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Fibrogenesis

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Introduction

Pulmonary fibrosis can be idiopathic or secondary to inflammatory states or injuries (Table 46.1). The tempo ranges from insidious to rapid, and the location of the fibrous tissue can be centered around or in the airways (bronchiolitis obliterans) or in the alveolar compartment (idiopathic pulmonary fibrosis [IPF]). In this chapter, we focus on IPF, the paradigmatic fibrosing lung disorder.

Early attempts to explain IPF emphasized inflammation ("alveolitis") and the effects of inflammatory and profibrotic cytokines.[1] The current consensus proposes instead that an initial epithelial injury provokes a wound repair response (Figure 46.1). Instead of restoring normal tissue architecture, in some cases the repair process goes awry, and persistent matrix deposition ensues; in turn, these events may produce signals that further damage epithelium.[2] Experimental models have allowed us insight into many cellular processes and molecular mediators that are triggered by injury and lead to fibrosis in susceptible animals. What remains elusive is the nature of the inciting injury in IPF and the host and environmental factors that determine whether the response to injury is adaptive or maladaptive.

Experimental Models of Lung Fibrosis

Animal models are valuable for testing the roles of specific molecules by using genetically altered mice or administering a specific factor or inhibitor; testing potential therapeutic compounds; distinguishing between the roles of resident lung cells and marrow-derived cells; and assessing the genetic factors through comparative genetics studies (e.g., linkage analysis using intercrosses of fibrosis-prone and fibrosis-resistant strains of mice).

Bleomycin, given either intratracheally or systemically, is the most commonly used model. Bleomycin interacts with oxygen and a transition metal to form an activated complex, which binds and cleaves DNA. Activated bleomycin can also damage proteins and lipids, in part by release of hydroxyl radicals. Bleomycin toxicity is most prominent in the lung; vascular injury, cell death, and inflammation occur acutely, followed by collagen deposition. The pathology is more similar to the acute respiratory distress syndrome (ARDS) than it is to IPF. Other fibrosis models utilize radiation exposure; intratracheal silica, asbestos, or fluorescein isothiocyanate; and adenoviral or transgenic expression of transforming growth factor (TGF)-β or interleukin (IL)-13.

Most experimental models consist of two components: an injury and a fibrotic response. In most models the injury is severe, associated with inflammation, and produces fibrosis rapidly. Hence, these are poor models of the postulated initial injury in IPF, which is so subtle that it has not yet been identified. Only one model has been reported that reproduces many of the histologic features of UIP/IPF: interferon-γ receptor knockout mice (which preferentially develop a Th2 cytokine response) chronically infected with murine γ-herpesvirus 68 (MHV68).[3] Using these models, in particular the murine bleomycin model, investigators have identified many factors involved in fibrosis. For example, around 40 different knockout mice have an altered fibrotic response in the bleomycin model (Table 46.2).

Fibroblastic Foci and the Fibroblast Phenotype

Ultimately, IPF is a disease of fibroblasts and the connective tissue they deposit. In seminal studies, Kuhn, McDonald, and colleagues used antibodies against procollagen I that stain cells actively synthesizing collagen.[4] These antibodies preferentially react with small clusters of fibroblasts in IPF lungs. These clusters, called *fibroblastic foci*, are one of the pathologic hallmarks of UIP/IPF. The

TABLE 46.1. Disorders associated with pulmonary fibrosis.

Usual interstitial pneumonia/idiopathic pulmonary fibrosis and other idiopathic pneumonias
Acute respiratory distress syndrome
Collagen vascular diseases
Bronchiolitis obliterans
Cryptogenic organizing pneumonia
Sarcoidosis
Langerhans cell histiocytosis
Dust exposures
Radiation injury
Asthma (subepithelial fibrosis)

extent of fibroblastic foci in IPF lung biopsy material predicts mortality (the extent of fibrosis and inflammation, in contrast, do not).[2]

Fibroblastic foci are relatively small collections of fibroblasts (as few as 3, up to 30 or more) located just below the alveolar gas–tissue interface (Figure 46.2). Inflammatory cells are rarely present. The fibroblasts are arranged parallel to the alveolar surface and are often hypertrophied. The fibroblasts stain for procollagen I and for cellular fibronectin, indicative of active matrix protein synthesis. The fibroblasts also express α-smooth muscle actin, indicating that they are contractile myofibroblasts. The remnants of a basement membrane, identifiable by collagen IV staining, are typically present *below* the fibroblastic focus, indicating that the fibroblasts have migrated into the alveolar space. If basement membrane can be identified at the epithelial layer rather than buried within the focus, it is frequently interrupted and distorted. Occasionally, two or three layers of basement membrane are detectable within a focus, suggesting repeated episodes of fibroblast invasion and epithelial repair. Fibrin is often present, indicating leakage of plasma.

