

REVIEW

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The novel molecular mechanism of pulmonary fibrosis: insight into lipid metabolism from reanalysis of single-cell RNA-seq databases

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Abstract

Pulmonary fibrosis (PF) is a severe pulmonary disease with limited available therapeutic choices. Recent evidence increasingly points to abnormal lipid metabolism as a critical factor in PF pathogenesis. Our latest research identifies the dysregulation of low-density lipoprotein (LDL) is a new risk factor for PF, contributing to alveolar epithelial and endothelial cell damage, and fibroblast activation. In this study, we first integrative summarize the published literature about lipid metabolite changes found in PF, including phospholipids, glycolipids, steroids, fatty acids, triglycerides, and lipoproteins. We then reanalyze two single-cell RNA-sequencing (scRNA-seq) datasets of PF, and the corresponding lipid metabolomic genes responsible for these lipids' biosynthesis, catabolism, transport, and modification processes are uncovered. Intriguingly, we found that macrophage is the most active cell type in lipid metabolism, with almost all lipid metabolic genes being altered in macrophages of PF. In type 2 alveolar epithelial cells, lipid metabolic differentially expressed genes (DEGs) are primarily associated with the cytidine diphosphate diacylglycerol pathway, cholesterol metabolism, and triglyceride synthesis. Endothelial cells are partly responsible for sphingomyelin, phosphatidylcholine, and phosphatidylethanolamines reprogramming as their metabolic genes are dysregulated in PF. Fibroblasts may contribute to abnormal cholesterol, phosphatidylcholine, and phosphatidylethanolamine metabolism in PF. Therefore, the reprogrammed lipid profiles in PF may be attributed to the aberrant expression of lipid metabolic genes in different cell types. Taken together, these insights underscore the potential of targeting lipid metabolism in developing innovative therapeutic strategies, potentially leading to extended overall survival in individuals affected by PF.

Keywords Pulmonary fibrosis, Lipid metabolism, Lipid metabolomic gene, Single-cell RNA-sequencing reanalysis

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Introduction

Pulmonary fibrosis (PF) is irreversible, with high mortality and few effective treatments [1]. The scarring causes the lung tissues to become thick and stiff, making it harder to absorb oxygen into the bloodstream. Clinically, dyspnoea, dry cough, fatigue and exhaustion are the main manifestations of patients with PF. Medical imaging studies suggest that PF lungs show high levels of collagen fiber deposition, severe alveolar loss and destroyed lung architecture.

The incidence of PF is approximately 3–18 per 100,000 people in idiopathic PF (IPF) and 3–24 per 100,000 people in autoimmune PF [2, 3]. Notably, global incidents of silicosis, a subtype of PF, have risen by 64.6%, from 84,821 cases in 1990 to 138,965 in 2019 [4]. PF encompasses various etiological subtypes, including IPF of unknown cause, connective tissue disease-related PF (notably systemic sclerosis and dermatomyositis patients, occupational PF (e.g., silica-related silicosis) due to environmental exposure, virus-induced PF (exemplified by SARS-CoV-2), and genetic spontaneous PF. Genetic spontaneous PF can be further classified into familial IPF, typically associated with surfactant proteins A and C (SP-A and SP-C) mutations, sporadic IPF linked to mutations in poly(A)-specific ribonuclease (PARN), telomerase reverse transcriptase (TERT), regulator of telomere elongation helicase 1 (RTEL1), telomerase RNA component (TERC), and gain-of-function mucin 5B (MUC5B)-induced IPF. Mechanistically, familial IPF mutations such as SP-C induce endoplasmic reticulum (ER) stress, and an acquired impairment in macroautophagy-dependent proteostasis and mitophagy increases alveolar epithelial type II (AT2) cell susceptibility to injury [5]. The sporadic mutations cause cells to suffer telomere DNA damage, while the MUC5B mutation induces distal airway epithelial distension [6–8]. Through decades of efforts investigating the etiology of the development of PF, complex underlying mechanisms of pathophysiological PF have been revealed, including cell senescence, alveolar epithelial injury, endothelial barrier disturbance, chronic inflammation, and activation of macrophages and fibroblasts [8–11]. Currently, the pharmacologic treatment options for PF are limited, with only the FDA-approved pirfenidone and nintedanib available [12]. However, both two drugs are highly toxic to the liver and kidneys. It requires a systemic-level approach to uncover detailed molecular and cellular alterations in PF toward understanding its pathology. Fortunately, recent advancements in scRNA-seq technology have enabled a more precise cell type-specific transcriptional analysis, offering promising opportunities for integrating and interpreting changes across cell types and genes, thereby aiding in the discovery of novel therapeutic targets.

Currently, increasing evidence suggests that lipid metabolism disorders are involved in PF, including abnormalities in low-density lipoprotein (LDL) metabolism [13], sphingosine-1-phosphate (S1P), and so on [14–18]. Epidemiological studies have indicated that patients with pneumoconiosis, often a result of environmental pollution, exhibit a distinctive serum metabolite profile. Metabolites such as phosphatidylethanolamine (22:6/18:1) and N-tetradecanoylsphingosine have been proposed as potential biomarkers, while 1,2-dioctanoylthiophosphatidylcholine, phosphatidylcholine (18:1/20:1) and indole-3-acetamide have been identified as potential indicators for the staging of pneumoconiosis [19]. Additionally, diseases with significant lipid metabolism abnormalities, like cardiovascular disease and obesity, have been notably linked with PF [20, 21]. For instance, lipid risk factors common in cardiovascular disease are also prevalent in IPF. Saturated fatty acid diets have been associated with an elevated prevalence of PF, and obesity doubles the risk of developing this condition. However, the specific roles and molecular mechanisms of lipid metabolic reprogramming in PF remain inadequately understood. Therefore, the investigation in PF lipidomics of detail PF-related cells is beneficial for understanding the underlying mechanisms of PF in-depth and providing clues for new therapeutic strategies.

Here, we first integratively summarized recent findings on dysregulated lipid metabolites in PF and then focused on the molecular regulation of these lipid metabolites by re-analysing two single-cell RNA-sequencing datasets of PF. We also investigate the importance of lipid and related metabolic mechanisms in PF pathophysiology. Our study reveals that lipid metabolism dysregulation is present in various PF-related cell types and mediates abnormal cellular functions. Furthermore, impaired lipid metabolism is closely correlated with clinical manifestations such as disease severity and survival. In addition, different cell types have an apparent predilection for lipid dysregulation, for example, phospholipid dysregulation is more likely to occur in AT2 cells. These insights suggest that understanding lipid metabolism dysregulation patterns at various disease stages and across different cell types could be beneficial for PF interventions.

Physiopathologic mechanisms underlying PF

Fibroblasts, known for their role in excessive extracellular matrix (ECM) deposition during wound healing, are pivotal effector cells in fibrosis [22]. In response to internal and external signals, the morphological stretches of fibroblasts are increased [3]. These cells secrete ECM components, primarily collagen, exacerbating fibrosis [23]. Indeed, the process of PF is far more than that

simple, and merely controlling fibroblast collagen metabolism cannot effectively inhibit PF.

The damage of endothelium, alveolar epithelium, and alveolar macrophage activation are essential for the initiation of PF [3]. During the early stage of PF, the injury of pulmonary capillary endothelial cells (ECs) initiates the apoptosis process and thus destroys the endothelial barrier. Meanwhile, the living apoptosis-resistant endothelial cells are activated and express many inflammatory cytokines and chemokines, contributing to inflammatory cell recruitment and infiltration. The activated ECs can also secrete profibrotic factors, especially transforming growth factor beta 1 (TGF- β 1) to induce fibroblast-like changes and activate perivascular fibroblasts. In the late stage of EC injury, the fibroblast repair processes are dominant. The abnormal repair processes collectively result in aberrant angiogenesis, vasculogenesis, tissue hypoxia, and fibrosis [24].

The alveoli, composed mainly of alveolar epithelial type I (AT1) and AT2 cells, characterized by their delicate and thin structure. Like ECs, some epithelial cells undergo apoptosis in response to injurious stimuli, while others become activated. The apoptotic loss of AT1 and AT2 cells results in the reduction of alveoli, thereby impairing pulmonary function. Though AT1 differentiates from AT2 to remedy the alveoli regeneration, this process is broken by persistent and chronic inflammation in the condition of PF. Instead, the physiological repair of AT2-AT1 differentiation is superseded by fibroblast repair. Finally, the injury loci are full of ECM accompanied by the alveoli disappearance. Besides apoptosis, the AT1 and AT2 also undergo the activation program by responding to the interleukins such as monocyte chemoattractant protein-1 (encoded by CCL-2), interleukin-8 (encoded by CXCL8), interleukin-6 (IL6), and TGF- β 1, leading to inflammation, accumulation of fibroblast-like AT1 and AT2 cells and fibroblast activation [8].

