PERSPECTIVE

Matrix Remodeling in Pulmonary Fibrosis and Emphysema

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Abstract

Pulmonary fibrosis and emphysema are chronic lung diseases characterized by a progressive decline in lung function, resulting in significant morbidity and mortality. A hallmark of these diseases is recurrent or persistent alveolar epithelial injury, typically caused by common environmental exposures such as cigarette smoke. We propose that critical determinants of the outcome of the injury-repair processes that result in fibrosis versus emphysema are mesenchymal cell fate and associated extracellular matrix dynamics. In this review, we explore the concept that regulation of mesenchymal cells under the influence of soluble factors, in particular transforming growth factor-β1, and the extracellular matrix determine the divergent tissue remodeling responses seen in pulmonary fibrosis and emphysema.

Keywords: extracellular matrix; myofibroblasts; transforming growth factor-β; pulmonary fibrosis; emphysema

Clinical Relevance

Pulmonary fibrosis and emphysema are chronic lung diseases that result from dysregulated injury-repair responses to chronic lung injury. This review explores the concept that the distinct clinical-radiological-pathological presentation of these diseases is determined by a central role of the extracellular matrix and the associated fate/function of extracellular matrix–producing mesenchymal cells.

Pulmonary fibrosis and emphysema represent distinct clinical-radiological-pathological syndromes that result from injury-repair responses to chronic lung injury, most notably cigarette smoke. The pathobiological mechanisms that result in these distinct remodeling responses in the lung are not well understood. Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and fatal form of interstitial lung disease that occurs primarily in older individuals; it is associated with disordered deposition of extracellular matrix (ECM) (1, 2). IPF is defined by the histopathological pattern of usual interstitial pneumonia characterized by spatial–temporal heterogeneity and the accumulation of myofibroblasts in fibroblastic foci (3, 4). Emphysema is a chronic, progressive, clinical phenotype of chronic obstructive pulmonary disease (COPD), with increasing morbidity, mortality, and economic burden worldwide. COPD is characterized by airway remodeling with increased ECM deposition in the bronchi and bronchioles; ECM degradation appears to be enhanced in the alveolar regions (5, 6). IPF and COPD are distinctive in their clinical presentations and physiology, yet they carry common risk factors and presumably distinct pathobiological mechanisms.

Cigarette smoke is the most common risk factor for both IPF and COPD. In addition to cigarette smoke, a number of potential environmental exposures have been linked to IPF; these include metal dusts (brass, lead, and steel), agricultural dust, and wood dust (7, 8). In addition, a substantial proportion of nonsmokers with emphysema from occupational exposure to mineral and biological dusts, gases, and fumes, in particular biomass fuel, in enclosed environments are being identified (9). These abnormalities in IPF and emphysema are typically associated with chronic inflammation from recurrent environmental insults and persistent tissue injury. However, emerging data support the concept that dysregulated lung repair after alveolar epithelial injury may give rise to these distinct clinical entities. Several common host response pathways after lung injury have been identified in

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both these chronic lung diseases, yet there are uncertainty and debate regarding theories for the pathogenesis of their phenotypes (10). In this review, we explore the concept that the distinct pathobiology of these clinical syndromes is determined by a central role for the ECM and the associated fate/function of ECM-producing mesenchymal cells.

Mechanisms of Lung Injury and Repair

Host responses to persistent tissue injury induce inflammatory and repair responses that involve myriad interactions among various cells and soluble factors in an attempt to restore normal organ structure and function. Disruption of this tightly orchestrated host response can lead to distinct pathological consequences: pulmonary fibrosis and emphysema.