TABLE 46.2. Gene products involved in bleomycin-induced fibrosis, revealed by use of knockout mice.

Profibrotic	Antifibrotic
Chemokines	
CCL11, CCR2	CXCR3, CXCL10
Cytokine and other signaling pathways	
IL-4, Smad3, IFN-γ, TGF-β, TNF receptors, angiotensin 1a receptor	Stat1, GM-CSF, IL12p40, CD73
Coagulation	
TAFI, PAR-1, PAI-1	
ROS	
p47phox, iNOS	Nrf2
Lipid mediators	
Leukotriene C(4) synthase, cytosolic phospholipase A_2, 5-lipoxygenase	CysLT2 receptor
Leukocyte function	
L-selectin, ICAM-1, perforin, CD28	
Proteases	
Matrix metalloproteinase-7	Cathepsin K
Apoptosis	
Bid, Fas, Fas ligand	
Other	
β6-Integrin (activates TGF-β1), C5, γ-glutamyl transpeptidase	SP-C, SPARC, Thy-1

Note: C5, complement factor 5; CysLT2, cysteinyl leukotriene 2; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; Nrf2, nuclear factor E2 p45-related factor 2; PAI-1, plasminogen activator inhibitor-1; PAR-1, protease-activated receptor-1; SPARC, secreted protein, acidic and rich in cysteine; SP-C, surfactant protein C; TAFI, thrombin-activatable fibrinolysis inhibitor; TGF, transforming growth factor; TNF, tumor necrosis factor.

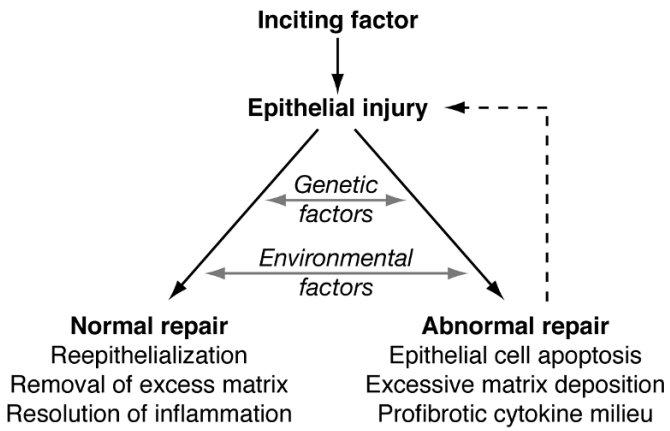

FIGURE 46.1. The current conceptual model of fibrogenesis in idiopathic pulmonary fibrosis. An inciting factor causes epithelial injury. Depending on the nature and repetitiveness of the injury, as well as genetic and environmental factors, the injury undergoes either normal repair or an abnormal repair process with associated fibrosis. Signals related to the abnormal repair process (e.g., oxidative stress or angiotensin II) may cause further epithelial injury.

The epithelium overlying fibroblastic foci is abnormal. The epithelial cells are cuboidal and hyperplastic. A basement membrane is often lacking, placing epithelial cells in direct contact with fibroblasts. Perhaps as a result, the epithelial cells are poorly adherent. In addition, by morphologic criteria and in situ end labeling, many epithelial cells are apoptotic or necrotic; dead or dying epithelial cells are preferentially located at fibroblastic foci.[5] Paradoxically, the underlying myofibroblasts are not apoptotic.

These observations suggest that an initial epithelial injury leads to a disruption of the normal barrier of the alveolus, with formation of a fibrin-rich exudate that is

