

Genetic studies provide clues on the pathogenesis of idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a progressive and often fatal lung disease for which there is no known treatment. Although the traditional paradigm of IPF pathogenesis emphasized chronic inflammation as the primary driver of fibrotic remodeling, more recent insights have challenged this view. Linkage analysis and candidate gene approaches have identified four genes that cause the inherited form of IPF, familial interstitial pneumonia (FIP). These four genes encode two surfactant proteins, surfactant protein C (encoded by *SFTPC*) and surfactant protein A2 (*SFTPA2*), and two components of the telomerase complex, telomerase reverse transcriptase (*TERT*) and the RNA component of telomerase (*TERC*). In this review, we discuss how investigating these mutations, as well as genetic variants identified in other inherited disorders associated with pulmonary fibrosis, are providing new insights into the pathogenesis of common idiopathic interstitial lung diseases, particularly IPF. Studies in this area have highlighted key roles for epithelial cell injury and dysfunction in the development of lung fibrosis. In addition, genetic approaches have uncovered the importance of several processes – including endoplasmic reticulum stress and the unfolded protein response, DNA-damage and -repair pathways, and cellular senescence – that might provide new therapeutic targets in fibrotic lung diseases.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease of unknown cause that typically leads to respiratory failure and death within 3-5 years of diagnosis. Cough, dyspnea and hypoxemia, restrictive pulmonary function tests with a diminished diffusing capacity for carbon monoxide (DLCO; see Box 1 for a glossary of clinical terms), and basilar predominant interstitial changes with honeycombing comprise the clinical syndrome of IPF (King et al., 2011). The histological correlate of IPF, usual interstitial pneumonia (UIP), is characterized by temporally heterogeneous lung architectural distortion and dense collagen and extracellular matrix (ECM)

deposition in the interstitium, alveolar collapse, and the presence of fibroblastic foci. Chronic inflammation is typically present but is not a prominent feature of UIP. 'Acute exacerbations' of IPF cause significant morbidity and mortality, and are not yet well understood. Despite decades of research, today there are no effective pharmacological therapies for IPF.

For many years it has been recognized that some cases of IPF seem to be inherited (Table 1). Early reports suggested that less than 5% of patients with IPF have a first-degree relative with the disease (Marshall et al., 2000; Hodgson et al., 2002). More recently, several groups have suggested that this is an underestimate and that up to 20%

of cases of IPF might be familial (Loyd, 2003; García-Sancho et al., 2011). This syndrome of heritable interstitial lung disease is called familial interstitial pneumonia (FIP), and has been the focus of intense research into the pathogenesis of IPF. A breakthrough occurred in 2001, when Noguee and colleagues identified a mutation in *SFTPC*, the gene encoding surfactant protein C (SP-C), in a mother and child with FIP (Noguee et al., 2001). Subsequently, other groups identified additional FIP-associated mutations in *SFTPC* (Thomas et al., 2002; van Moorsel et al., 2010; Ono et al., 2011) and *SFTPA2* [encoding surfactant protein A2 (SP-A2)] (Wang et al., 2009). *SFTPC* and *SFTPA2* are expressed exclusively by type II alveolar epithelial cells (AECs) in the lungs, suggesting that AEC dysfunction is a feature of IPF (see Box 2 for a summary of cell types involved in IPF). In 2007, two independent groups (Armanios et al., 2007; Tsakiri et al., 2007) studying FIP kindreds described disease-causing heterozygous mutations in *TERT* (encoding telomerase reverse transcriptase) and *TERC* (encoding the RNA component of the telomerase complex). Notably, pulmonary fibrosis has also been recognized as a feature of certain multisystem inherited diseases, including dyskeratosis congenita (DC; also caused by mutations in components of the telomere maintenance pathway; see below) (Armanios, 2012). It now appears that ~10-15% of FIP cases are caused by mutations in the telomerase pathway, in the absence of classical manifestations of DC. Interestingly, short telomeres are frequently found in individuals with sporadic IPF (Alder et al., 2008), suggesting that telomere dysfunction is a common feature of fibrotic lung disease even in the absence of telomerase mutations.

