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Genetic and non-genetic risk factors of idiopathic pulmonary fibrosis: A review

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Abstract

Idiopathic pulmonary fibrosis (IPF) is the most common form of fibrosis of internal organs. The etiology and pathogenesis of IPF are still not well understood. However, a growing line of evidence shows that both genetic and non-genetic factors contribute to IPF development. The release of pro-inflammatory cytokines activates the immune cells. The enhanced synthesis of interleukins and cytokines, especially transforming growth factor β1 leads to the proliferation of fibroblasts, increased extracellular matrix formation, and epithelial-mesenchymal transformation of the lung tissue. These pathological changes could lead to fibrosis. Polymorphisms of genes responsible for the function of mucociliary clearance (*MUC5B*), telomerases (*TERT*, *TERC*), as well as signaling pathway related-genes such as Sonic hedgehog, *Wnt,* and some other genes are also risk factors for IPF development. Epigenetic regulatory mechanisms, such as methylation and acetylation of DNA and histones, may also influence the development and progression of this disease. At present, the role of non-coding RNAs, in particular long non-coding RNAs (lncRNA) in the development of fibrotic processes, is actively studied. LncRNA is an RNA that is longer than 200 base pairs and does not code for any proteins. LncRNAs perform various functions in the cell, from nuclear compartmentation to epigenetic regulation of gene expression and post-translational modification of proteins. In this review, we present the important aspects in the pathogenesis of IPF.

Keywords: Long non-coding RNAs; Idiopathic pulmonary fibrosis, COVID-19-induced pulmonary fibrosis

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1. Introduction

Fibrosis is a pathological process of wound healing in which connective tissue replaces the normal parenchymal tissue by increasing extracellular matrix synthesis and proliferation of fibroblasts. This leads to a considerable level of tissue remodeling and formation of permanent scar on tissues and organs, contributing to the remodeling of organ and damages to its histo- and cyto-architecture. In clinical setting, signs of functional disorders or even organ failure could be observed in patients with this condition. In short, fibrosis is the outcome of chronic inflammation, frequent tissue damage followed by proliferation processes, systemic connective tissue diseases, autoimmune diseases, tissue necrosis, and atrophy due to ischemia and metabolic disorders[1,2].

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease characterized by the destruction of the acinar structure, the growth of the extracellular matrix, and other properties of the fibrotic disease, and is histologically similar to interstitial pneumonia. Respiratory failure develops in patients with IPF, which in some cases could be fatal. IPF shares some similarities with COVID-19-induced pulmonary fibrosis. However, the mechanisms of the occurrence of both IPF and COVID-19-induced pulmonary fibrosis are poorly understood. Of particular scientific interest are the mechanisms that are somehow associated with the function of long non-coding RNA (lncRNA), the role of which in the occurrence and progression of fibrotic processes in various organs is being actively studied[3,4]. The discovery of new lncRNA-associated molecular mechanisms that are responsible for the pathogenesis of IPF and COVID-19-induced pulmonary fibrosis would facilitate the development of diagnostic systems for predicting the risk and severity of the disease and the formulation of therapeutic measures which can target key pathogenic factor with minimal side effects.

IPF is the most common form of visceral fibrosis. According to meta-analyses, the incidence of IPF in Europe and North America is 3 – 9 cases/100,000 population per year (according to other sources, up to 18 cases[5]), whereas <4 cases/100,000 population per year were reported in South America and East Asia. The prevalence of the disease in North America is 10 – 60 cases/100,000 population per year. In total, there are about 3 million patients with IPF worldwide, while there is an increase in the number of patient visits to hospitals and the frequency of deaths. In addition, IPF is more common in men, with a median age of 65 years[6,7].

The goal of the review is to summarize the genetic and non-genetic risk factors that contribute to IPF development and the role of lncRNA in this disease.