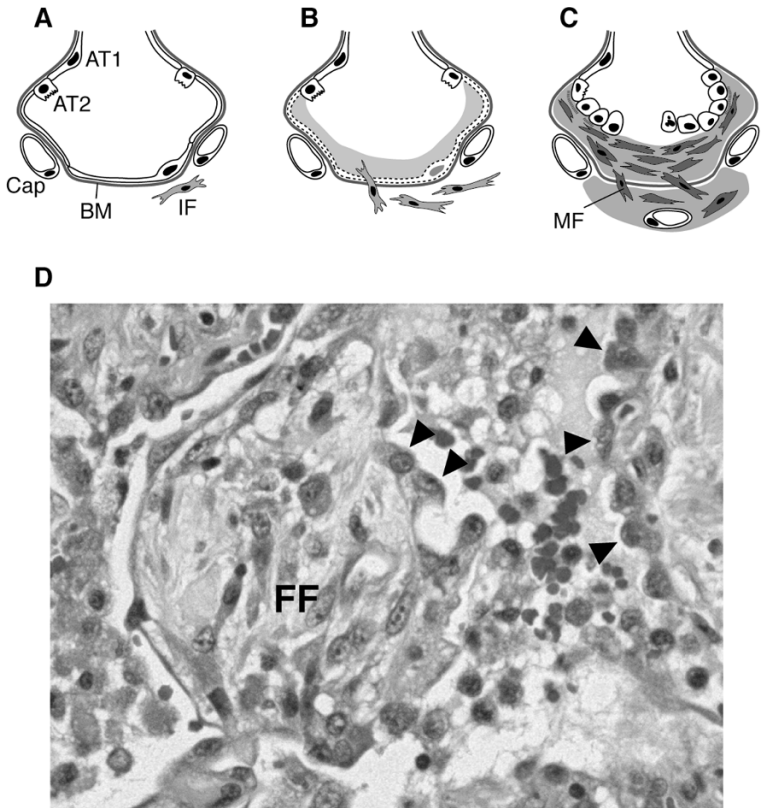

FIGURE 46.2. The fibroblastic focus. **(A)** A normal alveolus with alveolar type 1 (AT1) and type 2 (AT2) cells, capillaries (Cap), and an interstitial fibroblast (IF). BM, basement membrane. **(B)** Epithelial injury is followed by formation of a fibrin-rich provisional matrix on the alveolar surface. Fibroblasts are activated, and an inflammatory response may occur (not shown). **(C)** A fibroblastic focus with numerous myofibroblasts (MF) forms. The alveolar epithelium is hyperplastic and poorly adherent. A collagen-rich matrix is formed, and neovascularization occurs. **(D)** Photomicrograph of a fibroblastic focus (FF). Hyperplastic epithelial cells are indicated by arrowheads. (Courtesy of Herman Yee, PhD, MD.)

then invaded by activated, contractile fibroblasts (see Figure 46.2). The perpetuation of fibroblast activation, matrix synthesis and contraction, accompanied by epithelial cell death, defines the pathobiology of IPF.

Many laboratory investigators have compared IPF and normal lung fibroblasts. These studies consistently find that IPF fibroblasts behave differently in culture—but not always in the same way. For example, IPF fibroblast growth rates have been reported as increased and decreased compared with normal fibroblasts.[2] Several proteins are selectively expressed by IPF fibroblasts, including discoidin domain receptor 1 (a receptor tyrosine kinase that binds collagen),[6] fibroblast activation protein α (a membrane protease).[7] and Thy-1.

Thy-1 is a glycosylphosphatidylinositol-anchored cell surface protein expressed on many cells, including some fibroblasts.[8] It is a ligand for several integrins, interacts with signaling molecules such as the Src family kinase c-fyn, and localizes to lipid rafts. Cross-linking Thy-1 with antibodies triggers apoptosis. The activated fibroblasts in fibroblastic foci are Thy-1 negative, whereas most fibroblasts in normal human lung are Thy-1 positive. Thy-1 knockout mice are generally healthy; however, upon challenge with bleomycin, Thy-1 knockout mice develop increased fibrosis. In addition, absence of Thy-1 expression in lung fibroblasts correlates with higher TGF-β expression, the ability to activate latent TGF-β in response to fibrogenic stimuli, increased expression of the platelet-derived growth factor-α receptor, enhanced migration, and increased α-smooth muscle actin expression.[8]

Sources of Fibroblasts

Three sources of the increased number of fibroblasts in interstitial lung disease have been proposed: proliferation of existing interstitial fibroblasts, transdifferentiation of epithelial cells into fibroblasts (epithelial–mesenchymal transition), and bone marrow–derived precursor cells referred to as *circulating fibrocytes*. The relative contribution of each source is not known for lung fibrosis models, but in a model of kidney fibrosis (unilateral ureteral

obstruction) it was estimated that approximately one third of interstitial fibroblasts derived from epithelial–mesenchymal transition and one sixth originated from bone marrow.[9]

Circulating fibrocytes constitute <1% of circulating leukocytes and express a distinctive set of proteins, including collagens I and III, vimentin, CD34, CD45, CXCR4, CXCR7, major histocompatibility complex class II, and CD86.[10,11] When cultured, fibrocytes adopt a spindle shape, continue to express extracellular matrix proteins, and become α-smooth muscle actin positive; TGF-β enhances these changes. The fibrocytes undergo chemotaxis in response to the chemokines CCL21 and CXCL12 and are recruited from the circulation to sites of tissue injury, including the lung.