Subsequent work exploring the effects of these genetic mutations has implicated several unanticipated pathways in IPF pathogenesis, including endoplasmic reticulum (ER) stress and the unfolded protein response (UPR), cellular senescence, the DNA-damage response, and potentially

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Case study

A 53-year-old man with obstructive sleep apnea and gastroesophageal reflux disease presented to a pulmonary clinic with a 6-month history of progressive exertional dyspnea and non-productive cough. He had been treated for community-acquired pneumonia in the preceding months without improvement of his symptoms. He had retired following a 20-year career in the military and denied exposure to asbestos, silica or other particulates. He reported a 30 pack/year history of cigarette smoking but had been abstinent for 9 years. His family history was remarkable for two sisters with idiopathic pulmonary fibrosis (IPF) diagnosed at age 46 and 52. Another brother was deceased from complications of myelodysplasia, and a niece had died from aplastic anemia at age 24. His oxygen saturation was 97% breathing ambient air. There were faint bilateral inspiratory crackles and mild clubbing. Pulmonary function tests showed a mild restrictive defect with a moderately reduced diffusing capacity for carbon monoxide (DLCO). High-resolution CT of the chest showed scattered areas of basilar predominant peripheral interstitial prominence with several small areas of honeycomb change. Serological screening for connective tissue disease was negative. A prominent family history of pulmonary fibrosis coupled with his clinical course and radiographic pattern suggested he most likely had familial interstitial pneumonia (FIP), the inherited form of IPF. Genetic sequencing identified a heterozygous mutation in telomerase reverse transcriptase (*TERT*) that was subsequently confirmed in other affected family members.

Wnt- β -catenin signaling. As we discuss in this review, these pathways seem to converge in epithelial cells, suggesting that epithelial cell injury and dysfunction are crucial in the evolution of lung fibrosis. Clarifying the role of these pathways in epithelial cells should help to answer some of the many outstanding questions related to IPF.

ER stress and IPF Early genetic clues: surfactant protein mutations

Surfactant proteins have long been recognized as crucial in establishing and maintaining lung alveolar structure and function. Surfactant therapy has been a mainstay of treatment of neonatal respiratory distress syndrome for decades, and deficiency of surfactant protein B (SP-B) (Nogee et al., 1994) or SP-C (Amin et al., 2001) has been described as a cause of pediatric interstitial lung disease with high morbidity and mortality in childhood. In 2002, our group reported heterozygous inheritance of an *SFTPC* mutation (L188Q) that was associated with interstitial lung disease in 14 individuals from a single large FIP kindred (Thomas et al., 2002). Affected individuals in this cohort showed abnormal localization of SP-C pro-peptide (see below), which was distributed diffusely in the cytoplasm of atypical-appearing type II AECs. Transfection of L188Q *SFTPC* into mouse lung epithelial cells reduced cellular proliferation and enhanced cytotoxicity in vitro, suggesting that the deleterious effect of mutant SP-C was mediated through a gain-of-function mechanism. Subsequent work indicated that ER stress with activation of the UPR was probably a key mechanism

in interstitial lung disease associated with *SFTPC* mutations (reviewed by Tanjore et al., 2012).

SFTPC mutations cause ER stress

SP-C is a small secreted hydrophobic protein produced exclusively by type II AECs. SP-C is translated as a 21 kDa pro-peptide and requires the C-terminus for initial folding steps in the ER before undergoing multiple proteolytic cleavages prior to secretion of the highly hydrophobic mature peptide. Truncated forms of pro-SP-C lacking the C-terminus, including the Δ exon4 variant originally described by Nogee and colleagues (Nogee et al., 2001), fail to undergo appropriate proteolytic cleavage for targeting to cytoplasmic vesicles and remain in the ER (Beers et al., 1998; Wang et al., 2003). In

addition, some mutations in the C-terminus of pro-SP-C interfere with disulfide bond formation, resulting in protein misfolding and aggregation in the ER (Kabore et al., 2001). These C-terminal mutations lie in the BRICHOS domain of *SFTPC*. BRICHOS domains are conserved across various proteins that have diverse expression patterns and functions, and typically facilitate the appropriate folding of secreted proteins. BRICHOS domain mutations have been reported in other disease-associated genes; for example, mutations of this domain in β -amyloid precursor protein lead to improper protein folding, accumulation of misfolded protein in the ER and activation of the UPR, and might contribute to the pathogenesis of Alzheimer's dementia (Willander et al., 2011). Further work on *SFTPC* mutations demonstrated that expression of mutant *SFTPC* in a human lung epithelial cell line (A549 cells) led to increased expression of the ER chaperone protein glucose related peptide-78 (GRP78; also known as Bip) and X-box-protein 1 (XBP-1), a transcription factor that is upregulated in response to ER stress (Mulugeta et al., 2005). Cells expressing mutant *SFTPC* also had increased activation of caspase-4, an ER-specific caspase that triggers apoptosis in the setting of overwhelming ER stress (Mulugeta et al., 2005).

ER stress is not exclusive to familial IPF

Although in vitro evidence suggested that ER stress was an important mechanism in FIP

Box 1. Clinical terms

Acute exacerbation: subacute to acute worsening of symptoms in IPF characterized by an increase in oxygen requirement, ground glass opacities on radiography and absence of a recognized infectious etiology.

Clubbing: fingernail deformities seen in chronic pulmonary disease and other diseases that frequently develop in the setting of chronic hypoxia.

Crackles: adventitial lung sounds (also known as rales), typically exhibiting a 'dry' quality in patients with pulmonary fibrosis.