Current concepts of the pathogenesis of pulmonary fibrosis center primarily on dysregulated wound repair and altered epithelial–mesenchymal communication after repetitive epithelial injury. A number of factors that contribute to this dysregulation have been identified and include impaired fibrinolysis, imbalance of tissue inhibitor of metalloproteinases (TIMPs)–matrix metalloproteinases (MMPs), oxidative stress, elaboration of profibrotic cytokines/chemokines, and eicosanoid imbalance (11). Epithelial and inflammatory cells release several profibrotic mediators, including transforming growth factor (TGF)-β1, platelet-derived growth factor (PDGF), connective tissue growth factor, IL-1β, and tumor necrosis factor-α (12–16). Various MMPs expressed by different cell types in the alveolar microenvironment mediate diverse pro- and antifibrotic functions (17). A consistent feature of the pathobiology of IPF is the accumulation of myofibroblasts that acquire an apoptosis-resistant and invasive phenotype, thus perpetuating alveolar collapse and ECM remodeling (18, 19) (Figure 1).

In emphysema, inflammatory cells accumulate in the airways and alveolar spaces in response to chronic exposure to cigarette smoke or environmental toxins. They release proteolytic enzymes including elastases (such as neutrophil elastase and macrophage metalloelastase [MMP-12]) and collagenases/gelatinases (such as MMP-8 and MMP-9) (20–22). Proteolytic cleavage of elastin and other ECM components by these proteases results in degradation of the ECM, leading to loss of elastic recoil and the generation of matrikines, which may perpetuate inflammatory responses. Cleaved elastin fragments and collagen-derived peptide Pro-Gly-Pro (PGP) from ECM degradation mediate chemotactic activity and promote airway inflammation, thus precipitating a self-propagating cycle of inflammation and ECM proteolysis (23, 24). In addition, proteases may inactivate TIMPs, thus reducing the antiproteolytic activity within the matrix and leading to a protease–antiprotease imbalance (22, 25). Furthermore, the normal repair functions of fibroblasts may be suppressed, in part because of their reduced responsiveness to TGF-β1 signals (26) (Figure 2). Thus, dysregulated inflammation and repair may result in distinct fibroblast–ECM remodeling responses that account for the variability in the clinical–pathological presentations seen in pulmonary fibrosis and emphysema.

Fibroblast Biology

Fibroblasts are versatile cells that undergo differentiation to myofibroblasts and secrete ECM proteins during physiological tissue repair in almost all organ systems studied (27). Myofibroblasts are defined by their capacity to contract and by the expression of contractile proteins, particularly, α-smooth muscle actin (α-SMA) stress fibers (28). The highly controlled synthetic capacity of myofibroblasts is an important feature of tissue repair and ECM remodeling in the lung. The deletion of PDGF-A–expressing myofibroblasts results in developmental emphysema caused by a failure of alveolar septation (29). Aberrant fibroblast responses in the injured alveolar microenvironment may be a key mechanism driving the distinct clinical phenotypes in pulmonary fibrosis and emphysema. Pulmonary fibrosis is characterized by persistent and heightened myofibroblast activity, whereas emphysema appears to be associated with reduced fibroblastic activity.

Fibroblastic foci (FF), the histopathological hallmark of usual interstitial pneumonia, comprise aggregates of fibroblasts and myofibroblasts. Previous studies suggested that myofibroblasts originate from multiple different sources (30–32); however, more recent studies indicate that almost all myofibroblasts originate from the differentiation of resident fibroblast progenitors (33). The regulation of myofibroblast differentiation in pulmonary fibrosis is complex and involves the action of growth factors/cytokines such as TGF-β1 (34, 35) in concert with ECM-derived biochemical and biomechanical signals (36–42). The acquisition of an apoptosis-resistant myofibroblast phenotype by both soluble mediators and the ECM may drive the nonresolving nature of IPF (43, 44). In addition, the acquisition of invasive properties by myofibroblasts (45) may lead to disruption of the basement membrane, which is a distinctive feature of IPF. In addition to enhanced synthetic and invasive capacity, activated myofibroblasts exert isometric contractile activity. This activity is maintained by a single-cell lock-step model via the combined actions of intracellular calcium and Rho/Rho kinase (Rho/ROCK)-mediated calcium-independent contraction (46). The contractile activity of myofibroblasts results in macroscopic tissue contracture (28), which likely contributes to alveolar collapse and the observed reduction in total lung capacity in IPF.

