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Inflammation and dysregulated fibroblast proliferation – new mechanisms?

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Abstract. Idiopathic pulmonary fibrosis (IPF) is a devastating, age-related lung disease of unknown cause that has few treatment options. Once thought to be a chronic inflammatory process, current evidence indicates that the fibrotic response may primarily be driven by abnormally activated alveolar epithelial cells and the underlying mesenchyme. The mediators produced and present in this microenvironment induce the formation of fibroblast foci through the proliferation of resident mesenchymal cells, attraction of circulating fibrocytes, and stimulation of epithelial to mesenchymal transition. The fibroblast and myofibroblast foci secrete excessive amounts of extracellular matrix, mainly collagens, resulting in scarring and destruction of the lung architecture. The detailed mechanisms that link IPF with ageing and aberrant epithelial activation are unknown, but some evidence suggests that the abnormal recapitulation of developmental pathways and epigenetic changes may play a role. This review provides a brief synopsis of highlights in the current understanding of the pathophysiology of IPF, as well as novel therapeutics being explored in clinical trials for the treatment of this devastating disease. *(Sarcoidosis Vasc Diffuse Lung Dis 2013; 30 Suppl 1: 21-26)*

Key words: extracellular matrix, fibrosis, pathophysiology, mediators, myofibroblast

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic interstitial lung disease associated with a decline in lung function that increasingly restricts routine physical activity of the patient and has a median survival of 2-5 years from diagnosis (1, 2). It has long been assumed that the pathogenesis of IPF involves a process of chronic repair that results from persistent pulmonary inflammation with attendant activation of inflammatory cells, cytokines and growth factors capable of activating mesenchymal cells, with enhanced matrix production and deposition leading to fibrosis (3). However, the majority of IPF patients show little or no evidence of ongoing inflammation, raising questions as to whether IPF is truly associated with chronic inflammation.

While the mechanisms that result in IPF are still not fully understood, there is a strong suggestion of the involvement of alveolar epithelial cell death and alveolar collapse in the pathogenesis of usual interstitial pneumonia (UIP) (4, 5). Research over the past 10 years suggests that IPF may be an intrinsic fibroproliferative disorder involving an aberrant wound healing cascade where ongoing epithelial cell damage and/or activation results in abnormal mesenchymal cell activation, derivation of myofibroblasts, and excess matrix deposition (Figure 1) (3). The current paradigm of the pathophysiology of IPF has therefore shifted from a chronic inflammatory process towards alveolar epithelial cell dysfunction and disordered fibroproliferation being centre stage (3-8).

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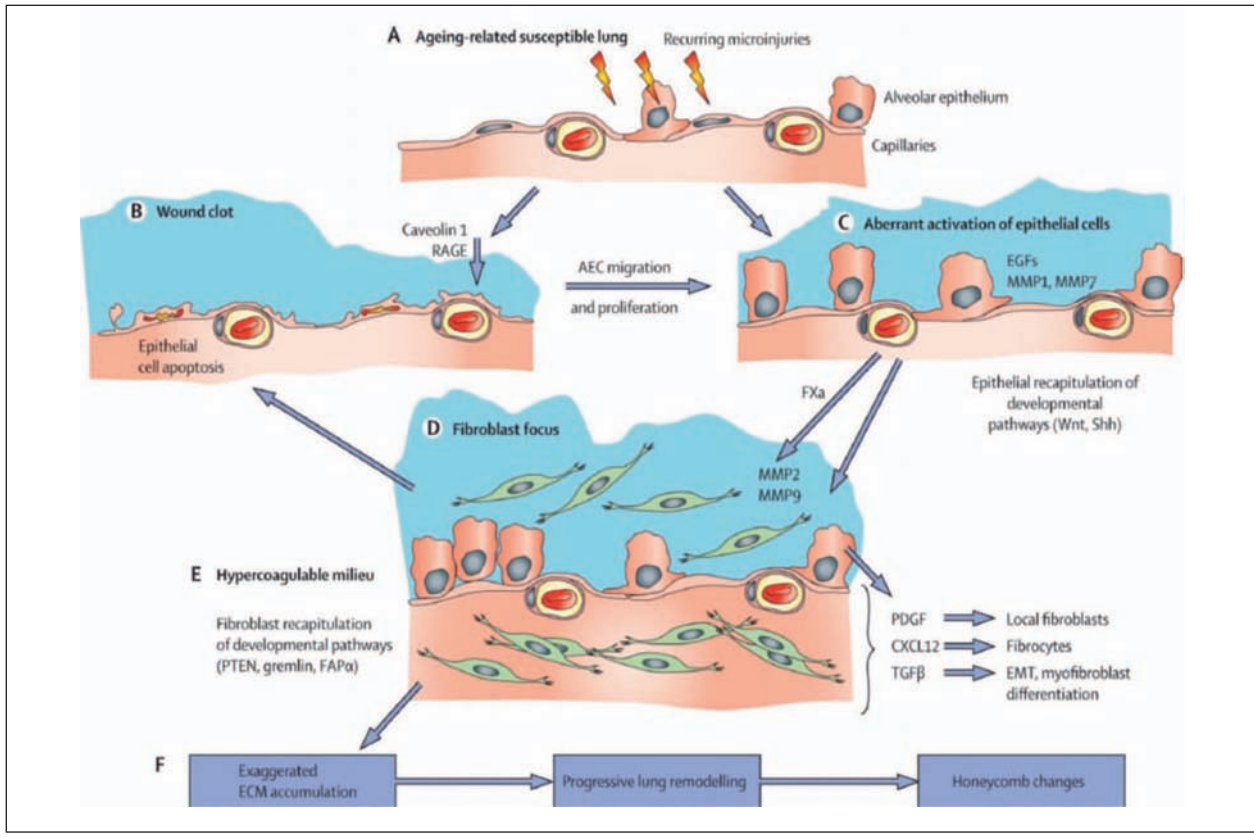