Diffusing capacity for carbon monoxide (DLCO): a measure of gas exchange in the lung that quantifies the extent to which carbon monoxide passes from the alveoli into the blood; reduced DLCO is associated with impaired oxygen delivery owing to pulmonary or cardiac insufficiency.

Dyskeratosis congenita (DC): inherited syndrome characterized by skin hyperpigmentation, nail dystrophy, oral leukoplakia and bone marrow abnormalities; caused by mutations in components of the telomerase complex.

Familial interstitial pneumonia (FIP): the inherited form of interstitial lung disease, typically with an autosomal dominant inheritance pattern and incomplete penetrance.

Idiopathic pulmonary fibrosis (IPF): the most common of the idiopathic interstitial pneumonias, commonly leading to respiratory failure and death within 3-5 years of diagnosis.

Usual interstitial pneumonia (UIP): the histological correlate of IPF characterized by temporally heterogeneous architectural distortion, and collagen and matrix deposition, with expansion of the inter-alveolar interstitium and fibroblastic foci.

Table 1. IPF-associated genes

Disease	Gene	% cases associated with mutation	Reference
Sporadic IPF (80% of IPF cases)	Unknown	–	–
FIP (20% of IPF cases)	<i>TERT</i>	8-15	Armanios et al., 2007; Tsakiri et al., 2007
	<i>TERC</i>	<1	Armanios et al., 2007; Tsakiri et al., 2007
	<i>SFTPC</i>	2-25	Thomas et al., 2002; van Moorsel et al., 2010
	<i>SFTPA2</i>	<1	Wang et al., 2009
	Unknown	75-80	–

caused by *SFTPC* mutations, a further advance occurred in 2008 when our group and others demonstrated evidence of increased ER stress in non-*SFTPC*-related interstitial lung disease. We found that expression of Bip and XBP-1 in hyperplastic type II AECs was common in lung tissue in both familial and sporadic IPF (Lawson et al., 2008). Furthermore, by comparing lung samples from patients with IPF to those of patients with chronic obstructive pulmonary disease (COPD) and healthy controls, Korfei and colleagues found that IPF was associated with increased expression of both activating transcription factor-6 (ATF-6), which increases expression of ER chaperone proteins, and C/EBP homologous protein (CHOP), a transcription factor that mediates ER-stress induced apoptosis (Korfei et al., 2008). Although observational, these striking findings suggest that activation of the ER stress response is a key general mechanism of IPF pathogenesis.

Other sources of ER stress in IPF

Recently, one FIP kindred was found to carry a heterozygous mutation in the gene encoding SP-A2 (*SFTPA2*) (Wang et al., 2009) that results in ER stress (Maitra et al., 2010), although the mechanism is not yet completely characterized. In addition to inherited mutations in *SFTPC* and *SFTPA2*, several other IPF risk factors are known to increase ER stress. Herpesviruses are found nearly ubiquitously in IPF lungs (Tang et al., 2003) and might contribute to IPF pathogenesis through multiple mechanisms (Kropski et al., 2012). For example, herpesviridae are known to activate the UPR (Isler et al., 2005), and herpesvirus antigens colocalize with ER stress markers in type II AECs in IPF lungs (Lawson et al., 2008). In a mouse model of aging (>18 months), infection with a murine herpesvirus led to upregulation of multiple components of the ER stress pathway and was accompanied by the development of lung fibrosis (Torres-González et al., 2012).

Additionally, tobacco smoking is a recognized risk factor for IPF, and cigarette smoke extracts have been shown to activate multiple arms of the UPR pathway (Hengstermann and Müller, 2008; Geraghty et al., 2011; Zhao et al., 2011).

ER stress and IPF pathogenesis

Although there is solid evidence associating ER stress with IPF, further work is needed to clarify the principal mechanisms involved. A thorough review of ER stress in IPF pathogenesis is available elsewhere (Tanjore et al., 2012) and is beyond the scope of this manuscript; however, several potential mechanisms warrant comment. First, it has been shown that antecedent ER stress worsens fibrosis in the commonly used bleomycin mouse model. We showed that induction of ER stress by expression of mutant human L188Q *SFTPC* in type II AECs or by tunicamycin administration led to fibrotic remodeling in response to low-dose bleomycin that was more severe than in mice without ER stress (Lawson et al., 2011). In this study, L188Q *SFTPC* mice showed an increased number of TUNEL-positive type II AECs and increased expression of caspase-3 following bleomycin exposure, suggesting that ER-stress-mediated induction of AEC apoptosis contributes to the development of fibrosis.

