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Review

Genomic Fingerprint Associated with Familial Idiopathic Pulmonary Fibrosis: A Review

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a severe interstitial lung disease; although the recent introduction of two anti-fibrosis drugs, pirfenidone and Nidanib, have resulted in a significant reduction in lung function decline, IPF is still not curable. Approximately 2–20% of patients with IPF have a family history of the disease, which is considered the strongest risk factor for idiopathic interstitial pneumonia. However, the genetic predispositions of familial IPF (f-IPF), a particular type of IPF, remain largely unknown. Genetics affect the susceptibility and progression of f-IPF. Genomic markers are increasingly being recognized for their contribution to disease prognosis and drug therapy outcomes. Existing data suggest that genomics may help identify individuals at risk for f-IPF, accurately classify patients, elucidate key pathways involved in disease pathogenesis, and ultimately develop more effective targeted therapies. Since several genetic variants associated with the disease have been found in f-IPF, this review systematically summarizes the latest progress in the gene spectrum of the f-IPF population and the underlying mechanisms of f-IPF. The genetic susceptibility variation related to the disease phenotype is also illustrated. This review aims to improve the understanding of the IPF pathogenesis and facilitate his early detection.

Key words: Familial idiopathic pulmonary fibrosis, Sporadic idiopathic pulmonary fibrosis, Telomerase-associated gene, Mucin 5B, Surfactant-related gene.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a form of idiopathic interstitial pneumonia (IIP) characterized by progressive lung scarring, persistent decline in lung function, and poor prognosis (1). Among all IIPs, IPF is the most common and fatal type with the worst prognosis. Approximately 80% of all patients with familial IIP are diagnosed with IPF (2). Patients with IPF have a shorter disease course than those with other types of IIP. The median survival of patients with IPF after diagnosis is only 2–4 years (3), and the clinical course and survival rates vary greatly (4). Pirfenidone and Nidanib are anti-fibrotic drugs approved by the US Food and Drug Administration (5), which can delay, but not prevent or improve, the decline of lung function. To date, lung transplantation is the only known life-prolonging intervention (6). The etiology of PF has not been fully established; however, it is thought to be caused by environmental factors and genetic susceptibility (7).

IPF occurs in sporadic, familial, or syndromic forms (8). The familial form accounts for 20% of all IPF cases, hereafter referred to as f-IPF (9). However, 80% of occurrences of IPF (the great majority) have not been divided among the two other forms. One of them could be rare, or they could be equally common. Sporadic IPF (s-IPF) is multifactorial; in addition to environmental risk factors, it is occasionally associated with polymorphic variants of various genes. Risk factors include smoking, inhaling metal and wood dust particles, and exposure to particular medications, such as methotrexate (2). Moreover, males have been identified to be more at risk than females. The clinical symptoms of f-IPF and s-IPF are similar; however, f-IPF is caused by gene variation

and characterized by the earlier onset of symptoms (8). An increasing number of reports on PF in familial clusters and some rare genetic diseases suggest that genetic factors play an important role in IPF (7). The incidence of IPF and rate of disease progression differ in patients with the same levels of exposure, indicating the influence of genetic background heterogeneity.

Furthermore, IPF may occur in a familial setting, and patients with IPF show familial aggregation in different environments, suggesting a genetic cause (10). IPF has been reported in monozygotic twins raised in different environments from an early age (11). Additionally, IPF has been detected in successive generations within families (12) and family members separated at an early age (13). The known familial incidence of IPF provides evidence of the genetic background of the disease (14). Genetic testing may reveal the necessity of screening for other etiopathogenesis factors of IPF (15), including multisystem genetic disorders such as dyskeratosis congenita (DC) (16), neuroendocrine cell hyperplasia of infancy (17), Niemann–Pick disease (18), Hermansky-Pudlak syndrome, and short telomere syndrome (14).

Family cases of IPF were first described in 1907 by Sandoz (19) in Germany, who described a pair of 18-year-old twin sisters who both died of slowly progressive respiratory failure and whose autopsies revealed terminal honeycomb. Between 2% and 20% of patients with IPF have a first-degree relative with DPLD (20). Since the earliest description of IPF, familial clusters of similar cases have been identified as familial PF or f-IPF, which is defined by at least two biological family members with clinical and histological features of IPF (21). The occurrence of familial cases of PF raises the possibility of a genetic basis, and the search for the underlying genetic determinants of this disease through familial aggregates can better elucidate the pathogenesis of familial and more common sporadic diseases.

However, it must first be speculated whether the sporadic and familial forms of IPF are indeed distinct manifestations of a single clinical entity, or whether they represent two distinct disease processes. Among relatives with f-IPF, the most common phenotype of interstitial lung disease (ILD) is usual interstitial pneumonia (UIP); however, in at least 50% of known familial cases, affected family members develop more than one ILD phenotype (14). The Mayo Clinic series further concluded that f-IPF and s-IPF share the same clinical, radiological, and pathological features (12), except that the average age of onset of f-IPF (55 years) is lower than that of s-IPF (68 years). In addition, the average age of diagnosis for f-IPF (58 years) is lower than that for s-IPF (65 years) (22).

Genetic factors play an important role in both s-IPF and f-IPF (23), especially in patients with f-IPF. This finding suggests that a single autosomal dominant mechanism of reduced penetration cannot exclude autosomal recessive inheritance or heterogeneous coexistence of genetic traits (24); however, the genetic pattern of f-IPF remains unclear. Current data suggest that at least one-third of the risks of s-IPF or f-IPF can be attributed to common genetic variants (25), some of which have prognostic significance for patients with IPF (26). In addition, more rare genetic variants that affect susceptibility to f-IPF have been identified (23).

Susceptibility gene variation is informative for clarifying the pathogenesis of f-IPF and determining its key disease-causing targets. To date, there are no clinical guidelines on when and which gene tests should be performed in patients with IPF. Previous research has examined genetic associations, common single nucleotide polymorphisms (SNP) and rare gene mutations are associated with their development (25). Rare mutations in genes telomerase reverse transcriptase (*TERT*) (27), telomerase RNA component (*TERC*) (28), dyskeratosis congenita 1 (*DKC1*) (29), telomere repeat binding factor 1-interacting nuclear factor 2 (*TINF2*) (30), regulator of telomere length 1 (*RTEL1*), zinc finger CCHC-type containing 8 (*ZCCHC8*), poly(A)-specific ribonuclease (*PARN*) (31), surfactant protein C (*SFTPC*) (32), surfactant protein A2 (*SFTPA2*) (33), the ATP-binding cassette-type family A member 3 transporter (*ABCA3*) (34)), and common variants of 10 loci (3q26, 4q22, 5p15, 6p24, 7q22, 10q24, 11p15, 13q34, 15q14-15, and 19p13) (25) are associated with f-IPF.

Telomere-associated genes

Telomeres are cap-like structures at the ends of chromosomes comprising multiple repeated 5′-TTA GGG-3′ DNA sequences that protect chromosome integrity and stability during cell division (35). As the cells divide, the telomere length progressively shrinks (36). Patients with familial and, less commonly, sporadic IPF have extremely short telomeres. TERC and TERT are the two main components that constitute the telomerase complex (37), a specialized ribonucleic protein and major component of the mechanism that maintains telomere lengthening (38). Telomerases are found in germlines, stem cells, tissue cells, and most cancer cells, with the ability to proliferate actively, but are scarce or absent in normal somatic cells (39). Mutations in genes that regulate telomere lengthening can negatively affect their maintenance, which can lead to gradual telomere shortening. When telomeres are shortened to a certain

critical point, DNA damage reaction is activated, mitochondria cannot divide, and apoptosis ensues (40) (Fig. 1). Telomere shortening is related to aging and senile diseases (41), as telomere length decreases with age (36). Telomere mutations are found in 25% of patients with f-IPF and 1–3% of those with s-IPF (42).