Pulmonary macrophages include alveolar macrophages (AMs) and interstitial macrophages (IMs) [25]. When the alveolar epithelium is injured, macrophages are activated to become activated macrophages and alternatively activated macrophages (AAMs), which consist of four subtypes, including AAM2a, AAM2b, AAM2c, and AAM2d [26, 27]. Macrophages are highly heterogeneous and complex to classify. The traditional categories are M1, M2, or AM, AAM. Recently scRNA-seq analyses have divided lung macrophages into inflammatory macrophages, airspace macrophages, and profibrotic macrophages or other specific gene high-expressing macrophages, including secreted CCL2, inhibin subunit beta A (INHBA), fatty acid binding protein 4 (FABP4), phosphoprotein 1 (SPP1), serpin family G member 1 (SERPING1), interleukin 1 receptor type 2 (IL1R2), interleukin

1 beta (IL1B), and ficolin 1 (FCN1) macrophages [25, 28–32]. Both activated macrophages and AAMs have been reported to mediate PF by releasing pro-inflammatory and profibrotic factors to activate continuous fibroblasts and promote myofibroblast proliferation [33].

Senescent cells undergo cell cycle arrest and phenotypic changes. However, they are metabolically active and contribute to many diseases. The contribution of cellular senescence to PF, and in particular its acceleration in this condition, is being increasingly recognized. The hallmarks of senescence, including telomere shortening, genomic instability, mitochondrial dysfunction, impaired autophagy, defective nutrient sensing, and epigenetic alterations, are significantly enriched in PF. Cell senescence is an important phenomenon affecting endothelial cells, AT2 cells, fibroblasts, and macrophages. Senescent cells drive the senescence-associated secretory phenotype (SASP) to affect surrounding cells. This secretion leads to the activation of proinflammatory and profibrotic pathways, ultimately contributing to PF.

Taken together, PF involves abnormalities in the endothelium, alveolar epithelium, fibroblasts, and macrophages (Fig. 1).

Impacts of lipid metabolism during PF

Phospholipids

Phospholipids are a predominant component of pulmonary surfactants, comprising about 10% specific surfactant proteins and 90% lipids. Pulmonary surfactants primarily function to reduce surface tension within the alveoli. Phosphatidylcholine (PC), with dipalmitoylphosphatidylcholine (DPPC) comprising half of it, makes up 70–75% of surfactant phospholipids and provides surface-active properties. These surfactant phospholipid components are critical to the functionality and stability of the alveoli and are reprogrammed in PF [34]. A recent plasma lipidomics study has shown that almost all the surfactant phospholipid species are reduced in IPF in comparison to controls, including PC, phosphatidylserine (PS), phosphatidylethanolamines (PE), phosphatidylinositol (PI), and phosphatidylglycerol (PG) [34, 35]. The decline of surfactant phospholipids has also been validated in lung biopsy samples from irradiation-induced PF patients [36]. Interestingly, Shabarinath Nambiar et al. observed higher plasma PC levels in progressive IPF than in stable cases, potentially linked to severe epithelial cell damage in advanced IPF [37]. These divergent PC patterns may be intimately associated with disease progression and could provide essential insights for diagnosis and treatment.

Phospholipids play an important role in fibrogenesis. Luis G. Vazquez-de-Lara et al. recently reported that PE could inhibit collagen deposition by promoting

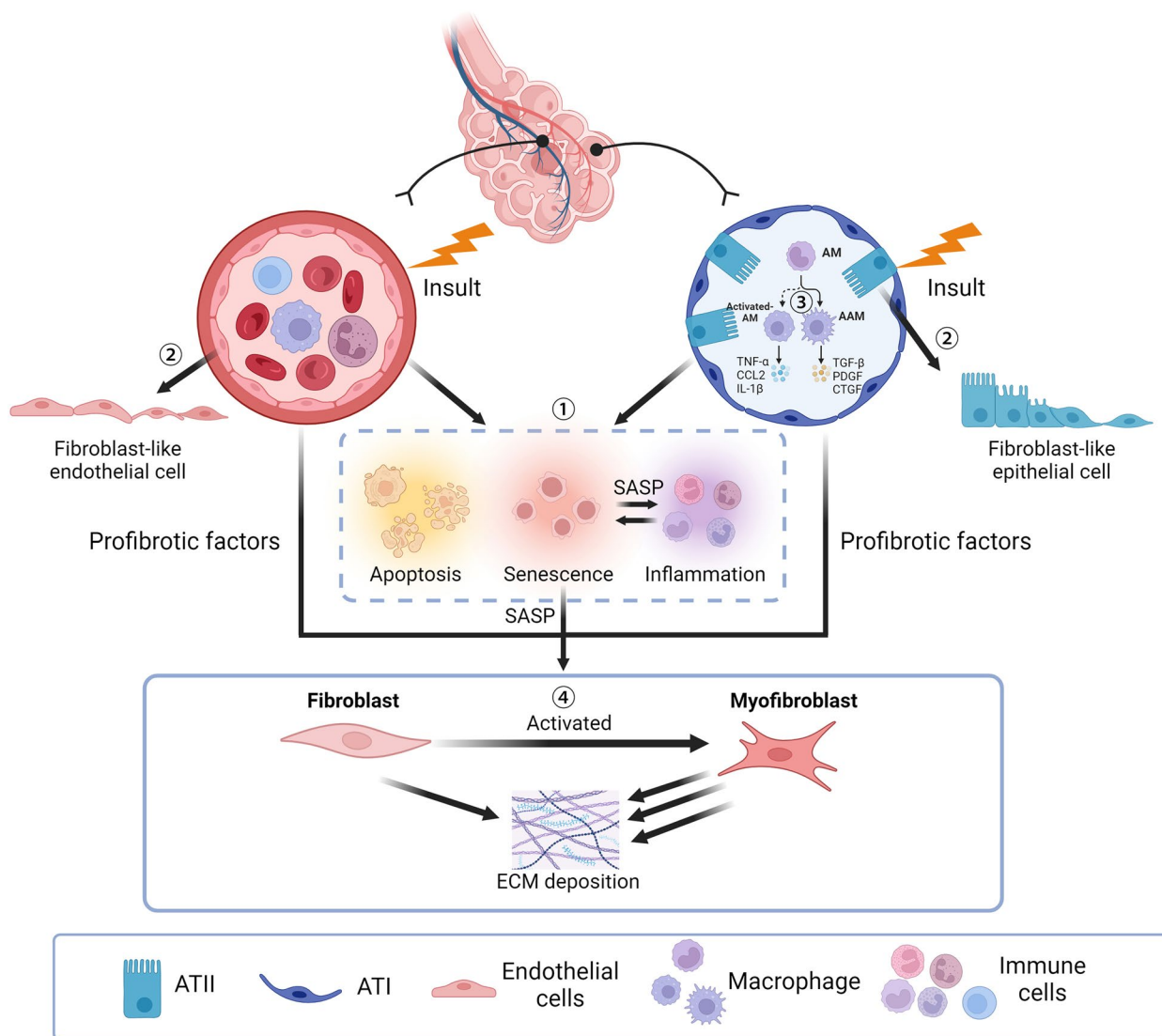


Fig. 1 The mechanism of PF. ① Excessive apoptosis of endothelial and epithelial cells leads to lung injury and releases various pro-inflammatory and profibrotic factors. Injured EC and alveolar epithelial cells also undergo senescence, inducing an SASP phenotype, which further enhances the pro-inflammatory and profibrotic effects. ② The apoptosis-resistant endothelial and epithelial cells undergo an activated process to obtain fibroblast-like properties. ③ The polarisation of macrophages confers AM and IM cells to differentiate into activated macrophages, which produce abundant pro-inflammatory and profibrotic factors and further promote PF. ④ Over-proliferation and hyperactivation of fibroblast lead to ECM deposition and fibrosis formation

apoptosis, inducing a dose-dependent Ca^{2+} signaling and mitigating bleomycin (BLM)-induced PF in mice [38]. In this study, PE treatment was started 1 day after BLM injection in mice and continued 6 times, and the degree of fibrosis was assessed on day 21. It was found that PE mainly decreased collagen expression in fibroblasts. Since fibroblast activation is a late effect of PF, we suggest that the remission effect of PE on PF may be therapeutic rather than preventive. Another study reported by Stefanie Preuß et al showed that PG could prevent fibrosis by inhibiting alveolar epithelial injury and fibrosis

responses by reducing secretory phospholipase A2 [39]. PG was administered ex vivo to the lungs of 2–6 days old domestic piglets. The results showed that PG could not only inhibit alveolar damage but also inhibit the TGF- β 1 secretion and other fibrotic factors, suggesting a potential role in PF prevention and treatment.