In two models, the degree of lung fibrosis correlated with the extent of fibrocyte recruitment. Phillips et al. showed that fibrocytes are recruited to lung in the bleomycin model, that the kinetics of recruitment parallel collagen deposition, and that blocking recruitment of fibrocytes with anti-CXCL12 antibody reduces fibrosis.[12] Similarly, Moore and colleagues found, in the fluorescein isothiocyanate–induced lung fibrosis model, that fibrocytes are recruited to the lung in a CCL12- and CCR2-dependent manner and that CCR2 knockout mice have reduced lung fibrosis.[13]

Epithelial–mesenchymal transition may also be a source of fibroblasts in lung fibrosis. The fact that TGF-β, a key mediator of fibrosis, induces epithelial–mesenchymal transition in a variety of epithelial cells in cell culture, including alveolar type 2 (AT2) cells,[14,15] lends appeal to this idea. Willis and colleagues have presented evidence that epithelial–mesenchymal transition occurs in human IPF lung based on the identification, by three-dimensional deconvolution microscopy, of cells that coexpress epithelial and mesenchymal markers.[14]

Mechanisms of Fibrosis

Oxidative Stress

Lung cells must deal with a significant oxidant load derived from high oxygen tension, inhaled substances (e.g., tobacco smoke, ozone, and particulates), and activated leukocytes.[16] Endogenously generated oxidants include superoxide (O_2^-), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and hydroxyl radical ($OH^\cdot$). Oxidants modify biologic macromolecules, potentially leading to cellular dysfunction and death. In addition, the redox state can alter gene expression via effects on redox-sensitive transcription factors such as nuclear factor-κB and activator protein-1. Defenses against oxidants include superoxide dismutases, which convert O_2^- to H_2O_2; catalase and other enzymes that decompose H_2O_2; and antioxidants such as glutathione, a tripeptide derived from glutamate, cysteine, and glycine. Glutathione concentrations in epithelial lining fluid (≈400 μM) are approximately two orders of magnitude higher than in nonlung extracellular fluids and plasma, and within cells glutathione constitutes ≈90% of the nonprotein thiol groups.[16]

Circumstantial evidence suggests that alterations in redox balance promote fibrosis. Many agents that induce fibrosis (e.g., asbestos, silica, bleomycin, ionizing radiation) produce reactive oxygen species (ROS) directly. Inflammatory cells from IPF lungs produce higher concentrations of oxidants than normal cells, and lavage fluid from IPF patients contains a high level of 8-isoprostane, a product of lipid peroxidation widely used as a marker of oxidative stress. Epithelial lining fluid from IPF patients has a reduced glutathione concentration (≈25% of normal).[17]

There are compelling links between redox biology and the fibrotic process. Transforming growth factor-β, a key profibrotic cytokine, causes reduced expression of γ-glutamyl cysteine synthetase, the rate-limiting enzyme for glutathione production, and induces the production of ROS by myofibroblasts isolated from IPF lung.[18] In addition, the latent form of TGF-β is activated by ROS.[19] Mice deficient in the redox-regulated transcription factor Nrf2, which is involved in the expression of several antioxidant enzyme systems, have increased fibrosis after bleomycin injury.[20] Reactive oxygen species can increase proliferation of fibroblasts and cause epithelial cell death.

There is also evidence that enhancing lung antioxidant functions reduces fibrosis. Administration of superoxide dismutase, or small molecules with superoxide dismutase activity, reduces fibrosis in several models. Because the availability of cysteine is rate limiting for glutathione synthesis, N-acetyl cysteine, which is converted to cysteine, has been used to augment glutathione levels. N-acetyl cysteine attenuates bleomycin-induced lung inflammation and subsequent fibrosis in mice and stabilizes lung function in IPF patients.[21]

Apoptosis

Apoptosis is a form of programmed cell death characterized by activation of a set of cysteine proteases (caspases), packaging of the cell remnants into membrane-bound vesicles that are taken up by surrounding cells, and lack of an inflammatory response.[22] Apoptosis is conceptually distinct from another form of programmed cell death (autophagy) and from nonprogrammed cell death (necrosis), although in practice these forms of cell death are often not clearly distinguishable. Triggers of apoptosis involve extrinsic signals that trigger tumor necrosis factor receptor superfamily members with a death domain (e.g., the Fas receptor) and/or intrinsic signals such as DNA damage that cause release of mitochondrial

cytochrome c and subsequent activation of a caspase cascade. Apoptosis can be induced by interruption of normal cell adhesion to the extracellular matrix, a process referred to as *anoikis*.

Increased numbers of apoptotic epithelial cells are present in IPF lungs, particularly adjacent to fibroblastic foci, but also in relatively normal areas of lung.[5,22] In animal models, apoptosis is causally related to fibrosis. Inhibition of apoptosis with the broad-spectrum caspase inhibitor zVADfmk inhibits fibrosis in the bleomycin model. The role of apoptosis has also been studied in mice with an inducible *TGF*-β transgene expressed in lung epithelium; *TGF*-β expression in these mice causes a wave of apoptosis, followed by fibrosis, both of which are blocked by zVADfmk.[23,24] Finally, intratracheal administration of an activating anti-Fas antibody, which binds to Fas expressed on epithelial cells, leads to lung epithelial cell apoptosis and fibrosis.[22]

