

Chapter 4

Pathogenesis of IPF

Is Abnormal Repair of Epithelial Damage Involved in the Basic Pathogenesis of This Disease?

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Abstract The molecular pathogenesis in IPF is not fully understood. However, epithelial injury and subsequent aberrant wound healing, rather than chronic inflammation, are thought to play central roles in the recent hypothesis. Alveolar epithelial cells predisposed with genetic mutations may involve in the abnormal responses subsequent to injury. Growth factors, such as transforming growth factor- β and platelet-derived growth factors, are critical mediators that control the growth and differentiation of lung fibroblasts. The novel topics in pulmonary fibrosis include the origin of lung fibroblasts, alveolar epithelial integrity, and resolution of extracellular matrixes. The further studies are required to clarify the mechanisms involved in the fibrogenesis in the lungs of IPF.

Keywords Epithelial injury • Fibroblasts • Growth factors • Epithelial integrity

4.1 Hypothesis of the Molecular Pathogenesis of IPF: From Inflammation to Epithelial Injury

IPF is believed to be caused by chronic inflammation in the lungs. In 2001, a novel pathogenesis was advocated, in which epithelial injury and subsequent aberrant wound healing, rather than chronic inflammation, play central roles [1, 2]. As a result of injury, alveolar epithelial type II cells (AECII) secrete several mediators capable of triggering profibrotic responses. Among these factors, growth factors, such as transforming growth factor (TGF)- β , platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), possess the ability to stimulate the migration, proliferation, and collagen production of lung fibroblasts, indicating that the interaction between AECII and fibroblasts is fundamental to the onset of

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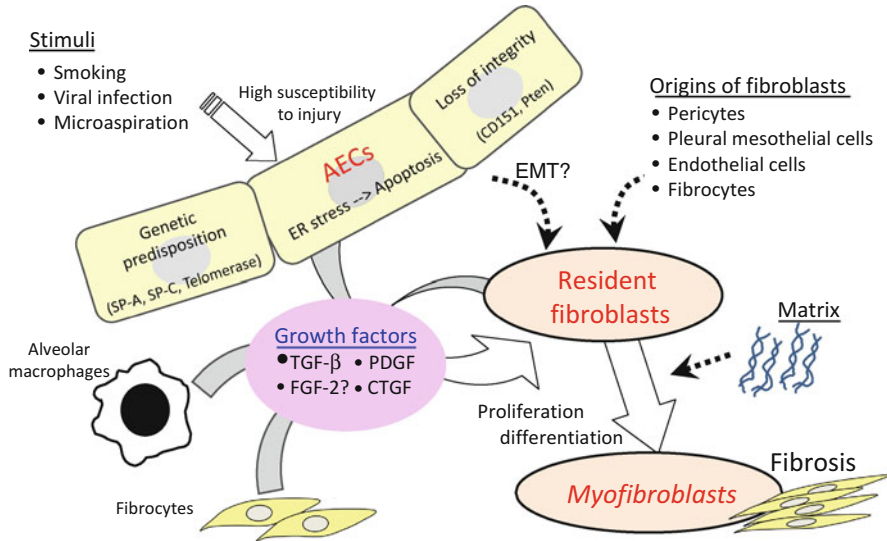


Fig. 4.1 Schematic drawing of the proposed molecular pathogenesis of IPF. AEC, alveolar epithelial cell; EMT, epithelial-mesenchymal transition; ECM, extracellular matrix

fibrogenesis in the lungs [3, 4]. In addition, growth factors are produced by alveolar macrophages and fibrocytes [3, 4]. Once the profibrotic pathway is stimulated, activated fibroblasts differentiate into myofibroblasts and cause excessive extracellular matrix (ECM) deposition, leading to remodeling of the lungs, with a so-called “honeycomb” appearance [1–4]. The updated hypothesis for the pathogenesis of IPF is summarized in Fig. 4.1.

4.2 Epithelial Injury and Genetic Background

Studies of the genetic backgrounds of patients with familial pulmonary fibrosis have revealed the existence of germline mutations in several genes. Surfactant proteins SP-A, SP-B, SP-C, and SP-D are produced from AECII and play roles in preserving lung homeostasis [5]. SP-B and SP-C are extremely hydrophobic and essential for reducing surface tension, whereas SP-A and SP-D belong to the collectin family of proteins and are involved in the host defenses. Among these genes, the SP-C gene mutation was first reported in children with familial pulmonary fibrosis [6, 7]. A mutation in the SP-A2 gene was subsequently identified in cases of familial disease [8, 9]; these mutations in the SP genes cause the accumulation of misfolded proteins in AECII. Other mutations have since been reported in telomerase genes [10, 11]. These mutations are believed to be associated with a reduced enzyme activity of telomerase and inversely correlate with the length of the telomere. An analysis of *terc*^{-/-} mice also showed a reduced number of AECII in

the distal lung epithelium [12]. Furthermore, the ATP-binding cassette protein A3 (ABCA3) is highly expressed in AECII and thought to be involved in the transport of surfactant proteins. The mutation in the ABCA3 gene was originally reported to cause the fatal interstitial lung disorder, whereas a later report showed an association with pediatric interstitial lung disease [13, 14]. More recently, a common MUC5B promoter polymorphism was identified to be associated with familial interstitial pneumonia, although the role of MUC5B in the pathogenesis of IPF remains unclear [15].

The above gene mutations are thought to render AEC susceptible to various stimuli, such as genetic predispositions. However, the frequency of these gene mutations appears to be rare in sporadic cases of IPF [16].