Conversely, lysophospholipids have a profibrotic effect by inducing apoptosis of alveolar cells, vascular permeability, migration and activation of fibroblasts [40]. Surfactant phospholipids are beneficial for lung hemostasis. However, the modified phospholipids are pathogenetic

and mainly removed by alveolar macrophages (AMs) in PF, especially the oxidized phospholipids (ox-PLs). Increasing evidence indicates that ox-PLs actively contribute to the commencement and advancement of PF. Freddy Romero et al. showed that ox-PLs accumulate in AMs of human patients and mouse models of PF and induce an M2 phenotype transition of AMs, secreting high levels of TGF- β 1, ultimately exacerbating BLM-induced PF [41]. Moreover, recent studies further showed that ox-PLs could induce ferroptosis and thus promote PF [42–44]. The uptake of ox-PLs is mediated by CD36 molecule (CD36). Amounts of studies demonstrated that CD36 promoted ER stress, cell death in AT2 cells, and PF [45, 46].

By reanalyzing scRNA-seq datasets (GSE136831 and GSE135893) of endothelial, AT2, fibroblast and macrophage cells, we found that the reduced surfactant lipids are partly attributed to the downregulated surfactant lipid metabolism-related genes in PF. Among those downregulated genes, CHK (choline kinase, α/β), choline phosphotransferase 1 (CHPT1), phosphate cytidyltransferase 1A, choline (PCYT1A), and phosphatidylethanolamine N-methyltransferase (PEMT) are involved in the PC biosynthetic process through the Kennedy pathway (Fig. 2). Besides, the generation of PE is also mediated by the Kennedy pathway, and the critical genes are ethanolamine kinase (ETNK1/2), PCYT2 (phosphate cytidyltransferase 2), and choline/ethanolamine phosphotransferase 1 (CEPT1). In contrast, lecithin-cholesterol acyltransferase (LCAT) and phospholipase are responsible for the decomposition of PC

and PE. The scRNA-seq datasets show that a series of surfactant genes are dysregulated in AT2 cells and macrophages of PF (Table 1). In addition, the expressions of CDP-diacylglycerol synthase (CDS1/2), phosphatidylglycerol phosphate synthase 1 (PGS1), phosphatidylserine synthase 1 (PTDSS1/2), CDP-diacylglycerol-inositol 3-phosphatidyl transferase (CDIPT), which are responsible for the synthesis of PS, PI, and PG by CDP-DAG pathway (Fig. 2), are altered as well (Table 2). Besides the *de novo* synthesis, phospholipids can also be converted from their corresponding lysophospholipids, which are mediated by the lysophosphatidylcholine acyltransferases family (LPCATs). In turn, phospholipids can be converted to lysophospholipids in the presence of phospholipase A2s (PLA2s).

Although lung fibroblasts, macrophages, and ECs are not primary sources of surfactant phospholipids, alterations in phospholipid gene expression in these cells merit attention. For instance, it was found that AT2 cells can uptake cholesterol from extracellular LDL via the LDL receptor (LDLR) [13]. Pulmonary lipofibroblasts are characterized by their lipid droplets and are located in the alveolar interstitium. They contain cortical contractile filaments and are related to contractile interstitial cells and are beneficial for alveolar development [47]. These lipofibroblasts can transport lipids to AT2 cells via the parathyroid hormone-related protein (PTHrP) signaling pathway, which is activated by stretch-sensitive AT2 cells and directs the differentiation of mesenchymal and alveolar epithelial cells [48, 49]. In addition to these signaling pathways, other cells can also deliver

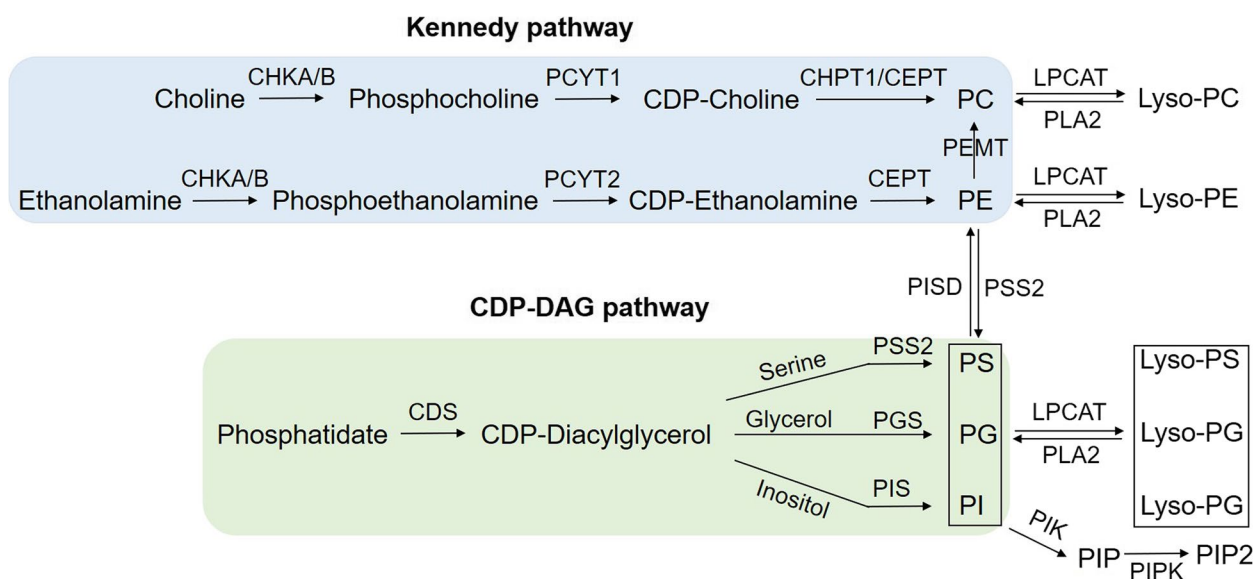


Fig. 2 The metabolites and genes involved in the Kennedy pathway and CDP-DAG pathway. The synthesis of PC and PE via the Kennedy pathway, and the synthesis of PS, PG and PI through the CDP-DAG pathway

Table 1 DEGs of Kennedy pathway in PF cells

Cell Type	AT2		Fibroblast		Macrophage		Endothelium	
	Fold Change	P value	Fold Change	P value	Fold Change	P value	Fold Change	P value
<i>CHKA</i>	/	/	0.57 (FB)	2.67 E-02	0.06 (IM) -0.15 (AM)	3.97 E-05 9.64 E-10	/	/
<i>CHKB</i>	/	/	/	/	-0.79 (AM)	2.73 E-09	/	/
<i>PCYT1A</i>	/	/	/	/	-0.20 (AM)	5.84 E-18	/	/
<i>CHPT1</i>	-0.95	4.80 E-84	-0.41 (FB) -1.90 (MyoF)	1.15 E-02 8.82 E-55	0.17 (IM) -0.27 (AM)	2.52 E-08 8.89 E-13	-1.58	1.09 E-58
<i>ETNK2</i>	/	/	/	/	-0.90 (AM)	1.71 E-02	/	/
<i>PCYT2</i>	-0.78	1.04 E-45	-1.13 (FB)	1.45 E-02	/	/	-0.58	6.21 E-04
<i>CEPT1</i>	0.47	1.51 E-03	/	/	-0.16 (IM) -0.77 (AM)	6.90 E-09 2.55 E-140	-0.55	1.92 E-05
<i>PEMT</i>	-0.24	2.68 E-03	/	/	0.17 (IM) 0.24 (AM)	5.00 E-05 2.34 E-04	0.29	4.97 E-03
<i>LCAT</i>	1.36	5.96 E-19	/	/	-0.39 (AM)	4.38 E-02	-0.69	6.71 E-03
<i>PLA2G4C</i>	/	/	/	/	-0.67 (AM)	3.36 E-22	/	/
<i>PLA2G16</i>	-0.44	4.91 E-54	/	/	-0.27 (IM) -0.18 (AM)	3.26 E-35 3.29 E-51	-0.28	1.42 E-09

phosphatidylcholine to epithelial cells in the form of exosomes [50]. These lipids likely contribute to the synthesis of AT2 surfactant lipids, underscoring the importance of considering the phospholipid synthesis capacity of other cells. Taken together, all of these results suggested that surfactant phospholipids were reduced in PF and thus exacerbated the PF course. Targeting the surfactant phospholipids metabolism by mediating the activity of Kennedy and cytidine diphosphate-diacylglycerol (CDP-DAG) pathways is a promising strategy for PF.

