inhibition of PI3K/AKT[80].

Additionally, endothelial cells may undergo mesenchymal transformation to promote the development of fibrosis[81], and mesothelial−mesenchymal transition also contributes to BLM-induced pulmonary fibrosis. Previous studies have shown that tissue factor-dependent exogenous coagulation pathways are central to the pathogenesis of IPF, and both plasmin and thrombin are considered effective activators of the PI3K/AKT signaling pathway. Moreover, pleural mesothelial cells have been found to undergo thrombin-mediated PI3K/AKT activation through protease-activated receptor-1 activation to obtain mesothelial−mesenchymal transition, leading to increased expression of α-SMA and a characteristic fibrotic phenotype. Additionally, PI3Kβ is considered to be associated with thrombus formation, and PI3Kβ plays various roles in downstream G protein-coupled receptor-mediated thrombin and ADP signals, as well as in integrin and glycoprotein receptors; thus, it is speculated that β isoform is related to the coagulation cascade process of IPF[82].

2.3. *Disease maintenance: Immune activation*

The immune response is divided into innate and adaptive immunity, both of which seem to be activated in IPF. Numerous studies have confirmed that inflammatory cells and lymphocytes and their related signals affect the pathophysiology of IPF[83]. Inflammation occurs in the early stage of wound healing and is characterized by continuous infiltration of inflammatory cells[84]. In IPF, macrophages and neutrophils are the most studied innate immune cells, while the role of lymphocytes in fibrosis is poorly understood and remains controversial[85]. This controversy mainly lies in the failure of IPF to improve in response to lymphocyte modulation therapies, but lymphocyte subsets and activation of lymphocytes are indeed found in the lungs and blood of IPF patients with abnormal prognosis[83]. Moreover, in patients with IPF, lymphocytes aggregate in lung tissue and autoantibodies are present in the serum, indicating that lymphocytes should still be regarded as a treatment target of IPF[86].

2.3.1. *Macrophage plasticity*

Macrophages are the origin of tissue inhibitors of metalloproteinase, which can antagonize the degradation of the ECM mediated by metalloproteinase[87]. Macrophages have two phenotypes: M1 (classical activation) and M2 (alternative activation). According to the polarization, local microstructure, and fibrosis stage of alveolar macrophages, M1 and M2 play different roles in the process of fibrosis and exhibit obvious phenotypic plasticity[88,89]. At the early stages of inflammation, acute lung injury promotes an M1 phenotype, leading to the secretion of proinflammatory cytokines. The continuous inflammatory response serves as a trigger to initiate fibrotic responses in the lung[90]. However, M2 macrophages are important in wound healing processes and in terminating inflammatory responses in the lung[91]. The mechanism by which M2 macrophages improve IPF may be *via* the generation of TGF-β and PDGF, or by enhancing ECM degradation through matrix metalloproteinase (MMP) activity[92,93]. It is well known that interleukin (IL)-4 is the major inducer of M2 polarization *via* activation of one of its major downstream signals PI3K/AKT[94]. Studies have shown that IL-13 produced by M2 macrophages also plays a key role in the homeostasis control of normal lungs and the pathogenesis of pulmonary fibrosis. AKT1 regulates pulmonary fibrosis by inducing M2 macrophages to produce IL-13, suggesting that targeting AKT1 blocks the fibrotic process of IPF[95]. The O subclasses of the Forkhead box (FOXO) family, such as FOXO1, FOXO3, and FOXO4, are directly phosphorylated by AKT, causing them to be exported to the cytoplasm and degraded through the ubiquitin-proteasome pathway. AKT2 regulates pulmonary fibrosis by up-regulating the production of pro-fibrotic cytokines, TGF-β1, and IL-13, *via* the AKT2/FOXO3a signaling pathway[18]. It has also been reported that myeloid PTEN deficient mice induced by BLM exhibit sustained PI3K activation to enhance macrophage M2 polarization, which leads to increased morbidity[96].