2. Non-genetic factors in the development and progression of IPF

2.1. Risk factors and pathogenesis

Approximately 1/3 of new IPF cases and the progression cases are etiologically linked to non-genetic factors. The main risk factors for IPF are older age, male sex, smoking, and living in unfavorable environmental surroundings. Aging of type 1 alveolocytes and fibroblasts, metabolic dysfunction of cellular proteins, and damage of subcellular structures are potential risk factors of lung tissue fibrosis[6]. Such alterations lead to the release of mediators from epithelium, endothelium and connective tissue cells that activate immunocompetent cells, such as polymorphic nuclear leukocytes, lymphocytes, monocytes, and macrophages (Figure 1). When the vascular wall is impaired and the blood coagulation cascade is activated, factor X is able to induce the differentiation of lung myofibroblasts, and thrombin (factor II) activates the production of the chemokine (C-C motif) ligand 2, along with low molecular weight hyaluronic acid (LMWHA) and inflammatory mediators, such as interleukin (IL)-1β, IL-6, IL-25, IL-33, which are chemoattractant for myelocytes and monocytes that could further differentiate into macrophages[8].

2.2. The role of macrophages in fibrosis development

Under the influence of LMWHA, interferon gamma (IFN-γ), ligands of toll-like receptors, macrophages are activated along the classical pathway. The secreted active forms of nitrogen, oxygen, tumor necrosis factor alpha (TNF-α) and IL-1β are strong tissue pro-inflammatory agents. IL-1β is responsible for the transition of epithelial cells to mesenchymal cells, as well as for the induction of myofibroblasts. TNF-α stimulates the expression of IL-6, an autocrine stimulator of fibroblast growth. There is also an alternative pathway for macrophage activation, which is induced by granulocyte-macrophage colony-stimulating factor, IL-4, and IL-13. As a result, the expression of the enzyme arginase-1 (Arg1) increases, as demonstrated in in vitro and murine experiments. This leads to the increase of L-proline concentration, which is necessary for the synthesis of collagen fibers[9].

2.3. The role of other immune cells in fibrosis development

Fibroblast growth factor (FGF)-2, FGF-10, FGF-9, and FGF-18 may also contribute to the development of IPF[10]. The role of eosinophils in the development of pulmonary fibrosis in allergy as well as IPF is attributed to their ability to synthesize transforming growth factor beta 1 (TGF-β1) and IL-13, which was confirmed in a study on mice[8,9]. According to a meta-analysis by Wynn and Ramalingam[1],