There are several possible causes for increased numbers of apoptotic epithelial cells in IPF. Epithelial cells overlying a fibroblastic focus may undergo anoikis. Li and colleagues showed that IPF myofibroblasts release angiotensin II, which triggers epithelial cell apoptosis, and captopril prevents fibrosis in several rodent models.[25] Oxidant stress and TGF-β signaling may also contribute to epithelial apoptosis. In patients with mutations in the gene encoding surfactant protein-C (SP-C), cellular dysfunction and the endoplasmic reticulum stress response may cause apoptosis of AT2 cells, and one can speculate that protein misfolding or trafficking abnormalities in AT2 cells might occur in IPF due to environmental and/or genetic factor(s). Vandivier and colleagues suggest that impaired apoptotic cell removal may also be a factor.[26]

How epithelial cell apoptosis leads to fibrosis is not known. A simple explanation is that loss of epithelial barrier function leads to formation of a fibrinous exudate followed by an abnormal fibroblast response. Also, apoptotic cells or the cells that engulf them can release the profibrotic cytokine TGF-β,[26] triggering other cellular and biochemical changes that promote development of fibrosis.

Transforming Growth Factor-β

Transforming growth factor-β is a multifunctional cytokine involved in development, the immune system, matrix production, angiogenesis, and control of proliferation. Essentially all cells express TGF-β and have TGF-β receptors. Overwhelming evidence implicates TGF-β as a major factor in fibrosis.[27] Transforming growth factor-β levels are increased in IPF lung. In animal studies, overexpression of TGF-β in lung causes fibrosis,[24,28] and interruption of TGF-β signaling prevents fibrosis in lung,[29,30] kidney, skin, and liver models. There are three isoforms of TGF-β; TGF-β1 is the main isoform involved in the pathogenesis of fibrosis.

The activated TGF-β receptor complex has serine/threonine kinase activity, and its major substrates are the receptor Smad (R-Smad) proteins Smad2 and Smad3. Phosphorylated R-Smads combine with a Co-Smad (Smad4), translocate to the nucleus, and, in conjunction with cell- and context-specific coactivators and corepressors, alter transcription of target genes.[31] Smad3 knockout mice have impaired fibrosis and wound healing responses.

Transforming growth factor-β is secreted in a latent form that results from noncovalent interaction between TGF-β and its propeptide (latency-associated peptide [LAP]). Latency-associated peptide also forms a disulfide linkage with proteins of the latent TGF-β binding protein (LTBP) family. Latent TGF-β binding proteins, in turn, are covalently attached to extracellular matrix proteins.[32]

Latent TGF-β must be released from LAP before it can bind to its receptors. This process, called *latent TGF-β activation*, is highly regulated and can be accomplished in several ways.[32] Proteases (plasmin and several matrix metalloproteinases) can degrade LAP and release TCFβ-1. Reactive oxygen species activate latent TGF-β1 by denaturing LAP. Thrombospondin-1 binds to LAP, triggering a conformational change that releases active TGF-β. Finally, the integrins αvβ6 and αvβ8 activate TGF-β1 by binding to an integrin binding motif (arginine-glycine-aspartic acid) near the C terminus of LAP.

Sime et al. demonstrated the critical importance TGF-β activation in lung fibrosis.[28] Using an adenoviral system, they expressed either wild-type TGF-β1 or a mutated form bearing mutations in the LAP domain that eliminate the inhibitory function of LAP. Only the constitutively active form of TGF-β1 produced fibrosis, despite the presence of high lung levels of total TGF-β1 protein in both cases. This result indicates that TGF-β activating capacity, rather than the amount of substrate (latent TGF-β), can be the limiting factor in TGF-β–mediated fibrotic lung reactions.

αvβ6-mediated TGF-β activation appears to be a particularly important activation mechanism in the lung. Integrins are heterodimeric cell surface receptors, each composed of an α- and a β-subunit. αvβ6 is one of 24 mammalian integrins, 8 of which bind arginine-glycine-aspartic acid sequences. αvβ6 is mainly expressed in epithelial cells. Activation of TGF-β1 by αvβ6 requires an intact actin cytoskeleton and tethering of latent TGF-β1 to the extracellular matrix by LTBP1.[33] Protease-activated receptor-1 signaling enhances actin contractility and αvβ6-mediated TGF-β1 activation.[34]

In the lung, αvβ6 normally is expressed at a low level but is sharply upregulated by inflammatory stimuli. β6-

integrin knockout mice develop lung inflammation and ultimately emphysema, are protected from acute lung injury, and are resistant to fibrosis.[35,36] All these effects are due to, or at least consistent with, reduced TGF-β signaling. Bleomycin-treated β6 knockout mice have more lung inflammation than wild-type controls, indicating that inflammation and fibrosis can be dissociated. Other mechanisms of TGF-β activation in the lung may also come into play; for example, IL-13–dependent lung fibrosis is dependent on TGF-β signaling, and proteases (plasmin and matrix metalloproteinases) appear to be the activators.[29,30] Also, bleomycin-stimulated alveolar macrophages activate TGF-β using plasmin and thrombospondin-1.[37]