2.3.2. *Formation of neutrophil extracellular traps*

Neutrophils produce various proteases, especially serine proteases (neutrophil elastase [NE]) and MMPs, which degrade matrix components, but can also activate TGF-β and produce inhibitory factors through NE, thus promoting the accumulation of ECM[97−99]. NE can promote fibroblast proliferation and myofibroblast differentiation *in vitro*, while NE-deficient mice are protected from asbestos-induced pulmonary fibrosis[100]. Neutrophil extracellular traps (NETs) are released by neutrophils and consist of decolorized chromatin filaments and granular proteins[101]. The release of NETs may cause local tissue damage and inflammation, and plays a significant role in cystic fibrosis and acute virus-mediated lung injury[102,103]. If the damage is sustained, neutrophils and monocytes are recruited, and promote the production of reactive oxygen species (ROS) to intensify epithelial damage. PI3Kγ and PI3Kδ are mainly expressed in leukocytes, which leads to speculation that these are the primary isoforms in PI3K-mediated innate and adaptive immune response signals[104]. Neutrophils primed with tumor necrosis factor-α (TNFα) can be divided into two phases: PI3Kγ mainly mediates PIP3 accumulation at the leading edge of the cell in the chemokinetic phase, while the subsequent chemotactic phase depends on PI3Kδ[105,106]. PI3Kγ and PI3Kδ have also been shown to affect neutrophil degranulation and superoxide production[107]. Inhibition of PI3Kγ and PI3Kδ can restore the effective directionality of neutrophil movement to reduce the release of NETs and minimize potential diseases due to immunosuppression[108].

2.3.3. *Lymphocyte aggregates*

Studies have confirmed that lymphocyte factors are related to IPF, but the role of lymphocytes is controversial. In IPF lungs, lymphocytes usually aggregate near the fibroblast foci. These aggregates are composed of $CD3^+$ T lymphocytes and mature dendritic cells[109,110]. Th2 and Th17 cells promote pulmonary fibrosis by inducing elevated levels of IL-4, IL-13, and TGF-β1[111−114], while Th1, Th22, and $\gamma\delta$-T cells inhibit fibrosis by inducing IFN-γ and IL-12, IL-9, and CXCL10, respectively[115−118]. Regulatory T cells and Th9 cells have been associated with anti-fibrosis effects[116,117,119,120]. An increase in $CD20^+$ B cells has also been detected in the lungs of patients with IPF, and represent an important subset of aggregation. Additionally, many soluble factors that promote the growth and differentiation of B cells have been observed in the blood of patients with IPF, including B cell activating factor (also known as B lymphocyte stimulator), IL-6, and IL-13[121]. The inhibition of the PI3K/AKT pathway can inhibit the overproduction of pro-inflammatory cytokines, including TNFα, IL-1β, and IL-6, in bronchoalveolar lavage fluids

and enhance the release of the anti-inflammatory cytokine IL-10. The specific loss of PTEN in myeloid cells is sufficient to reduce leukocyte recruitment, promote pathogenic inflammation, and drive TGF-β1 activation and subsequent expression of pro-fibrotic molecules[96].

2.4. *Disease evolution: Fibroblast accumulation/myofibroblast differentiation*

Fibroblasts are tissue-derived mesenchymal cells whose central features are to secrete ECM proteins, providing an environment for regular repair events such as epithelial cell migration[122]. During the pathogenesis of IPF, activated fibroblasts secrete pro-fibrotic mediators to enhance the fibrotic environment, leading to excessive production of ECM and transdifferentiation to myofibroblasts[123]. Myofibroblasts have shrinkable attributes similar to smooth muscle cells and express α-SMA[124]. Compared to fibroblasts, myofibroblasts survive longer in damaged tissues and synthesize more ECM[125]. The strict connection between endothelial cytokines and interstitial cells is conducive to abnormal crosstalk and augments the role of the TGF-β1, PDGF, and WNT pathways, amplifies the fibrotic environment, and leads to a higher rate of transdifferentiation[126]. One unique pathological characteristic of IPF is the existence of fibroblastic foci, that is, an active synthetic cluster of fibroblasts in the vicinity of the air-tissue interface, which can be regarded as the site of new fibrosis[127]. Therefore, fibroblasts and myofibroblasts are deemed the potential therapeutic targets for IPF.