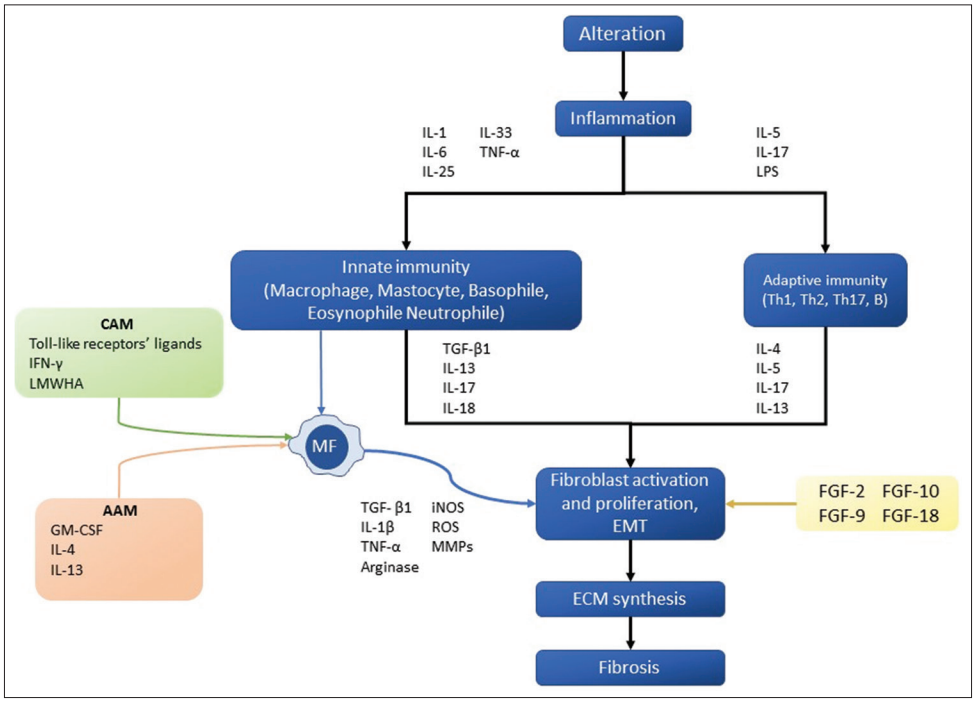

Figure 1. Immune mechanisms of fibrosis development. During inflammation, the release of interleukins, cytokines and other substances that activate both innate and acquired immunity occurs. The corresponding immune cells also synthesize interleukins and cytokines that stimulate the differentiation and proliferation of fibroblasts, ECM synthesis, and EMT of alveolocytes. An important role is played by classically or alternatively activated macrophages, since they are able to increase inflammation not only due to released cytokines, but also due to metalloproteinases, iNOS, ROS, and other enzymes that damage normal lung tissue. In summary, these factors lead to lung fibrosis. AAM: Alternatively activated macrophage; CAM: Classically activated macrophage; FGF: Fibroblast growth factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL: Interleukin; iNOS: Inducible nitric oxide synthase; LMWHA: Low molecular weight hyaluronic acid; LPS: Lipopolysaccharide; MF: Macrophage; MMPs: Metalloproteinases; ROS: Reactive oxygen species; TGF-β1: Transforming growth factor beta 1; TNF-α: Tumor necrosis factor α.

lymphocytes have a significant impact on the development of fibrotic processes. Thus, a subpopulation of $CD4^+$ Th17 cells secretes IL-17A, which has a significant effect on the synthesis of TGF-β and causes persistent neutrophilia. Th2 produces IL-13, which activates TGF-β synthesis and fibroblast proliferation. IL-4 and IL-5 also have a profibrotic effect. In contrast, IFN-γ produced by Th1 cells inhibits TGF-β and fibroblast proliferation.

2.4. TGF-β and fibrosis development

TGF-β is a polypeptide expressed in many organs and tissues during ontogenesis. TGF-β regulates the processes of cell proliferation, differentiation and apoptosis. In this case, the signal is transmitted to the nucleus with the help of SMAD 2/3 proteins. There are three isoforms of TGF-β, with TGF-β1 isoform being the most active in the pathogenesis of IPF. Specifically, the overexpression of *TGF-β1* in type 2 alveolocytes leads to hyperplasia, while in fibroblasts, the overexpression influences cell proliferation and increases synthesis of extracellular matrix. Of note, the level of TGF-β sharply increases with age[10]. The induction of epithelial-mesenchymal transformation (EMT) in mouse type 1 alveolocytes was also proven *in vitro* when they were co-cultivated with M2 alveolar macrophages[11].

3. Genetic factors in the development and progression of IPF

3.1. Profibrotic genes

Genetic factors account for up to a third of the risk factor for the development of IPF. The role of *TERT* and *TERC* encoding telomerase and its various components that are responsible for telomere synthesis has been well studied. The excessive shortening of telomeres in mesenchymal cells and type 2 alveolocytes accelerated their death, which in turn increases the risk of developing pulmonary fibrosis[1,5-7]. Mutations in the *TINF2*, *DKC1*, *RTEL1*, *PARN,* and *NAF1* genes that regulate telomerase function have been found in 25% of patients with IPF[7]. Polymorphism in the promoter of the mucin *(MUC5B)* gene, which is responsible for the function of mucociliary clearance, results in an increased risk of developing sporadic and hereditary pulmonary fibrosis[5,6]. Variants of the Toll-interacting protein *(TOLLIP)* gene (which encodes an inhibitor of *TGF-β*, a regulator of the toll-