Once activated, TGF-β sets in motion a wide range of profibrotic processes (Figure 46.3). In addition to its direct effects, TGF-β upregulates other profibrotic molecules such as platelet-derived growth factor, ROS, and connective tissue growth factor. Connective tissue growth factor, a member of the CCN family of matricellular proteins, is a downstream mediator of TGF-β effects. Its signaling function is not well understood but may derive in part from its ability to enhance binding of TGF-β to TGF-β receptors.[38] Transforming growth factor-β signaling causes autoinduction of the *TGFβ-1* gene and upregulation of TGF-β activators (αvβ6 and ROS), suggesting the possibility of a positive feedback loop in TGF-β signaling. Endogenous negative regulators of TGF-β signaling include an inhibitory Smad (Smad7), which is induced by TGF-β, and extracellular molecules such as decorin and α_2-macroglobulin. Impaired Smad7 expression may contribute to scarring in scleroderma.[39]

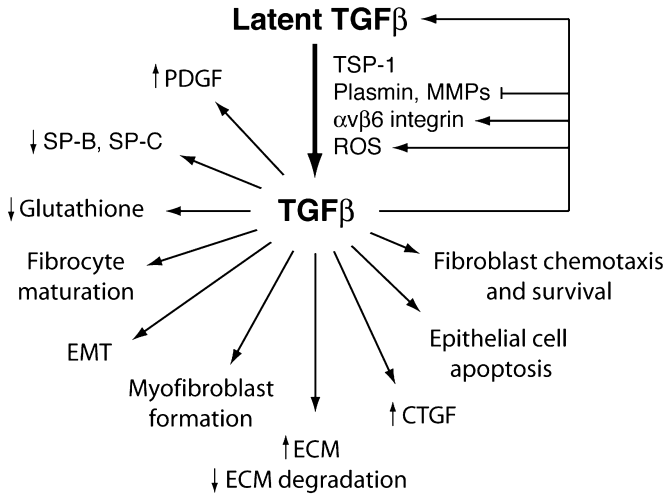

FIGURE 46.3. The central role of transforming growth factor-β (TGF-β) in lung fibrosis. Latent TGF-β is converted to active TGF-β by thrombospondin-1 (TSP-1), αvβ6 integrin, proteases, or reactive oxygen species (ROS). Transforming growth factor-β may positively regulate its own signaling by upregulating two activation mechanisms (ROS and αvβ6 expression) and autoinducing transcription of the TGF-β1 gene. Transforming growth factor-β signaling also positively regulates multiple fibrogenic factors and processes. CTGF, connective tissue growth factor; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; MMPs, matrix metalloproteinases; PDGF, platelet-derived growth factor; SP, surfactant protein.

Viral Infection

Accumulating evidence supports the intriguing hypothesis that chronic viral infection of alveolar epithelial cells triggers fibrosis in susceptible hosts.[40] Tang et al.[41] found that 97% of IPF biopsy specimens were positive for one or more of four herpesviruses tested compared with only 36% of controls, and others have reported the presence of actively replicating Epstein-Barr virus (EBV) in IPF lung.[42] Although EBV DNA can be detected in the blood of most people, it is less commonly detected in the lung; EBV can be detected in lungs of IPF patients about four times more frequently than in controls. Furthermore, a specific form of EBV (the Wzhet rearrangement) that favors active replication was found to occur much more frequently in IPF patients (59%) than in controls (2%).[42]

Causality cannot be proved in such studies but can be assessed in animal models. The murine γ-herpesvirus 68 (MHV68), analogous to EBV, has been used in several studies.[40] BALB/c mice, normally fibrosis resistant, develop fibrosis if infected with MHV68 prior to bleomycin injury. Also, MHV68 infection of interferon receptor knockout mice results in lung fibrosis with many of the pathologic features of human UIP/IPF.

Angiogenesis

Idiopathic pulmonary fibrosis lungs have abnormal anastomoses between the systemic and pulmonary circulations, and neovascularization also occurs in animal models. In addition, extracts of fibrotic lungs have angiogenic activity when tested in the corneal pocket assay. Strieter and colleagues showed that the abnormal angiogenic activity of fibrotic lung is due to an imbalance between angiogenic CXC chemokines (e.g., CXCL5 and CXCL8, which signal through the receptor CXCR2) and interferon-inducible angiostatic CXC chemokines (CXCL9, CXCL10, and CXCL11, which signal through CXCR3).[43,44] Increasing angiostatic activity (e.g., by administration of recombinant CXCL10 or CXCL11) or reducing angiogenic activity (e.g., in CXCR2 knockout mice) reduces fibrosis in the bleomycin model, supporting the concept that neovascularization may be causally connected to the fibrotic process.