In contrast to pulmonary fibrosis, there appears to be an impaired fibroblast response to epithelial injury in emphysema. Fibroblasts obtained from patients with COPD demonstrate decreased proliferation, migration, and contractility (26). This effect occurs potentially through increased expression of prostaglandin E2, a known inhibitor of fibroblast repair function, with a resultant decrease in synthesis and deposition of ECM components (47–49). Importantly, fibroblasts in emphysema have reduced responsiveness to transcriptional regulation by TGF-β1 and myofibroblast differentiation (26). Neutrophil elastase, which is highly expressed in emphysema, releases epidermal growth factor from the cell surface and initiates epidermal growth factor receptor/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (EGFR/MEK/ERK) signaling to down-regulate elastin synthesis in rat lung fibroblasts (50, 51); this is mediated at least in part by stabilization of the Smad

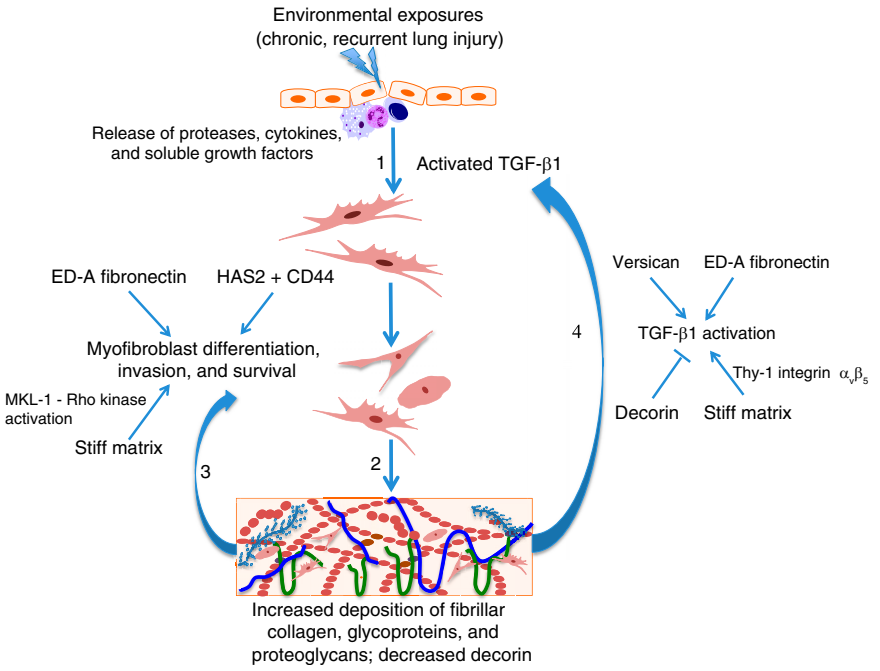

Figure 1. Loss of cellular and extracellular matrix (ECM) homeostasis in the pathogenesis of pulmonary fibrosis. Exposure to inhaled toxins such as cigarette smoke leads to epithelial cell injury, inflammation, and release of proteases and soluble growth factors. Latent transforming growth factor-β1 (TGF-β1) may be activated by multiple mechanisms such as integrin $\alpha_v\beta_6$ (*1*). TGF-β1 signaling in fibroblasts mediates myofibroblast differentiation and survival with enhanced ECM synthesis (*2*). ECM deposition and remodeling results in matrix stiffening, which activates GTPase RhoA and its effector Rho kinase via induction of MKL-1, perpetuating myofibroblast activation (*3*). Extradomain A (ED-A) fibronectin is essential for myofibroblastic differentiation, whereas HAS2 and CD44 are essential for fibroblast migration/invasion. Positive feedback loops maintain TGF-β1 activation via ECM-derived signals that include Thy-1 integrin $\alpha_v\beta_5$, ED-A fibronectin, and versican; in contrast, decorin may inhibit TGF-β1 activity by direct binding (*4*). HAS2, hyaluronan synthase 2; MKL-1, megakaryoblastic leukemia factor-1.

