Escape from the Matrix

Multiple Mechanisms for Fibroblast Activation in Pulmonary Fibrosis

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Lung fibrosis is a recognized feature of many chronic lung diseases and is central to the pathogenesis of idiopathic pulmonary fibrosis, a disease that carries a prognosis worse than many cancers. Current research into this condition is defining the key pathways of activation either in resident fibroblasts, matrix-producing cells derived from circulating fibrocytes, or epithelial cells that appear to transdifferentiate to fibroblast-like cells. The downstream signaling pathways are also being delineated as well as the gene interactions leading to altered cell phenotype. These studies have led to an appreciation that multiple pathways, including inflammatory and coagulation cascades, are involved in the pathogenesis of idiopathic pulmonary fibrosis. As these facts come to light, we are exploring promising new approaches to treat fibrosis and halt the inexorable progression that is a feature of these disorders. This article reviews these findings and our current concepts of the key molecular events leading to tissue damage and excessive matrix deposition in lung fibrosis. It also highlights the need for new studies to delineate alternative pathogenetic mechanisms and integrate these pathways so we have a framework to better understand their importance in individual patients.

Keywords: fibroblasts; lung fibrosis; matrix

Chronic lung diseases are a major cause of morbidity and mortality and an enormous burden on world health systems. We now recognize that there are often common mechanisms in these chronic diseases and that new treatments developed in one setting may have application to others. A good example of this is the fibrosis that is central to idiopathic pulmonary fibrosis (IPF) but is now recognized to be an important feature of asthma and chronic obstructive pulmonary disease (1, 2). IPF has a poor prognosis (median survival, 3–5 yr) and an increasing incidence, and current therapies are ineffective (3, 4). A central feature of this disease is the destruction and remodeling of the lung's supportive matrix, leading to severely compromised gas exchange. This article reviews our current concepts of the pathogenesis of IPF and in so doing highlights the emerging approaches to treat IPF and other chronic lung diseases in which fibrosis is a component.

FIBROBLASTS AND THE FIBROTIC PHENOTYPE

It is now well recognized that the lung is actively synthesizing and degrading a diverse group of matrix components and the rates of these processes are rapid in normal tissues (5, 6). Many cell types are involved, but predominantly mesenchymal cells (fibroblasts, myofibroblasts, and smooth muscle cells) are responsible for this turnover (reviewed in References 1 and 7). Fibroblasts are widely distributed in all lung structures and play a key role in matrix homeostasis. For example, in response to growth factors or mechanical stimuli, these cells are capable of generating more than 5,000 molecules of procollagen per cell per minute (8, 9). They are also in communication with a large number of different cell types and respond to a host of cytokines and growth factors (Figure 1). More recent studies using microarray technologies have further reiterated the diversity of this response. We have recently profiled human fetal lung fibroblast global gene expression in response to transforming growth factor (TGF)- β_1 using oligonucleotide microarrays and reported that at least 150 genes are up-regulated across several major functional categories, including genes involved in cytoskeletal reorganization, matrix formation, metabolism and protein biosynthesis, cell signaling, proliferation, survival, and gene transcription (10). This included 80 that were not previously known to be TGF- β_1 responsive. Such a profound and diverse transcriptional response is also reflected *in vivo*, with studies of pulmonary fibrosis in human and animal models reporting that almost 500 genes are differentially expressed more than twofold, including a large cluster of diverse matrix and matrix-related genes (11, 12).

PROFIBROGENIC CYTOKINES AND PULMONARY FIBROSIS

A large number of mediators produced by many different cell types are known to promote fibroblast proliferation, collagen synthesis, migration, and differentiation (for review, *see* References 1, 13, and 14, and Figure 1). TGF- β is the most potent profibrotic mediator characterized to date and is a central player in several remodeling diseases, including asthma (15) and pulmonary fibrosis (16). Blocking the action of TGF- β by a number of strategies has been shown to ameliorate experimental pulmonary fibrosis. On this basis, pharmaceutical companies are developing and testing several classes of TGF- β blockers, including inhibitors of latent TGF- β activation, TGF- β blocking antibodies, and receptor kinase inhibitors, in the hope of better treating human fibrosis. A serious caveat, however, is the role TGF- β plays as an inhibitor of immune responses or as a tumor suppressor (17). For this reason, there are concerns that blocking TGF- β may lead to undesired side effects but thus far these reservations appear to be unfounded (reviewed in Reference 18).