Lysophosphatidic acid (LPA) has been identified as a key fibroblast chemokine in experimental lung fibrosis, and LPA1 receptor KO mice are protected in this model[128]. Studies have found that PI3Kβ is a key downstream target of LPA *in vivo*. The activation of PI3Kβ downstream of LPA may help fibroblasts chemotax to sites of tissue damage *in vivo*[129]. Additionally, the increase in PI3Kγ expression in fibroblasts and basal cells is thought to be related to IPF[14]. In fibroblasts, activated AKT regulates the production of collagens I and III, and promotes human liver fibrosis and BLM-induced lung fibrosis in mice. In addition, AKT can maintain the low autophagy activity of fibroblasts by activating downstream mTOR signaling so that it can preserve the characteristics of high proliferation and anti-apoptosis[130]. Importantly, AKT inhibitors can effectively inhibit the expansion of fibroblasts and the formation of fibronectin matrix in lung tissue, reducing the levels of collagen I and collagen III and retaining lung compliance[64]. Because the fibroblasts in the fibrotic foci express low levels of PTEN, PI3K/AKT activity is enhanced in IPF fibroblasts[131]. Studies have found that PTEN inhibition and AKT/mTOR activation desensitize IPF fibroblasts from collagen matrix-induced cell death.

TGF-β is a member of a large family of polypeptides that modulate various cellular functions[132]. There are three subtypes of TGF-β (β1, β2, and β3), and TGF-β1 performs an essential function in the pathogenesis of IPF. It can promote the recruitment of fibroblasts, the differentiation and survival of myofibroblasts, and deposition of ECM, which is a powerful fibrotic promoting medium[133–136]. Previous studies have shown that the PI3K/AKT signal pathway is involved in the TGF-β1 regulating pathway, and TGF-β1 could induce the activation of the PI3K/AKT pathway[137]. Furthermore, studies have confirmed that PI3K/AKT functions upstream of ER stress, affecting the proliferation of lung fibroblasts, leading to BLM-induced pulmonary fibrosis[138]. However, the relationship between ER stress and the PI3K/AKT pathway in regulating the proliferation of fibroblasts has not yet been elucidated. Moreover, pharmacological inhibition of ER stress reduces TGFβ1-induced myofibroblast differentiation, α-SMA expression, and collagen production in patients with IPF. ER stress-induced autophagy is partly attributable to the down-regulation of the AKT/mTOR pathway, and TGF-β1 inhibits autophagy of fibroblasts, at least in part, by activating mTORC1[139–141], indicating that the activation of PI3K/AKT contribute to fibroblast accumulation and myofibroblast differentiation induced by TGF-β.

WNT protein is a secreted glycoprotein that can signal paracrine or autocrine through its frizzled receptors, low-density lipoprotein receptor-related protein 5 and 6, and disheveled to stabilize β-catenin and cause its nuclear translocation[142]. Activation of the WNT/β-catenin pathway is closely related to apoptosis resistance and proliferation[76], and it is connected with EMT and fibrogenesis after initiation by TGF-β1, sonic Hedgehog, gremlin-1, and PTEN. It is worth noting that both TGF-β1 and the canonical WNT/β-catenin pathway can stimulate each other through PI3K/AKT signaling, and the WNT/β-catenin pathway is considered an upstream activator of the PI3K/AKT/mTOR pathway. More importantly, WNT/β-catenin signaling has been found to be activated in patients with IPF, in whom several WNT/β-catenin-dependent are up-regulated simultaneously[143–145]. Additionally, WNT target genes, such as stromelysin (*MMP-7*) and fibronectin, contribute to the transdifferentiation of fibroblasts in the development of pulmonary fibrosis[143]. Therefore, the WNT/β-catenin pathway and the PI3K/AKT pathway mediate each other to promote the pathogenesis of IPF.