corepressor (TG-interacting factor), which inhibits TGF-β signaling (52). In addition to reduced synthetic capacity, fibroblasts exposed to cigarette smoke may induce higher proteolytic activity in the extracellular milieu by production/release of proteases. Increased expression of MMP-1 and MMP-2 in fibroblasts exposed to cigarette smoke has been reported (53, 54). Furthermore, low concentrations of cigarette smoke have also been shown to induce fibroblast apoptosis (55), which may favor an emphysema phenotype. Collectively, these studies highlight a divergence of tissue repair responses after lung injury, leading to myofibroblast activation in pulmonary fibrosis, whereas this activity is inhibited in emphysema. These abnormal mesenchymal responses further contribute to the alterations in the biochemical and biomechanical properties of the ECM that are emerging as newly appreciated contributors to the pathogenesis of these two chronic pulmonary diseases.

ECM Composition and Dynamics

The ECM is an interconnected lattice of secreted proteins that forms a scaffold and functions as bidirectional signals for cells within tissues of all adult organs. The ECM is composed of almost 300 proteins forming the core "matrisome," together with glycosoaminoglycans (GAGs), polysaccharides, ECM-binding growth factors, and ECM-modifying enzymes (56, 57). ECM is a highly dynamic structure that is constantly remodeled either enzymatically or nonenzymatically. It confers unique biochemical and mechanical properties to each organ through its interactions with the cellular components such as fibroblasts and epithelial and endothelial cells.

ECM remodeling is a highly regulated process in tissue homeostasis and injury repair (58). In this broad context, ECM remodeling in lung injury repair is defined as dynamic changes in the quantity and quality of ECM components that result in restoration of normal tissue structure/function or pathological changes such as fibrosis or emphysema. Thus, dysregulated ECM remodeling may result from an imbalance in ECM secretion, degradation, organization, and/or post-translational modifications. The extent to which each of these contribute to fibrosis versus emphysema is not well understood. In addition, whether altered lung mechanics resulting from changes in the quantity or quality of ECM proteins contribute to distinct clinical phenotypes has not been established. In this section, we briefly review the role of fibrous proteins (collagen, elastin), glycoproteins (fibronectin [FN], tenascin), proteoglycans (decorin, versican [VCAN]), and glycosaminoglycans (hyaluronan [HA]) in the development of lung fibrosis and emphysema.

Collagens

Collagens are proteins composed of different types of chain-forming helical regions via repeating sequences of three amino acids (Gly-x-y). Fibrillar collagens (types I, II, III, V, and XI) are the most commonly found collagen in the lungs and are critical in maintaining tensile strength and distensibility of the lungs. Type IV collagen, a nonfibrillar collagen, is present in the basement membrane of the lungs and plays an important role at the blood–gas barrier (57). Ultrastructural studies have shown an increased deposition of collagen in ECM surrounding the myofibroblasts in FF in patients with IPF (3). In addition, the expression of lysyl oxidase-like 2, an enzyme that catalyzes cross-linking of fibrillar collagens, is increased in IPF (59). Further investigation is needed to evaluate whether alterations in the mechanical properties of the lung are caused by the increased deposition and/or abnormal structural modifications of collagen. In addition to its structural properties, fibrillar type I collagen limits fibroblast proliferation in normal lung repair by interacting with the cell surface receptor $\alpha_2\beta_1$ integrin (60). This interaction leads to suppression of the phosphoinositide 3-kinase signal