Inherited Forms of Interstitial Lung Disease

Surfactant Protein C Mutations

Surfactant protein C (SP-C) mutations are the best-characterized genetic cause of IPF-like lung disease. Surfactant protein C is produced by AT2 cells and contributes to the surface tension–reducing properties of surfactant and to innate immune defense. Because of its hydrophobicity and tendency to form insoluble aggregates, including amyloid fibrils in aqueous solution, SP-C poses a processing challenge to the AT2 cell. The 3.7-kDa mature protein is generated from a 21-kDa precursor (Figure 46.4). Surfactant protein C processing involves translocation of the proprotein to the endoplasmic reticulum, folding, and addition of two palmitic acid residues, a total of four proteolytic cleavages of the N- and C-terminal propeptide domains to generate mature SP-C, release of SP-C into the lumen of the lamellar body where it associates with surfactant phospholipids and proteins, and secretion into the alveolar space.[45]

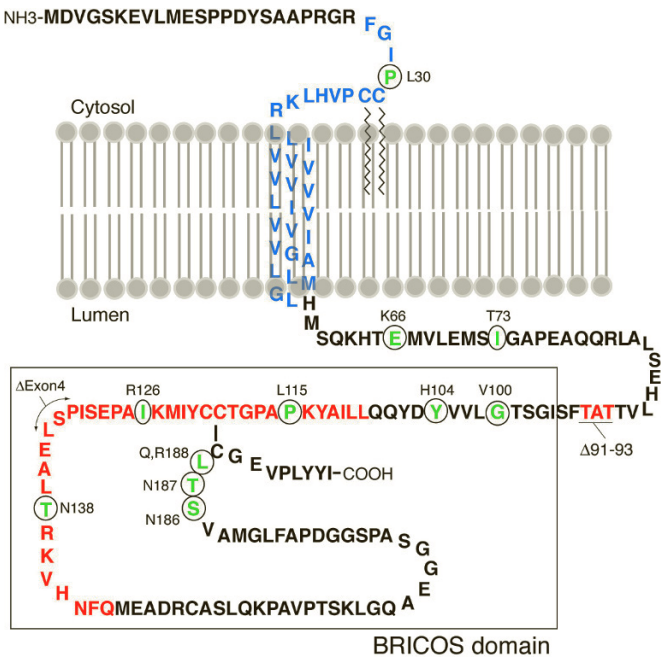

FIGURE 46.4. Schematic diagram of prosurfactant protein C and mutations that cause interstitial lung disease. Mature surfactant protein C (SP-C) is shown in blue. Pro-SP-C associates with the cell membrane via a transmembrane domain and two palmitic acid residues before it is translocated into the lumen of the lamellar body. After removal of the N- and C-terminal prodomains, mature SP-C is translocated to the lumen of the lamellar body where it associates with the other constituents of surfactant. Lamellar bodies are lysosome-related organelles unique to AT2 cells. Amino acid substitutions causing interstitial lung disease are shown in green. Two deletions causing interstitial lung disease are shown in red. The mutations interfere with normal processing, causing protein aggregation and in some cases impaired secretion of mature SP-C.

Surfactant protein C mutations in patients with interstitial lung disease occur in several locations and act in an autosomal dominant fashion. Almost all mutations occur in the luminal part of the proprotein, and most are within the ≈100 amino acid C-terminal region (Phe94–Ile197) that forms a so-called BRICHOS domain. The BRICHOS domain affects targeting, processing, and folding of proteins that contain them.

The clinical manifestations of these mutations are varied. Some patients present within weeks of birth, whereas others manifest disease as adults. Pathologic findings range from UIP and nonspecific interstitial pneumonia to pulmonary alveolar proteinosis.

The molecular basis of the lung disease appears to be loss of normal SP-C levels in surfactant and/or toxic effects of misfolded mutant SP-C within the AT2 cell. The toxic "gain-of-function" effects of mutant proteins have been studied by expressing mutant SP-C in epithelial cells. Depending on the mutation and level of expression, mutant protein accumulates in the endoplasmic reticulum, in perinuclear collections termed *aggresomes* or in endosomal vesicles. Misfolded protein can induce the endoplasmic reticulum stress response, leading to apoptosis or increased expression of inflammatory mediators.[46] Also, accumulations of mutant protein may be directly toxic to the cell, as postulated for other proteins that form amyloid.

Mutant SP-C acts in a dominant-negative fashion to block release of wild-type, mature SP-C. This effect appears to be restricted to SP-C mutations within the BRICHOS domain; patients with these mutations fail to secrete mature SP-C. Amin and colleagues described a patient with interstitial lung disease who had no detectable mature SP-C within the lung despite the absence of mutations within the SP-C gene coding regions, suggesting that absence of SP-C may cause lung disease.[47] However, patients with SP-C mutations outside the BRICHOS domain produce mature SP-C, suggesting that toxic gain-of-function effects may be the dominant mechanism leading to lung disease.