FB Fibroblasts, *MyoF* Myofibroblasts, *IM* interstitial macrophages, *AM* alveolar macrophages.

FB Fibroblasts, *MyoF* Myofibroblasts, *IM* interstitial macrophages, *AM* alveolar macrophages.

Sphingomyelin (SM) is synthesized via SM synthases (SMSs, SGMS1). In the presence of sphingomyelinases (SMases), which are encoded by sphingomyelin phosphodiesterases (SMPD1–4) genes, SM is hydrolyzed to ceramide (Cer). Previous studies have demonstrated

Cer-mediated cell infection, inflammation, and death susceptibility in cystic fibrosis [51]. N-acyl sphingosine amidohydrolases (ASAH1/2) facilitate the degradation of ceramide into sphingosine, which could be phosphorylated by sphingosine kinase 1 (SPHK1) to generate S1P. The actions of S1P are predominantly mediated by S1P receptors (S1PRs), including S1PR1, S1PR2, and S1PR3 [52]. SM is the most abundant sphingolipid and has particularly high levels in the brain [53]. Indeed, moderate levels of SM were also found in the lung [54], and the metabolic pathway was disrupted in PF. Decreased sphingolipid metabolites in IPF have been reported by Yidan D Zhao [55]. In addition, a series of sphingolipid metabolism-related genes are reduced in IPF lungs, including SMPD1, SMPD4, SPHK1, S1PR1, S1PR4, and S1P lyase (SGPL1) [55]. A report by Long Shuang Huang et al. showed that S1P lyase (S1PL, encoded by SGPL1), an enzyme that catalyzes S1P to

Table 2 DEGs of CDP-DAG pathway in PF cells

Cell Type	AT2		Fibroblast		Macrophage		Endothelium	
	Fold Change	P value	Fold Change	P value	Fold Change	P value	Fold Change	P value
<i>SELENO1</i>	/	/	/	/	0.09 (IM)	4.28 E-02	/	/
<i>CDS1</i>	-0.22	6.50 E-05	/	/	-0.28 (AM)	2.08 E-03	/	/
<i>CDS2</i>	/	/	/	/	-0.24 (AM)	6.78 E-26	/	/
<i>PTDSS1</i>	-0.34	1.54 E-03	/	/	-0.21 (AM)	4.76 E-15	/	/
<i>PTDSS2</i>	/	/	/	/	-0.34 (IM)	1.59 E-12	/	/
					-0.39 (AM)	5.77 E-07		
<i>PISD</i>	0.43	2.36 E-03	-0.84 (MyoF)	2.80 E-02	-0.14 (IM)	7.56 E-06	/	/
					-0.45 (AM)	1.35 E-37		
<i>PGS1</i>	-0.28	3.27 E-05	/	/	-0.33 (IM)	1.16 E-121	-0.31	4.47 E-04
					-0.43 (AM)	1.62 E-48		
<i>CDIPT</i>	/	/	/	/	0.19 (IM)	2.35 E-10	/	/
					0.18 (AM)	1.93 E-04		

phosphoethanolamine, is negatively correlated with PF severity but positively correlated with survival rate. Moreover, overexpression of *S1PL* reduces *S1P* levels, enhances fibroblast autophagy, attenuates lung fibroblast activation, and effectively inhibits BLM-induced PF [16]. Researches have also shown that inactivation of the *SPHK1/S1P/S1PR* signaling attenuates mouse PF by reducing ECM deposition in fibroblasts [14, 17, 56–58]. However, Rachel S. Knipe et al. recently found that endothelial-specific *S1pr1* deletion suppresses sphingosine-1-phosphate metabolism and shows increased peripheral lymphocyte numbers by increasing vascular permeability and exacerbating BLM-induced PF [59]. The distinct roles of *S1P* in different PF-related cell types may explain this discrepancy. We then analyzed the scRNA-seq datasets and found that the changing trends of these SM metabolism-related genes in different cell types were inconsistent (Table 3). Nevertheless, it gives us a hint and suggests that the function of *S1P* metabolism could be considered in a manner that is dependent on the cell type, and results obtained from whole lung tissues should be carefully interpreted. In summary, these results underscore the important role of SM metabolism in PF, with the processes involved depicted in Fig. 3.

Glycolipids

Miguel Arias-Guillen et al. discovered that glycosphingolipids mediate profibrotic TGF- β /SMAD signaling in human lung fibroblasts. Suppression of glycosphingolipid synthesis was found to decrease ECM deposition and myofibroblast transformation. Similarly, Toru Kimura et al. demonstrated that a glycolipid derived from marine sponges, α -galactosylceramide, attenuates BLM-induced PF. This attenuation occurs through the regulating of several cytokines, including TGF- β , interferon-gamma (IFN- γ), connective tissue growth factor (CTGF), and macrophage inflammatory protein-2 released by natural killer T cells [60]. The metabolism and the roles of glycolipids in PF remain largely unknown.

Steroids

Steroids include sterols (e.g., cholesterol), bile acids, steroid hormones (e.g. adrenal corticosteroids, androgens, estrogens), etc.

Cholesterol metabolism

(1) Alterations of cholesterol and its derivatives in PF

Table 3 DEGs of SM metabolism pathway in PF cells

Cell Type	AT2		Fibroblast		Macrophage		Endothelium	
	Fold Change	P value	Fold Change	P value	Fold Change	P value	Fold Change	P value
<i>SGMS1</i>	/	/	/	/	-0.18 (IM) 0.06 (AM)	8.54 E-46 1.66 E-03	/	/
<i>SGMS2</i>	0.49	1.06 E-24	/	/	0.40 (IM) 0.29 (AM)	2.25 E-176 7.56 E-117	/	/
<i>SMPD1</i>	/	/	/	/	-0.29 (AM)	2.54 E-09	-0.49	7.33 E-08
<i>SMPD4</i>	/	/	/	/	-0.24 (IM) -0.37 (AM)	1.87 E-13 1.23 E-17	-0.59	3.98 E-06
<i>ASAH1</i>	-0.37	6.67 E-121	/	/	-0.05 (IM) -0.02 (AM)	4.47 E-13 1.62 E-02	-0.36	1.86 E-13
<i>ASAH2</i>	/	/	/	/	-0.81 (AM)	1.82 E-04	/	/
<i>SPHK1</i>	/	/	/	/	-0.94 (IM) -0.89 (AM)	2.30 E-301 7.96 E-12	0.23	2.84 E-02
<i>SPHK2</i>	0.54	1.18 E-04	/	/	/	/	/	/
<i>S1PR1</i>	/	/	/	/	0.82 (AM)	3.93 E-02	-0.31	1.32 E-30
<i>S1PR3</i>	/	/	/	/	-0.72 (IM)	4.05 E-08	/	/
<i>S1PR4</i>	1.00	3.73 E-04	/	/	/	/	-0.66	3.74 E-05
<i>SGPL1</i>	0.74	6.80 E-08	/	/	-0.50 (IM) -0.11 (AM)	3.67 E-291 2.75 E-03	/	/

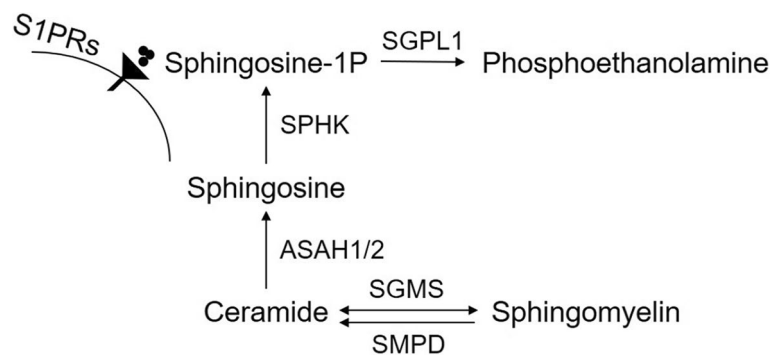


Fig. 3 The metabolites and genes involved in SM metabolism. ASAHS catalyze ceramide to sphingosine, which is phosphorylated by SPHK to produce S1P. S1P can bind to S1PRs on the cell surface to regulate cell function or be degraded by SGPL1

As early as 1996, E Fireman et al. reported that cholesterol was deposited in the bronchoalveolar lavage fluid (BALF) of IPF patients [61]. Besides, increased cholesterol is observed in BLM-induced PF [41]. In addition to cholesterol levels, Tomohiro Ichikawa et al. reported that one cholesterol's derivative, 25-hydroxycholesterol, could promote myofibroblast differentiation and ECM deposition via a TGF- β /nuclear factor kappa B (NF- κ B) dependent manner [62]. Feng Yan et al. observed lower plasma levels of the sterol lipid 20:1-Glc-Sitosterol in IPF patients than in healthy donors. Moreover, three sterol lipids (16:1 stigmaterol ester, 3-hydroxyvitamin D3, and 20:1-glc-sitosterol) have been identified as correlating with IPF [35]. These results demonstrate that abnormal cholesterol metabolism is a risk factor in the pathogenesis of PF.