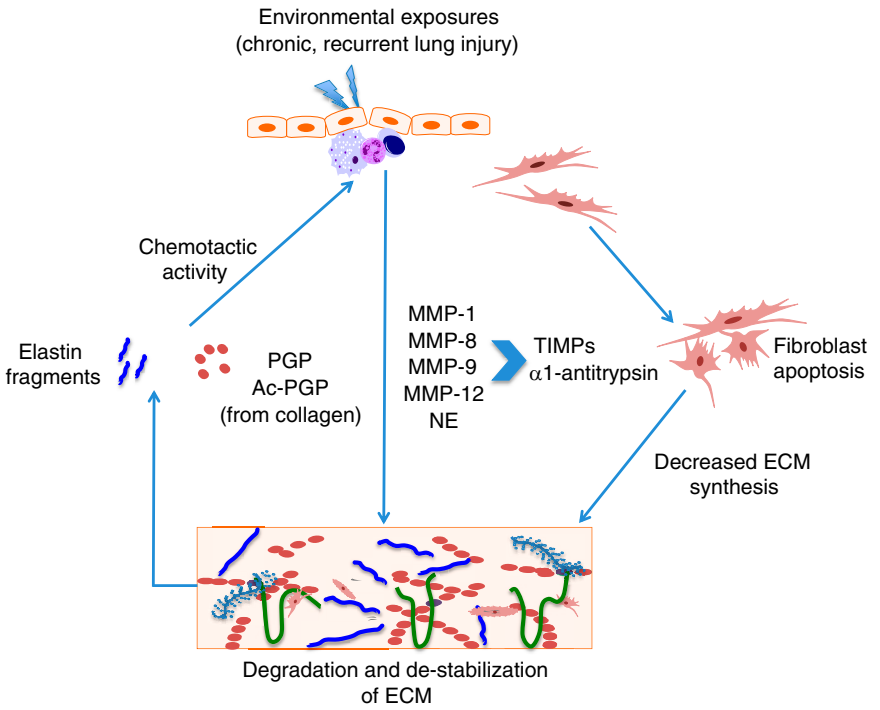

Figure 2. Loss of cellular and ECM homeostasis in the pathogenesis of emphysema. Exposure to inhaled toxins such as cigarette smoke and environmental dust leads to epithelial cell injury and recruitment of inflammatory cells. Inflammatory cells release proteolytic enzymes that include elastases such as NE, macrophage metalloelastase (MMP-12), and collagenases/gelatinases (e.g., MMP-8 and MMP-9). Elastase and MMP-12 cleave elastin to release elastin peptide fragments. The coordinated action of MMP-8 and MMP-9 in the presence of PE results in specific cleavage of collagen to release Pro-Gly-Pro (PGP) and its acetylated form, Ac-PGP. Cleaved elastin fragments and the collagen-derived peptides mediate chemotactic activity for monocytes and neutrophils, respectively, and perpetuate a self-propagating cycle of inflammation and ECM proteolysis. Impaired fibroblast proliferation and survival, with reduced responses to transforming growth factor-β1, down-regulate ECM production. Ac-PGP, N-acetyl Pro-Gly-Pro; MMP, matrix metalloproteinases; NE, neutrophil elastase; PE, propyl endopeptidase; TIMP, tissue inhibitor of metalloproteinase.

pathway caused by maintenance of high phosphatase activity of the tumor suppressor phosphatase and tensin homolog. Loss of this restraint because of a pathologically altered fibroblast–integrin interaction may contribute to their abnormal proliferation in pulmonary fibrosis (60, 61).

In contrast, there is an increase in collagenase activity in emphysema, with weakening of the collagen fiber network and loss of the mechanical forces that appear to be a prerequisite for the development of emphysema (62). In addition to their structural roles, proteolytic products of collagen may have profound effects on repair responses and the perpetuation of inflammation. Neutrophil-derived MMP-8 and MMP-9 cleave whole collagen in fragments of 30 to 100 amino acids followed by specific cleavage to release tripeptides, PGP, and its acetylated form, Ac-PGP by the action of propyl endopeptidase (23, 63). The multistep pathway in the degradation of collagen, in the presence of MMPs and propyl endopeptidase, was shown in murine models of cigarette smoke (CS)-induced emphysema, and further *in vitro* studies reported the breakdown of whole collagen to PGP and Ac-PGP (23, 63, 64). These collagen fragments induce neutrophil chemotaxis through CXC-receptors 1 and 2 on neutrophils, thus precipitating an inflammatory cycle (65).