Second, increased ER stress might underlie pathological changes in cellular phenotype and function in IPF. Interactions between epithelial cells and mesenchymal cells (including fibroblasts) are thought to contribute to IPF pathogenesis. Myofibroblasts (mesenchymal cells with features of both fibroblasts and smooth muscle cells) are activated in IPF and are thought to be prominent sources of collagen and ECM, which contribute to fibrotic remodeling in the lung (Chapman, 2011) (see Box 2). Although there is some controversy surrounding the issue (Rock et al., 2011), most evidence suggests that epithelial-mesenchymal transition (EMT), whereby

epithelial cells acquire functional and phenotypic characteristics of mesenchymal cells, occurs in IPF lungs (Willis et al., 2005; Marmai et al., 2011). However, the role of EMT in IPF pathogenesis remains incompletely understood, and is an area of intense ongoing study (reviewed by Chapman, 2011). Thus far, it has been shown that ER stress can contribute to EMT in vitro (Tanjore et al., 2012). For example, following induction of ER stress by tunicamycin or thapsigargin, AECs undergo morphological changes, and show increased alpha smooth muscle actin (α -SMA) and decreased E-cadherin expression, consistent with a shift to a mesenchymal-like phenotype (Zhong et al., 2011). Overexpression of mutant *SFTPC* in lung epithelial cell lines largely recapitulated these effects (Zhong et al., 2011). We reported similar findings following induction of ER stress by overexpression of L188Q *SFTPC* (Tanjore et al., 2011). In this study, ER-stress-induced EMT could be abrogated by inhibition of Smad2/3 or Src. Together, these studies suggest that reducing ER stress might represent a novel therapeutic approach to inhibiting EMT and fibrogenesis, although clarification of the role of EMT-derived fibroblasts, and the relative contribution of ER-stress-induced EMT, in IPF is needed.

Finally, the role of ER stress and immune responses in the lung remains underexplored. Activation of the UPR has been shown to decrease the expression of major histocompatibility complex I (MHC-I) in various epithelial cell types (de Almeida et al., 2007; Ulianich et al., 2011) and to alter the expression of numerous immune regulatory genes (Bartoszewski et al., 2011). Furthermore, ER stress caused by mutant SP-C enhanced susceptibility to virus-induced cell death and was associated with impaired proteasome function (Bridges et al., 2006). Thus, local dysregulation of immune responses in the alveolus might heighten susceptibility to recurrent epithelial cell injury and contribute to IPF pathogenesis.

Box 2. Cell types involved in IPF pathogenesis

Type II alveolar epithelial cells (AECs): epithelial cells residing in the 'corners' of alveoli; responsible for the production of surfactant proteins; thought to serve as progenitors of type I AECs, which make up the respiratory gas exchange surface.

Fibroblasts: mesenchymal cells that reside in the pulmonary interstitium and produce collagen and extracellular matrix (ECM).

Myofibroblasts: contractile mesenchymal cells expressing alpha-smooth muscle actin; it is thought that these cells have an activated phenotype, and contribute to exuberant collagen and ECM production in IPF.

Beyond ER stress

Although there is compelling evidence that ER stress plays a role in IPF pathogenesis, it is also clear that other factors must be involved. Induction of ER stress by tunicamycin or by expression of L188Q *SFTPC* does not lead to spontaneous lung fibrosis in mice (Lawson et al., 2011). Furthermore, I73T *SFTPC*, the most commonly described *SFTPC* mutation, does not seem to directly induce ER stress (Mulugeta et al., 2005). In one FIP kindred carrying the I73T *SFTPC* mutation, a genetic variant in ATP-binding cassette A3 (*ABCA3*), which encodes a molecule involved in intracellular trafficking and is linked to pediatric interstitial lung disease (Bullard et al., 2005), seemed to affect disease penetrance (Crossno et al., 2010). Vesicular trafficking defects have also been implicated in Hermansky-Pudlak syndrome (HPS; discussed below), a genetic disease in which pulmonary fibrosis is also a feature (Gahl et al., 1998; Di Pietro and Dell'Angelica, 2005). Therefore, the impact of these trafficking pathways in AEC dysfunction warrants further investigation in adult fibrotic lung disease.

Telomeres and IPF

For years, pulmonary fibrosis has been recognized in some patients with DC. DC is an inherited disorder classically characterized by skin hyperpigmentation, nail dystrophy and oral leukoplakia, along with aplastic anemia. The most prominent manifestations of DC affect rapidly dividing tissues, including epithelial cells and bone marrow. DC is most commonly caused by mutations in dyskerin (*DKC1*), a component of the telomerase complex, but mutations in genes encoding other members of the telomerase complex (including *TERT* and *TERC*) have also been reported (Calado and Young, 2009). Pulmonary fibrosis occurs in ~20% of individuals with DC (Armanios, 2012).