(2) Abnormal regulation of cholesterol metabolism

The sterol regulatory element-binding protein 2 (SREBP2) tightly regulates de novo cholesterol synthesis. In sterol-deficient cells, SREBP2 increases cholesterol synthesis by generating oxysterol ligands for LXR α / β (encoded by NR1H3 and NR1H2, respectively) [63]. Interestingly, although excessive cholesterol has been observed to be deposited in the BALF of PF, the expressions of cholesterol-synthesis-related genes decrease in PF lungs, including hydroxymethyl-glutaryl coenzyme A reductase (HMGCR) and SREBPs [41]. Moreover, overexpression of SREBP2 could suppress lung fibroblast proliferation, ER stress, and attenuates PF [64, 65]. Among the target genes of SREBP2, many of them can regulate non-sterol lipid metabolism, and are antifibrotic in PF, such as LDLR, fatty acid synthase (FASN), SCD, etc. [66]. In contrast, another study reported that SREBP2 is markedly increased in IPF lung specimens. Endothelial-specific transgenic of SREBP2 activated the TGF- β and Wnt signaling and fibrotic genes such as smooth muscle (α -SMA), vimentin, snail family transcriptional repressor 1 (Snai1), neural cadherin, and actin alpha 2. This led to EC overgrowth, ECM deposition, stress fiber formation, and exacerbated BLM-induced PF [24].

The uptake of extracellular cholesterol is mediated by LDLR. LDLR governs the uptake of cholesterol packaged with apolipoprotein B (ApoB), especially LDL-C particles, from the blood [67–69]. In lung tissue, specifically on AT2 cells, LDLR takes up peripheral LDL particles for surfactant synthesis, a process that is impaired in acute and chronic lung injury [67, 68, 70]. Mice lacking LDLR (*Ldlr*^{-/-}) exhibit impaired lung development compared to wild-type (WT) mice [71]. Consistent with our and other previous studies, a disrupted LDL-LDLR metabolic axis was found in PF patients [13, 72]. Further in vivo and

in vitro studies of these aberrations revealed their contributions and mechanisms in PF. We develop a combined treatment with a statin and an anti-proprotein convertase subtilisin/kexin type 9 (PCSK9) antibody that significantly reduces the severity of PF, more effectively than either treatment alone, by increasing LDLR and lowering LDL in mice.

For cholesterol homeostasis, lung cells either expel excess cholesterol or store it as cholesteryl esters in lipid droplets. ATP-binding cassette subfamily A member 1 (ABCA1) is expressed widely throughout the body, with the lung having the second highest expression after the liver [73]. ABCA1 in macrophages facilitates the removal of cholesterol and prevents excessive cholesterol deposition in the lungs [74]. Lipid-free circular apolipoprotein A-I (apoA-I) receives cholesterol effluxed via ABCA1 and forms the nascent high-density lipoprotein (HDL) particles on the cell membrane. Nascent HDL matures after acquiring cellular cholesterol effluxed through ATP-binding cassette subfamily G member 1 (ABCG1) and ABCA1 [75]. In the liver, circular HDL binds to hepatic scavenger receptor class B type I (SR-BI) and is cleared, whereas cholesteryl ester transfer protein (CETP)-mediated cholesterol transfer from HDL to LDL is cleared by hepatic LDLR. This process of cellular cholesterol disposal is termed reverse cholesterol transport (RCT) [67]. To prevent intracellular free cholesterol accumulation, acetyl-CoA acetyltransferase 1 (ACAT)-mediated cholesterol esterification directs cholesterol toward storage [76]. Esterification is also necessary to balance free cholesterol and cholesteryl esters.

Alessandro Venosa et al. found that reduced ABCA1 and ABCG1 in macrophages from nitrogen mustard-induced PF mice [77]. Disabled cholesterol efflux and esterification were found in the BLM-induced PF model. Moreover, deleting the lipid efflux transporter ABCG1 could reduce pulmonary lipid clearance and worsen lung fibrosis [41]. Besides, our previous study revealed that plasma HDL levels are both decreased in PF patients and mice, further indicating dysfunctional cholesterol efflux in PF. In addition, HDL particles are negatively correlated with the death of IPF [78]. scRNA-seq dataset of PF further shows these cholesterol effluxes and esterification genes are decreased in macrophages and AT2 cells compared to healthy individuals (Table 4). The exact role of ACATs in PF remains to be determined, although increased ACATs have been associated with atherosclerosis [79].

The current discourse on cholesterol homeostasis has received considerable attention owing to its crucial role in an expanding spectrum of diseases, extending beyond traditional cardiovascular disorders to include pulmonary diseases [13], various cancers [80], and

Table 4 DEGs of cholesterol metabolism in PF cells

Cell Type	AT2		Fibroblast		Macrophage		Endothelium	
	Fold Change	P value	Fold Change	P value	Fold Change	P value	Fold Change	P value
Significance								
<i>SREBF1</i>	/	/	−0.57 (FB)	4.15 E-03	−0.20 (AM)	1.66 E-03	−0.58	1.91 E-06
<i>SREBF2</i>	−0.14	1.27E-02	/	/	0.23 (IM)	3.51 E-42	/	/
					0.07 (AM)	4.19 E-02		
<i>NR1H2</i>	−0.38	5.61 E-24	/	/	−0.13 (IM)	7.63 E-10	−0.24	5.95 E-04
					−0.30 (AM)	8.00 E-37		
<i>NR1H3</i>	/	/	/	/	0.55 (IM)	2.55 E-144	/	/
					−0.20 (AM)	1.08 E-14		
<i>LDLR</i>	−0.43	3.18 E-44	/	/	0.21 (IM)	1.14 E-18	/	/
					0.56 (AM)	9.63 E-59		
<i>FASN</i>	−0.59	5.43 E-130	/	/	/	/	/	/
<i>SCD</i>	/	/	−1.04 (FB)	7.92 E-03	0.32 (IM)	3.42 E-61	/	/
					−0.14 (AM)	2.80 E-21		
<i>PCSK9</i>	−1.03	5.02 E-44	/	/	/	/	/	/
<i>ABCA1</i>	/	/	−1.11 (FB)	3.04 E-33	−0.63 (IM)	0.00 E+00	/	/
			−0.54 (MyoFB)	5.29 E-03	−0.66 (AM)	5.32 E-233		
<i>ABCG1</i>	/	/	/	/	−0.04 (IM)	3.56 E-03	−0.25	6.74 E-03
					0.11 (AM)	5.00 E-28		
<i>ACAT1</i>	−0.52	7.51 E-55	/	/	0.07 (IM)	1.66 E-02	−0.17	1.44 E-02
					−0.17 (AM)	7.01 E-17		
<i>ACAT2</i>	−1.15	1.79 E-130	/	/	/	/	/	/

Alzheimer's disease [81]. Intriguingly, our research has shown that in addition to cardiovascular disease, cholesterol reduction may be a viable therapeutic approach for PF. Nevertheless, numerous critical inquiries regarding cholesterol metabolism in PF persist, particularly concerning the underlying mechanisms. The question of whether cholesterol levels are regulated in a manner akin to that in the lungs remains unresolved. Unraveling this uncertainty is imperative for a full understanding of the relationship between cholesterol and PF.