BLM-induced pulmonary fibrosis depends on the production of ROS, and ROS can participate in lung injury through the PI3K/AKT signaling pathway. Studies have found that ROS can cause fibroblast proliferation and collagen production by activating the PI3K/AKT/HIF-1α pathway[64]. Additionally, AKT can act on fibroblasts after activation, causing the release of hydrogen peroxide and subsequent damage to adjacent type II AECs to participate in pulmonary fibrosis[146]. By inhibiting catalase and other products produced by ROS, the activation of the PI3K/AKT signaling pathway is inhibited to mediate anti-pulmonary fibrosis.

2.5. *Disease formation: Tissue remodeling and decomposition*

After the completion of the proliferative phase, wound repair enters its ultimate remodeling state, which can take several years. A major feature of this stage is the remodeling of the ECM into a structure resembling normal tissue[147]. The abnormal tissue remodeling is represented by considerable ECM accumulation. With the development of fibrosis, the biological characteristics of the ECM have changed, such as increased tissue elasticity (stiffness) and changes in matrix composition[148]. Increasing matrix stiffness increases the ability of myofibroblasts to differentiate, and, in turn, myofibroblasts can extend matrix stiffness, which may be accomplished by collagen synthesis and cross-linking to create a feedforward loop that drives the fibrosis process[149]. Studies on the ECM of IPF lungs have shown that type III collagen is mainly present in the alveolar septum and interstitial fibrosis area, while type I collagen is dominant in mature fibrosis area[150,151]. ECM turnover is tightly regulated by several protease families and their respective inhibitors[152]. MMPs include a family of proteases that target collagen and other matrix components for degradation. However, during fibrosis, collagen in the ECM is insoluble, and it is relatively resistant to deterioration by

Table 1 Potential PI3K/AKT inhibitor for IPF treatment.

Agent	Mechanism/target	Function description	Phase of development and status	Common adverse event	Ref.
GSK2126458	PI3K/mTOR inhibitor	Reduce TGF-β-induced fibroblast proliferation and collagen I synthesis	Phase I completed (NCT01725139)	Diarrhoea, hyperglycaemia, nausea	166
HEC68498	PI3K inhibitor	Anti-fibrosis and anti-inflammation	Phase I Active, not recruiting (NCT03502902)	Not described	/
Rapamycin	mTOR inhibitor	Inhibit TGF-α and EGFR signaling	NA, completed (NCT01462006)	Hyperglycemia, hypophosphatemia, anemia	167
PX-866	pan-PI3K inhibitor	Inhibit TGF-α	Pre-clinical	Rash, hyperglycemia, transaminase elevations	168
Derivatives of 4-methylquinaz-oline	PI3K inhibitor	Anti-fibrosis and anti-inflammation	Pre-clinical	Not described	169
LY294002	AKT inhibitor	Inhibit fibroblasts expansion and fibronectin matrix formation	Pre-clinical	Not described	49
ASV	TGFβ1/PI3K/AKT pathway inhibition	Inhibit EMT	Pre-clinical	Raised total bilirubin and rash	170,171
Hyp	AKT/GSK3β pathway inhibition	Inhibit inflammation, oxidative stress and EMT	Pre-clinical	Not described	172
Ligustrazine	PI3K/AKT/mTOR pathway inhibition	Reduce ROS	Pre-clinical	Edema, hypertension, gastrointestinal bleeding	62,173
Quercetin	PI3K/AKT pathway inhibition	Anti-oxidation and anti-aging	Pre-clinical	Gastrointestinal effects, rash	174−176

PI3K, phosphatidylinositol-3-kinase; mTOR, mammalian target of rapamycin; TGF-α, transforming growth factor-α; EGFR, epidermal growth factor receptor; TGF-β1, transforming growth factor-β1; ASV, astragaloside IV; EMT, epithelial−mesenchymal transition; Hyp, hyperin; GSK3β, glycogen synthase kinase 3β; ROS, reactive oxygen species.
NA, not available.

proteases[153]. Therefore, an understanding of this process is conducive to determining new targets for the treatment of IPF.