In a cohort of 73 kindreds with FIP who had no manifestations of DC, 8% were found

to harbor heterozygous mutations in *TERT* or *TERC*, which encodes the RNA component of the telomerase complex, and these mutations followed an autosomal dominant inheritance pattern (Armanios et al., 2007). Mutation carriers had short telomeres when adjusted for age, suggesting that the mutations resulted in functional deficits in telomere maintenance. Another group also identified *TERT* or *TERC* mutations in 7 of 44 FIP families, although in this cohort some family members had other features of DC (Tsakiri et al., 2007). Together, these studies indicate that isolated interstitial lung disease can be caused by mutations in the same genes as are associated with DC, and that *TERT* and *TERC* mutations are the most common cause of FIP identified to date. Furthermore, because many of the manifestations of DC reflect aberrant epithelial cell function, the discovery of telomerase mutations in FIP points to the importance of the alveolar epithelium in disease pathogenesis.

Additional evidence implicating telomere dysfunction in IPF came from the recognition that short telomeres are not exclusive to patients with telomerase-mutation-associated FIP. In a study of individuals with idiopathic interstitial lung disease, a striking 97% of subjects had telomeres that were shorter than the median length of telomeres in age- and sex-matched controls (Alder et al., 2008). Telomere length was similar in sporadic IPF subjects and carriers of DC-associated mutations and, in both groups, telomeres were significantly shorter than those in normal subjects. Using a more stringent definition of short telomeres (<10th percentile for age and sex), another group found that 25% of sporadic IPF subjects and 24% of FIP cases not associated with *TERT* or *TERC* mutations had short telomeres (Cronkhite et al., 2008). In the same study, this group also reported that all subjects who carried a mutation in *TERT* or *TERC* and had pulmonary fibrosis had telomere lengths below the 10th percentile.

Short telomeres have been associated with subclinical pulmonary fibrosis as evidenced by a low DLCO and radiographic changes in a cohort of asymptomatic *TERT* mutation carriers (Diaz de Leon et al., 2011). Finally, short telomeres and decreased dyskerin expression were found in an FIP kindred without a detectable coding mutation in *TERT*, *TERC*, *DKC1* or other known genes causing DC, suggesting that regulation of components of the telomerase complex might be another mechanism causing short telomeres in patients with lung fibrosis (Parry et al., 2011).

From short telomeres to epithelial cell dysfunction

Compared with *SFTPC* mutations, the mechanism by which telomerase dysfunction and *TERT* and *TERC* mutations lead to lung fibrosis is less well characterized; however, several lines of evidence again implicate epithelial cell dysfunction as a key feature of lung fibrosis in this setting. Specifically, defects in telomere maintenance have been linked to epithelial cell senescence and an impaired response to epithelial injury (Armanios and Blackburn, 2012).

The telomerase complex is a multi-component polymerase that adds six-nucleotide telomere repeats to the ends of chromosomes; an important function of these telomere 'caps' is to protect chromosomes from deterioration. During successive cycles of cell division, telomere shortening occurs owing to incomplete replication of telomere caps on the ends of chromosomes. When telomeres shorten to reach a critical threshold (known as the Hayflick limit), they can be sensed as double-stranded DNA breaks, triggering a DNA-damage response. Activation of DNA-damage pathways can then lead to apoptosis or growth arrest (senescence) (Armanios et al., 2007). Although in certain circumstances cellular senescence is probably an adaptive response (Minagawa et al., 2011), premature senescence can impair normal lung epithelial homeostasis and injury response mechanisms. Notably, loss of telomerase activity diminishes the plasticity and proliferative capacity of stem cell populations (Batista et al., 2011), and has been implicated in syndromes of accelerated aging (Armanios, 2012).

There is evidence of increased epithelial cell senescence in IPF. Minagawa and colleagues demonstrated extensive β -

galactosidase staining (a marker of senescence) in epithelial cells lining fibroblast foci, suggesting a potential pathogenic role of senescent epithelial cells in IPF progression (Minagawa et al., 2011). Interestingly, in vitro and in vivo, after exposure to bleomycin there is increased β -galactosidase activity that is associated with increased expression of p21, which promotes cell cycle arrest (Aoshiba et al., 2003). Furthermore, bleomycin exposure seems to induce a biphasic response in telomerase expression: in A549 cells exposed to bleomycin, both *TERT* mRNA expression and telomerase activity increase after 24 hours, but by 72 hours both *TERT* expression and telomerase activity are decreased compared with untreated cells (Fridlender et al., 2007). A decrease in telomerase activity was also found in bleomycin-treated mice at 7 and 14 days after exposure, and was associated with increased epithelial cell apoptosis.