like receptor-mediated signaling pathway of innate immunity) also contribute to the onset of the disease[6,7]. The role of the group of *Wnt* genes encoding a family of 19 glycoproteins has been studied. Glycoproteins bind to Frizzeled-receptors on the cytoplasmic cell membrane and activate the β-catenin-mediated signaling pathway, leading to the upregulation of the expression of T-cell factor genes, matrix metalloproteinases (MMPs), oncogenes, cell cycle regulators, and angiogenic growth factors. The second signaling pathway, which is calcium- and protein kinase C-dependent, regulates the differentiation of red bone marrow cells, cell migration and their polarity, which are modulators of embryonic development of tissues and their regeneration in response to damage. An association has been found between activation of the β-catenin-mediated signaling pathway and increased expression of IL-1β in type 2 alveolocytes, MMP-7 activation, increased profibrotic activity, and fibroblast proliferation. The latter was also found in the *Wnt5a* gene when it activated the second signaling pathway. There are scientific data on the contribution of Wnt1, Wnt7b, Wnt10b, and Frizzeled-2 and -3 receptors in the development of human IPF, which was further confirmed in murine experiments[10]. In mice, Wnt1-induced protein 1 increased proliferation of type 2 alveolocytes, EMT of lung and renal epithelial cells, and enhanced synthesis of extracellular substance[1]. The Sonic hedgehog (SHH) gene family is responsible for the morphogenesis of many organs, including the lungs. Signal transduction into the cell is carried out using SHH protein and three groups of receptors, which are cell-surface receptors Ptch1 and Ptch2, transmembrane protein Smo, and DNA-binding proteins-"zinc fingers" Gli1, Gli2, Gli3. Increased *SHH* signal transduction exacerbates the course of pulmonary fibrosis; an increase in fibroblast proliferation and resistance to apoptosis was confirmed *in vitro*[12] (Table 1).

Table 1. Genetic factors that contribute to IPF pathogenesis

Genetic factors	Examples
Genes	*TERT, TERC*[1,5-7] *TINF2, DKC1, RTEL1, PARN, NAF1*[7] *MUC5B*[5,6] *TOLLIP*[6,7] *Wnt gene family*[10] *SHH gene family*[12] *NOCH1, FBXO32*[19]
DNA methylation	Histones: H3K9[17], H3K27[25], MBD2[18] Genes and other DNA sequences: Thy-1 promoter region[20], *COX-2*[21], *14ARF*[22], promoters of *SFRP1* and *SFRP4*[23], *SMAD7, NOCH1, FBXO32*[19]
DNA acetylation	HDAC2, HDAC4[30] HDAC3[31] HDAC8[32]
LncRNAs	*PFAR*[3] *MALAT1*[4] *H19*[50-52] *MEG3*[53] *TERRA*[54] *PVT1*[55] *HOXAAS3*[56] *PFAL*[57] *DNM3OS*[58] *ZFAS1*[59] *FENDRR*[51]

HDAC: Histone deacetylase

3.2. DNA methylation

DNA methylation is the most common form of epigenetic modification, which may not only regulate gene expression, but also play a role in various life activities, such as embryonic development, aging and tumor formation. In recent years, DNA methylation has been proven to be involved in the process of multiple organ fibrosis. The in-depth studies of methylation in pulmonary fibrosis[13], renal fibrosis[14], and myocardial fibrosis[15] have confirmed that DNA methylation plays an important role in fibrosis development by regulating the expression of key genes[16].

It has been reported that changes in DNA methylation in CPG islands are related to the pathogenesis of IPF. DNA methylation can block the binding of transcription factors to cognate DNA sequences, thereby preventing the transcriptional repression of fibrotic gene expression. Coward *et al.*[17] found that histone H3 lysine 9 (H3K9) methylation inhibits the expression of the anti-fibrotic gene C-X-C motif chemokine ligand 10. Methylated DNA can also specifically bind to methyl-binding-proteins and recruit joint repressors, thereby inhibiting fibrosis-related genes. For example, Wang *et al.*[18] found that methyl-CPG-binding domain 2 (MBD2) stimulates the differentiation of fibroblasts to muscle tissue by binding to the methylated *CPG* site, the Erdr1 promoter, and inhibiting the expression and transcription of its downstream genes. Differentiation of fibroblasts leads to fibrotic processes. Clinical data have shown that fibroblast genes in patients with pulmonary fibrosis are abnormally methylated at multiple *CPG* sites in the genome of patients compared with those in healthy subjects, and the degree of abnormal DNA methylation in patients with fibrosis at different stages is different: Hypomethylation was associated with increased expression of profibrotic genes (*NOCH1*, *FBXO32*, and *TOLLIP*), whereas hypermethylation was associated with decreased expression of profibrotic genes[19].