Elastin

Elastin, which is the primary component of elastic fibers, is an insoluble polymer of the monomeric soluble precursor, tropoelastin. Elastic fibers are found interspersed together with collagen in the ECM, and they contribute to the elastic recoil properties of the lung (66). Lysyloxidase-catalyzed cross-linking of lysine residues on tropoelastin monomers is essential for elastic fiber assembly. There is a paucity of studies investigating the role of elastin in lung fibrosis. Increased deposition of elastin was demonstrated in a mouse model of bleomycin-induced lung fibrosis (39). Elastin was also shown to enhance TGF-β1–induced myofibroblast differentiation with increased gene expression of α-SMA and type I collagen in human lung fibroblasts (39). In contrast, the role of elastin in the pathogenesis of emphysema is well established. Elastin and collagen degradation by neutrophil and macrophage proteases results in ECM proteolysis *in vitro* and emphysema *in vivo*. There is a protease–antiprotease imbalance, as shown by early studies in patients with α-1 antitrypsin deficiency, a major inhibitor of elastases (67). In addition, intratracheal administration of elastase induces emphysema, and both macrophage-elastase (MMP-12) and neutrophil-elastase–null mice were protected against CS-induced emphysema (22, 68). However, this protection against CS in MMP-12 and neutrophil-elastase–null mice may not be solely caused by a lack of elastase production. Similar to collagen fragments, elastin fragments generated from the elastin polymer by proteolysis have increased chemotactic activity, resulting in macrophage accumulation and further ECM degradation by MMP-12 (24). Elastin fragment antagonism with a monoclonal antibody abrogated this feedback loop of macrophage accumulation and ECM elastin degradation in a mouse emphysema model (24), suggesting a potential target for therapeutic intervention.

Glycoproteins

FN is a dimeric glycoprotein that mediates cell–matrix adhesions through the binding of ECM proteins such as collagen and cell-surface integrins (69). Partly through its effects on reorganization of the cytoskeleton, FN facilitates cell migration and differentiation during the process of wound healing. Alternative splicing of the type III domains results in polymorphism of FN, particularly extradomain-A (ED-A)–FN, which is

essential for wound healing (70) and fibrogenesis (41, 42). The role of alternative splicing of multiple genes in the regulation of the immune response is better appreciated (71) than its role in regulating wound healing and fibrosis. Studies on the role of alternative splicing involving other ECM genes and their receptors (e.g., CD44) are likely to provide novel insights into the diversity of the wound-healing responses.

Tenascin-C (TNC) is another large hexameric glycoprotein that is up-regulated during tissue injury. ED-A–FN and TNC are present in abnormally larger quantities in fibrotic lung with increased synthesis of these glycoproteins by fibroblasts from patients with IPF compared with fibroblasts from normal subjects (41, 72). They have been shown to colocalize with collagen and VCAN in fibroblastic foci, which are active sites of fibrogenesis (72). Expression of these glycoproteins is stimulated by several soluble factors such as TGF-β1 and PDGF. TGF-β1 preferentially promotes the accumulation of ED-A–FN (73, 74), the predominant isoform in pulmonary fibrosis. ED-A–FN and TNC, in turn, have been shown to be essential for myofibroblast differentiation, cellular motility, and collagen production (41, 42, 75, 76). The mechanism of ED-A–FN–mediated fibroblast differentiation is incompletely understood; the involvement of $\alpha_4\beta_7$ integrin and the activation of focal adhesion kinase and mitogen-activated protein kinase–associated signaling pathways have been reported (77). ED-A–FN–null mice exposed to bleomycin demonstrate diminished pulmonary fibrosis with fewer myofibroblasts compared with wild-type mice; this correlated with diminished activation of TGF-β1 (41). Similarly, TNC-null mice exposed to bleomycin demonstrate reduced fibrosis from impaired TGF-β1 responsiveness (78). These data suggest that glycoproteins, released from fibroblasts, may play an important role in TGF-β1–mediated myofibroblast differentiation, resulting in a feed-forward loop. Increased peribronchial deposition of collagen, α-SMA, TNC, and FN in patients with emphysema suggests a similar mechanism of aberrant repair after epithelial injury, with resultant airway remodeling and airflow obstruction in COPD (79, 80). However, our current understanding of the role of specific glycoproteins in the matrix remodeling associated with emphysema is limited.