Modeling telomerase deficiency

Several groups have attempted to develop lung fibrosis models in mice by using telomerase deficiency. Through successive generations, *Tert*- or *Terc*-null mice have shortened lifespans, weight loss and impaired responses to injurious stimuli (Rudolph et al., 1999; Liu et al., 2007; Lee et al., 2009). There is some evidence suggesting that *Tert* or *Terc* haploinsufficiency can result in shortened telomeres and manifestations that are similar to those seen in null mice. By breeding *Tert* heterozygous mice through successive generations, progressive telomere shortening was observed in parallel with that seen in *Tert*-null mice (Strong et al., 2011). Furthermore, *Tert*^{+/-} mice manifested dysplastic changes in bone marrow and intestinal villous atrophy; these defects were similar to, but less severe than, those observed in *Tert*^{-/-} mice, suggesting that telomere length, as opposed to enzyme activity, was mainly responsible for the observed phenotype.

Telomerase deficiency has been reported to cause a decrease in lung architectural complexity and impaired regenerative capacity after partial pneumonectomy. In fourth generation (F4) *Terc*-null mice, alveolar size was reportedly increased, which was associated with thinning of alveolar walls and decreased numbers of type II AECs (Lee et al., 2009). AECs isolated from *Terc*-null mice had evidence of DNA damage and activation of pro-apoptotic pathways. This

group also showed that the proliferation of type II AECs was impaired 3-7 days after partial pneumonectomy in *Terc*-null mice (Jackson et al., 2011). Given these findings, it was anticipated that telomerase-deficient mice would have more severe lung injury and fibrosis than wild-type mice in response to profibrotic stimuli. However, results thus far have been inconclusive. In contrast to what might have been expected based on findings in humans, Liu and colleagues reported that early generation (F1-F3) *Tert*-null mice were protected from bleomycin-induced lung fibrosis and showed a less proliferative fibroblast phenotype than wild types (Liu et al., 2007). However, using early (F1) or late (F4-F6) generation *Tert*- or *Terc*-null mice, our group did not detect any impact of telomerase deficiency on lung fibrosis after single-dose or repetitive bleomycin exposure (Degryse et al., 2012). The two groups employed similar mouse models and methodology, so it is not clear what led to these contradictory findings. Regardless, it is evident that deletion of *Tert* or *Terc* in mice does not cause spontaneous lung fibrosis, and the mice do not display a consistent and robust lung fibrosis phenotype following bleomycin exposure. There are several potential reasons for these unexpected findings. One possibility is that mouse telomeres are substantially longer than human telomeres, and that other organ systems in mice might suffer life-limiting complications before telomeres in lung epithelial cells are sufficiently short to cause or exacerbate fibrosis in late generation telomerase-null mice. In addition, bleomycin-induced fibrosis might not be the best model to study lung fibrosis in the setting of telomerase deficiency. Further investigations are needed to clarify the role of telomere maintenance and cellular senescence in lung fibrosis.

Beyond telomeres

One impact of short telomeres in cells is the activation of DNA-damage pathways. Because other sources of DNA damage – including radiation and various chemotherapeutic agents – are known to cause lung fibrosis, defects in DNA repair might play a role in IPF pathogenesis. Interestingly, there are reports of lung fibrosis developing in pediatric patients with ataxia telangiectasia (Schroeder et al., 2005) and other disorders caused by deficiencies in DNA-repair pathways (Vece et al., 2012).

Although TERT and TERC complex to act primarily as a polymerase, it has recently been recognized that TERT also carries out a non-telomeric function as a transcriptional regulator. For example, one recent report demonstrated that telomerase-deficient mice have marked metabolic abnormalities owing to downregulated expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha and beta (PGC-1 α and PGC-1 β) (Sahin et al., 2011). Another intriguing target of TERT regulation is the Wnt- β -catenin pathway. Several mechanisms of IPF pathogenesis converge on developmental pathways, particularly the Wnt- β -catenin signaling cascade. Activation of the Wnt- β -catenin pathway in human IPF samples was first demonstrated in 2003 (Chilosi et al., 2003) and, subsequently, two more reports have implicated this pathway in the pathogenesis of IPF (Königshoff et al., 2008; Königshoff et al., 2009). Transforming growth factor- β (TGF β) signaling and activation of the Wnt- β -catenin pathway promote EMT (Zhou et al., 2012) as well as myofibroblast differentiation (Carthy et al., 2011) in vitro. Furthermore, co-administration of siRNA directed against β -catenin decreased bleomycin-induced lung fibrosis in mice (Kim et al., 2011). Although not yet studied specifically in the context of IPF, there seems to be a bidirectional interaction between TERT and Wnt- β -catenin signaling. For example, in mouse embryonic stem cells and Wnt reporter mice, TERT can act as a transcriptional activator of Wnt signaling by complexing with β -catenin (Park et al., 2009). In embryonic stem cells, *TERT* expression is significantly decreased in the absence of β -catenin, and overexpression of β -catenin increases *TERT* expression and lengthens telomeres (Hoffmeyer et al., 2012). Future studies will hopefully clarify the role of this pathway in lung injury response and fibrotic remodeling.