Telomere shortening occurs gradually throughout the life cycle as cells undergo continuous mitosis, especially in individuals genetically susceptible to familial IPF who have significantly shorter telomeres than patients with sporadic IPF. The mutation of the telomerase complex accelerates telomere shortening. When telomere shortening reaches a critical value, DNA damage is activated, and mitochondria fail to divide, resulting in cell senescence, apoptosis, or cell division cycle termination.

Half of IPF families with known mutations carry TERT or TERC mutations (43). Half member might carry mutations in one or more of the other telomere maintenance genes, such as *RTEL1*, *PARN*, nuclear assembly factor 1 (*NAF1*), *TINF2*, *ZCCHC8*, and *DKC1*(Fig. 2). Four genes that affect *TERC* mRNA deadenylation, TERC trafficking, and stability are *PARN*, *NAF1*, *ZCCHC8*, and *DKC1*. Fig. 2 shows how they indirectly affect telomere maintenance.

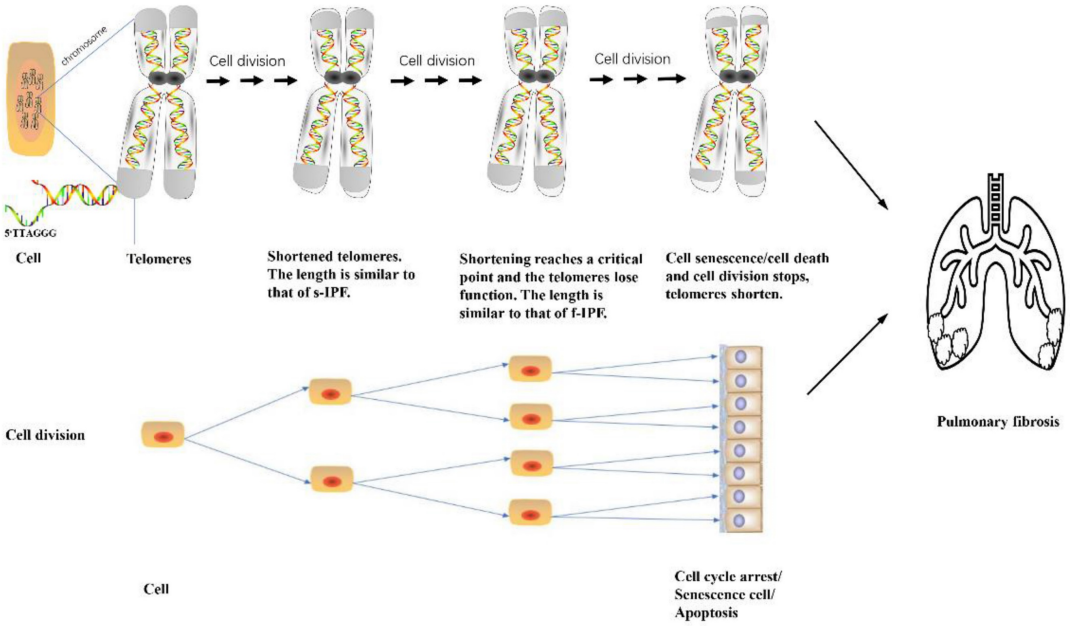

Fig. 1. Role of short telomeres in PF.

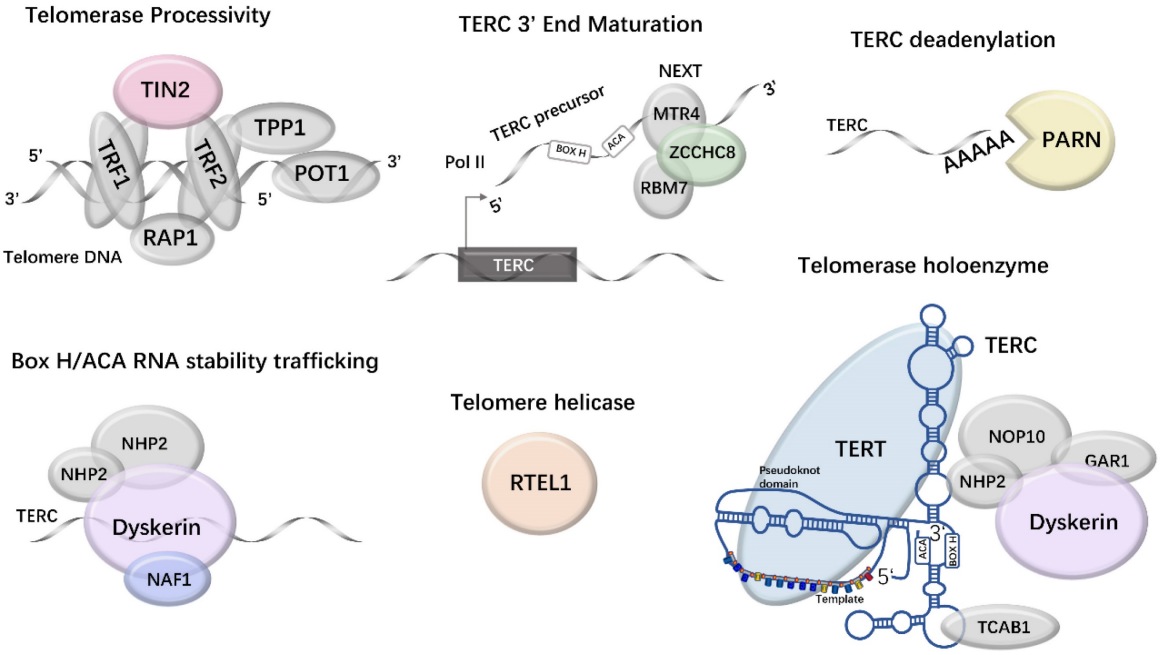

Fig. 2. Eight telomerase and telomere maintenance genes are mutated in f-IPF. The mutated components are shown in color.

Telomerase

Telomerase inactivation is due to the silence of TERT expression (44). TERT mutations are associated with short telomere syndrome and a wide range of clinical phenotypes, such as congenital dyskeratosis, bone marrow failure, liver disease, and PF (45). Age-related PF is usually the first indicator of genetic defects in families with TERT mutations (46), with an age at onset of 60–70 years (47), and mutations in telomerase genes are the most common inherent risk factors for f-IPF (48). Families with telomerase gene mutations for f-IPF show an expected genetic predisposition (49). Germline *TERT* mutations lead to haploinsufficiency, which in turn leads to telomere shortening (50). These shorter telomeres are inherited over generations. This is why TERT mutations are considered pathogenic; however, it may take over 300 years before the cumulative effect of hereditary telomere shortening leads to PF (51). Ultimately, in these families, the onset of the disease is anticipated. This is followed by successive generations with increased symptoms of an earlier onset (52). This finding explains the clinical complexity of *TERT* mutations, which are based on randomly occurring mutations and hereditary genetic defects.

The non-coding RNA component of telomerase *TERC* provides a structural scaffold for the formation and action of telomerase complexes (53). It is used for the synthesis of template regions and progressive telomere repeats (54). *TERC* RNA is made up of 451 nucleotides, but longer transcripts can be identified by reverse transcription-polymerase chain reaction (55) and cDNA 3' end (56).