Bile acid metabolism in PF

Bile acids (BAs) are synthesized by cytochrome P450 family 7 subfamily A member 1 (*CYP7A1*) and subfamily B member 1 (*CYP8B1*). BAs serve as crucial mediators of inflammation and fibrosis, exerting their effects via the activation of both nuclear and membrane G protein-coupled receptors [82]. Activation of farnesoid X receptor (FXR, encoded by the *NR1H4* gene) occurs upon its interaction with BAs or their derivatives. Notably, Obeticholic acid (OCA), a BA-derived agonist of FXR, is clinically advanced in its ability to suppress BA production in

hepatocytes and enhance bile acid transport from hepatocytes, thus reducing hepatic exposure to BAs [83].

BAs have been detected in cystic fibrosis lungs [84], with their levels being closely associated with lung function parameters [85]. Yidan D. Zhao et al. found elevated bile acid metabolites in pulmonary arterial hypertension (PAH) patients, suggesting that pulmonary vascular endothelial cells of CYP7B1 protein may partly drive the de novo bile acid synthesis process [86]. Importantly, BAs could increase intracellular reactive oxygen species (ROS) production and subsequently induce EMT of AT2 and lung fibroblast activation in vitro through TGF- β /Smad3 signaling-dependent manners [87]. Consistently, microaspiration of BAs induces lung fibrosis through activating VEGF, CTGF, bFGF, and TGF- β pathways in rats [88]. Though BAs are profibrotic, FXR mediates inhibitory effects of inflammation and fibrosis in FXR-expressing organs [89, 90]. Indeed, FXR is confirmed to be expressed in AT2 cells [91]. In vivo treatment with OCA has been shown to effectively ameliorate BLM-induced pulmonary function loss and reverse lung fibrosis by attenuating EMT, reducing IL-6 and IL-1 β , and downregulating profibrotic SNAI1 and TGF- β 1 expression [92], even superior to those obtained with pirfenidone [93], highlighting FXR as a novel PF therapeutic target. The mechanisms of the opposite effect of BAs and FXR on PF need to be further investigated. In addition to FXR, the roles of other receptors of BA, like pregnane X receptor (PXR), Takeda G protein-coupled receptor (TGR5), and sphingosine-1-phosphate receptor 2 (S1PR2) in the lungs, remain unexplored. Collectively, these findings suggest that BA receptor agonists may be promising for alleviating inflammation- and fibrosis-related diseases.

Steroid hormones

Steroid hormones are broadly divided into two categories: corticosteroids and sex steroids, which are generally synthesised in the adrenal glands and gonads or placenta, respectively. These categories include glucocorticoids, mineralocorticoids, estrogens, androgens, and progestins-five types based on the receptors they bind.

The lungs can respond to hormones through steroid hormone receptors expressed in the lungs. The classical estrogen receptors include estrogen receptor alpha (ER α) and estrogen receptor beta (ER β). Studies have indicated a high expression of ER β in both alveolar and bronchiolar epithelial cells. Interestingly, both female and male ER β knockout (ER β -/-) mice exhibited decreased caveolin-1, while increased metalloproteinases, and TIMP metalloproteinase inhibitor 2 (TIMP2), and manifested defective alveogenesis, reduced lung volume, unexpanded alveoli, systemic hypoxia, and spontaneous fibrosis [94]. More

recent work by Sharon Elliot has shown elevated pulmonary ER α levels in PF patients and mice. Mice harboring inactivated estrogen receptors develop BLM-induced lung fibrosis [95]. Additionally, progesterone receptor (PR) is positively stained in myofibroblasts in the scarred areas of IPF, implying PR could be a potential target in PF [96]. Steroid hormones can transfer to the lung through a circular system and thus act on lung fibrosis. For instance, plasma dehydroepiandrosterone (DHEA) is reduced in IPF patients and has been shown to significantly inhibit PF characteristics. DHEA decreases fibroblast proliferation and increases apoptosis, likely through the intrinsic pathway involving caspase-9 activation. It also significantly inhibits fibroblast-to-myofibroblast differentiation, collagen production and fibroblast migration [97]. Conversely, male sex hormones, or androgens, appear to exacerbate lung fibrosis following BLM administration [98]. Testosterone and 5 α -dihydrotestosterone (DHT) are decreased significantly in the IPF group [99], but their roles in PF remain unclear.

Vitamin D (VitD) functions as a steroid hormone with inhibitory effects on inflammation and fibrosis, largely by modulating TGF- β , MAPK, and NF- κ B pathways [100, 101]. IPF and other types of interstitial lung disease (ILD) patients display decreased serum VitD concentrations and lung Vitamin D receptor (VDR). VitD was also positively correlated with the diffusion capacity of the lungs for carbon monoxide (DLCO)% and predicted forced vital capacity (FVC)%, and negatively correlated with mortality of IPF [102].

In 2011, international guidelines for IPF recommended glucocorticoids in acute exacerbation IPF patients [103]. In vivo and in vitro experiments consistently demonstrate glucocorticoids enter lung cells through glucocorticoid receptors (GR α and GR β , two isoforms encoded by nuclear receptor subfamily 3 group C member 1, *NR3C1*), and then suppress PF by blocking fibroblast TGF- β production [104, 105]. However, the contents of glucocorticoid receptors in IPF patients are lower than those in normal volunteers. What's worse is that IPF patients with lower glucocorticoid receptor levels are resistant to glucocorticoid treatment [106, 107]. In the realm of vascular diseases, such as cardiovascular disease and PAH, the mineralocorticoid receptor (MR) has been identified as a contributory factor. As a result, MR antagonism is considered a promising therapeutic approach [108, 109]. However, it has been observed that MR antagonism does not significantly alter the outcomes of COVID-19-related PF treatment [110]. Presently, there is a dearth of data regarding the potential involvement of mineralocorticoid hormones and their receptors in IPF and other forms of PF. DEGs of bile acid and steroid hormones metabolism-related genes were listed in Table 5.

Table 5 DEGs of bile acid and steroid hormones metabolism in PF cells

Cell Type	AT2		Fibroblast		Macrophage		Endothelium	
	Fold Change	P value	Fold Change	P value	Fold Change	P value	Fold Change	P value
Significance								
<i>NR1H4</i>	/	/	/	/	-0.72 (IM)	7.53 E-10	/	/
					-0.77 (AM)	9.47 E-04		
<i>ESR2</i>	/	/	/	/	-0.52 (IM)	3.54 E-10	3.32	3.83 E-07
					-0.44 (AM)	6.70 E-03		
<i>ESR1</i>	/	/	/	/	0.14 (IM)	2.67 E-04	/	/
					-0.58 (AM)	6.78 E-86		
<i>VDR</i>	/	/	/	/	-0.23 (IM)	5.89 E-32	/	/
					-0.50 (AM)	9.39 E-38		

In summary, steroid hormones and their receptors are involved in PF and may prove to be effective therapeutic targets in PF.

Triglyceride (TG)

Lipofibroblasts (LFs) received more and more attention as they widely participate in various lung disorders, including PF [111]. Cell fate tracing experiments revealed that LFs originate from fibroblasts, and will transdifferentiate to myofibroblasts when exposed to a stimulus [112]. TG may act as a pivotal modulator of lung fibroblast homeostasis by contributing to the assembly of lipofibroblasts, which are characterised by lipid droplets (LDs) that undergo continuous cycles of synthesis and degradation. We found that diacylglycerol O-acyltransferase 1 (DGAT1), an essential enzyme responsible for the last step of TG synthesis, is specifically enriched in perilipin 2 (PLIN2)-positive LFs, rather than fibroblast, myofibroblast, or any other types of lung cells [112]. This suggests that TG is crucial for LF maintenance by regulating LD synthesis and degradation. The schematic diagram of lipid droplet metabolism lipid droplet metabolism was shown in Fig. 4, and the DEGs in PF responsible for LD metabolism are listed in Table 6. Furthermore, LDs function as a multifaceted organelle involved in various physiological and pathological processes, including ER stress, insulin resistance, autophagy, mitochondrial and nuclear function regulation, inflammatory response, and viral infection [113].

Studies have shown that lipid droplets can maintain the high activation of the lipogenic pathway of lipofibroblasts and convert myofibroblasts into fibroblasts with weak collagen-producing ability [114]. Moreover, lipid

droplets store large amounts of lipids, and lipid droplet-rich lipofibroblasts are physically adjacent to the alveoli and play a crucial role in alveolarisation [115]. Therefore, LDs may be significant in the resolution of PF and potentially in alveolar regeneration.