4.3 Biological Alteration in Injured AEC in IPF

Gene mutations in SP-C or SP-A2 cause the production of misfolded proteins that are not secreted or accumulated in the endoplasmic reticulum (ER) of AECs [17, 18]. Subsequently, the response to ER stress is induced, thereby upregulating the expressions of XBP-1, GRP78/BiP, and HDJ-2/HSP40, followed by the activation of caspase3 and apoptosis. Increased ER stress is seen in epithelial cells in the lungs of patients with IPF [19, 20], and apoptosis is reported to be increased in AECs in IPF lungs [21]. ER stress is enhanced by numerous factors. For example, viral infections, such as that with the influenza virus and cytomegalovirus, stimulate ER stress in AECs [20, 22]. In addition, environmental exposure to toxins, including cigarette smoke, upregulates ER stress in AECs [23], and aging is related to enhanced susceptibility to ER stress induced by murine herpesvirus [24]. These findings suggest that ER stress and apoptosis in AECs may be critical in both sporadic and familial cases of IPF and that similar mechanisms leading to ER stress, such as SP genes, may function in sporadic cases.

How do AECs under ER stress or apoptosis promote pulmonary fibrosis? The answer is not fully understood. However, it was recently demonstrated that the expression of TGF- β is mediated by ER stress associated with the SP-A2 gene mutation and influenza infection in AECs [20, 25]. ER stress may also generate EMT of AECs [26]. Since AECs have various biological functions, further research is required to clarify the link with epithelial injury and pulmonary fibrosis.

4.4 Critical Roles of Growth Factors in the Progression of Pulmonary Fibrosis

As mentioned above, injury to AECs is believed to be the first event triggering the onset of pulmonary fibrosis. However, growth factors are produced by various cells in addition to AECs in the IPF lungs [27]. Alveolar macrophages are known to produce numerous soluble factors under conditions of fibrogenesis in the lungs. Furthermore, emerging cells called fibrocytes also have the ability to produce profibrotic growth factors.

Among profibrotic mediators, transforming growth factor (TGF)- β and PDGF are thought to play a critical role in the onset of fibrogenesis of the lungs due to the activation of lung fibroblasts. Despite the limited evidence, other growth factors, including fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor (IGF)-1, etc., are also believed to contribute to the pathogenesis of pulmonary fibrosis.

Representative growth factors with a profibrotic activity are summarized in Table 4.1.

4.4.1 PDGF and PDGF Receptors

PDGF is a homo- or heterodimeric molecule with a molecular weight of 30 kDa [28]. PDGF genes consist of four different genes, including PDGF-A, PDGF-B, PDGF-C, and PDGF-D, which are located on chromosomes 7, 22, 4, and 11, respectively [29, 30]. There are two types of PDGF receptors (PDGFRs), α and β , which have a molecular weight of 170–180 kDa and are composed of homo- or heterodimers. Possible PDGF-PDGFR interactions are multiple and complex according to an in vitro study [7]. However, an in vivo study showed a limited pattern of binding of PDGF-AA or -CC to PDGFR- α and PDGF-BB to PDGFR- β [30].

PDGFs are expressed in many types of cells, including fibroblasts, vascular endothelial cells, macrophages, and platelets/megakaryocytes [30]. It is also known that the expression of PDGFs is upregulated by various inflammatory cytokines and growth factors, including both TGF- β and PDGF. Moreover, PDGFRs are expressed in various cells, although the classical targets of PDGF are fibroblasts and smooth muscle cells, and the expression of PDGFRs is induced by various stimuli. In contrast to PDGFs, the expression of PDGFR in some cells is limited to PDGFR- α or PDGFR- β , not both.

PDGF is known to be a major mitogen for mesenchymal cells. In fact, PDGF is the strongest stimulus of the proliferation of fibroblasts [28, 30], and the depletion of the PDGF-A gene in mice has been shown to be homozygous and lethal, with two different restriction points, one located prenatally and one located postnatally [31]. Postnatally surviving PDGF-A-deficient mice develop lung emphysema

Table 4.1 Major growth factors related to IPF and their biological effects

Growth factor	Producing cells						Biological activities				
	Endothelial cells	Epithelial cells	Fibroblasts	Fibrocyte	Myofibroblasts	Proliferation	Migration	Collagen production	Fibrosis		
TGF- β	+	+	+	+	+	→	↑	↑↑	↑		
PDGF	+	+	+	+	+	↑↑	↑↑	↑	↑		
FGF2	-	-	+	+	+	↑	↑	↑	↑?		

secondary due to the loss of alveolar myofibroblasts containing PDGFR- α . On the other hand, PDGFR- α null mice show cranial malformations and deficiency of myotome formation [32]. Mice deficient for PDGF-B or PDGFR- β exhibit renal, cardiovascular, and hematological, but not pulmonary, abnormalities [33, 34]. In summary, PDGF-A/PDGFR- α pathway plays a role in the secondary septation process, since PDGFR- α -expressing cells located in the alveolar entry ring have characteristics of myofibroblasts [35].

PDGF is known to be a growth factor that plays a role in the pathogenesis of pulmonary fibrosis [28, 30]. In animal models, the induction of pulmonary fibrosis with bleomycin (BLM) has been used to assess the molecular pathogenesis. For example, Maeda et al. reported that the expression of the PDGF-A gene is increased in mice showing BLM-induced pulmonary fibrosis using semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) [36]. Walsh et al. also examined the bronchoalveolar lavage fluid of rats treated with BLM and found 38- to 40-kDa and 29-kDa peptides detected with anti-PDGF-BB and anti-PDGF-AA antibodies, respectively, demonstrating the growth-promoting activity of lung fibroblasts [37]. In that study, the growth-promoting activity was neutralized 64 % by the anti-PDGF-BB antibody and 15 % by the anti-PDGF-AA antibody. In contrast, Zhuo et al. showed that the PDGF-C, but not the PDGF-A, PDGF-B, or PDGF-D, gene is induced in the lungs of mice treated with BLM according to a Northern blot analysis [38]. Moreover, Shimizu et al. reported that PDGF-A and PDGF-B are induced and elevated in the BLM-treated lungs of mice at both the mRNA and protein levels [39]. Additionally, the adoptive transfer of an adenovirus expressing the PDGF-B gene into the lungs induces severe fibrosis in mice [40]. Based on these reports, the expression of PDGF isoforms is enhanced in the setting of fibrogenesis of the lungs, although the details require a further analysis.