Diaz de Leon et al. assessed 134 patients with f-IPF from 21 families with PF (all with telomerase *TERT* mutations) and found that 18% of the patients had heterozygous *TERT* mutations, covering 18 mutation sites, and some patients may have several telomerase mutations (57). Telomerase gene mutations have been reported in approximately 10% of patients with s-IPF, and in approximately 25% of patients with f-IPF (48). Armanios et al. observed 73 families with f-IPF, six of whom (8%) had shorter telomeres in peripheral blood leukocytes than those observed in their peers, and *TERT/TERC* heterozygous mutations encoding RNA ligands of the telomerase complex in an autosomal dominant inheritance pattern (43). *TERT* and *TERC* mutations have also been validated in another study in families with f-IPF (58).

RTEL1

RTEL1 is a DNA helicase that unlocks the T-ring structure of telomeres at the end of chromosomes (59). Mutations in *RTEL1* result in the cleavage of telomere ends near the T-loop by the structure-specific endonuclease subunit (SLX4), which leads to the release of telomeric repeats (T-circles). These successive DNA cycle mutations gradually generate shortened telomeres (60). *RTEL1* mutants have very short telomeres, whose length is similar to those of peripheral blood mononuclear cells (PBMCs) in individuals with *TERT*, *TERC*, and *DKC1* mutations (61). Damage to *RTEL1* also leads to genomic instability and replication defects (62), which result in disruption of cell cycle progression. Short telomeres are present in approximately one-third of patients with familial ILD, and rare variations in *RTEL1* are associated with the development of familial ILD, especially in patients with short telomeres in PBMCs (61).

PARN

PARN is a widely expressed enzyme that removes poly(A) tails by cap-dependent deadenylation of mRNAs and, in doing so, typically plays a role in regulating global mRNA levels during development (63), as well as other more specialized functions, including Dicer-independent end pruning of microRNA miR-451 (64) and deadenylation of small nucleolar RNAs (snoRNAs) (65). It contributes to the poly(A)-specific maturation and telomere length maintenance in fibroblasts and induces pluripotent stem cells (66). *PARN* haploinsufficiency may lead to telomere shortening through a decrease in small nucleolar RNAs of the H/ACA box or *TERC* RNA composition. *PARN* mutations cause diseases that have been related to telomere length, including f-IPF (67) and DC (68). Carriers of *PARN* and *RTEL1* mutations have shortened leukocyte telomere lengths, and family members who did not inherit *PARN* or *RTEL1* mutations have shorter mean telomere lengths than unrelated individuals (i.e., spouses) but longer telomere lengths than carriers of *PARN* or *RTEL1* mutations, with epigenetic inheritance of short telomeres observed in family members (67). Together, these genes explain approximately 7% of f-IPF and highlight the link between PF and telomere dysfunction.

DKC1

The DC gene variant, X-linked gene *DKC1*, is another component of the telomerase complex and is also important for its stability and maintenance (69). In 1995, the first link between DKC1 and pulmonary fibrosis was found in 46 families recruited at Hammersmith Hospital which occurs in 19% of cases and is a congenital telomere disease characterized by skin abnormalities, bone marrow failure, and lung fibrosis (70). DNA analyses of two siblings who died

of IPF revealed significantly shortened peripheral blood telomeres, undetectable alveolar epithelial telomeres, and a shared new A-to-G transition near the TERC-binding domain in *DKC1*; this domain would encode an amino acid substitution for Thr405Ala, a mutation that destabilizes TERC and impairs telomerase function (71). The X-linked *DKC1* variant represents a telomere-associated gene for the genetic cause of f-IPF.

TINF2

TINF2 encodes a protein that is involved in telomere protection and maintenance (72). The shelterin complex, made up of six proteins, protects chromosome ends from DNA damage and regulates signaling and repair mechanisms as well as telomerase entry and telomere activity (73). TERF1-interacting nuclear factor 2, the protein product of *TINF2*, is a subunit of the shelterin complex. Mutations in *TINF2* lead to telomere shortening and the impaired recruitment of tripeptidyl-peptidase 1 (TPP1)-dependent telomerase to telomeres (74). Epithelial DNA damage response activation at chromosome ends in *TPP1*-null mice increases telomere fusion and fragility. This confirms the role of TPP1 in telomere protection, which is required for TERT recruitment to telomeres and telomere elongation *in vivo* (75). Jonathan et al. identified *TINF2* mutations in a family with PF by exome sequencing. They confirmed that *TINF2* mutations are a rare cause of f-IPF and explained the genetic risk in approximately 1% of the reported cases (30). However, *TINF2* mutations have also been reported in s-IPF (72).

ZCCHC8

ZCCHC8 is a component of the nuclear exosome targeting complex in mammalian cells (76). It drives the decay of predominantly short, unprocessed RNA with non-adenylated 3' termini (77), which is associated with short telomere disease (78). In single cells and model organisms, *ZCCHC8* loss leads to the accumulation of short-extended TERC forms while sacrificing mature TERC, possibly at an early stage, before PARN deadenylation (79). A genome-wide analysis identified *ZCCHC8* mutations in an adult with IPF who exhibited typical features of short telomere syndrome and a family history consistent with autosomal dominant pulmonary fibrosis; 13 affected family members were deceased.

Their TERC levels could not be measured to determine preclinical status; however, the proband's two children, who had abnormally short telomeres (shorter than those of the proband), had 50% lower TERC levels than healthy controls, and the remaining relatives had similar TERC levels to healthy controls. No *ZCCHC8* variants or other short telomere syndrome phenotypes were found in 42 genetically-unidentified families that had also been screened for PF. This suggests that these mutations are rare (1 out of 43.2%). Furthermore, this result is consistent with a loci heterogeneity found in short telomere syndrome (79). *ZCCHC8* is essential for TERC 3′-terminal maturation and telomerase function, and a loss in heterozygosity causes f-IPF (79).

NAF1

NAF1 is a non-coding RNA that encodes an essential protein for the biogenesis of H/ACA box small nucleolus RNA. NAF1 affects telomere activity by combining with other proteins (such as TERT) to form telomerase, which plays an important role in telomeric DNA synthesis (80). Mammalian NAF1 is essential, probably because of its conserved role in rRNA modification (81). In contrast to other box H/ACA, NAF1 has its own transcriptional regulation and is not protected by an intron lariat, so TERC may be particularly susceptible (82). Naf1 +/- mice have reduced H/ACA RNA levels, and their telomerase RNA levels were only half those of the control group, but snoRNA-guided rRNA pseudouridylation still occurred (83). Thus, NAF1 deficiency selectively disrupts telomere length homeostasis by reducing telomerase RNA levels and preserving rRNA pseudouridylation. Loss of *NAF1* function leads to short telomere syndrome, manifested as PF emphysema and telomere-mediated extrapulmonary disease, which is common in patients with s-IPF and f-IPF (83).

Short telomeres and PF

The association between telomere abnormality and PF is not fully understood (84). Notably, short telomeres can lead to apoptosis, senescence, and a combination of phenotypic DNA damage responses (85). Senescent cells have altered gene expression profiles and secrete a range of cytokines and growth factors (senescence-related secretory phenotypes) that may play a direct role in pathogenesis. It is hypothesized that the loss of regenerative potential of alveolar type II (AT2) epithelial cells after injury underlies telomere-associated PF, accompanied by excessive proliferation of airway cells exhibiting abnormal phenotypes (86). A study using mouse models, in which telomere repeat factor 1 was knocked out in alveolar and bronchiolar epithelial cells, has shown that the preferential activation of cellular senescence leads to inflammatory responses, upregulation of immune signaling pathways,

increased mortality, lung remodeling, and spontaneous fibrosis compared with controls (87).