Mice lacking SP-C or overexpressing mutant SP-C have lung abnormalities but do not recapitulate the findings found in humans. Surfactant protein C knockout mice are viable and develop lung inflammation and emphysema; when exposed to bleomycin they have an exaggerated fibrotic response.[48] Mice overexpressing a mutant *SFTPC* transgene lacking much of the BRICHOS domain have a lethal disruption of branching morphogenesis.[49]

Hermansky-Pudlak Syndrome

Hermansky-Pudlak syndrome (HPS) is another inherited disorder leading to interstitial lung disease, and, interestingly, the defect also targets AT2 cells. Hermansky-Pudlak syndrome is due to mutations in one of eight genes in humans *(HPS1–8)*[50–52]; corresponding mutations occur in mice (Table 46.3). Patients manifest reduced skin and eye pigmentation, a bleeding diathesis due to platelet dysfunction, and, in some cases, progressive lung fibrosis in middle age. The defect underlying these abnormalities is impaired genesis or function of lysosome-related organelles.[51] These organelles are variants of lysosomes that perform specialized functions. The affected organelles are melanosomes in melanocytes, cytotoxic granules in lymphocytes, platelet dense granules, and lamellar bodies in AT2 cells. Biopsy specimens reveal proliferative, enlarged AT2 cells containing giant lamellar bodies packed with surfactant. The AT2 cells appear degenerative and often desquamated. There is inflammation centered on respiratory bronchioles and fibrosis with honeycombing.

The disorder is autosomal recessive and involves two classes of genes. One group encodes proteins with known roles in protein trafficking (e.g., adaptor protein-3). The second group encodes proteins that are subunits of protein complexes called BLOCs (biogenesis of lysosome-related organelles complex). The BLOCs are ubiquitously expressed, but the cellular localization and precise function of these multiprotein complexes have not been defined.

While the mouse strains with HPS mutations have pigmentation and platelet defects that mimic HPS, the lung pathology is quite different.[52] The HPS-like strains in general have reduced life spans and develop emphysema. Lyerla and colleagues generated a double homozygous mutant (*HPS1* and *HPS2* genes); the AT2 cells in these mice are enlarged and contain enlarged lamellar bodies engorged with surfactant, as well as significant inflammation, as seen in humans with HPS lung disease.[53]

The mechanism connecting the genetic defects and lung fibrosis in humans is not known. Toxicity to AT2 cells and/or reductions in surfactant components may be involved. Toxicity to AT2 cells is readily apparent in biopsy tissues of patients. Alterations in surfactant secretion in patients have not been defined. In *HPS1/HPS2* double mutant mice, total lung phospholipid, SP-B, and SP-C levels are increased (and SP-C is processed to the 3.7-kDa form), but alveolar levels of these components are reduced and isolated AT2 cells from these mice have reduced basal and stimulated phospholipid secretion.[52,53]

Table 46.3. Hermansky-Pudlak syndrome (HPS) genes in humans and mice.

Mouse	Human	Product	Lung fibrosis
Pale ear	*HPS1*	BLOC-3 subunit	Yes
Pearl	*HPS2*	AP-3 β3A subunit	
Cocoa	*HPS3*	BLOC-2 subunit	
Light ear	*HPS4*	BLOC-3 subunit	Yes
Ruby eye-2	*HPS5*	BLOC-2 subunit	
Ruby eye	*HPS6*	BLOC-2 subunit	
Sandy	*HPS7*	BLOC-1 subunit	
Reduced pigmentation	*HPS8*	BLOC-1 subunit	
Pallid		BLOC-1 subunit	
Muted		BLOC-1 subunit	
Cappuccino		BLOC-1 subunit	
Buff		Vps33A	
Mocha		δ-Subunit of AP-3	
Gunmetal		Rab geranylgeranyl transferase α-subunit	

Note: AP, adaptor protein; BLOC, biogenesis of lysosome-related organelles complex.

Genetic Studies of Sporadic and Familial Interstitial Lung Disease

Case–control studies have been carried out to test the association of polymorphic variants of candidate genes with the risk for IPF. Positive results have been reported for genes encoding angiotensin-converting enzyme, complement receptor-1, IL-1 receptor antagonist, TNF-α, and SP-A and SP-B.[54] Interestingly, the SP-A1 variant ($6A^4$) encodes a protein with an altered amino acid (tryptophan rather than arginine) that appears to affect aggregation.[55]

Sometimes interstitial lung disease cases cluster within families. Inheritance is autosomal dominant with reduced penetrance. In about half the families the disease is uniformly UIP/IPF, and in the others there are various presentations (as occurs in families with SP-C mutations).

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