On the other hand, an enhanced expression of PDGF in epithelial cells and alveolar macrophages in the lungs of patients with IPF has been reported [41, 42]. However, the mechanisms involved in the enhanced expression and actions of PDGF in the fibrotic lung are poorly understood. Recently, Gochuico et al. examined growth factors in the alveolar lining fluid of patients with rheumatoid arthritis complicated with pulmonary fibrosis and reported that PDGF-AB and PDGF-BB, but not TGF- β or PDGF-AA, are associated with the progressive stage of pulmonary fibrosis [43], indicating the importance of PDGF-B in the onset of fibrogenesis of the lungs.

The evidence described above suggests that targeting the PDGF/PDGFR signaling pathway may have therapeutic effects against pulmonary fibrosis. This hypothesis has been investigated using animal models of pulmonary fibrosis with specific inhibitors of PDGFR [44]. Rice et al. first reported that AG1296, a tyrosine kinase inhibitor (TKI) for PDGFR, prevents the development of pulmonary fibrosis induced by vanadium pentoxide (V_2O_5) in rats [45]. In addition, imatinib mesylate (Gleevec in the United States, Glivec in Europe) has been applied, as imatinib inhibits PDGFR in addition to bcr-abl and c-kit [46]. The antifibrotic effects of imatinib in various pulmonary fibrosis models have been extensively examined, and it has been reported that imatinib strongly inhibits fibrogenesis in the lungs

[47–49]. In addition, Yoshida et al. reported that the *in vivo* gene transfer of an extracellular domain of PDGFR- β reduces the onset of BLM-induced pulmonary fibrosis [50]. Recently, nilotinib, another compound with a similar profile to that of imatinib, was also reported to show a higher antifibrotic activity than imatinib [51]. These observations suggest that the PDGF/PDGFR axis is a potential therapeutic target for pulmonary fibrosis.

4.4.2 FGF and FGF Receptors

FGF and FGF receptor (FGFR) are thought to be involved in the onset of fibrogenesis in the lungs. The FGF/FGFR family is composed of 18 FGF ligands and four FGFRs [52, 53]. Alternative splicing of the domain III of FGFR1–3 yields two different isoforms, IIIb in epithelial tissue and IIIc in mesenchymal tissues. Heparan sulfate glycosaminoglycan (HSGAG) binds to both FGF and FGFR in order to stabilize the binding of FGF to FGFR by facilitating dimerization. The specificity of different FGFs for different receptor isoforms has also been reported [54].

The physiological functions of FGF are diverse and complex, and studies have evaluated the roles of FGF proteins *in vitro* and in genetically modified mice *in vivo* [52–54]. Since FGFs originally possess mitogenic, chemotactic, and angiogenic activities, the biology of FGF has been examined in cancer research. In addition, knockout mice have been used to demonstrate the role of FGFs in the embryonic development of various organs. However, the physiological roles of most FGFs/FGFRs remain unclear. Both *Fgf1*^{-/-} and *Fgf2*^{-/-} mice are viable and fertile, with the exception of some abnormalities in the vascular system, although exogenous FGF-2 induces the proliferation of endothelial cells, smooth muscle cells, and fibroblasts.

The profibrogenic activities of FGFs have been observed in FGF-2 (basic FGF). For example, Hetzel et al. reported the proliferative activity of FGF-2 for lung fibroblasts [55], and fibroblasts derived from IPF show a reduced response to FGF-2, IGF-1, and EGF, but not PDGFs, as compared with normal fibroblasts. Meanwhile, Kanazawa et al. reported the activity of these compounds in promoting the migration of skin fibroblasts [56]. However, FGF-2 does not stimulate the production of fibronectin by lung fibroblasts. In IPF patients, there are scant data regarding the localization of FGF-2 in the lungs. However, the expression of FGF-2 mRNA has been reported to be upregulated in BLM-treated lungs in mice [57]. Although immunohistochemical staining has been reported to show the expression of FGF-2 in inflammatory cells, the role of the FGF-2/FGFR axis in mouse models of lung fibrosis remains unclear. Ju et al. reported that the administration of soluble FGFR2c ectodomain significantly reduces the extent of lung fibrosis in TGF- β -induced lung fibrosis mice [58]. However, Guzy et al. recently reported that *Fgf2*^{-/-} mice show increased mortality due to epithelial injury induced by BLM without any effects on lung fibrosis [57]. At present, it is not

possible to draw firm conclusions regarding the role of FGF-2 in the pathogenesis of pulmonary fibrosis in mice or in humans.

In contrast to FGF-2, FGF-1 has been reported to be an antifibrotic factor. FGF-1 reverts the profibrogenic effects of TGF- β such as α -SMA induction [59]. In addition, FGF-1 reduces the expression of collagen-I and induces the apoptosis of lung fibroblasts [60, 61].

It is currently difficult to determine the overall roles of FGF/FGFR in the pathogenesis of IPF. Further studies with an inhibitor specific for FGFR in pulmonary fibrosis models may help to clarify the roles of FGF/FGFR, although each isoform of FGF/FGFR cannot be analyzed minutely.

4.4.3 VEGF and VEGF Receptors

VEGF and VEGF receptors play a central role in both physiological and pathological angiogenesis [62]. The VEGF family is composed of seven members, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, PlGF, and svVEGF, while VEGF receptors include three members, VEGFR1, VEGFR2, and VEGFR3. Within the VEGF family, VEGF-A apparently serves as a key player in angiogenesis. Ebina et al. reported that the number of CD34-positive vessels is increased in the early phase of IPF of the lungs [63]. The role of angiogenesis in the development of lung fibrogenesis has not yet been determined, although Antoniou showed elevated VEGF levels in the BAL in patients with IPF [64]. There is limited evidence concerning the relationship between VEGF and lung fibrosis. Farkas et al. reported the direct effects of VEGF-A on lung fibroblasts and found that VEGF-A enhances the collagen-I expression induced by TGF- β [65]. In addition, Hamada et al. demonstrated that gene therapy with the soluble flt-1 gene reduces pulmonary fibrosis in a BLM-induced model in mice [66]. On the other hand, Ou et al. reported that the VEGFR-2 antagonist SU5416 attenuates BLM-induced lung fibrosis in mice [67]. These reports suggest the possibility of the profibrotic effects of VEGF, although the effects are unknown with respect to the role of each isoform of VEGF/VEGFR.