In the absence of telomerase mutations, s-IPF is often associated with telomere shortening; 5.71–68.2% of s-IPF patients present shorter blood leukocyte telomeres than age-matched controls, most of whom had no identifiable telomerase gene mutation, suggesting that the pathways involved in familial disease contribute to sporadic disease (88). The telomere length in PBMCs and alveolar epithelial cells is shorter in patients with f-IPF than in controls (88) and is significantly longer in the non-fibrotic region than in the fibrotic region (89) in patients with f-IPF after controlling for age and ethnicity. There is a significant difference in age at onset between the younger and older generations of patients with IPF (90), probably owing to shorter telomeres across generations. Short telomeres are disproportionately frequent in patients with s-IPF and are associated with worse survival (91).

Telomere length in peripheral blood is considered to be a marker of biological age (92); the survival of patients with IPF may be predicted by the genomic DNA telomere length. Regardless of the underlying diagnosis, faster disease progression has been observed in patients with ILD with fibrotic telomere gene mutations, suggesting that telomere length affects disease severity. Short telomeres are disproportionately present in patients with s-IPF and associated with worse survival in those with IPF (91). Observations in families with telomerase mutations suggest that emphysema is the first manifestation of telomere-associated lung disease in telomerase mutation carriers, either alone or in conjunction with PF presenting as mixed lung injury (93). No significant lung phenotypic abnormalities were found in mice with short telomeres (93). However, when mice with short telomeres were chronically exposed to cigarette smoke, they developed features of emphysema (93).

Like IPF, emphysema is associated with cumulative DNA damage in alveolar epithelial cells, with age and smoking as major risk factors (94). It follows that short telomeres represent a heterogeneous group of age-related lung diseases, of which IPF is the most common, that may be accompanied by some forms of emphysema. Patients with IPF have an annual decline in forced vital capacity of 130–210 mL, short telomeres caused by mutations in telomere-related genes, and forced vital capacity is reduced by an average of 300 mL per year (47). Short telomeres are associated with increased fibrosis, histological patterns, and mortality (95). These data support the notion that telomere function is involved in the development and progression of PF.

Short telomeres and lung transplantation

Regarding lung transplantations, lung epithelial cell renewal rates are high. This leads to accelerated telomere wear, senescent reprogramming of airway progenitor cells, and ultimately, airway remodeling, and fibrosis (96). In a lung transplant study of 20 patients with fibrotic ILD, eight patients presented f-IPF, and telomere shortening was found in five of them. Moreover, four patients with f-IPF had rare mutations in the telomere-related T cell receptor gamma locus (*TRG*) gene (potential risk of pathogenicity: *TERT*, *TERC*, *DCK1*, *PARN*, *RTEL1*, and *TINF2*), suggesting that telomere dysfunction is associated with adverse outcomes after transplantation. Despite that, the 1-year survival rate after transplantation was above 80%, and the procedure was beneficial to the survival of patients with fibrotic ILD, regardless of telomere dysfunction (97). Conversely, Swaminathan et al. reported a significantly increased risk of death after lung transplantation in patients carrying *TERT*, *RTEL1*, or *PARN* variants (98).

Telomere length can be inherited independently of rare genetic variants, and inheriting short telomeres (and disease risk) without inheriting the mutation is possible; this is called "occult hereditary disease" (57). Among the 75 asymptomatic first-degree relatives of patients with f-IPF, telomere length was shorter than in healthy controls, with 36% having telomere lengths less than the 10th percentile of age (99). One study showed that 97% of patients with IPF have shorter telomeres than age- and sex-matched controls (100), suggesting that short telomeres are not only found in patients with telomerase-related gene mutations associated with f-IPF. PF may be one of the few clinical symptoms of some inherited multisystem diseases, and f-IPF is the only manifestation of the disease in some DC families, members of which lack typical skin and mucosal characteristics (43).

A family history of short telomeres may be associated with neonatal respiratory distress, childhood ILD, aplastic anemia, and cryptogenic cirrhosis (101). Short telomeres and reduced keratinoid-associated undesirable protein expression are found in families with f-IPF (102); however, no mutations in *TERT*, *TERC*, *DKC1*, or other genes known to induce DC were detected (103), suggesting that the abnormal regulation of the telomerase complex is another mechanism of telomere shortening in patients with PF. However, the proportion of telomerase mutations in f-IPF was not high, indicating that other factors contributing to telomere shortening should be studied.

Mucin 5B genes

In normal lungs, the airway secretion of mucus is a defense mechanism that traps pathogens, particles, and toxic chemicals floating in the air. The mucus also assists in the removal of endogenous debris, including dying epithelial cells and leukocytes, which are expelled from the airways by cilia and cough (104). Extracellular gels made of water and mucins (highly glycosylated proteins) are the most important components of mucus. Paradoxically, although the lack of a mucus barrier makes the lungs vulnerable, excess mucus or an impaired clearance thereof is the pathogenesis of many common airway diseases, including PF (104). Excessive mucin secretion or surface fluid volume imbalance may increase the solid concentration by up to 15%, resulting in sticky and elastic mucus that is not easily removed (105). Mucin 5B (*MUC5B*) encodes major gel-forming mucin, which is mainly secreted by the proximal submucosal gland and distal airway, and plays a key role in mucociliary clearance (106). This protein mainly affects the rheological properties of airway mucus and participates in airway defense.

Expression of MUC5B in IPF

IPF has long been thought to involve mainly the alveolar region (107). However, abnormal distal airways may also contribute to IPF pathogenesis. In addition to consistent abnormal physiological changes in the distal airways, they have been implicated in ILD (108). A recent histological study showed increased bronchiolar and peribronchiolar inflammation, fibrosis, and thickened bronchial walls in patients with IPF compared with controls (109). The ERBB-YAP axis is considered a driver of distal airway epithelial cell obstruction. This signaling pathway dynamically interacts with MUC5B to increase the degree of distal airway obstruction and is specific to IPF. Distal airway epithelial dysfunction is sufficient to drive primary lung fibroblast activation (108). Visually, the characteristic changes of fibrotic lesions can be seen as subpleural honeycomb-like cysts and predominantly reticular infiltrations in the lower region, as well as a microscopic honeycomb, which represents the airway expanded by the traction of the fibrotic process. They are filled with MUC5B protein and chronic inflammatory cells (110).

MUC5B is co-expressed in respiratory bronchiolar airway epithelia (111) and type 2 alveolar epithelial cells (112). In mice, the MUC5B concentration in bronchoalveolar epithelial cells is directly related to the degree and persistence of bleomycin-induced PF and mortality (113). In normal mouse airways that resemble the distal human airway, MUC5B is generated in surface secretory cells in the airways (114). This suggests that it mediates baseline barrier and clearance functions in mice, and that it might have the same role in the human distal airways (115). *MUC5B* expression was increased in tissue sections of distal airways from subjects with PF (116). These data suggest that the distal airways are involved in the pathogenesis of IPF.