There is a substantial body of literature that support that PI3K/AKT can greatly regulate ECM[154]. Studies have found that when IPF fibroblasts interact with collagen-rich matrix, integrin receptors signal down-regulation of PTEN, which is followed by activation of the PI3K/AKT/mTORC1 phosphorylation cascade[155]. Additionally, focal adhesion kinase (FAK) plays a key role in regulating the integrin-mediated ECM signal[156]. The signaling between FAK and PI3K, which are also important in the focal adhesion pathway, could regulate cell survival, apoptosis, and cell cycle progression[157,158].

CTGF (also known as CCN2) belongs to the connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. As an essential downstream mediator of TGF-β signaling, CTGF is also regarded as a component of the profibrotic matrix. CTGF is usually expressed at low levels in healthy individuals, but is strongly upregulated in mechanically stressed tissues[159,160]. In patients with IPF, high CTGF expression has been proven to promote ECM deposition, fibroblast activation, cell adhesion, and invasion, which are key in tissue remodeling and fibrosis[161,162]. Rapamycin can regulate the expression of CTGF in lung fibroblasts and epithelial cells through the PI3K signaling pathway, leading to excessive accumulation of ECM[163]. CCN5, another member of the CCN family, acts as a dominant-negative protein to suppress CCN2-mediated fibrogenesis. Emerging evidence has demonstrated that when CCN5 is overexpressed, it can down-regulate CCN2 to inhibit the PI3K/AKT signaling pathway and alleviate pulmonary fibrosis[164].

3. Potential drugs targeting PI3K/AKT for IPF treatment

Based on this understanding of IPF, numerous therapeutic targets have been realized and the development of anti-IPF drugs has been improved. While several of these anti-IPF drugs have entered clinical trials, only nintedanib and pirfenidone are approved for IPF treatment, and the effects of these two drugs are limited to slowing down the disease process[165]. Three drugs target the PI3K signaling pathway, and many other experimental trials have also achieved promising results. A summary of these drugs is shown in Table 1[49,62,166–176].

3.1. *Clinical trials of PI3K/AKT inhibitors for IPF treatment*

Based on the significance of PI3K/AKT in the pathogenesis of IPF, clinical trials began to reposition some PI3K/AKT inhibitors originally used for cancer treatment for the treatment of IPF. Although current drugs targeting PI3K to treat IPF are still in the early stages, their therapeutic effects are impressive. These clinical trials could provide important insights into the treatment of IPF and identify more PI3K/AKT inhibitors for lung fibrotic disorders.

3.1.1. *Omipalisib (GSK2126458)*

Omipalisib (GSK2126458), an effective small molecule inhibitor of the PI3K/mTOR pathway, was developed as an anti-tumor treatment and has been evaluated in phase I clinical trials in subjects with solid tumors and lymphomas[177,178]. The efficacy of omipalisib in IPF has also been evaluated[179]. In primary human lung fibroblasts derived from IPF lung tissue, omipalisib can reduce TGF-β-induced fibroblast proliferation and collagen I synthesis *in vitro*[179]. Additionally, omipalisib has an anti-fibrotic effect in IPF fibroblasts by inhibiting AKT phosphorylation. Moreover, some studies have shown that omipalisib changes the glycolysis in the IPF lung and fibroblasts isolated from fibrotic tissue, and can also reduce abnormal glucose signaling in IPF lung fibrosis areas[180]. A dose-finding, double-blind, placebo-controlled study (NCT01725139) to detect omipalisib in IPF subjects demonstrated the safety of the drug[166]. The results showed that orally dosed omipalisib exerts a measurable dose- and exposure-dependent inhibition of the PI3K/mTOR pathway in the systemic circulation and lungs of individuals with IPF. Reported treatment-related AEs mainly include diarrhea, hyperglycemia, and nausea, and no serious AEs were reported, as well as no AEs that led to early termination of treatment. However, there are some weaknesses in this study that limit its further research. The study only recruited a small number of subjects, and no formal evaluation of anti-fibrosis efficacy was performed due to the short duration of the study. Furthermore, it is designed to establish the pharmacological properties and biological relevance of the use of a PI3K inhibitor to alleviate IPF, and the evaluation of IPF therapeutic effects of this trial is inadequate. Further clinical trials are needed to determine more clinically relevant effects of GSK2126458 on attenuating IPF.