Many other cytokines in addition to TGF- β are believed to play roles in the pathogenesis of IPF (14). Based on a modified Koch's postulate, we proposed some time ago a criterion to

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Figure 1. Fibroblasts and factors. CGRP = calcitonin gene-related peptide; ECP = eosinophil chemotactic protein; EGF = epidermal growth factor; ET-1 = endothelin-1; FGF2 = fibroblast growth factor 2; GM-CSF = granulocyte-macrophage colony-stimulating factor; IGF-1 = Insulin-like growth factor-1; LTB₄ = leukotriene B₄; MCP-1 = monocyte chemotactic protein-1; PAF = platelet activating factor; PDGF = platelet-derived growth factor; PGD₂ = prostaglandin D₂; PGE₂ = prostaglandin E₂; PGF_{2α} = prostaglandin F_{2α}; PGI₂ = prostacyclin; TGF-α = transforming growth factor-α; TGF-β = transforming growth factor-β; TNF-α = tumor necrosis factor-α. Reprinted by permission from Reference 1.

evaluate the likelihood of any one individual cytokine/mediator playing a role in IPF (19). These criteria include the presence in the lung of patients with IPF, profibrotic properties *in vitro*, and amelioration when the action of the cytokine is blocked in animal models. Many cytokines/mediators, including tumor necrosis factor (TNF)-α, endothelin (ET)-1, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF)-1, IL-1, and IL-13 fulfill these criteria and inhibitors are currently being tested in humans.

ENDOTHELIN AND ANGIOTENSIN: VASOCONSTRICTORS THAT REGULATE REMODELING AND FIBROSIS

It has been observed that vasoconstrictors often induce remodeling and fibrosis, whereas vasodilators are inhibitors. For example, the vasoconstrictors ET-1 and angiotensin II both exhibit profibrotic features *in vitro* (20, 21), and receptor antagonists of these agents have shown some success in blocking fibrosis in animal models. Furthermore, a common polymorphism in angiotensin converting enzyme has been shown to influence outcome in patients with acute respiratory distress syndrome (22), a disease in

which fibrosis is often a component (23). The relevance of these studies to humans remains uncertain and will await the results of ongoing trials with drugs blocking these pathways.

CYTOKINES AND LIPID MEDIATORS AS INHIBITORS OF FIBROBLAST FUNCTION AND FIBROSIS

Fibrosis occurs if the normal homeostatic balance is perturbed, resulting in an excessive production of profibrotic mediators that then activate fibroblasts (14). This concept has led us to consider ways of changing this balance in patients with fibrotic disorders. The focus has mostly been on inhibition of profibrotic cytokines, but there is also considerable interest in strategies to up-regulate antifibrotic molecules. This was given impetus by the reports in a small group of patients that IFN-γ was an effective treatment for IPF (24), although this early promise was not borne out in a recent large multicenter trial (25). Another molecule of interest is prostaglandin E₂ (PGE₂), a product of cyclooxygenase (COX) catalyzed arachidonic acid metabolism, which is an inhibitor of fibroblast proliferation and collagen deposition (26, 27). PGE₂ production is reduced in fibroblasts

Figure 2. Sources of fibroblasts and myofibroblasts in pulmonary fibrosis. Blood-borne fibrocytes, derived from bone marrow stem cells, target to the lung where they may play roles in elaborating matrix. Fibroblasts may also be derived from the transdifferentiation of epithelial cells, a process termed epithelial-mesenchymal transition (EMT). Finally, resident fibroblasts are also capable of transdifferentiation into myofibroblasts, which exhibit both a contractile and matrix-producing phenotype. The relative importance of these pathways in human disease is yet to be elucidated. ECM = extracellular matrix. Adapted by permission from Reference 71.

from patients with lung fibrosis after stimulation with mediators such as IL-1 (28) or TGF- β (29), and this is due to a decreased capacity to up-regulate COX2. Furthermore, COX2-deficient mice are more susceptible to bleomycin-induced pulmonary fibrosis (30), supporting the hypothesis that there is a defect in the COX2-PGE₂ axis in fibrosis. We have also recently shown that a functional promoter polymorphism in the COX2 gene (PTGS2), which reduces gene expression (31), is associated with susceptibility to sarcoidosis (32). Moreover, the association is most common in those patients with persistent progressive disease who are most likely to develop pulmonary fibrosis (32). These data highlight the importance of gene interactions and also suggest that therapeutic strategies to overexpress anti-fibrotic molecules might be fruitful. One possibility may be a gene delivery approach, although there are clearly concerns about safety of this approach when viral vectors are used. To circumvent these concerns, we have explored an integrin-targeting gene delivery system, composed of a cationic liposome, an integrin binding peptide, and plasmid DNA, which shows high delivery efficiency while avoiding the immune and inflammatory effects associated with the use of adenoviral vectors (33).

PROTEASES IN THE REGULATION OF FIBROBLAST FUNCTION AND REMODELING

The serine and matrix metalloproteinases have long been assumed to play key roles in emphysema in which degradation of matrix and destruction of parenchymal lung structures are a feature. However, there is increasing evidence that these molecules are also important in the pathogenesis of acute lung injury and pulmonary fibrosis (34). In this context, inhibitors of neutrophil elastase have been shown to inhibit lung injury and fibrosis (35–37), and we have recently demonstrated that mice deficient for this proteinase are protected from lung fibrosis (38).