4.4.4 TGF- β and TGF- β Receptors

The signaling pathway via the TGF- β /TGF- β receptor complex is complicated [68, 69]. Human TGF- β ligand has three isoforms TGF- β 1, TGF- β 2, and TGF- β 3, among which TGF- β 1 has been reported to be a predominant isoform in cases of pulmonary fibrosis [70]. The TGF- β ligand binds heterodimeric serine/threonine kinase receptors of the TGF- β receptor I (T β RI) (ALK5) and T β RII. The binding of TGF- β to these receptors is regulated by several molecules, including latent TGF- β binding protein (LTBP) and integrins α V β 6. The signaling pathway in the

cytoplasm is also complex. The classical Smad pathway and non-Smad pathway differentially regulate the profibrotic effects of TGF- β . The biological activities of TGF- β are extremely pleiotropic and dependent on the type of cell and pathological condition. Representative activities are known to inhibit cell proliferation, regulate the extracellular matrix, suppress the immune response, and induce the epithelial-mesenchymal transition (EMT) [69]. Therefore, TGF- β is believed to play a central role in the pathogenesis of lung fibrosis.

The expression of TGF- β has been reported to be upregulated in the IPF lungs, particularly in AECs [71]. TGF- β stimulates the migration and production of collagen in lung fibroblasts [68, 69]. In addition, TGF- β inhibits the apoptosis of myofibroblasts. The involvement of TGF- β in lung fibrosis was recently demonstrated using BLM models of pulmonary fibrosis [72–74]. However, Andorianifahanana et al. showed that profibrotic TGF- β responses require the cooperative actions of PDGF and ErbB receptor tyrosine kinases [75]. Of course, the TGF- β function is partly mediated by the productions of CTGF and FGF-2 [76, 77]. Based on these reports, the profibrotic effects of TGF- β are mediated by several other growth factors, including PDGF, EGF, CTGF, and FGF-2, indicating that the role of TGF- β in lung fibrogenesis may have been overestimated. The other concern in targeting therapy for TGF- β is the paradoxical induction of persistent inflammation by blocking TGF- β signals [78, 79]. Therefore, further evidence is required to completely understand and control TGF- β signals.

4.4.5 Origin of Lung Fibroblasts and Their Contribution to Pulmonary Fibrosis

Lung fibroblasts are thought to be differentiated from the mesoderm during embryonic development and exist in the interstitium of the lungs as resident fibroblasts [80]. However, recent findings have demonstrated other origins of lung fibroblasts [81]. In 2004, Hashimoto et al. clearly showed the existence of bone marrow-derived fibroblasts in the lungs treated with BLM in mice [82]. The typical phenotype of these cells was both CD45 and collagen positive, suggesting that the characteristics of these cells are compatible with those of so-called fibrocytes [83]. The presence of fibrocytes in the BLM model was also reported by Phillips et al., who confirmed that the trafficking of fibrocytes is dependent on the CXCL12/CXCR4 axis [84]. In the setting of IPF, patients with progressive fibrosis or acute exacerbation show increased numbers of fibrocytes in the peripheral blood [85]. However, the role of fibrocytes is currently moving to a supportive role for resident fibroblasts via the production of growth factors as a result of the direct contribution of collagen production in mice [86–89]. The significance of fibrocytes in IPF should be further examined.

The other source of these cells is believed to be EMT-derived fibroblasts. In 2005, Willis et al. reported the existence of the EMT in AECs in vitro as well as in

IPF lungs [90]. According to their data, AECs expressing both pro-SP-B and α -SMA are present in cases of IPF. Kim et al. also reported the existence of EMT-derived fibroblasts expressing both pro-SP-C and α -SMA in a BLM model [91], and Tanjore found EMT-derived cells (S100A4⁺SP-C+) in a BLM model in mice, although the myofibroblasts expressing α -SMA were not derived from an EMT origin [92]. Subsequently, Rock et al. demonstrated no contribution of the EMT in α -SMA cells or S100A4⁺ cells using a confocal microscopic analysis [93]. Although these results suggest the negligible contribution of the EMT in the pathogenesis of pulmonary fibrosis, we cannot rule out the possibility that the EMT is partially generated or that the incomplete differentiation of AECs still contributes by producing several mediators, albeit not ECM.

Pleural mesothelial cells (PMCs) are reported to transform into fibroblasts in response to TGF- β in vitro [94]. In recombinant mice for GFP driven by the Wilms tumor-1 promotor, PMCs trafficking into the lungs are found to express α -SMA [95]. Although PMC-derived myofibroblasts are detected in the subpleural area of the IPF lungs, the number of these cells is reduced, indicating that PMCs are not a major source of myofibroblasts in IPF [95]. Meanwhile, endothelial cells are a major cellular component of the lungs. Hashimoto et al. reported that endothelial cells differentiate into myofibroblasts in vitro as well as in mice [96]. Although further research is required in humans, the endothelial-mesenchymal transition (EndoMT) may contribute to the onset of fibrogenesis in the case of IPF. More recently, pericytes have become a center of attention as a novel origin of lung myofibroblasts. Pericytes are known to be specialized mesenchymal cells that share a common basement membrane with endothelial cells. Using Foxd1-mapping system in genetically modified mice, Hung et al. demonstrated that 45–68 % of myofibroblasts are derived from pericytes in the lungs of BLM-treated mice [97]. Further analyses of pericytes in the IPF lungs are expected in future studies.