MUC5B promoter single nucleotide polymorphism and IPF

The gain-of-function promoter variant, rs3570 5950, is 3 kb upstream of the *MUC5B* transcriptional start site and is the strongest risk factor for the development of f-IPF (117). The conversion from G to T in the 5′ region interferes with the binding site of DNA enzymes to transcription factors and upregulates the expression of *MUC5B* (118). The downstream 32 bp of rs35705950 is a highly conserved forehead box A2 (*FOXA2*) binding motif, which is differentially methylated in IPF (119), resulting in increased *MUC5B* expression and rs35705950 being considered a risk allele. This hypermethylation may lead to increased occupancy of *FOXA2* in the binding motif, resulting in increased expression of *MUC5B* (120). The rs35705950 variant enhances *MUC5B* transcription. The resulting post-transcriptional processing can produce a pro-fibrotic response, induce endoplasmic reticulum (ER) stress, and activate the unfolded protein response (UPR) (121). The UPR attempts to restore normal protein folding through three reactions: IRE1/XBP1, PERK/ATF4, and BiP/ATF6 (122).

When protein homeostasis is abnormal, UP accumulates in the ER, leading to increased ER pressure, which results in apoptosis. ER stress has been observed in patients with IPF (123). In experimental models of PF, the activation of the UPR in alveolar epithelial cells leads to an epithelial-mesenchymal transition (124) and fibroproliferative pulmonary disease (125). ER stress and apoptosis interfere with normal epithelial responses to injury and repair, which are hallmarks of IPF (126). Whole-genome sequencing analysis has identified an SNP in the promoter region of the *MUC5B* gene, i.e., the rs35705950 variant. It poses a major risk factor for developing IPF, accounting for 30–35% of the risk (127).

Plantier et al. studied the correlation between morphology and transcription factors in the signal transduction system in IPF and found that the uncontrolled expression of *MUC5B* is involved in PF formation (110). Both the homozygous mutation (T/T) and heterozygous mutation (G/T) of the *MUC5B* rs35705950 variant significantly increase the risk of developing f-IPF and s-IPF. By scanning the

entire gene sequence of the chromosome 11 p-terminal, 19 SNPs were found to be associated with f-IPF. Finally, rs35705950 located in the promoter region of *MUC5B* was confirmed to be the most strongly associated with familial lung stromal disease and IPF, present in 34% of familial lung stromal disease, 38% of IPF, and only 9% of normal controls, and was considered a risk allele for both diseases (117). The 4-kb promoter region gene of *MUC5B* has three CpG islands and other regulatory sites, suggesting that the expression of *MUC5B* is affected by both genetic and non-genetic factors.

In 2011, two laboratories simultaneously reported that the *MUC5B* promoter variant rs3570 5950 is the strongest risk factor for the development of f-IPF and s-IPF (128) and appears to be similarly prevalent in patients with f-IPF and s-IPF (117) (approximately 50–60% of individuals with FIP or IPF). This factor increases the risk of heterozygosity by six-fold and that of homozygosity by 20-fold, suggesting that both forms of the disease share common genetic variations. IPF is caused by excessive and continuous lung injury or repair abnormalities (122).

In addition, common exposure and basic biological processes can affect *MUC5B* expression (Table 1).

Pathological role of MUC5B in IPF

In addition to the association between the SNP of the *MUC5B* promoter and IPF, its protein may play a direct role in the pathogenesis of IPF (117). Persistent bronchiolar epithelial injury and the overproduction of MUC5B by airway progenitor cells lead to honeycomb cysts and IPF (116).

First, excessive expression of *MUC5B* can impair mucosal host defense and leads to excessive lung injury caused by inhaled substances. Over time, a reduced clearance rate may lead to the formation of scar tissue, which replaces normal lung tissue. It also results in persistent fiber proliferation, which in turn develops into IPF (117). Exposure to cigarette smoke increases *MUC5B* expression in the lung through the dysregulation of classical signaling pathways (e.g., TGF-β and Wnt), resulting in a fibroblast-like phenotype in alveolar epithelial cells, as demonstrated in mouse (MLE-12) and rat (RLE-6TN) epithelial cells (148). Additionally, several clinical and epidemiological studies have shown that f-IPF and s-IPF are more common in smokers (148). In IPF model mice infected with H1N1, distal airway stem cells proliferate and express keratin 5-positive (*Krt5*+), ablates the intrabronchial region, and assemble into alveolar structures (149). Persistent notch signaling after H1N1 injury leads to failure of regeneration and formation of cellular cysts. KRT5+ cells are present in the fibrotic stroma of IPF and are closely associated with MUC5B-rich cellular cysts (150).

Alveolar stem cells are mainly derived from the distal airway epithelium (151), and MUC5B and dry/progenitor cells participate in the fiber proliferation response. When stem cells attempt to regenerate damaged bronchioles and alveolar epithelium, *MUC5B* overexpression interferes with the interaction between AT2 cells and the underlying matrix, inhibiting alveolar basement membrane re-epithelization. This may exacerbate the ongoing alveolar collapse and fibrosis of adjacent bronchoalveolar units, resulting in chronic fiber proliferation and regeneration process disorders and cellular cyst formation (151). These structures inhibit normal alveolar homeostasis and promote fibroproliferative activity in persistent foci.

Second, under normal conditions, mucociliary clearance depends on effective ciliary movement, adequate hydration of the fluid layer around the cilia, and complete cough (152). The overexpression and accumulation of MUC5B prevent efficient mucus hydration and ciliary function (111) and cause excessive retention of inhalants. Endogenous inflammatory fragments at the bronchoalveolar junction are associated with changes in the osmotic gradient, water moving out of the periciliary layer and into the airway lumen (153), and a reduction in mucociliary clearance. They may also physically affect cilial function (150), enhance inhalation retention. Finally, when sticky protein expression relies on the cystic fibrosis transmembrane conductance regulator anion secretion separation, abnormally thick mucus may be beyond the influence of airway dehydration, which can lead to abnormal host defense (154). The common defects of mucous membranes of inhaled particles may exaggerate the host defense. Facilitate recurrent injury/repair/regeneration mechanism disruption (106), and lead to microscopic scarring and progressive fibrous proliferation in the lungs, lung structural destruction, and IPF development over time (3).

Third, IPF is characterized by the co-overexpression of *MUC5B* and cilium-associated genes (155), which are expressed and operate in ciliated airway epithelial cells of the distal airways and are related to microscopic cellular structures. Matrix metalloproteinase 7 (*MMP7*) is a WNT/β-catenin target gene encoding for a metalloproteinase, which is overexpressed in IPF proliferative epithelial cells. Based on the discovery that MMP7-deficient mice are protected from bleomycin-induced PF, it is speculated that *MMP7* plays a role in promoting fibrosis (156). Plasma *MMP7* concentration in IPF was correlated

with two single nucleotide polymorphisms in gene promoter regions, suggesting a potential genetic basis for *MMP7* upregulation.

A known biomarker of IPF can indicate the presence, severity, and prognosis of the disease (157). Plasma *MMP7* has been proposed as a univariate predictor of early interstitial lung disease in first-degree relatives of patients with familial interstitial pneumonia (158). Ciliary *MUC5B* gene expression is associated with *MMP7* concentration and attenuation of ciliary cell differentiation in the airways (159). However, *MUC5B* promoter mutations cause mucus accumulation in the distal region of the lung and impair mucociliary function. Abnormal repair processes after distal airway injury and failure of terminal airway regeneration are activated by cellular cysts.

All three mechanisms are reasonable and can act alone or together to cause IIP associated with the *MUC5B* promoter SNP (Fig. 3).