3.1.2. *HEC68498*

As a class I isoform inhibitor of PI3K and mTOR, HEC68498 has convincing and highly selective properties. It has a robust activity against fibrosis and inflammation, which can, at a lower effective dose, achieve a superior therapeutic effect. To study the application of HEC68498 IPF, a phase I, double-blind, placebo-controlled, single oral dose study is underway to assess safety, tolerability, and pharmacokinetics (NCT03502902). As of now, there has been no progress in related experiments and no relevant research results made public.

3.1.3. *Rapamycin*

Rapamycin, also known as sirolimus, is an mTOR inhibitor. Because of its anti-inflammatory and anti-immune effects, rapamycin is mainly used for immunosuppressive therapy. With the deepening of research on mTOR, the role of this important target in anti-fibrosis therapy has become increasingly clear. Rapamycin has antifibrotic properties in BLM-induced fibrosis in mice[181]. Moreover, previous studies have found that rapamycin can prevent and inhibit the progression of progressive pulmonary fibrosis caused by the expression of TGF-α and increased epidermal growth factor receptor (EGFR) signaling[182,183]. A double-blind, placebo-controlled trial is currently underway to evaluate the ability of rapamycin to act as a fibrosis inhibitor (NCT01462006). The results of the study have not been made public, but related studies have observed grade 3 or 4 AEs related to rapamycin, including hyperglycemia, hypophosphatemia, and anemia[167]. However, studies have also shown that rapamycin can effectively promote CCN2 expression in a PI3K-dependent manner to produce direct fibrotic activity[163]. Considering the controversial role of rapamycin for the treatment of IPF, there is no recent progress in the clinical treatment of IPF. It may due to the negative feedback of PI3K/AKT pathway by rapamycin as a single-target inhibitor of mTOR. Interestingly, the combination therapy of mTOR and PI3K/MAPK inhibitors showed superior anti-tumor activity[184], suggesting that this combination therapy could be considered for treating IPF.