4.4.6 Loss of Alveolar Epithelial Integrity and Pulmonary Fibrosis

The concept of epithelial cell integrity may have an effect on the pathogenesis of IPF. Tsujino et al. found that CD151^{-/-} mice spontaneously develop age-related pulmonary fibrosis and display increased susceptibility to BLM-induced lung fibrosis [98]. CD151 is a tetraspanin protein expressed on the basolateral surface of AECs that exhibits binding with integrin α 3 β 1 and maintains epithelial integrity to support the adhesion of AECs to the basement membrane. In the setting of IPF, the expression of CD151 is significantly downregulated in AECs. A similar phenomenon is observed in mice deficient for phosphatase and tensin homolog deleted from chromosome 10 (*Pten*). *Pten* is a multifunctional phosphatase that negatively regulates the PI3K/Akt pathway. Miyoshi et al. showed that the knockdown of *Pten* in AECs induces the dysfunction of tight junctions and exacerbates lung fibrosis

induced by BLM [99]. Furthermore, the phosphorylation of Akt is enhanced in the lungs of *Pten*-deleted mice, and the blockade of Akt by the inhibitor reduces pulmonary fibrosis. Moreover, a decreased expression of Pten and enhanced phosphorylation of Akt are observed in the lungs of IPF animals, and the AECs in *Pten*-deficient mice show reduced expressions of surfactant proteins [100]. However, it remains undetermined whether the loss of epithelial cell integrity is a causative factor in IPF. In contrast to that observed in CD151-deficient mice, lung fibrosis in *Pten*-deficient mice is not dependent on TGF- β signals. Therefore, disorders in epithelial integrity may be crucial and fundamental for the progression of lung fibrosis in the case of IPF.

4.4.7 Resolution of ECM and Fibrosis

The resolution of pulmonary fibrosis is dependent on the degradation of ECMs. The irreversible fibrosis noted in cases of IPF may be related with the abnormalities in the resolution phase. It is known that the balance between matrix metalloproteases and its inhibitors is theoretically crucial for ECM metabolism. The gene expression profile determined using a microarray analysis has demonstrated that several MMPs are associated with pulmonary fibrosis in mice and IPF patients [101–103]. However, mice deficient for MMP-3 or MMP-7 are protected from the onset of BLM-induced lung fibrosis. On the other hand, MMP-19^{-/-} mice show marked enhancement of pulmonary fibrosis, indicating that the role of MMPs is complicated due to the interaction of both direct and indirect effects. Recently, LeBleu et al. identified a novel proteinase inhibitor, a human epididymis protein (HE)-4, of the novel serine proteases Prss35 and Prss23 in a renal fibrosis model [104]. Furthermore, the neutralizing antibody for HE-4 significantly improves renal fibrosis in mice. Therefore, specific molecules related to ECM metabolism in the lungs may be important for regulating pulmonary fibrosis.

Another interesting point in terms of the matrix is the degree of stiffness of the IPF lungs. Booth et al. reported the significance of the matrix, but not cells (fibroblasts), in regulating the profibrotic phenotype [105].

4.4.8 Conclusions

Recent understanding with respect to the molecular pathogenesis of IPF was summarized in this chapter. However, the etiology of IPF is not fully understood. In addition, IPF is undoubtedly heterogeneous in clinical phenotype as well as pathogenesis. It is therefore difficult to fully explore the characteristics of fibrogenesis in the case of IPF. However, updated technology in the fields of genetics, proteomics, and mechanics (i.e., microscopy) may help to clarify the

remaining problems, particularly issues pertaining to the fundamental topics “what is IPF?,” in the future.

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References

1. Gross TJ, Hunninghake GW. Idiopathic pulmonary fibrosis. *N Engl J Med*. 2001;345:517–25.
2. Selman M, King Jr TE, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. *Ann Intern Med*. 2001;134:136–51.
3. White ES, Lazar MH, Thannickal VJ. Pathogenetic mechanisms in usual interstitial pneumonia/idiopathic pulmonary fibrosis. *J Pathol*. 2003;201:343–54.
4. King Jr TE, Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet*. 2011;378:1949–61.
5. Wright JR. Immunoregulatory functions of surfactant proteins. *Nat Rev Immunol*. 2005;5:58–68.
6. Noguee LM, Dunbar 3rd AE, Wert SE, et al. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med*. 2001;344:573–9.
7. Brasch F, Griese M, Tredano M, et al. Interstitial lung disease in a baby with a de novo mutation in the SFTPC gene. *Eur Respir J*. 2004;24:30–9.
8. Wang Y, Kuan PJ, Xing C, et al. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am J Hum Genet*. 2009;84:52–9.
9. Maitra M, Cano CA, Garcia CK. Mutant surfactant A2 proteins associated with familial pulmonary fibrosis and lung cancer induce TGF- β 1 secretion. *Proc Natl Acad Sci U S A*. 2012;109:21064–9.
10. Armanios MY, Chen JJ-L, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med*. 2007;356:1317–26.
11. Tsakiri KD, Cronkhite JT, Kuan PJ, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc Natl Acad Sci U S A*. 2007;104:7552–7.
12. Lee J, Reddy R, Barsky L, et al. Lung alveolar integrity is compromised by telomere shortening in telomerase-null mice. *Am J Physiol Lung Cell Mol Physiol*. 2009;296:L57–70.
13. Shulenin S, Noguee LM, Annilo T, et al. ABCA3 gene mutations in newborns with fatal surfactant deficiency. *N Engl J Med*. 2004;350:1296–303.
14. Bullard JE, Wert SE, Whitsett JA, et al. ABCA3 mutations associated with pediatric interstitial lung disease. *Am J Respir Crit Care Med*. 2005;172:1026–31.
15. Seibold MA, Wise AL, Speer MC, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med*. 2011;364:1503–12.
16. Garcia CK. Idiopathic pulmonary fibrosis: update on genetic discoveries. *Proc Am Thor Soc*. 2011;8:158–62.
17. Mulugeta S, Nguyen V, Russo SJ, et al. A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and caspase 3 activation. *Am J Respir Cell Mol Biol*. 2005;32:521–30.
18. Maitra M, Wang Y, Gerard RD, et al. Surfactant protein A2 mutations associated with pulmonary fibrosis lead to protein instability and endoplasmic reticulum stress. *J Biol Chem*. 2010;285:22103–13.