MUC5B is mainly produced by submucosal glands and mucous cells on the surface of the airway. SNP were found in the promoter region of *MUC5B*. The abnormal expression of *MUC5B* can be induced by environmental exposure and *MUC5B* SNP. The mucus in the airways can be adjusted by the ability to clear, eventually leading to acute events or pulmonary fibrosis.

Table 1. Regulation of MUC5B expression in airway epithelial cells.

Species	Effector	Pathway	Transcriptional factors	Secretion	References
Bacteria spp	LPS/staphylococcus enterotoxin	MAPKs	CREB/AP1/SP1/ NF-κB	+	(129-137)
Cytokines	IL-1 β		NF-κB	+	(132, 138, 139)
	IL-17A		NF-κB	+	(132, 139, 140)
	IL-6	MAPKs	CREB/AP1/SP1/ NF-κB	+	(131-135, 140, 141)
		STAT3	FOX	Decrease	(119, 142, 143)
	IL-13	STAT6	FOXA2	Decrease	(119, 144, 145)
Lipid Mediator	PGD2	MAPKs	CREB/AP1/SP1/ NF-κB	+	(119, 131-135, 146)
Oxidation factors	ROS	MAPKs	NF-κB	+	(147)

Note: Different stimuli in airway epithelial cells can regulate *MUC5B* expression, including certain bacterial components, some interleukins, oxidative factors, lipid mediators, which may induce *MUC5B* overexpression through MAPK, STAT3, or STAT6 pathways. Some important transcription factors are also directly or indirectly involved in *MUC5B* overexpression.

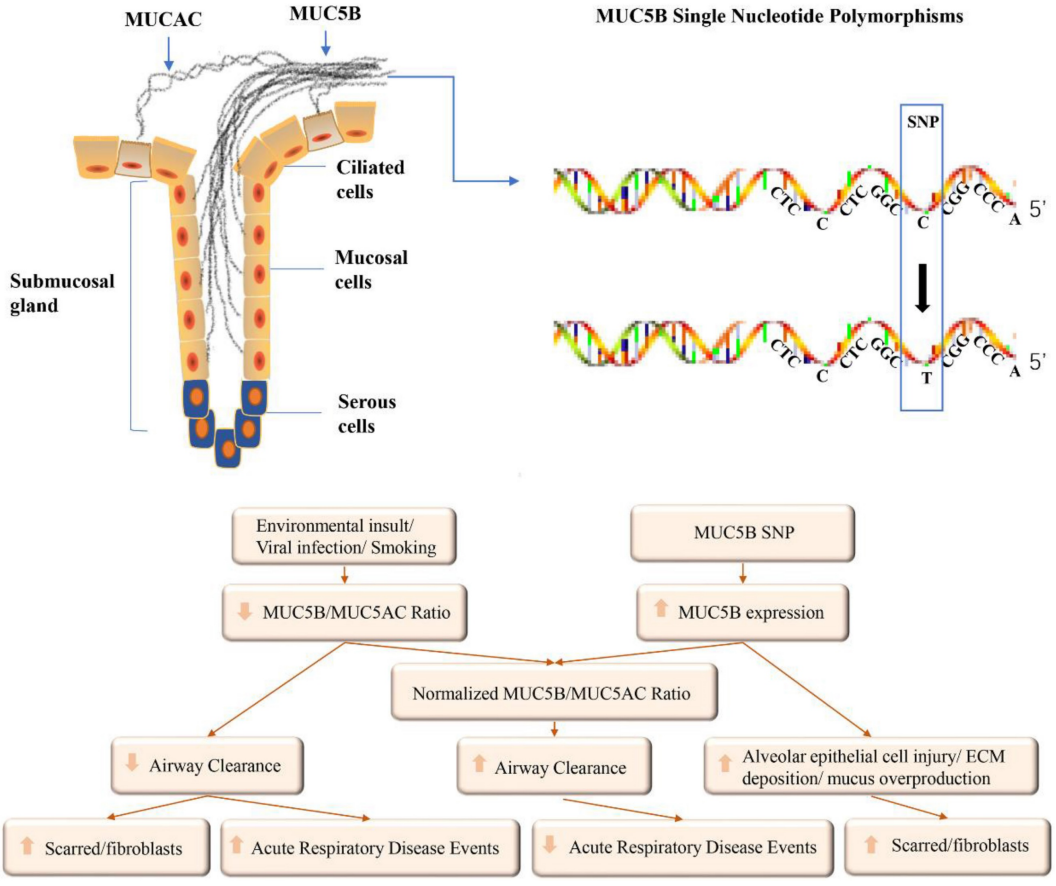

Fig. 3. Possible influencing factors of MUC5B expression and its role in airways.

MUC5B and *TOLLIP* reside at the same genetic locus. If IPF is also associated with the *TOLLIP* promoter SNP, the risk of death is higher (160). The independence of the association signals at 11p15.5 suggests that multiple variants of this locus may be influencing disease susceptibility and course (127). However, this does not prove a relationship between the two genes.

In a meta-analysis of 28 studies, the overall IIP and FIP risk of the TT genotype and the T allele of rs35705950 was significantly increased across all genetic models. These results suggest that *MUC5B* rs35705950 may be a predictor for IIP and FIP susceptibility (161). Preclinical PF (PrePF) is frequently reported in smokers and patients with f-IPF (162), and the polymorphism of the *MUC5B* promoter can be used as an indicator of IPF susceptibility for identifying individuals with PrePF (163) and predicting the radiological progression of PrePF, as well as the prognosis of IPF (164). Participants affected by the IPF variant rs35705950 showed better survival than those who were not (26).

For early asymptomatic f-IPF, the increased MUC5B levels can appear years before the symptoms of PF, and it can be detected in patients with mild PF (165); the variant can serve as an effective new molecular target for intervention during the early stages of IPF. The *MUC5B* promoter variant rs35705950 is predictive of PrePF; however, rs35705950 is present in approximately 19% of the population (117), and IPF rarely occurs (& LT, 0.1%) (166). Therefore, the observed differential expression of *MUC5B* in cases and controls cannot be fully explained (117), and additional biomarkers are needed to identify individuals with PrePF in high-risk populations.

Surfactant-related genes

Dysfunction and repair of alveolar epithelial cells are considered important components of the pathogenesis of IPF and childhood ILD (167). Nogee et al. first reported a case of nonspecific interstitial pneumonia with exon 4 and amino acid deletion at position 37, which was caused by mutations in the carboxy-terminal region of the gene encoding the surfactant protein of alveolar epithelial cells; the mother of the patient had desquamative interstitial pneumonia (168). F-IPF may also be present in children, accounting for a larger proportion of children with ILD (169).