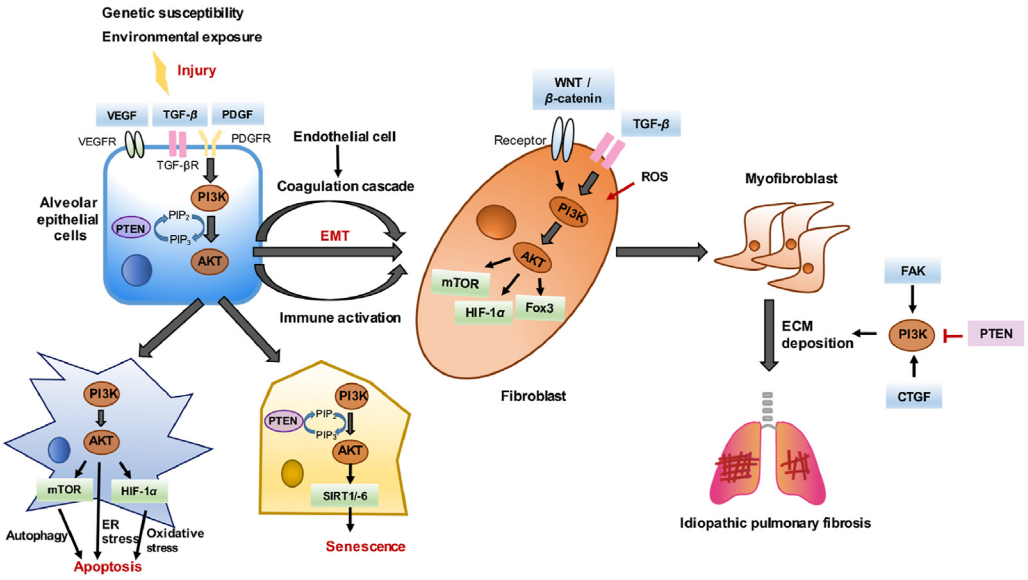

Figure 3 PI3K/AKT in regulating IPF. The activation of PI3K/AKT can directly participate in the formation of IPF, and cooperate with other pathways including TGF, VEGF, PDGF, FAK, WNT, and mTOR, thus contributing to the pathogenesis of IPF. Specifically, activated PI3K/AKT is involved in aging, autophagy, EMT, immunity and other essential processes to promote IPF. Besides, endoplasmic reticulum stress (ER) and the release of reactive oxygen species (ROS) also contribute to the activation of PI3K/AKT to promote the deposition of excess extracellular matrix (ECM) in the lung fibrosis.

3.2. PI3K/AKT inhibitor in the treatment of IPF

Several PI3K/AKT inhibitors are being investigated in pre-clinical research and have shown positive progress. The advent of these inhibitors has profoundly expanded the treatment landscape of IPF, and novel PI3K/AKT inhibitor drugs are currently under evaluation. We believe that more PI3K pathway-targeted drugs will be available for the treatment of IPF in the future.

3.2.1. PX-866

PX-866, a pan-PI3K inhibitor, can down-regulate tumor phosphorylation of AKT and has anti-tumor activity in many human tumor xenograft models[185]. Recent studies indicate that PX-866 can prevent the progression of TGF-α-induced lung fibrosis *in vivo*[168]. Rash, hyperglycemia, and transaminase elevation are considered common AEs related to the treatment of PX-866[186]. At present, there has been no new progress in the use of PX-866 for the treatment of IPF.

3.2.2. Derivatives of 4-methylquinazoline

Rationally designed chemical derivatives of 4-methylquinazoline can be used as high-efficiency PI3K inhibitors for the potential treatment of IPF[169]. They have excellent resistance to proliferate mouse lung fibroblasts, and can significantly improve the lung function of BLM-induced pulmonary fibrosis mice by reducing the levels of α-SMA and hydroxyproline and exerting anti-fibrosis and anti-inflammation effects. These derivatives are expected to become popular drugs for IPF treatment, largely due to their limited adverse event reports.

3.2.3. LY294002

LY294002, a specific PI3K/AKT inhibitor, has been reported to significantly ease PI3K/AKT-mediated cellular processes by suppressing AKT phosphorylation. Numerous studies have confirmed that LY294002 can inhibit the expansion of fibroblasts and the formation of fibronectin matrix in lung tissue in the BLM-induced pulmonary fibrosis model, as well as reduce the content of collagens I and III[49,187]. These findings suggest that AKT inhibitors have anti-inflammatory and anti-fibrotic effects in pulmonary fibrosis, and no related AEs have yet been described.

3.3. Promising natural products targeting PI3K/AKT in IPF treatment

Recent studies have shown that active ingredients in natural compounds have anti-fibrotic effects[188]. These natural compounds may provide promising drug candidates for treating pulmonary fibrosis.

3.3.1. Astragaloside IV (ASV)

ASV is a natural saponin derived from astragalus, which has anti-fibrotic properties in BLM-induced pulmonary fibrosis[189]. The therapeutic effect of ASV is *via* the activation of FOXO3a by inhibiting the TGF-β1/PI3K/AKT pathway, thereby preventing EMT in BLM-induced pulmonary fibrosis[170]. Few studies have been conducted on the toxicity and AEs of AS-IV *in vivo* and *in vitro*, although preclinical trails indicate that ASV is safe and well tolerated, and that the AEs, such as raised total bilirubin and rash, were mild and resolved spontaneously[171].

3.3.2. Hyperin (Hyp)

Hyp is extracted from rhododendron and has various biological effects, including anti-inflammatory, anti-oxidant, anti-fibrosis, and anti-cancer effects[190,191]. Hyp has been shown to reduce the development of pulmonary fibrosis in mice, potentially due to the inhibition of BLM-induced inflammation, oxidative stress, and EMT through the AKT/glycogen synthase kinase 3β (GSK3β) pathway[172]. Moreover, few treatment-related AEs have been reported following the use of Hyp.