19. Korfei M, Ruppert C, Mahavadi P, et al. Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2008;178:838–46.
20. Lawson WE, Crossno PF, Polosukhin VV, et al. Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. *Am J Physiol Lung Cell Mol Physiol.* 2008;294:L1119–26.
21. Kuwano K, Kunitake R, Kawasaki M, et al. P21Waf1/Cip1/Sdi1 and p53 expression in association with DNA strand breaks in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 1996;154:477–83.
22. Roberson EC, Tully JE, Guala AS, et al. Influenza induces endoplasmic reticulum stress, caspase-12-dependent apoptosis, and c-Jun N-terminal kinase-mediated transforming growth factor- β release in lung epithelial cells. *Am J Respir Cell Mol Biol.* 2011;46:573–81.
23. Jorgensen E, Stinson A, Shan L, et al. Cigarette smoke induces endoplasmic reticulum stress and the unfolded protein response in normal and malignant human lung cells. *BMC Cancer.* 2008;8:229.
24. Torres-Gonzalez E, Bueno M, Tanaka A, et al. Role of endoplasmic reticulum stress in age-related susceptibility to lung fibrosis. *Am J Respir Cell Mol Biol.* 2012;46:748–56.
25. Marita M, Cano CA, Garcia CK. Mutant surfactant A2 proteins associated with familial pulmonary fibrosis and lung cancer induce TGF- β 1 secretion. *Proc Natl Acad Sci U S A.* 2012;109:21064–9.
26. Tanjore H, Cheng DS, Degryse AL, et al. Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress. *J Biol Chem.* 2011;286:30972–80.
27. Gharaee-Kermani M, Gyetko MR, Hu B, et al. New insights into the pathogenesis and treatment of idiopathic pulmonary fibrosis: a potential role for stem cells in the lung parenchyma and implications for therapy. *Pharm Res.* 2007;24(5):819–41.
28. Herdin CH, Westermarck B. Mechanism of action and in vivo role of platelet-derived growth factor. *Physiol Rev.* 1999;79:1283–316.
29. Li X, Eriksson U. Novel PDGF family members. PDGF-C and PDGF-D. *Cytokine Growth Factor Rev.* 2003;14:91–8.
30. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. *Genes Dev.* 2008;22:1276–312.
31. Boström H, Willetts K, Pekny M, et al. PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell.* 1996;14:863–73.
32. Soriano P. The PDGF α receptor is required for neural crest cell development and for normal patterning of the somites. *Development.* 1997;124:2691–700.
33. Levéen P, Pekny M, Gebre-Medhin S, et al. Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev.* 1994;8:1875–87.
34. Soriano P. Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. *Genes Dev.* 1996;8:1888–96.
35. McGowan SE, Grossmann RE, Kimani PW, et al. Platelet-derived growth factor receptor-alpha-expressing cells localize to the alveolar entry ring and have characteristics of myofibroblasts during pulmonary alveolar septal formation. *Anat Rec (Hoboken).* 2008;291:1649–61.
36. Maeda A, Hiyama K, Yamakido H, et al. Increased expression of platelet-derived growth factor A and insulin-like growth factor-I in BAL cells during the development of bleomycin-induced pulmonary fibrosis in mice. *Chest.* 1996;109:780–6.
37. Walsh J, Absher M, Kelley J. Variable expression of platelet-derived growth factor family proteins in acute lung injury. *Am J Respir Cell Mol Biol.* 1993;9:637–44.
38. Zhou Y, Zheng J, Laboy M, et al. Modulation of PDGF-C and PDGF-D expression during bleomycin-induced lung fibrosis. *Am J Physiol Lung Cell Mol Physiol.* 2004;286:L182–8.
39. Shimizu S, Gabazza EC, Taguchi O, et al. Activated protein C inhibits the expression of platelet-derived growth factor in the lung. *Am J Respir Crit Care Med.* 2003;167:1416–26.