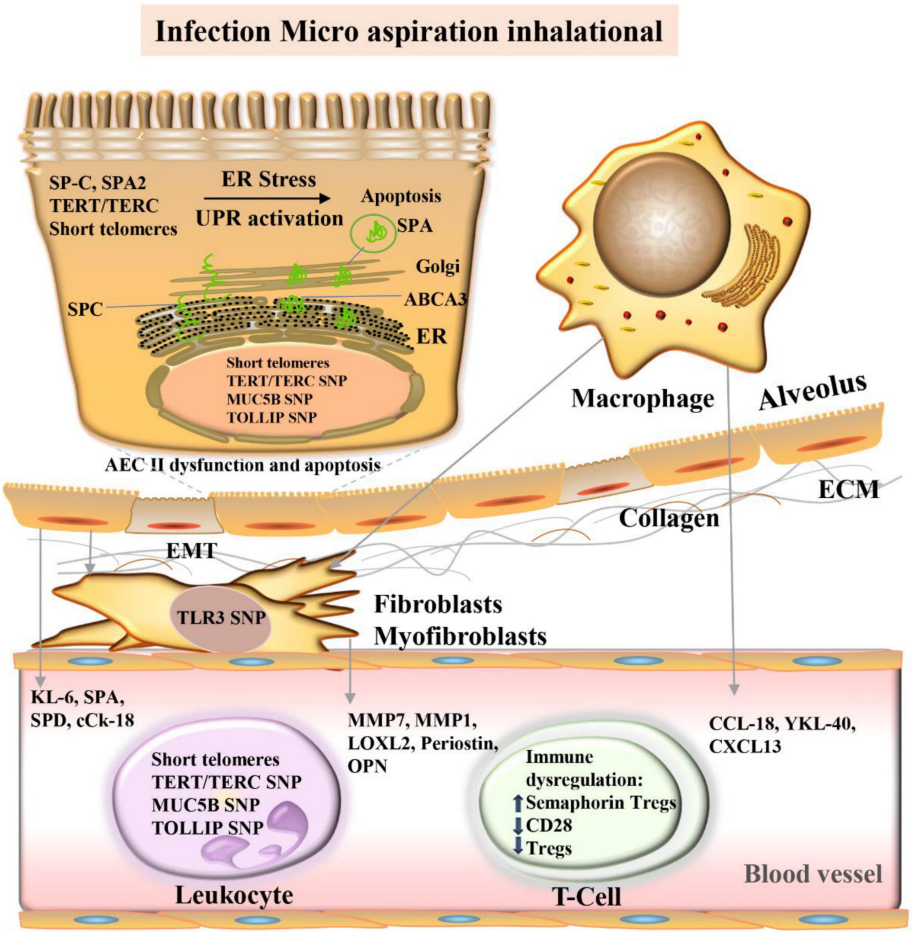

Fig 4. Core mechanisms and candidate molecular biomarkers of idiopathic pulmonary fibrosis.

The central role of AT2 cells in IPF is to generate pulmonary surfactant (170), and AT2 cells self-renew as progenitor cells and transdifferentiate into AT1 cells to maintain alveolar stability (171). Rare mutations in the components of the surfactant system, including surfactant-associated protein C (SP-C), SP-A, and ABCA3 lipid transporters, provide new clues for IPF caused by AT2 cell dysfunction (Fig. 4). More than 60 rare mutations in the SP-C gene (*SFTPC*) have been identified in affected children and adults combined (168), two mutations in the SP-A2 gene (*SFTPA2*), although present in less than 5% of s-IPF, and 150 mutations in *ABCA3* (172). Protein aggregation, ER stress, proinflammatory/profibrotic cytokine processing, altered macroautophagy, and apoptosis occur in model systems with *SFTPC*, *SFTPA*, and *ABCA3* mutations. In humans, they occur in lung and AT2 epithelial cells of patients with f-IPF and s-IPF alike (173).

Pictured are the alveolar lumen (top), epithelial cell layer (tan), interstitial space (white), and capillaries (bottom, pink). Damage to alveolar epithelial cells by multiple stimuli (blue boxes) leads to epithelial dysfunction, fibrosis/extracellular matrix (ECM) deposition, and immune dysregulation. Proposed molecular biomarkers for these processes include blood proteins, genomic markers, and blood cells (see light purple/light green boxes). *ABCA3*, ATP-binding cassette class A3 lipid transporter; AEC, Alveolar epithelial cells; cCK18, cleaved cytokeratin 18; CCL-18, CC chemokine ligand-18; CXCL, chemokine ligand; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; ER, endoplasmic reticulum; MMP, Matrix metalloproteinase; KL-6, Krebs von den Lungen-6; *LOXL2*, Lysyl oxidase-like 2; *MUC5B*, mucin 5B; SNP Single Nucleotide Polymorphism; OPN, osteopontin; SPA, surfactant protein A; SPA2, surfactant protein A2; SPC, surfactant protein C; SPD, surfactant protein D; TERT telomere RNA component; TERC, telomere reverse transcriptase; *TLR3*, Toll-like receptor 3; TOLLIP, Toll-interacting protein; Treg, regulatory T cells; UPR, unfolded protein response.

Rare mutations of surfactant system components (*SP-C*, *SP-A*, and *ABCA3*) cause stress and activate the unfolded protein response, AT2 dysfunction, and apoptosis, thereby promoting epithelial-mesenchymal transformation and the development of PF. ER, endoplasmic reticulum; TERT, telomere reverse transcriptase; TERC, telomere RNA component; MUC5B, Mucin 5B; SNP, single nucleotide polymorphism; TOLLIP, toll-interacting protein.

SP-C

SP-C is a highly hydrophobic protein present in lung surfactants and is produced by ER integration of membrane protein precursors in AT2. The gene encoding *SFTPC* is located on chromosome 8P and organized into six exons (encoded from I to V, with VI untranslated) and five introns, which produce an mRNA that encodes a 21-kDa proprotein (pro-SP-C 21) at either 191 or 197 amino acids. The pro-SP-C 21 COOH terminal, known as the BRICHOS domain (residue 90–197), is the carboxy-terminal region of the pro-SP-C proprotein. Most *SFTPC* mutations are located in the intrapeptide disulfide bond within the pro-SP-C COOH terminal (BRICHOS) domain (174). *SFTPC* BRICHOS mutants are retained by the ER, inhibit the proteasome, and activate the UPR (175), a set of protective biochemical pathways aimed at matching the protein capacity of the ER. The resulting cytokine processing, apoptosis, and functional disruption of progenitor cells lead to PF under AT2 and AEC lumen ER stress (176).

Although *SFTPC* mutations occur in less than 5% of patients with sporadic IPF, these rare mutants enhance our understanding of AT2 cell and AEC cavity biology in PF. Lawson et al. demonstrated evidence of UPR activation markers (including heavy-chain- binding protein (BiP), ER degradation enhancing α-mannosidase-like proteins (EDEM), and XBP1) in patients with f-IPF expressing the L188Q *SFTPC* mutation and samples from non-SFTPC f-IPF and s-IPF cases (177). Korfei et al. extended these findings by reporting molecular signatures of multiple UPR pathways and apoptosis in AT2 cells from patients with s-IPF and UIP pathologic features (178). These included activating transcription factor 6 (ATF6), ATF4, XBP1, C/EBP-homologous protein (CHOP), and caspase 3.

Chronic ER stress in the alveolar epithelium may represent a broad mechanism of ILD pathogenesis (125). In a transgenic mouse model, the diphtheria toxin receptor was expressed exclusively in AT2 cells, using the SFTPC promoter. The administration of the toxin induced AT2 cell death and increased lung collagen deposition, leading to spontaneous PF (179).

The missense substitution G.1286T>C, which replaces isoleucine at position 73 in the SP-C preprotein (pre-SP-C I73T) with threonine, is the most common SFTPC mutation associated with ILD (180). The deposition of pre-SP-C I73T in the plasma membrane and subsequent accumulation of misprocessed allotypes result in a dysfunctional cellular phenotype characterized by late macrophage arrest, impaired mitophagy, and defective cellular protein homeostasis (181). In the bleomycin-induced mouse model of PF, immunohistochemistry of pre-SP-C I73T confirmed that the proliferative cells in the fibrotic and tissue deformable areas are type II

AEC, similar to human UIP, and that the fibrotic areas in the AEC cavity are positive for the expression of pre-SP-C (182).