Fig. 1. Pathogenesis of IPF (3)

Alveolar epithelial cells

Alveolar epithelial cells (AEC) type II play a central role in regulating the fluid balance in the lung by the synthesis, secretion, and recycling of surfactant, thereby reducing surface tension and allowing alveolar ventilation, perfusion and gas exchange at normal transpulmonary pressures (9-12). The surfactant proteins SP-B and SP-C, and phospholipid dipalmitoylated phosphatidylcholine are key components of surfactant (12, 13). AEC type II also produce compounds of the innate immune defense system, such as defensins, collectins (of which the SP-A and SP-D are notable) and lysozyme, which contribute towards the prevention of infection. SP-A and SP-D, for example, bind to the surface of various pathogens facilitating their removal by alveolar macrophages (14-19).

AEC type II also have stem-cell-like or progenitor cell self-renewal characteristics and have a high proliferative potential (20-22) for trans-differentiating into AEC type I (22). Mutations in the genes for surfactant proteins SP-A and SP-C appear to be associated with an increased susceptibility of chronic AEC type II cell injury and apoptosis in familial forms of IPF and nonspecific interstitial pneumonia (23-28). More than 30 different mutations of SP-C have been reported to date (29). Abnormal telomere shortening in AEC types I and II is also associated with the development of IPF, suggesting that early exhaustion of the regenerative properties of the alveolar stem cell pool may contribute to the progressive fibrogenesis seen in IPF lungs (30).

Fibroblasts and matrix deposition

Fibrosis is a pathobiological process common to many human diseases and is characterised by the progressive replacement of normal tissue with a col-

lagen-rich extracellular matrix (31, 32). Myofibroblasts in IPF originate from trans-differentiation of interstitial fibroblasts, from AEC via epithelial mesenchymal transition, or from bone-marrow derived circulating progenitor cells, the fibrocytes (33, 34). Fibroblasts and activated myofibroblasts accumulate as small clusters (fibroblastic foci) in subepithelial areas (33, 34). The overlying epithelium consists of hyperplastic pneumocytes or columnar non-ciliated bronchiolar cells. These mesenchymal cells secrete excessive amounts of extracellular matrix molecules, primarily fibrillar collagens, but also many different proteoglycans and glycoprotein, resulting in extensive structural disorganisation of the lung microenvironment with loss of alveolar-capillary units with the development of scarring and cysts (honeycombing) (32).

The resulting tissue remodelling culminates in increased tissue mechanical stiffness (35-37). Relatively recent observations have highlighted the fact that variations in matrix stiffness can potently alter fibroblast morphology, proliferation, synthetic function, responsiveness to growth factor signalling (e.g. transforming growth factor-beta [TGF-β]) and myofibroblast activation (38-43). Thus, rather than simply an outcome of fibrosis, stiffening of the mechanical environment may directly affect cellular behaviours that promote, amplify, and perpetuate fibrosis in an autocrine activation loop (44).

Growth factors

Not only inflammatory cells but also aberrantly activated AEC type II and interstitial mesenchymal cells can produce many of the cytokines and growth factors responsible for migration and proliferation of local fibroblasts and their transition to myofibroblasts. Much attention has focused on the role that soluble inflammatory and fibrogenic mediators play in the initiation and progression of fibrosis (45). Whilst numerous, key factors include the pleiotropic growth factor TGF-β1, platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and interleukin (IL)-13 (45-47). Integrins such as αvβ6 are able to activate latent, matrix-bound TGF-β and thereby enhance profibrotic elements in the tissue even without synthesis of new molecules (48). TGF-β1 is expressed at low or undetectable levels in normal lungs but is upregulated within AEC in IPF. In fibroblasts isolated from patients with IPF, TGF-β1 promotes many of the pathogenic mechanisms of fibrosis and remodelling, such as enhanced collagen synthesis, extracellular matrix deposition and fibroblast to myofibroblast differentiation (45, 49). The lack of growth inhibition and delayed apoptosis showed by fibrotic cells might be also related to a modified response to tumour necrosis factor-alpha (TNF-α) due to a reduced expression of TNF receptor 1, known to mediate growth inhibition and resistance to apoptosis (50).