40. Yoshida M, Sakuma J, Hayashi S, et al. A histologically distinctive interstitial pneumonia induced by overexpression of the interleukin 6, transforming growth factor beta 1, or platelet-derived growth factor B gene. *Proc Natl Acad Sci U S A*. 1995;92:9570–4.
41. Antoniadou HN, Bravo MA, Avila RE, et al. Platelet-derived growth factor in idiopathic pulmonary fibrosis. *J Clin Invest*. 1990;86:1055–64.
42. Homma S, Nagaoka I, Abe H, et al. Localization of platelet-derived growth factor and insulin-like growth factor I in the fibrotic lung. *Am J Respir Crit Care Med*. 1995;152:2084–9.
43. Gochuico BR, Avila NA, Chow CK, et al. Progressive preclinical interstitial lung disease in rheumatoid arthritis. *Arch Intern Med*. 2008;168:159–66.
44. Nishioka Y, Azuma M, Kishi M, et al. Targeting platelet-derived growth factor as a therapeutic approach in pulmonary fibrosis. *J Med Invest*. 2013;60(3, 4):175–83.
45. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the abl tyrosine kinase on the growth of bcr-abl positive cells. *Nat Med*. 1996;2:561–6.
46. Rice AB, Moomaw CR, Morgan DL, et al. Specific inhibitors of platelet-derived growth factor or epidermal growth factor receptor tyrosine kinase reduce pulmonary fibrosis in rats. *Am J Pathol*. 1999;55:213–21.
47. Aono Y, Nishioka Y, Inayama M, et al. Imatinib as a novel antifibrotic agent in bleomycin-induced pulmonary fibrosis in mice. *Am J Respir Crit Care Med*. 2005;171:1279–85.
48. Daniels CE, Wilkes MC, Edens M, et al. Imatinib mesylate inhibits the profibrogenic activity of TGF- β and prevents bleomycin-mediated lung fibrosis. *J Clin Invest*. 2004;114:1308–16.
49. Abdollahi A, Li M, Ping G, et al. Inhibition of platelet-derived growth factor signaling attenuates pulmonary fibrosis. *J Exp Med*. 2005;201:925–35.
50. Yoshida M, Sakuma-Mochizuki J, Abe K, et al. In vivo gene transfer of an extracellular domain of platelet-derived growth factor beta receptor by the HVJ-liposome method ameliorates bleomycin-induced pulmonary fibrosis. *Biochem Biophys Res Commun*. 1999;265:503–8.
51. Rhee CK, Lee SH, Yoon HK, et al. Effect of nilotinib on bleomycin-induced acute lung injury and pulmonary fibrosis in mice. *Respiration*. 2011;82:273–87.
52. Turner N, Grose R. Fibroblast growth factor signaling: from development to cancer. *Nat Rev Cancer*. 2010;10:116–20.
53. Beenken A, Mohammadi M. The FGF family: biology, pathophysiology and therapy. *Nat Rev Drug Discov*. 2009;8:235–53.
54. Powers CJ, McLeskey SW, Wellstein A. Fibroblast growth factors, their receptors and signaling. *Endocr-Relat Cancer*. 2000;7:165–97.
55. Hetzel M, Bachem M, Anders D, et al. Different effects of growth factors on proliferation and matrix production of normal and fibrotic human lung fibroblasts. *Lung*. 2005;183:225–37.
56. Kanazawa S, Fujiwara T, Matsuzaki S, et al. bFGF regulates PI3-kinase-Rac1-JNK pathway and promotes fibroblast migration in wound healing. *PLoS One*. 2010;5, e12228.
57. Guzy RD, Stoilov I, Elton TJ, et al. Fibroblast growth factor 2 is required for epithelial recovery, but not for pulmonary fibrosis, in response to bleomycin. *Am J Respir Cell Mol Biol*. 2015;52:116–28.
58. Ju W, Zhihong Y, Zhiyou Z, et al. Inhibition of α -SMA by the ectodomain of FGFR2c attenuates lung fibrosis. *Mol Med*. 2012;18:992–1002.
59. Ramos C, Becerril C, Montano M, et al. FGF-1 reverts epithelial-mesenchymal transition induced by TGF- β 1 through MAPK/ERK kinase pathway. *Am J Physiol Lung Cell Mol Physiol*. 2010;299:L222–31.
60. Ramos C, Montano M, Becerril C, et al. Acidic fibroblast growth factor decreases α -smooth muscle actin expression and induces apoptosis in human normal lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol*. 2006;291:L871–9.
61. Becerril C, Pardo A, Montano M, et al. Acidic fibroblast growth factor induces an antifibrogenic phenotype in human lung fibroblasts. *Am J Respir Cell Mol Biol*. 1999;20:1020–7.

62. Shibuya M. Vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1): a dual regulator for angiogenesis. *Angiogenesis*. 2006;9:225–30.
63. Ebina M, Shimizukawa M, Shibata N, et al. Heterogeneous increase in CD34-positive alveolar capillaries in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med*. 2004;169(11):1203–8.
64. Antoniou KM, Soufla G, Proklou A, et al. Different activity of the biological axis VEGF-Flt-1 (fms-like tyrosine kinase 1) and CXC chemokines between pulmonary sarcoidosis and idiopathic pulmonary fibrosis: a bronchoalveolar lavage study. *Clin Dev Immunol*. 2009;2009:537929.
65. Farkas L, Farkas D, Ask K, et al. VEGF ameliorates pulmonary hypertension through inhibition of endothelial apoptosis in experimental lung fibrosis in rats. *J Clin Invest*. 2009;119:1298–311.
66. Hamada N, Kuwano K, Yamada M, et al. Anti-vascular endothelial growth factor gene therapy attenuates lung injury and fibrosis in mice. *J Immunol*. 2005;175:1224–31.
67. Ou X-M, Li W-C, Liu D-S, et al. VEGFR-2 antagonist SU5416 attenuates bleomycin-induced pulmonary fibrosis. *Int Immunopharmacol*. 2009;9:70–9.
68. Varga J, Pasche B. Transforming growth factor b as a therapeutic target in systemic sclerosis. *Nat Rev Rheumatol*. 2009;5:200–6.
69. Akhurst R, Hata A. Targeting the TGF β signaling pathway in disease. *Nat Rev Drug Discov*. 2012;11:790–812.
70. Coker RK, Laurent GJ, Shahzeidi S, et al. Transforming growth factors- β 1, - β 2, and - β 3 stimulate fibroblast procollagen production in vitro but are differentially expressed during bleomycin-induced lung fibrosis. *Am J Pathol*. 1997;150:981–91.
71. Khalil N, O'Connor RN, Unruh HW, et al. Increased production and immunohistochemical localization of transforming growth factor-beta in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol*. 1991;150:981–91.
72. Sime PJ, Xing Z, Graham FL, et al. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. *J Clin Invest*. 1997;100:768–76.
73. Wang Q, Wang Y, Hyde DM, et al. Reduction of bleomycin induced lung fibrosis by transforming growth factor beta soluble receptor in hamsters. *Thorax*. 1999;54:805–12.
74. Zhao J, Shi W, Wang YL, et al. Smad3 deficiency attenuates bleomycin-induced pulmonary fibrosis in mice. *Am J Physiol Lung Cell Mol Physiol*. 2002;282:L585–93.
75. Andrianifahanana M, Wilkes MC, Gupta SK, et al. Profibrotic TGF β responses require the cooperative action of PDGF and ErbB receptor tyrosine kinases. *FASEB J*. 2013;27:4444–54.
76. Li C-M, Khosla J, Pagan I, et al. TGF- β 1 and fibroblast growth factor-1 modify fibroblast growth factor-2 production in type II cells. *Am J Physiol Lung Cell Mol Physiol*. 2000;279:L1038–46.
77. Kucich U, Rosenbloom JC, Herrick DJ, et al. Signaling events required for transforming growth factor-beta stimulation of connective tissue growth factor expression by cultured human lung fibroblasts. *Arch Biochem Biophys*. 2001;395(1):103–12.
78. Horan GS, Wood S, Ona V, et al. Partial inhibition of integrin α β 6 prevents pulmonary fibrosis without exacerbating inflammation. *Am J Respir Crit Care Med*. 2008;177:56–65.
79. Puthawala K, Hadjiangelis N, Jacoby SC, et al. Inhibition of integrin α β 6, an activator of latent transforming growth factor- β , prevents radiation-induced lung fibrosis. *Am J Respir Crit Care Med*. 2008;177:82–90.
80. Dunsmore SE, Shapiro SD. The bone marrow leases its scar: new concepts in pulmonary fibrosis. *J Clin Invest*. 2004;113:180–2.
81. Garantziotis S, Steele MP, Schwartz DA. Pulmonary fibrosis: thinking outside of the lung. *J Clin Invest*. 2004;114:319–21.
82. Hashimoto N, Jin H, Liu T, et al. Bone marrow-derived progenitor cells in pulmonary fibrosis. *J Clin Invest*. 2004;113:243–52.