Despite the low expression, many genes are hypermethylated in IPF patients, such as Thy-1 promoter region[20], cyclooxygenase-2 (*COX-2*)[21], *P14ARF*[22], and so on. Significantly hypermethylated promoters of *SFRP1* and

SFRP4 have been reported to result in impaired transcription and decreased expression, which in turn affects fibrosis progression[23]. The reduction of hypermethylated genes in IPF patients promotes the activation of fibroblasts and accelerates the fibrosis process. Methyltransferase also plays an important role in the process of fibrosis. Elkouris *et al.*[24] found that the methyltransferase Set9 binds to E3 ligase by promoting *SMAD7* methylation, thereby inducing ubiquitin-dependent degradation of SRSF7 and enhancing TGF-β signaling. Inhibition of DNA methyltransferases can alleviate pulmonary fibrosis by reducing abnormal DNA methylation using a classic inhibitor, 5-aza-2′-deoxycytidine. Among the regulatory mechanisms of pulmonary fibrosis, methylations of histone H3 lysine 27 (H3K27) and histone H3 lysine 9 (H3K9) are the most common histone methylations. H3K27 methylation is mainly catalyzed by the histone-lysine N-methyltransferase EZH2 and inhibited by histone demethylases KDM5, KDM6A and KDM6B[25]. Methylation of H3K9 is catalyzed by G9a or G9a analogs[26].

A variety of disease-specific biomarkers that are used for detecting diseases clinically are based methylation; for instance, *SDC2* methylation detection kits are used to detect colorectal cancer[27], but there is no mature DNA methylation biomarker for diagnosis of pulmonary fibrosis. Therefore, in-depth exploration of the mechanism of DNA methylation in pulmonary fibrosis is of great significance for the diagnosis, treatment, and prognosis of pulmonary fibrosis.

3.3. DNA acetylation

Histone acetylation is one of the most common modifications of histone tails, which regulate DNA accessibility to various transcriptional factors to control gene expression[28]. Acetyl groups are conjugated to lysine by histone acetyltransferases and removed from lysine by histone deacetylases (HDACs)[29]. It has been reported that HDAC plays an important role in setting up the imbalance of histone acetylation/deacetylation, and is the main driving force for the progression of pulmonary fibrosis. HDAC2 is mainly involved in the chronic progression of pulmonary fibrosis, while HDAC4 is mainly involved in the early stress response of pulmonary fibrosis[30]. HDAC3 promotes EMT, inflammation, and pulmonary fibrosis development by activating Notch1 and STAT1 signaling[31]. Saito *et al.* observed an increased expression level of HDAC8 in IPF lung tissue as well as in TGF-β1-treated normal human lung fibroblasts, and HDAC8 inhibitors could be employed as potential treatment of IPF as well as other fibrotic lung diseases[32]. In addition, ERK5 plays a key role in TGF-β1-induced pulmonary fibrosis by enhancing Smad3 acetylation[33]. Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, is currently approved for clinical treatment of cancer. Meanwhile, SAHA was found to be able to induce apoptosis in IPF myofibroblasts[34]. Therefore, histone acetylation has a key epigenetic regulatory role in the pathogenesis of pulmonary fibrosis, which may facilitate the development of novel therapeutic strategies against IPF (Table 1).

3.4. Non-coding RNAs (ncRNAs) and pulmonary fibrosis

3.4.1. Classification and functions of non-coding RNAs

To date, according to the NONCODE (available from: http://www.noncode.org) database of non-coding RNA (ncRNA), approximately more than 170,000 ncRNAs and about 96,000 genes encoding the ncRNAs exist in humans. Conventionally, ncRNAs are classified as "housekeeping ncRNAs," which are directly involved in the processes of protein synthesis, splicing, telomerase activity (mRNA, tRNA, rRNA, small nuclear RNA [snRNA], etc.), and as "regulatory ncRNAs," such as microRNA (miRNA), small interfering RNA (siRNA), enhancer RNA (eRNA), circular RNA (circRNA), and lncRNA, which regulate transcription and post-transcriptional processes of cells. LncRNAs are single-stranded RNA containing more than 200 base pairs. They are divided into several groups: (i) Intergenic lncRNA, which is transcribed from both DNA strands in intergenic regions; (ii) intron lncRNA, which is transcribed from introns of protein-coding genes; (iii) overlapping lncRNA, which is transcribed from the sense strand of DNA, overlapping with protein-coding genes; and (iv) antisense lncRNA, which is transcribed from the antisense strand, overlapping with exon or intron regions[35]. LncRNAs can also be divided into cis-acting lncRNAs that regulate nearby genes and trans-acting lncRNAs that regulate distant genes. In addition, lncRNAs can be spliced to form both short ncRNAs, such as miRNA, piRNA, and lncRNA isoforms[36]. LncRNAs have a variety of functions, for example, nuclear lncRNAs are involved in the enhancement and silencing of transcription, chromatin remodeling, and compartmentalization of the nucleus. In the cytoplasm, lncRNAs inhibit miRNAs (being competing endogenous RNAs for miRNAs), post-translational modification of the protein structure and formation of a "framework" for proteins of various signaling pathways, maintenance of mitochondrial homeostasis, regulation of pre-mRNA splicing (lncRNA *NEAT1*), and stabilization of intercellular contacts through interaction with membrane complexes PECAM1, p120 catenin[1,36-39].