Role of a common genetic variant: Muc5B

In 2011, a large genome-wide linkage study of IPF patients identified a common polymorphism in the promoter of the gene encoding a mucin (*MUC5B*) that was associated with a 20-fold increased risk of IPF in subjects that were homozygous for the polymorphism and a 7-fold increased risk in heterozygous subjects (Seibold et al., 2011). At least one copy of the promoter polymorphism was present in 34-38% of IPF

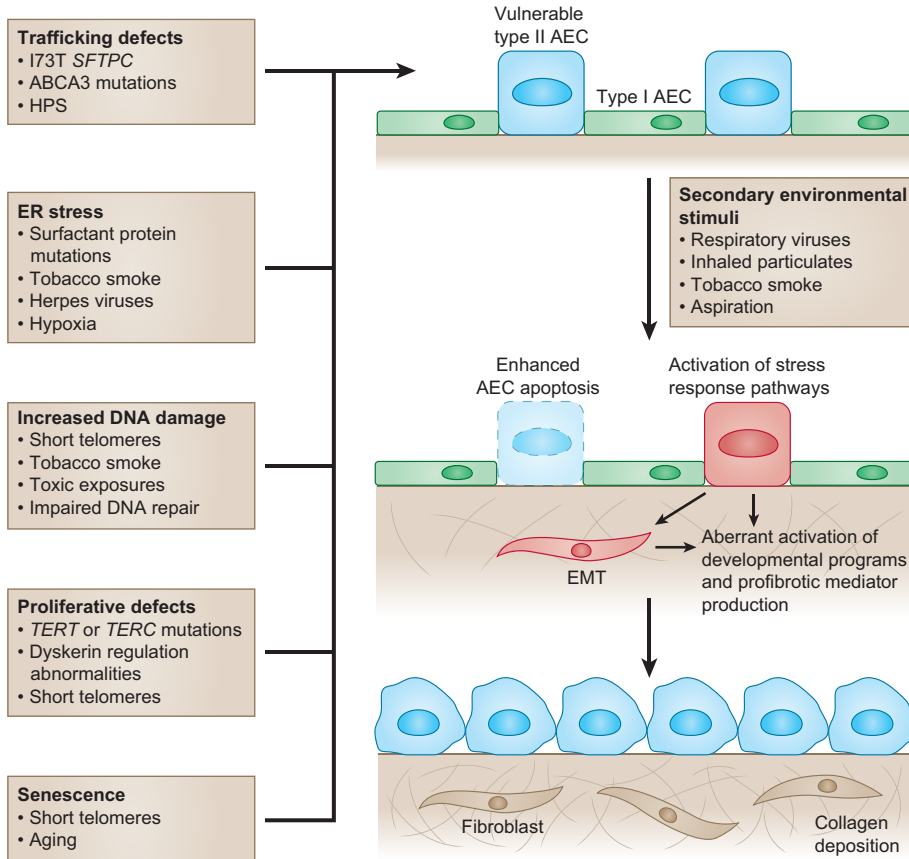


Fig. 1. Proposed events underlying IPF pathogenesis. Various genetic factors and environmental exposures act through pathways that converge to produce a ‘vulnerable’ alveolar epithelial cell (AEC) population. Exposure of vulnerable type II AECs to secondary recurrent or persistent injurious stimuli leads to enhanced AEC apoptosis, as well as aberrant activation of developmental programs and stress response pathways, including epithelial-mesenchymal transition (EMT). Ongoing type II AEC dysfunction impairs normal injury-response mechanisms, leading to scar formation and progressive loss of lung architectural complexity. Over time, this culminates in progressive fibrotic remodeling and clinically evident IPF. HPS, Hermansky-Pudlak syndrome.

subjects compared with 9% of healthy controls, and the polymorphism was shown to lead to markedly increased MUC5B expression in the lung. This robust association was observed in both familial and sporadic IPF cases, and confirmed in an independent cohort (Zhang et al., 2011). Interestingly, the same *MUC5B* promoter polymorphism was not associated with interstitial lung disease in scleroderma patients (Peljto et al., 2012), suggesting a specific role for this promoter polymorphism in IPF pathogenesis. It should be noted that, as is typical of common polymorphisms associated with rare diseases, the *MUC5B* promoter polymorphism has minimal positive predictive value for the future development of IPF in the general population. However, this polymorphism should be considered a risk factor for IPF, and there is active investigation into the mechanisms by which MUC5B influences lung fibrosis. Because mucins play a role in barrier function and innate immunity (Parker and Prince, 2011; Plantier et al., 2011), immune dysregulation might be one mechanism by which abnormal mucin expression contributes to IPF pathogenesis.