83. Bucala R, Spiegel LA, Chesney J, et al. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med.* 1994;1:71–81.
84. Phillips RJ, Burdick MD, Hong K, et al. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediates fibrosis. *J Clin Invest.* 2004;114:438–46.
85. Reilkoff RA, Bucala R, Herzog E. Fibrocytes: emerging effector cells in chronic inflammation. *Nat Rev Immunol.* 2011;11:427–35.
86. Abe S, Okazaki H, Kishi M, et al. Fibrocytes regulates lung fibroblast activation. *Am J Respir Crit Care Med.* 2012;185:A4460.
87. Kleaveland KR, Velikoff M, Yang J, et al. Fibrocytes are not an essential source of type I collagen during lung fibrosis. *Am J Respir Cell Mol Biol.* 2014;193:5229–39.
88. Aono Y, Ledford JG, Mukherjee S, et al. Surfactant protein-D regulates effector cell function and fibrotic lung remodeling in response to bleomycin injury. *Am J Respir Crit Care Med.* 2012;185(5):525–36.
89. Aono Y, Kishi M, Yokota Y, et al. Role of PDGF/PDGFR axis in the trafficking of circulating fibrocytes in pulmonary fibrosis. *Am J Respir Cell Mol Biol.* 2014;51(6):793–801.
90. Willis BC, Liebler JM, Luby-Phelps K, et al. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor- β 1. *Am J Pathol.* 2005;166:1321–32.
91. Kim KK, Kugler MC, Wolters PJ, et al. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci U S A.* 2006;103:13180–5.
92. Tanjore H, Xu XC, Polosukhin VV, et al. Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. *Am J Respir Crit Care Med.* 2009;180:657–65.
93. Rock JR, Barkauskas CE, Cronic MJ, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci U S A.* 2011;108:E1475–83.
94. Nasreen N, Mohammed KA, Mubarak KK, et al. Pleural mesothelial cell transformation into myofibroblasts and haptotactic migration in response to TGF- β 1 in vitro. *Am J Physiol Lung Cell Mol Physiol.* 2009;297:L115–24.
95. Mubarak KK, Montes-Worboys A, Regev D, et al. Parenchymal trafficking of pleural mesothelial cells in idiopathic pulmonary fibrosis. *Eur Respir J.* 2012;39:133–40.
96. Hashimoto N, Phan SH, Imaizumi K, et al. Endothelial–mesenchymal transition in bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol.* 2012;43:161–72.
97. Hung C, Linn G, Chow Y-H, et al. Role of lung pericytes and resident fibroblasts in the pathogenesis of pulmonary fibrosis. *Am J Respir Crit Care Med.* 2013;188:820–30.
98. Tsujino K, Takeda Y, Arai T, et al. Tetraspanin CD151 protects against pulmonary fibrosis by maintaining epithelial integrity. *Am J Respir Crit Care Med.* 2012;186(2):170–80.
99. Miyoshi K, Yanagi S, Kawahara K, et al. Epithelial Pten controls acute lung injury and fibrosis by regulating alveolar epithelial cell integrity. *Am J Respir Crit Care Med.* 2013;187:262–75.
100. Yanagi S, Kishimoto H, Kawahara K, et al. Pten controls lung morphogenesis, bronchioalveolar stem cells, and onset of lung adenocarcinomas in mice. *J Clin Invest.* 2007;117:2929–40.
101. Zuo F, Kaminski N, Eugui E, et al. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci U S A.* 2002;99:6292–7.
102. Yamashita CM, Dolgonos L, Zemans RL, et al. matrix metalloproteinase 3 is a mediator of pulmonary fibrosis. *Am J Pathol.* 2011;179:1733–45.
103. Yu G, Kovkarova-Naumovski E, Jara P, et al. matrix metalloproteinase 19 is a key regulator of lung fibrosis in mice and humans. *Am J Respir Crit Care Med.* 2012;186:752–62.
104. LeBleu VS, Teng Y, O’Connell JT, et al. Identification of human epididymis protein-4 as a fibroblast-derived mediator of fibrosis. *Nat Med.* 2013;19(2):227–31.
105. Booth AJ, Hadley R, Cornett AM, et al. Acellular normal and fibrotic human lung matrices as a culture system for in vitro investigation. *Am J Respir Crit Care Med.* 2012;186:866–76.