3.4.2. LncRNAs in pathogenesis of diseases

Given such an active role of lncRNA, it is not surprising that they are involved in the pathogenesis of many diseases, especially cancers. For example, increased level of lncRNA

HOXA expression was observed in breast, stomach, pancreatic cancer, and hepatocellular carcinoma[40]; lncRNA *LUCAT1* was observed in breast, ovarian, thyroid cancer, and renal cell carcinoma[41]; and lncRNA *MALAT1* was observed in cancer of the breast, prostate, colon, liver, and uterus[37], etc. An association was found between increased expression of lncRNA *TP53TG1* and increased migration and proliferation of hepatocellular carcinoma cells[42], progression of retinoblastoma (by *miR-33b* [miRNA-33b] binding)[43], and development of pancreatic duct adenocarcinoma[44]. Activation of lncRNA *LINC00342* resulted in inhibition of *miR-545-5p*, subsequent overexpression of *CNPY2*, and progression of gastric cancer[45]. A study of Shen *et al.*[46] observed an association between increased expression of *LINC00342*, sequestration of *miR-19a-3p*, and progression of colorectal cancer. Chen *et al.*[47] found an increase in *LINC00342* expression in non-small cell lung cancer tissue; the binding of this lncRNA to *miR-203a-3p* led to increased cell proliferation, migration, and invasion. Furthermore, *LINC00342* overexpression and *miR-384* binding led to the development of thyroid cancer[48]. In turn, in gastric cancer, a decrease in lncRNA *RP11-363E7.4* expression is observed, and functional experiments performed by Chen *et al.*[49] showed the presence of antitumor activity during overexpression of this lncRNA when it affects the signaling pathways tp53, Bax/Bcl-2, and β-catenin.

3.4.3. LncRNAs in pulmonary fibrosis pathogenesis

There are studies confirming the role of lncRNA in the development of pulmonary fibrosis (Table 2). Overexpression of lncRNA *PFAR* was observed in a mouse model of bleomycin-induced pulmonary fibrosis, with *PFAR* being the competing endogenous RNA (*ceRNA*) for *miR-138*. Knockdown of *PFAR* caused attenuation of TGF-β1-induced fibrosis[3]. Yan *et al.* demonstrated the antifibrotic role of *miR-503* in pulmonary silicosis and the profibrotic role of lncRNA *MALAT1* (ceRNA for *miR-503*)[4]. The *miR-29b*-binding *H19* expression was reported to be upregulated in mice with bleomycin-induced pulmonary fibrosis, which was accompanied by an increase in the expression of *COL1A1* (responsible for the synthesis of the collagen I alpha-1 chain). Furthermore, *H19* bound *miR-196a* and *miR-140 in vitro* and *in vivo*, which led to the progression of TGF-β1- and bleomycin-induced fibrosis[50]. An analysis of the role of some lncRNAs in the development of lung diseases yielded findings as follows: *H19* sequesters hsa-*miR-140* to cause an increase in the expression of *TGF-β1* and sequester *hsa-miR-196* to enhance the proliferation and migration of fibroblasts[51,52]. *MEG3* upregulates *TP63*, *STAT3*, *KRT14*, *YAP1*, *AXL*, *TP53*, *EZH2*, and *TGF-β* genes to promote the migration of young alveolar epitheliocytes and prevent their final differentiation, which can affect tissue remodeling in IPF[53]. *Lnc TERRA* contains telomerase sequences and reduces telomerase activity. This leads to mitochondrial dysfunction in alveolar epitheliocytes, thereby contributing to fibrosis[54]. *FENDRR* can inhibit fibroblast activation and reduce pulmonary fibrosis by taking up aconitase 1, thereby reducing iron levels and sequestering profibrotic hsa-miR-214[51]. LncRNA *PVT1*, which sequesters *miR-497-5p*, has a profibrotic effect in mouse models with lung silicosis[55]. LncRNA *Hoxaas3* induced by TGF-β1/SMAD 4 signaling pathway promotes the activation of fibroblasts and EMT by inhibiting the action of *miR-450b-5p*[56]. LncRNA *PFAL* was found to have profibrotic activity, which manifested itself upon the inhibition of *miR-18a*. There was an increase in migration, proliferation of fibroblasts, EMT, and synthesis of intercellular substance, which was proven in laboratory mice with pulmonary fibrosis and activated by TGF-β1 fibroblast as shown in cell culture experiments[57]. LncRNA *DNM3OS*, whose expression is activated by both TGF-β1- and Wnt-mediated signaling pathways is the precursor