Furthermore, the expression of mutant SFTPC alveolar epithelial cells of mice drives the outbreak of a spontaneous FP. This demonstrates that AT2 cells can promote the creation of characteristic endophenotypes of IPF/UIP, including abnormal tissue remodeling to collagen deposition, AT2 cell proliferation, α-smooth muscle actin-positive cells, and limiting lung physiology (183). SP-C null mice are motile at birth and grow normally in the absence of a pulmonary phenotype (184); however, they show a higher sensitivity to bleomycin-induced fibrosis (185) and respiratory syncytial virus infection (186). When the original SP-C-null-mouse strain was backcrossed to a different genetic background, the resulting loss of SP-C was associated with spontaneous inflammation and lung remodeling (187), suggesting that SP-C deficiency is a disease regulator.

SP-A

SP-A, the most abundant surfactant-associated protein, is an intra-luminal multifunctional sialic acid glycoprotein. It has a molecular mass of 28–36 kDa and contains a COOH-terminal C-type lectin motif, triple helix collagen domain, and carbohydrate recognition domain (188). Mutations in *SFTPA*, encoding one of the two isomers of surfactant proteins, are associated with the heritability of f-IPF (mutations *SFTPA1* and *SFTPA2*, 4.5 kb long, each, and located on chromosome 10) (189). *In vivo*, the expression of disease-associated SFTPA1 mutations leads to AT2 UPR activation, necrotizing apoptosis, and IPF, which are associated with ER stress-induced c-Jun N-terminal kinase (JNK) signaling (190).

Takezaki et al. detected a homozygous mutation of the *SFTPA1* locus in two Japanese patients with f-IPF (190). These patients exhibited a significant decrease in downstream SP-A secretion and an increased susceptibility to the influenza virus, which accelerated the progression of IPF. The overexpression of receptor interacting serine/threonine kinase 3 (*RIPK3*) and mixed lineage kinase-like (*MLKL*) was detected in AT2 of *SFTPA1* homozygous knockout mice, but both *RIPK3* and *MLKL* could significantly improve the progression of IPF, suggesting that the *SFTPA1* homozygous mutant reduced *SP-A* production.

Furthermore, we found that the loss of *SFTPA1* leads to ER stress, which results in the overexpression of inositol requiring enzyme 1α (*IRE1a*) and increases *JNK* phosphorylation, thereby upregulating *RIPK3* expression. The inhibition of *IRE1a* and *JNK* significantly inhibited *RIPK3* expression and prevented the progression of PF. In contrast, these processes can be reversed by *RIPK3* overexpression; this finding supports the idea that family genetic history can increase the susceptibility of IPF to external stimuli, aggravate the damage caused by the fibrotic process of AT2, and mediate the aggravation of IPF. Thus, *RIPK3* may be an important target to treat IPF.

ABCA3

Clinical mutations in *ABCA3*, encoding for an ATP-dependent transporter that converts phosphatidylcholine and cholesterol into lysosome-associated organelles and is critical for lamellar formation, link lamellar body dysfunction to fibrotic lung disease. Human *ABCA3* has been mapped to chromosome 16p13.3 and encodes a protein of 1,704 amino acids (191). Although *ABCA3* mRNA has been detected in many tissues, its mRNA is highly expressed in AT2 cells (192). Homozygous *ABCA3* mutations lead to lamellar body deletion and neonatal respiratory failure in both humans and mice (193), indicating its importance in the production of pulmonary surfactants. Heterozygous *ABCA3* mutations exist with partial loss of function owing to ER retention (type I), improper functioning of the lipid pump (type II), or both (type I/II compound heterozygotes). These mutants act as genetic modifiers of pulmonary disease associated with *SFTPC* mutations in children (194).

The heterozygous variants, I73T *SFTPC* and D123N *ABCA*, increase disease penetrance, suggesting an interaction between these genotypes; however, the molecular mechanism between them remains unclear (195). In addition to the loss of function, several clinical *ABCA3* mutations are associated with PF, and cell phenotypes derived from *ABCA3* mutations are found in isotypes, ER retention, and misallocation homotypes based on protein behavior (167). However, the mechanistic links among *ABCA3* mutants, changes in cell quality control, and fibrosis require further investigation.

Conclusions

F-IPF is rarely identified in patients before disease progression, which limits our ability to study mechanisms. Identifying diagnostic and prognostic biomarkers and mechanisms helps to understand the pathogenesis of early f-IPF. Recent studies have increased the understanding of the underlying genetic susceptibility to PF and the pathogenesis of this disease. Genomic factors can also influence the development of f-IPF and the observed heterogeneity in pathogenicity. Genetically susceptible bronchoalveoli may sustain disproportionate damage at different time points owing to different environ-

mental exposures and can eventually manifest as PF.

A positive gene diagnosis may be significant for the early detection, prognosis, and risk assessment of close relatives, and genetic testing may be beneficial for patients with high FIP and positive gene probability (101). If these changes predict disease progression, they will provide the strongest evidence to date that there is a long but identifiable pre-symptomatic period during which targeted therapies can prevent the progression of PF. There are no standardized guidelines for the timing of genetic tests for patients with IPF (3); however, experts generally agree that individualized genetic testing should be performed case by case.

The relationship between the mechanisms of rare and common genetic variations in FIP is not fully understood; they are thought to act synergistically to modulate disease-related phenotypes, such as telomere shortening. In addition, when considering lung transplantation, a positive genetic diagnosis in patients with f-IPF helps predict disease course and assess the risks. Further research should be aimed at revealing the underlying and pathogenic genetic factors and epigenetic changes in patients with f-IPF to improve the usefulness of precision therapies.

Abbreviations

ABCA: adenosine triphosphate binding box subfamily A3

AT2: alveolar type II

ATF: activating transcription factor 6

BRICHO: bond within the pro-SP-C COOH terminal

CHO: C/EBP-homologous protein

DC: dyskeratosis congenita

DDR: DNA damage response

DKC1: dyskeratosis congenita 1

EDEM: ER degradation-enhancing alpha-mannosidase-like proteins

ER: endoplasmic reticulum

f-IPF: familial idiopathic pulmonary fibrosis

ILD: interstitial lung disease

IIP: idiopathic interstitial pneumonia

IRE1α: inositol requiring enzyme 1α

JNK: c-Jun N-terminal kinase

MLKL: mixed lineage kinase-like

MMP7: matrix metalloproteinase 7

MUC5B: mucin 5B

NAF1: nuclear assembly factor 1

PARN: poly(A)-specific ribonuclease

PBMCs: peripheral blood mononuclear cells

PrePF: preclinical PF

RIPK3: receptor interacting serine/threonine kinase 3

RTEL1: regulator of telomere length 1

snoRNAs: small nucleolar RNAs

SNP: single nucleotide polymorphism

SFTPA2: surface-active protein A2

SFTPC: surface-active protein C

s-IPF: sporadic IPF

SP-A: surfactant protein A

SP-C: surfactant-associated protein C

TERC: telomerase RNA component

TERT: telomerase reverse transcriptase

TINF2: telomere repeat binding factor 1-interacting nuclear factor 2

TPP1: tripeptidyl-peptidase 1

TRG: T cell receptor gamma locus

UIP: usual interstitial pneumonia

UPR: unfolded protein response

ZCCHC8: zinc finger CCHC-type containing 8

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Competing Interests

The authors have declared that no competing interest exists.

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