Several matrix metalloproteases (MMPs) are upregulated in the lungs of IPF patients and have been shown to actively participate in the pathogenesis of the disease through extracellular matrix remodelling and basement membrane disruption (51, 52). MMPs can also break down molecules that mediate cell-cell and cell-extracellular matrix interactions, and can activate growth factors and growth factor receptors indicating that they likely contribute to other local biological processes such as apoptosis, migration, proliferation and angiogenesis (51).

Implications for treatment

Advances in the understanding of the pathogenetic processes involved in the development of IPF have led to novel therapeutic targets. An increasing number of compounds currently in preclinical and clinical development aim at these novel "fibrotic" mechanisms, as opposed to the more traditionally used anti-inflammatory strategies that have largely failed for IPF therapy (53). Pirfenidone, an orally administered pyridone derivative (5-methyl-1-phenyl-2-[1H]-pyridone), is the first anti-fibrotic agent to be approved for clinical use in the treatment of IPF (54). Its exact biological mechanisms are still unknown, but it has a number of properties that give it an attractive anti-fibrotic drug profile. Pirfenidone is an anti-inflammatory and anti-oxidant agent that inhibits TGF-β and TNF-α *in vitro,* both having a likely role in IPF progression (54, 55). Pirfenidone acts as an anti-fibrotic by altering the expression, synthesis, and accumulation of collagen, and inhibiting the recruitment, proliferation and possibly expression of the extracellular

matrix-producing cells, as shown in a variety of animal models (56).

Receptor tyrosine kinases are another potential therapeutic target for IPF. Several signalling pathways activated by these tyrosine kinase receptors are involved in lung fibrosis (57-59) and inhibition of specific receptors may slow the progression of IPF (47, 59-62). BIBF 1120 is a potent intracellular tyrosine kinase inhibitor that is in clinical development (phase III) for the treatment of IPF and a number of types of cancer. Its targets include platelet-derived growth factor receptors (PDGFR) α and β, vascular endothelial growth factor receptors (VEGFR) 1, 2, and 3, and fibroblast growth factor receptors (FGFR) 1, 2, and 3 (59, 63).

Another compound under investigation for IPF is serum amyloid P (SAP), a member of the pentraxin family of proteins. SAP interferes with bleomycin-induced lung fibrosis through inhibition of downstream TGF-β1 effects, and alters fibroblast apoptosis, tissue inflammation, pulmonary fibrocyte accumulation and collagen deposition, but does not affect levels of TGF-β1 themselves. SAP also appears to influence pulmonary macrophages and increase the anti-fibrotic chemokines IP10/CXCL10 in a SMAD 3-independent manner, which may contribute to its pronounced anti-fibrotic effects. Interestingly, circulating SAP concentrations are reduced in IPF patients (64).

A further potential target in fibrotic disorders is the NADPH oxidase (NOX) family of enzymes. NOX4, for example, catalyses the reduction of O_2 to form reactive oxygen species (ROS) and is upregulated in IPF lungs. NOX4-dependent generation of hydrogen peroxide (H_2O_2) is required for TGF-β1-induced myofibroblast differentiation, extracellular matrix production, and contractility. Initial proof-of-concept studies in two different murine models of lung injury have suggested that genetic or pharmacologic targeting of NOX4 abrogates fibrogenesis (65).

Collagen crosslinking is yet another important mechanism that is enhanced in IPF lungs and contributes to matrix accumulation and rigidity. Particular attention is currently on the lysyl oxidases and especially LOXL2, for which neutralising antibodies have been developed and shown excellent preclinical efficacy in animal models (66). The clinical usefulness of this compound in currently investigated in a large trial in IPF.

Discussion

Over the past 10 years, substantial advances have been made in the understanding of the pathophysiology of IPF. As new pathogenic pathways and mediators are discovered, new therapies in development are targeting the fibroblastic process and abnormal tissue remodelling, excessive extracellular matrix accumulation, and angiogenesis more directly. While it is likely that any effective treatment strategy for IPF will need to target more than one of the pro-fibrotic pathways associated with its complex pathogenesis, only one agent has been approved to date worldwide. Although the mechanisms underlying this disease remain poorly understood, the advances that have been made provide momentum for the discovery and development of additional and/or alternative effective treatment modalities.

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