Table 2. Molecular targets of lncRNAs and their effect on lung fibrosis

LncRNA	Molecular targets	Effect
PFAR[3]	miR-138	Profibrotic
MALAT1[4]	miR-503	
H19[50-52]	miR-29b	
	miR-196a	
	miR-140	
	hsa-miR-196	
MEG3[53]	TP63, STAT3, KRT14, YAP1, AXL, TP53, EZH2, TGF-β genes	
TERRA[54]	Telomerase	
PVT1[55]	miR-497-5p	
HOXAAS3[56]	miR-450b-5p	
PFAL[57]	miR-18a	
DNM3OS[58]	Predecessor of miR199a-5p/3p, miR-214-3p	
ZFAS1[59]	miR-150-5p	Antifibrotic
FENDRR[51]	hsa-miR-214	
	Aconitase 1	

DNM3OS: DNM3 (Dynamine 3) opposite strand/antisense RNA, FENDRR: FOXF1 (Forkhead Box F1) adjacent non-coding developmental regulatory RNA, H19:H19 imprinted maternally expressed transcript, HOXAAS3:HOXA (Homeobox A Cluster) antisense RNA 3, MALAT1:metastasis-associated lung adenocarcinoma transcript 1, MEG3: Maternally expressed 3, PFAL: Pulmonary fibrosis-associated lncRNA, PFAR: Pancreatic fibrosis-associated lncRNA, PVT1: Plasmacytoma variant translocation 1, TERRA: Telomeric-repeat-containing RNA, ZFAS1: ZNFX1 (Zinc Finger NFX1-Type Containing 1) antisense RNA 1

of three microRNAs: *miR199a-5p*, *miR199a-3p,* and *miR-214-3p*, which are formed during *DNM3OS* splicing and enhance TGF-β1 signaling through TGF-β1/SMAD and TGF-β1/β-catenin pathway, leading to the development and progression of fibrosis[58]. Inhibition of lncRNA *ZFAS1* resulted in a decrease in lipid peroxidation (LPO), TGF-β1-activated migration of HFL1 fibroblasts, and a more favorable course of bleomycin-induced pulmonary fibrosis in mice due to the sequestration of *miR-150-5p*, which has inhibitory activity against *SLC38A1* (LPO controller)[59]. Li *et al.*[60] identified groups of genes whose co-expression is associated with both SARS-CoV-2 infection and the development of IPF, while their expression is largely regulated by *m6A* (N6-methyladenosine), which is one of the most common mRNA modifications in mammalian cells.

Patients with IPF are more susceptible to COVID-19, and patients who have had this infectious disease are at an increased risk of developing pulmonary fibrosis, which, in addition to gene expression features, is due to the presence of immune cell infiltrate in the lung tissue, represented by natural killer cells, mast cells, M2-macrophages, and gamma delta T (γδT) cells that secret profibrotic cytokines. The development of pulmonary fibrosis after COVID-19 is also facilitated by high levels of IL-6, IL-1, TGF-β1, TNF-α, and other pro-inflammatory cytokines, which are secreted as a result of viral infection[61,62].