Taken together, the investigation focuses on the regulatory network and the roles of TG and LD metabolism in the lung may provide a new therapeutic approach for PF.

Fatty acids (FAs)

Recent studies have highlighted distinctive alterations in FA metabolism in PF, encompassing de novo synthesis, uptake, oxidation, and derivatization processes [116]. The initial step of de novo FA synthesis involves ATP citrate lyase (ACLY), which converts cytoplasmic citric acid into oxaloacetic acid and acetyl-CoA. Subsequently, acetyl-CoA carboxylase (ACC), a rate-limiting enzyme, allows acetyl-CoA to be carboxylated to malonyl-CoA. Finally, malonyl-CoA and acetyl-CoA are catalyzed by fatty acid synthase (FASN) to palmitic acid (PA). FAs can also be internalized via cell surface receptors, such as the CD36 receptor. Intracellular FAs bind to coenzyme A and are then shuttled to the mitochondria to start the FA oxidation (FAO) process, producing carbon dioxide and water in the presence of sufficient oxygen.

Yidan D Zhao et al. found increased free FAs but reduced carnitine shuttle, suggesting reduced mitochondrial β -oxidation in IPF. Hiroaki Sunaga et al. reported a significant downregulation of elongation of long-chain fatty acids family member 6 (Elovl6) in PF. Elovl6 knock-down altered the composition of oleic acid (OA), PA, and linoleic acid (LOA), resulting in heightened apoptosis, reactive oxygen species (ROS) production, and TGF- β 1

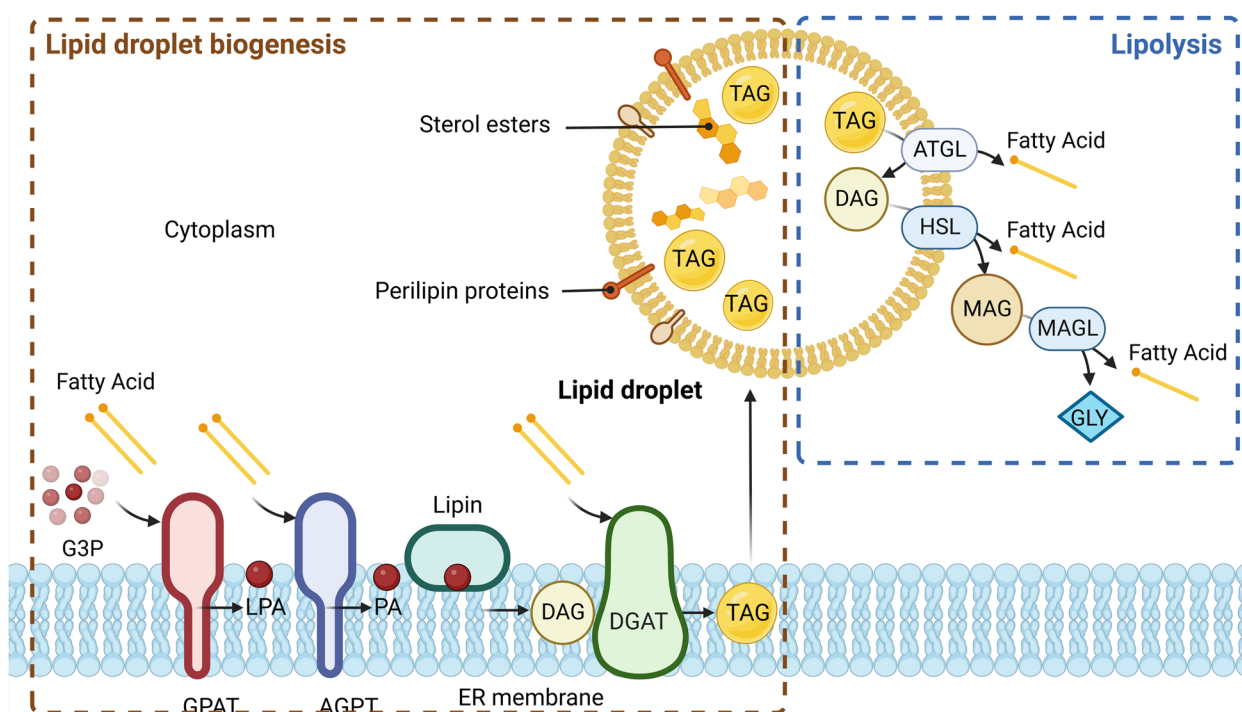


Fig. 4 Regulation of Lipid Droplet Formation by TG. The left panel of this figure depicts the multi-step synthesis of triglycerides (TAGs). Initially, glycerol-3-phosphate acyltransferase (GPAT) catalyzes the biosynthesis of lysophosphatidic acid (LPA) with a preference for saturated fatty acids and glycerol-3-phosphate (G3P) as substrates. GPATs exist in two forms, the mitochondrial isoform (GPAT1/2) and the endoplasmic isoform (GPAT3/4). Next, 1-acylglycerol-3-phosphate O-acyltransferases (AGPATs) convert LPA to phosphatidic acid (PA). Following this, the enzyme lipin, a magnesium-ion-dependent phosphatidic acid phosphohydrolase, dephosphorylates PA to yield diacylglycerol (DAG). Diacylglycerol O-acyltransferases (DGAT1/2) catalyze DAG and fatty acyl-CoA to TAG. The biogenesis of lipid droplets commences with TG synthesis, which accumulates between the ER membrane's two leaflets. Proteins bound to the lipid droplet surface, such as perilipins (PLINs), localize to the phospholipid monolayer, while the neutral lipid core comprises triacylglycerols and sterol esters. The right panel illustrates the hydrolysis of TG. Adipose triglyceride lipase (ATGL), encoded by the PNPLA2 gene, initiates TAG degradation to produce DAG, which is subsequently hydrolyzed to monoacylglycerol (MAG) by hormone-sensitive lipase (LIPE). LIPE also participates in steroid hormone synthesis by converting cholesterol esters to free cholesterol. Finally, monoglyceride lipase (MGLL) hydrolyzes MAG to free fatty acids and glycerol

secretion in AT2 cells, thereby exacerbating PF [117]. The pathogenic effect of PA on PF is also revealed by the Sarah G. Chu group [45]. In contrast, stearic acid markedly decreased p-Smad2/3 phosphorylation, ROS generation, and fibrosis [118]. The altered FA levels in IPF lung tissues have also been explored in this article and it found that PA, oleic acid, and LOA were elevated, while the level of stearic acid was significantly reduced compared to controls. PA, OA, or LOA significantly enhance the TGF- β 1 induced fibrosis, whereas stearic acid significantly reduces it. Studies have demonstrated that the FA synthetic activator nuclear receptor subfamily (LXR)/SREBP-1c axis is linked to fibrosis [64, 65, 119]. A role for altered FA metabolism through the activation of FASN via the rapamycin-sensitive TGF β 1/mTORC1 pathway [120]. As a downstream of the LXR/SREBP-1c axis, FASN is downregulated in AT2 cells. AT2 cell-specific loss of FASN impaired mitochondria biogenesis and promoted PF [23]. However, FASN is required for

TGF- β -induced profibrotic responses, and its inhibition not only mitigates fibrosis but also improves lung function. Stearoyl-CoA desaturase (SCD) desaturates saturated FA to prevent lipotoxicity, ER stress and apoptosis caused by saturated FA [121]. Therefore, it is a theoretical benefit for PF via SCD manipulation. The schematic diagram of the metabolism of fatty acid is shown in Fig. 5.