Proteinases of the coagulation cascade, including factor VIIa, factor Xa, and thrombin, exert proinflammatory and profibrotic effects, and likely play key roles in acute lung injury and remodeling disorders of the lung (39–42). These proteinases exert their cellular effects via interaction with four proteinase-activated receptors, PAR1–PAR4, with the coagulation proteinases of the extrinsic pathway targeting all four of these receptors. In terms of influencing fibroblast function, we and others have shown that PAR1, the high-affinity thrombin receptor, is the major receptor by which thrombin (39, 40) and factor Xa (43) exert their potent profibrotic effects. Thus, PAR1 has emerged as a promising new target to prevent fibrosis both in the setting of IPF and acute respiratory distress syndrome. Furthermore, thrombin inhibition partially blocks experimental fibrosis (44), and mice deficient for PAR1 are protected from lung inflammation, pulmonary edema, and lung collagen accumulation after bleomycin injury (45, 46).

MECHANICAL FORCES AND FIBROBLAST FUNCTION

The potent actions of mechanical forces on influencing fibroblast phenotype have long been recognized in studies of muscle (47), skin (48), and the cardiovascular system (49). More recently, the potential role for mechanical forces in altering lung cell phenotype has also been highlighted (50), although we still know little of the mechanisms of mechanosensing and the subsequent molecular pathways involved. In the context of fibrosis, it is of interest that mechanical forces promote matrix production and that this effect is mediated via multiple cell surface receptors and intracellular signaling pathways (51–53) and involves autocrine actions of TGF- β (9). The relevance of these pathways to lung fibrosis remains to be elucidated.

APOPTOSIS AND PULMONARY FIBROSIS

Apoptotic pathways are key to the resolution of inflammation and fibrosis after lung injury (54). In pulmonary fibrosis, there is some evidence that fibroblasts are resistant to apoptosis (55) and that epithelial cells may be more susceptible to this process (56). Thannickal and Horowitz (57) have reviewed the evidence for these changes and suggested that this “apoptosis paradox” is central to the pathogenesis of IPF. In experimental models of IPF, the data suggest that inhibitors of the proapoptotic molecules, Fas or the caspases, inhibit fibrosis (58, 59), consistent with epithelial cell apoptosis being an important event. The mechanisms driving these events are uncertain, although TGF- β is again emerging as a central player (60).

CELL PLASTICITY: EPITHELIAL–MESENCHYMAL TRANSITION AND BLOOD FIBROCYTES

The pathways leading to fibrosis are now recognized to involve considerable cell plasticity, with the excessive numbers of profibrotic cells potentially being derived from several sources (Figure 2). Epithelial–fibroblast interactions may be central to remodeling both in the airways (61) and in the fibrotic foci within the lung parenchyma of patients with IPF (62, 63). Epithelial cells can transdifferentiate into mesenchymal cells with a profibrotic phenotype (64, 65). They can also release many profibrotic cytokines, including TGF- β , IGF-1, and ET-1, which stimulate fibroblast proliferation and procollagen production. Furthermore, these cells may play key roles in the activation of growth factors via cell surface integrins. Considerable interest has focused on the epithelial cell integrin $\alpha_v\beta_6$. Mice deficient in the β_6 subunit are protected from pulmonary fibrosis and lack the ability to activate TGF- β (66). Furthermore, recent studies suggest that PAR1 activation leads to the generation of active TGF- β via an $\alpha_v\beta_6$ -dependent mechanism (46). These data, taken together with the paucity of inflammatory cells in late-stage IPF and the ineffectiveness of current antiinflammatory drugs, have led to the suggestion that remodeling and fibrosis may proceed independently of inflammation and that epithelial cells have a central role.

Recent studies have also suggested that fibrocytes, bone marrow–derived blood cells expressing type I collagen, are targeted to the lung both in experimental disease (67) and in human pulmonary fibrosis (68). Furthermore, several different chemokines have been implicated in the recruitment process (69). A better understanding of this process and the molecular interactions that underpin it are urgently needed.

CONCLUSIONS

This article outlines our current concepts for the pathogenesis of IPF, a disease characterized by excessive matrix deposition at fibrotic foci within the lung parenchyma. Studies from a large number of groups have led to an appreciation that there are multiple pathways involved (recently reviewed in Reference 70). Furthermore, in some cases, the molecular events are now well described, leading to new drugs that are being tested in clinical trials. Many challenges remain; at this point in time, we have no adequate drugs to treat IPF and undoubtedly new pathways remain to be discovered. It will also be vital in the future to develop ways to assess the importance of these pathways in individual patients and at different stages of the disease process. Studies of genetic and epigenetic mechanisms that increase susceptibility to fibrosis are still in the very earliest stages and more effort in this area is urgently needed. As these new studies are performed and new facts come in, we remain

hopeful that new avenues will emerge to treat lung diseases in which fibrosis is an important component.

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