4. IPF therapy and biomarkers

At present, two pharmacological drugs are used for the treatment of pulmonary fibrosis. However, they are only able to slow down the progression of the disease. Pirfenidone is a modified pyridine molecule that reduces collagen synthesis by fibroblasts, suppresses TGF-β1 and TNF-α, and has an antifibrotic and antioxidant effect. Nintedanib is an inhibitor of receptors with tyrosine kinase activity, namely receptors for endothelial growth factor 1 – 3 (EGF 1 – 3), receptors for FGF 1 – 3, and platelet-derived growth factor receptor a and b (PDGFRA, PDGFRB), which suppresses proliferation, fibroblast migration, and differentiation into myofibroblasts. Both of them have been proven to be effective during phase 3 clinical trials[63,64].

Pentraxin 2 is one of the plasma acute phase proteins. It suppresses the transformation of monocytes into macrophages and fibrocytes, as well as inhibits the synthesis of TGF-β1. The level of pentraxin 2 is reduced in patients with IPF; at present, the recombinant protein drug is at phase 3 clinical trials[63,65].

Pamrevlumab is a monoclonal antibody against connective tissue growth factor. The drug is now at phase 3 clinical trials. Based on earlier phases of the clinical trials, pamrevlumab was found to reduce mortality and improve lung function in patients taking this drug, as compared with the placebo group[64].

GLPG1690 is an inhibitor of autotoxin, an enzyme that hydrolyses lysophosphatidylcholine to lysophosphatidic acid, which has a profibrotic effect. Clinical trials of this inhibitor were terminated due to unsatisfactory safety profile[63].

TD139 is an inhibitor of galectin-3, a profibrotic protein receptor on the cell membrane of macrophages. This drug is undergoing phase 2b clinical trials[66].

There are a number of drugs that could inhibit the activity of TGF-β1, JAK 1, JAK 2, JAK 3, ROCK2, HSP47, JNK, NOX1, and NOX4 signaling pathways (drugs rhPTX-2/PRM-151, Jaktinib Dihydrochloride Monohydrate, KD025/SLx-2119, ND-L02-s0201/BMS-986263, CC-90001, GKT137831, respectively). These drugs are undergoing phase 2 clinical trials. The promising treatment is the use of small interfering RNAs, in particular TRK-250, which suppresses the expression of the TGF-β1 gene (Phase 1 clinical trials). The possibility of using monoclonal antibodies against ILs, lysyl oxidase, integrins, and leukotriene antagonists for IPF therapy is being studied[63,65].

Biomarkers to differentiate IPF patients from healthy people include Krebs von den Lungen (KL-6), a high-molecular weight glycoprotein on the surface of alveolar epithelium, chitinase-like protein (YKL40), surfactant proteins, mainly (SP)-A, -D, less -B, lysyl oxidase-like proteins, and genetic markers, such as polymorphisms of the *MUC5B*, *TERT*, and *TERC* genes. MMP1 and MMP7 are also prognostic markers; their concentration in the blood are correlated with the severity of the disease[65,67,68]. Circulating immune cells can also be a biomarker of IPF; a higher level of monocytes in blood is correlated with more severe IPF type and increased mortality risk[69].

5. Conclusion

The development of pulmonary fibrotic processes involves both genetic mechanisms (genes encoding signaling pathway proteins that activate fibroblast proliferation and extracellular matrix synthesis) and non-genetic mechanisms (immune) featuring the secretion of TGF-β, pro-inflammatory, and profibrotic cytokines. LncRNAs, being an important epigenetic regulator, also contribute to the development of pulmonary fibrosis, including the idiopathic variant, which is presented in the current review.

At present, our research group is studying the role of lncRNAs *TP53TG1*, *LINC00342*, *RP11-363E7.4* and others in the pathogenesis of IPF and COVID-19-induced

lung fibrosis. We are currently in the process of lncRNA extraction and studying their expression profile in peripheral blood leukocytes and lung tissue for further comparison with clinical parameters. The study of lncRNA-mediated mechanisms of fibrosis development is an important and urgent task, since many lncRNAs, including those we have studied, are potential disease biomarkers and targets for specific pharmacological therapy. In addition, they can serve as diagnostic and prognostic targets for early detection and prediction of the course of idiopathic and COVID-19-induced pulmonary fibrosis.

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Conflict of interest

None of the authors has conflicts of interest to report with regard to this manuscript.

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Not applicable.

Consent for publication

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Availability of data

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