3.3.3. *Ligustrazine*

Ligustrazine is extracted from the roots and stems of *Ligusticum chuanxiong* Hort. (Chuan Xiong), and has a protective effect by scavenging ROS, regulating the production of nitric oxide, and preventing the formation of peroxynitrite[192]. As ROS causes fibroblast proliferation and stimulates collagen synthesis, ROS play a pivotal role in IPF pathogenesis. Studies have found that ligustrazine can reduce pulmonary fibrosis by inhibiting PI3K/AKT/mTOR[62]. Common AEs reported following Ligustrazine use include edema, hypertension, and gastrointestinal bleeding[173].

3.3.4. *Quercetin*

Quercetin is a member of the flavonoid family and can provide direct protection in the development of pulmonary fibrosis by resisting oxidative damage and inflammation[193]. Additionally, quercetin and its regulate the activities of PI3K and other kinases, and selectively reduce the viability of senescent endothelial cells. The combination of dasatinib and quercetin effectively reduce senescence and SASP markers in isolated AEC2 from BLM-treated mice[174]. Intriguingly, a recent study reported that quercetin might render senescent IPF fibroblasts susceptible to pro-apoptotic stimuli *via* up-regulation of caveolin-1 and inhibition of PI3K/AKT[175]. Common AEs associated with quercetin tend to be gastrointestinal (constipation, heartburn, bloating, diarrhea, nausea, and vomiting) and skin associated (rash, dryness, flushing)[176].

4. Conclusions and future prospects

The PI3K/AKT pathway has gained growing recognition in the field of oncology due to its key roles in cell survival, growth, and proliferation. However, recent studies have found significant PI3K signaling activity in fibrotic lung lesions[179]. This review encapsulates the correlation between PI3K/AKT and IPF (Fig. 3) and explores potent PI3K/AKT inhibitors and novel anti-fibrotic agents in IPF. Although the pathogenesis of IPF remains largely unknown, the current findings are sufficient to deem the PI3K/AKT pathway a reasonable target for the treatment of IPF.

First, aside from regulating IPF alone, PI3K/AKT also has numerous crosstalk and interactions with signaling pathways, including TGF, VEGF, WNT, FAK, mTOR, Jun N-terminal kinase, CTGF, Hedgehog, and Notch pathway, thus participating in multiple links in the pathogenesis of IPF[194]. Second, there are few drugs that can directly target PI3K and AKT to treat pulmonary fibrosis, but these PI3K pathway-targeted drugs have entered the clinical research stage with encouraging results, and there is scope for improvement for treating IPF in the future. Some natural compounds also exhibit potent anti-fibrotic activity through the inhibition of PI3K/AKT, and are considered promising drug candidates for IPF treatment. It is worth noting that as the PI3K/AKT pathway contains a complex negative feedback system, compared to the suppression of specific isoforms in tumor treatment, inhibiting all four class I PI3K subtypes may produce better IPF treatment efficacy. This has higher requirements, that is, to reduce the systemic toxicity of pan-PI3K as much as possible while effectively treating IPF. Thus, it may be appropriate to design drugs that target pathological cells such as fibroblasts. Finally, the role of PI3K/AKT in IPF broadens the treatment horizons for IPF to establish novel targets to treat IPF, such as peroxisome proliferators-activated receptor γ and the eukaryotic translation initiation factor 4E-binding protein 1. Therefore, it is not difficult for us to conclude that the PI3K and AKT play a significant role in the pathogenesis of IPF. A clear understanding of the existing problems and finding new ways to cure IPF is still something that will require continued efforts for a long time to come.

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Author contributions

Qinjie Weng and Jiajia Wang designed the work. Jincheng Wang, Kaili Hu and Xuanyan Cai collected data and wrote the manuscript. Jincheng Wang and Kaili Hu designed and regenerated the conceptual pictures. Bo Yang and Qiaojun He gave some critical comments. Jincheng Wang, Kaili Hu, Jiajia Wang and Qinjie Weng in charge of checking and revision. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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