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Molecular Targets in Pulmonary Fibrosis*

The Myofibroblast in Focus

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Idiopathic pulmonary fibrosis (IPF) is one of a group of interstitial lung diseases that are characterized by excessive matrix deposition and destruction of the normal lung architecture. Long-term survival of IPF patients is poor, with a 5-year survival rate of only 20%. Despite a lack of evidence-based benefit, IPF has historically been treated with corticosteroids and/or cytotoxic agents such as prednisone. Given the poor efficacy of these drugs, novel therapeutic strategies are required for the management of IPF. This demands a better understanding of the molecular mechanisms underlying the pathogenesis and progression of this disease. The primary effector cell in fibrosis is the myofibroblast; these cells are highly synthetic for collagen, have a contractile phenotype, and are characterized by the presence of α-smooth muscle actin stress fibers. They may be derived by activation/proliferation of resident lung fibroblasts, epithelial-mesenchymal differentiation, or recruitment of circulating fibroblastic stem cells (fibrocytes). From a therapeutic viewpoint, interfering with the pathways that lead to myofibroblast expansion should be of considerable benefit in the treatment of IPF. This review will highlight some of the key molecules involved in this process and the clinical trials that have ensued.

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Abbreviations: ALK-5 = activin-like kinase receptor-5; CTGF = connective tissue growth factor; ECM = extracellular matrix; EMT = epithelial-mesenchymal transition; FIZZ = found in inflammatory zone; IFN = interferon; IL = interleukin; ILD = interstitial lung disease; INSPIRE = International Study of Survival Outcomes in Idiopathic Pulmonary Fibrosis With Interferon γ-1b Early Intervention; IPF = idiopathic pulmonary fibrosis; PAR = proteinase-activated receptor; PDGF = platelet-derived growth factor; SMA = smooth muscle actin; TGF = transforming growth factor; Th2 = T-helper type 2

The interstitial lung diseases (ILDs) comprise a group of acute and chronic lung disorders with varying degrees of inflammation and fibrosis. One of the most prevalent is idiopathic pulmonary fibrosis (IPF)/usual interstitial pneumonitis. Current epidemiology studies[1] suggests that IPF is more common in male subjects, with onset usually in middle or old age, but it has no distinct geographic distribution and does not distinguish between particular races or ethnic groups. In all cases, however, it is an insidious, progressive disease with a median survival of only 2 to 3 years following diagnosis. Although the precise etiology is unknown, a number of risk factors may contribute to disease development, including smoking, drug exposure, infectious agents, and genetic predisposition.[1,2]

Diagnosis of IPF remains problematic, although the recent joint consensus statement from the American Thoracic Society and the European Respiratory Society aims to standardize the criteria for diagnosis and subsequent therapeutic approaches.[2] Histologically, IPF lungs have alternating regions of normal lung parenchyma, interstitial inflammation, fibrosis,

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and "honeycombing." These features are a result of failed alveolar reepithelialization, fibroblast persistence, and excessive deposition of collagen and other extracellular matrix (ECM) components, leading to irreversible loss of lung function. Aberrant vasculogenesis may also contribute to the disease process in a manner similar to that seen during tumorigenesis.[3] The specific molecular and cellular mechanisms that lead to disease progression are unknown, although considerable effort is being made to delineate these pathogenic processes. Since the current treatments for IPF are largely ineffective, the identification of pathways that may provide novel therapeutic targets is absolutely crucial. This review will focus on the pathogenesis of IPF, although the paradigms and potential molecular targets described may be relevant to a number of other fibrotic conditions, including sarcoidosis and systemic sclerosis.

Current Therapy

Historically, IPF was believed to result mainly from chronic inflammation, leading to persistent epithelial and vascular injury and activation/expansion of the lung mesenchyme. Established treatments based on this assumption involve the use of antiinflammatory or immunosuppressive drugs such as prednisone, azathioprine, or cyclophosphamide.[1] Unfortunately, none of these agents have been shown to unequivocally alter the inflammatory process in IPF or reduce severity or progression of the disease; prospective placebo-controlled randomized clinical trials have not been performed, yet clinicians continue to prescribe these drugs primarily because there are no recommended alternatives (see Raghu[4] for more information). Recent experimental evidence suggests that inflammation is not necessary or sufficient for the progression to fibrosis[5]; overexpression of the potent profibrotic mediator, transforming growth factor (TGF)-β1, for example, leads to progressive fibrosis in mice, without any significant inflammatory component.[6] In the human condition, antiinflammatory treatment during the end stage of fibrosis may well be somewhat ineffective, but this does not preclude a pathogenic role for inflammation in the earlier stages of the disease.[3]

Myofibroblasts

Irrespective of the uncertainty regarding the precise etiology of IPF, it is generally accepted that aberrant wound healing and epithelial-mesenchymal cross-talk are major components of the pathogenic process. Ongoing damage to the alveolar epithelium and/or capillary endothelium leads to apoptotic events that culminate in the initiation of repair mechanisms; in IPF, these repair mechanisms are apparently dysregulated. In response to a variety of growth factors and cytokines such as TGF-β1 and platelet-derived growth factor (PDGF), the subsequent hyperproliferation of type II alveolar epithelial cells, recruitment of fibroblasts, and formation of fibroblastic foci are the hallmarks of the disease; an increase in the number of these fibrotic foci is associated with disease progression and a worsened prognosis.[7] Examination of individual tissue sections would suggest that fibrotic foci are isolated lesions, potentially arising from localized injury. However, recent data from Cool et al[8] demonstrate that these foci actually form a highly complex, interconnected, and continuous fibrotic reticulum, arising from polyclonal fibroblast proliferation.

The key effector cell in fibrogenesis is the myofibroblast; these spindle- or stellate-shaped cells share features with smooth muscle cells in that they are contractile and contain α-smooth muscle actin (SMA) stress fibers. They localize to fibrotic foci and other sites of active fibrosis, and are the primary cell type responsible for the synthesis and deposition of ECM and the resultant structural remodeling that leads to the loss of alveolar function. When considering potential therapeutic approaches, understanding the pathways that lead to fibroblast proliferation, activation, and differentiation should provide a number of molecular targets that may be worthy of intense investigation for the treatment of IPF.

Current opinion suggests that myofibroblasts have at least three possible origins, although the relative contribution of each of these pathways in IPF is currently unknown (Fig 1). The most straightforward suggestion is that resident lung fibroblasts differentiate directly under the influence of the profibrotic microenvironment to form myofibroblasts.[9] For example, evidence from the bleomycin model of lung fibrosis in rats certainly suggests that the initial α-SMA–positive myofibroblasts arise in the adventia of the distal airways from peribronchiolar/perivascular fibroblasts[10]; it is highly likely that interstitial fibroblasts can respond in a similar manner.

The second possibility is that epithelial cells undergo transdifferentiation to form fibroblasts and thence myofibroblasts by a process termed *epithelial-mesenchymal transition* (EMT); epithelial cells lose their characteristic markers such as E-cadherin and zona occludens-1 and acquire mesenchymal markers such as fibroblast-specific protein-1 and α-SMA.[11] The concept of EMT has been recognized for > 20 years, and evidence is now accumulating to support a role for EMT in IPF. Alveolar epithelial cells *in vitro* can undergo EMT in response to