Abnormal expression of MUC5B in distal airway epithelial cells of IPF lungs has been reported, suggesting that ectopic expression of MUC5B also plays a role in the development of IPF (Plantier et al., 2011). Animal models of this polymorphism are in development and should soon offer new insights into the role of MUC5B in IPF. In addition, a large genome-wide association study (GWAS) is ongoing and offers hope of identifying additional common genetic variants associated with IPF.

Clues about mechanisms of pulmonary fibrosis from HPS

Interstitial lung disease can occur in various multisystem genetic disorders, including HPS, neurofibromatosis, Gaucher’s disease and Niemann-Pick disease. Of these disorders, the features of HPS share the most similarity with those of FIP and IPF; therefore, studies of HPS might offer insight into the fundamental pathways underlying lung fibrosis. HPS is characterized by ocular albinism, platelet dysfunction, colitis and lung fibrosis (Gahl et al., 1998; Brantly et al., 2000; Gochuico et al., 2012). Nine genetic loci have been associated with HPS in humans,

and pulmonary fibrosis has been associated with only some genotypes, including HPS1, HPS2 and HPS4 (Gahl et al., 1998; Brantly et al., 2000; Shotelersuk et al., 2000; Huizing et al., 2009; Cullinane et al., 2011; Gochuico et al., 2012). HPS2 is caused by autosomal recessive mutations in the β -subunit of adaptor protein-3 (AP3 β 1), which is involved in intracellular trafficking of secretory products and lysosome-related organelles. Mouse models of HPS1 and HPS2 have AECs with abnormal appearance, constitutive activation of lung macrophages (Young et al., 2006) and basal lung inflammation (Atochina-Vasserman et al., 2011). Interestingly, following bleomycin exposure, HPS mice have increased AEC apoptosis and lung fibrosis, but no significant differences in the number of inflammatory cells in bronchoalveolar lavage fluid, compared with wild-type mice (Young et al., 2007), suggesting that defective epithelial injury-repair mechanisms underlie repair pathology. Using a bone marrow transplantation approach, our group recently demonstrated that both constitutive activation of alveolar macrophages and increased susceptibility to bleomycin-

induced fibrosis in HPS mice were conferred by the genotype of the stromal cells, rather than the bone-marrow-derived cells, in the lungs. Furthermore, transgenic epithelial-specific correction of the genetic defect in HPS2 mice attenuated alveolar epithelial apoptosis, macrophage activation and susceptibility to fibrosis (Young et al., 2012). It has been demonstrated that ER stress is associated with advanced fibrotic lung disease in individuals with HPS, and that ER stress increases in an age-dependent manner in HPS1/2 double-mutant mice (Mahavadi et al., 2010). However, other data suggest that HPS-associated intracellular trafficking defects do not directly result in ER stress (Young et al., 2012). Thus, impaired intracellular trafficking pathways in AECs might result in epithelial cell dysfunction that contributes to lung fibrosis through mechanisms other than ER stress.

Going forward

Over the past decade, there has been tremendous progress in understanding IPF pathogenesis. The identification of surfactant protein mutations has revealed a role for ER stress in this disease, and the identification of telomerase mutations has highlighted a potential role for aging-associated pathways and senescence that were not previously linked with in IPF. The fact that the mechanisms affected by both types of mutations converge in epithelial cells has refocused efforts to understand how these cells determine onset and progression of this deadly disease.

It seems likely that progression to lung fibrosis involves both genetic background and environmental exposures (analogous to carcinogenesis), and that 'multiple hits' might be required to induce overt fibrotic lung disease (Fig. 1). Based on the current evidence, we propose that genetic and acquired or environmental insults converge to render AECs vulnerable to subsequent injury. When subjected to repeat or persistent injurious stimuli (such as aspiration, inhaled particulates, tobacco smoke and respiratory viruses), normal alveolar repair mechanisms fail and culminate instead in persistent collagen deposition, scar formation and progressive distortion of lung architecture. Over time, clinically evident pulmonary fibrosis develops. Incomplete disease penetrance in FIP supports this 'multiple hit' model, and it is likely that as-yet-unidentified secondary genetic factors and/or specific

Clinical and basic research opportunities

- To identify additional genes associated with familial (and possibly sporadic) IPF
- To characterize the role of common genetic variants (including *MUC5B* variants) in IPF pathogenesis
- To further investigate the role of AEC dysfunction in IPF
- To develop an animal model that recapitulates the histopathology and progressive nature of IPF
- To test new potential therapies for IPF based on advances in understanding the genetics of this disease.

environmental exposures are needed to develop clinically significant lung disease. Further studies with patients and experimental models are needed to identify such modifiers. In this context, the role of the recently described *MUC5B* promoter polymorphism in IPF remains to be clarified. With further advances in genomic science, additional genes responsible for FIP and/or IPF are likely to be identified in the coming years that illuminate new pathways for further investigation.

COMPETING INTERESTS

The authors declare that they do not have any competing or financial interests.

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