Lipoproteins

Lipids can be stored in lipoproteins and transported to peripheral tissues. Altered lipoprotein levels have been reported to be associated with PF. Our and other groups consistently revealed that plasma HDL is decreased in SSc-PF and IPE. Moreover, total serum HDL particles have been negatively correlated with mortality or the necessity for lung transplantation in IPF patients [78]. In contrast to HDL, LDL levels are elevated in PF. We have clarified the role of LDL in PF by acting on apoptosis in endothelial and AT2 cells,

Table 6 DEGs of lipid droplet metabolism in PF cells

Cell Type	AT2		Fibroblast		Macrophage		Endothelium	
	Fold Change	P value	Fold Change	P value	Fold Change	P value	Fold Change	P value
<i>GPAT3</i>	-0.72	3.40 E-04	/	/	-0.57 (IM) -1.15 (AM)	1.01 E-06 3.20 E-03	-0.40	5.89 E-21
<i>AGPAT4</i>	0.79	2.46 E-02	/	/	0.31 (IM)	2.47 E-10	/	/
<i>AGPAT5</i>	/	/	/	/	/	/	0.80	5.41 E-09
<i>AGPAT3</i>	0.47	3.33 E-09	/	/	-0.35 (AM)	1.50 E-06	/	/
<i>AGPAT1</i>	0.73	2.91 E-12	/	/	/	/	/	/
<i>AGPAT2</i>	-0.35	8.81 E-49	/	/	/	/	/	/
<i>PNPLA2</i>	-0.25	9.30 E-09	/	/	-0.40 (IM) -0.35 (AM)	1.92 E-03 4.01 E-07	/	/
<i>LPIN2</i>	/	/	/	/	-0.50 (AM)	3.34 E-31	/	/
<i>LPIN3</i>	1.29	8.21 E-16	/	/	/	/	/	/
<i>MGLL</i>	/	/	/	/	-0.46 (AM)	3.33 E-15	1.07	5.22 E-40
<i>PLIN2</i>	/	/	-0.37 (FB)	2.43 E-03	-0.40 (IM) -0.36 (AM)	0.00 E+00 0.00 E+00	/	/
<i>PLIN5</i>	/	/	/	/	-0.49 (IM) -1.65 (AM)	1.13 E-05 3.63 E-02	/	/
<i>DGAT1</i>	0.84	5.96 E-20	/	/	-0.34 (IM)	1.01 E-19	/	/
<i>DGAT2</i>	-1.10	2.05E-10	/	/	-1.32 (IM) -1.75 (AM)	4.56 E-80 4.05 E-73	/	/

and activation of fibroblasts. Chylomicrons (CMs) and other lipoproteins have not been studied in PF, neither the changes nor the roles. The uptake and utilization of these lipoproteins are involved in multiple receptors and enzymes; thereby, the whole metabolic processes in fibrotic lungs are demanded. Considering that lipoproteins are primarily produced by extrapulmonary tissues, such as the liver and the intestine, the contribution of these organs to PF, and the regulatory network between lung and extrapulmonary tissues are needed to investigate further.

Strengths and limitations

The reconstructed lipid metabolite profiles in ECs, AT2 cells, macrophages, and fibroblasts contribute to collagen deposition and the lung architecture remodeling observed in PF through various mechanisms. Single-cell RNA-sequencing reanalysis helps to further clarify cell type-specific metabolism changes in PF. Moreover, uncovering the function of the metabolite-metabolic gene axis in specific cell types is useful for explaining the dysfunction of these cells and, eventually fibrosis. We suggest that abnormal lipid metabolism may be a strong

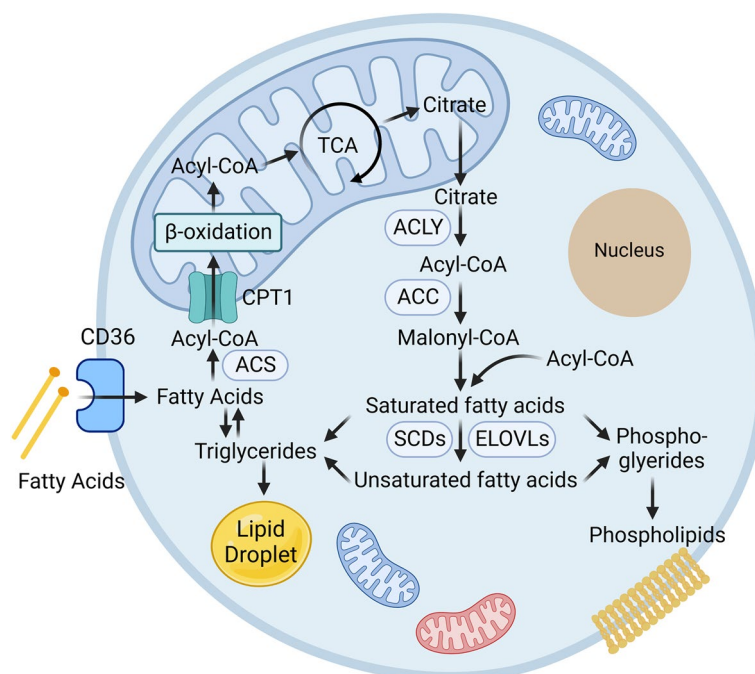


Fig. 5 Regulation of FA Metabolism Pathways. ACLY utilizes cytoplasmic citrate to produce cytosolic acetyl-CoA, which is then used as a substrate by ACC to produce malonyl-CoA. FASN could convert acetyl-CoA and malonyl-CoA into long-chain saturated fatty acids for palmitate synthesis. SCD plays a critical role in the synthesis of unsaturated FAs, particularly oleic acid. The ELOVLs are involved in fatty acid elongation. In FA catabolism, acyl-CoA synthetases (ACSLs) first degrade long-chain FAs (LCFAs) to fatty acyl-CoA esters. Subsequently, mitochondrial carnitine palmitoyltransferases (CPT1/II), in conjunction with a carnitine-acylcarnitine translocase, induce the oxidation of LCFAs, which are ultimately broken down to acetyl-CoA via the β -oxidation pathway in the mitochondria

risk factor for PF. When individuals are exposed to any external or internal stimulus, these lipid metabolism disorders may exacerbate the process of PF.

Single-cell metabolomics can more accurately reflect the level of single-cell metabolism in PF than transcriptomics or proteomics. However, at present, only single-cell transcriptomics is being studied in PF research, and there is no single-cell proteome or single-cell metabolome. In addition, many metabolic gene changes at the cellular transcriptional level align well with the metabolomic results of PF tissues or blood, suggesting that the single-cell transcriptome is powerful in revealing metabolism. In many other areas of disease research, single-cell transcription is also used to summarise single-cell metabolism [122–124]. In conclusion, single-cell transcription can reflect, at least in part, the level of single-cell metabolism. Changes in lipid metabolism in other cell types also need to be investigated, in particular, bronchioalveolar stem cells (BASCs) located at the junction of bronchioalveolar [125]. Depending on the location of the injury, BASCs can differentiate unidirectionally into airway epithelial cells or alveolar epithelial cells, playing a role in lung regeneration and the management of PF [126, 127]. Moreover, lipid metabolism has an impact

on stem cell function through the induction of fatty acid oxidation [128, 129]. Therefore, understanding the regulatory network of metabolic programs in BASCs is necessary to clinically restore tissue homeostasis post-injury by manipulating the regenerative machinery.

Conclusion

This review summarizes the various lipid species' metabolite changes and reanalyzes their corresponding lipid metabolomic genes at the single-cell level found in PF. It sheds light on the pathogenesis of PF from the perspective of abnormal lipid metabolism and identifies potential targets. Consistently, recent articles have also demonstrated that the important role of metabolism in pulmonary fibrosis from the genomic data, pathogenesis data and reports of pulmonary fibrosis [130–138]. Abnormal lipid metabolism is strongly associated with FVC%, DLCO%, disease severity, progression and survival in PF [16, 34, 56, 139]. It seems that PF can also be viewed as a disease due to abnormal lipid metabolism induced by risk exposures. In patient care, it is imperative to assess both undernutrition and overnutrition in PF patients [140]. The wide spectrum of disrupted lipid metabolism in PF necessitates a precise approach that considers

interventions specific to cell types, disease stages, and nutrient cycling. Collectively, these results highlight the clinical relevance of metabolic expression and provide an informative metric for the care of patients.

Datasets reanalysis and statistical analysis

Public scRNA-seq datasets reanalyzed in this study can be found on GEO using accession numbers GSE135893 and GSE136831. According to the cell numbers, data in endothelial and AT2 cells were derived from dataset GSE135893, and data in fibroblasts and macrophages were derived from GSE136831. For normally distributed parameters, the independent sample t-test was employed in cases of homogenous variance; if not, the non-parametric Mann-Whitney test was utilized. A *P* value of less than 0.05 was considered statistically significant. Except for the DEGs related to lipid metabolism, other DEGs were listed in Supplemental Table 1.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12944-024-02062-8>.

Supplementary material 1.

Supplementary material 2.

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Authors' contributions

XS, JC and JW conceived the study concept and wrote the main manuscript text; LH and JD were in charge of revising the manuscript; YC, MS and FG prepared figures and tables. WW, DW, CS, XX, NS and XC summarized the published literature. YY and CL analysed and interpreted the data. All authors reviewed the manuscript.

Availability of data and materials

Data available upon request.

Declarations

Competing interests

The authors declare no competing interests.

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