Proteoglycans

Proteoglycans consist of core proteins to which highly charged GAGs are covalently linked. They are critical constituents of the hydrated gel or "ground substance" in which collagen and elastin fibers are embedded (81). The lung tissue comprises sulfated (chondroitin sulfate, heparan sulfate, and keratin sulfate) and nonsulfated (hyaluronic acid) GAGs (82). The PGs normally contribute to the mechanical stability of the collagen–elastin network and prevent collapse of alveolar structures caused by electrostatic interactions from the negatively charged GAGs (81). Structural remodeling of the lung resulting from abnormal expression of PG-GAG complexes and structural changes to the GAG side chains can contribute to the development of pulmonary diseases (45, 83–88).

VCAN is a chondroitin sulfate/dermatan sulfate attached PG that is important for matrix assembly and regulation of collagen fibrillation. Increased deposition of VCAN has been demonstrated around active areas of fibrogenesis in the fibrotic lungs of patients with IPF (72). Increased expression of VCAN and enzymes essential for the synthesis and sulfation of associated GAGs was demonstrated in bleomycin-instilled rats and in fibrotic lung fibroblasts (88). Furthermore, this study showed that the up-regulation of VCAN in rat lung fibroblasts was mediated by the TGF-β type I receptor/activin receptor-like kinase 5/p38 mitogen-activated protein kinase signaling pathway. Interestingly, recombinant VCAN-expressing mouse fibroblasts were shown to display a TGF-β1–mediated myofibroblast-like phenotype with increased proliferation and synthetic activity in association with enhanced collagen gel contractility (40). Data exploring the role of VCAN in the pathogenesis of emphysema are limited. Increased deposition of VCAN from fibroblasts in the distal airways and alveolar walls of patients with COPD has been demonstrated previously (89, 90). However, this is negatively correlated with the amount of elastin and elastin-binding protein in COPD, suggesting that modulation of VCAN could influence the resynthesis of elastin fibers through its activity on elastin-binding protein.

Decorin, biglycan, and fibromodulin are small, leucine-rich PGs with complex roles in the structural and signaling functions of the ECM. In rat models of pulmonary fibrosis, increased levels of biglycan and fibromodulin with reduced levels of decorin have been reported (86). Interestingly, intratracheal delivery of recombinant decorin by adenovirus gene transfer was shown to decrease subpleural fibroproliferation in a bleomycin mouse model (91). Similarly, intranasal administration of a decorin-encoding adenoviral vector resulted in transient increase in decorin that ameliorated bleomycin-induced lung fibrosis in mice (92). The antifibrotic effects of decorin have been attributed to inhibition of TGF-β1 activity through binding of the patent peptide to leucine-rich repeats in the core protein (92, 93). There is no difference in peribronchial or parenchymal deposition of decorin in mild/moderate COPD compared with normal lungs; however, decreased expression of peribronchiolar decorin has been shown in patients with severe emphysema (79, 94). In addition, TGF-β1 has been shown to down-regulate decorin expression in fibroblasts from patients with severe emphysema compared with fibroblasts from patients with mild emphysema (95). Interestingly, it was shown that fluticasone induced decorin production in airway fibroblasts from patients with severe COPD (96). From these studies, it appears that restoration of decorin may result in an improvement in airway obstruction by ameliorating the negative effects of TGF-β1 on airway remodeling.

Glycosaminoglycans

HA synthesized by membrane-bound HA synthases is a large, nonsulfated GAG that has been shown to accumulate at sites of tissue injury and play a role in normal wound healing (97, 98). In a mice model of bleomycin-induced lung injury, mice with myofibroblast-specific overexpression of membrane-bound HA synthases (HAS2) were shown to develop severe fibrosis; silencing of the *HAS2* gene in fibroblasts inhibited matrix invasion and myofibroblast accumulation (45). The absence of CD44, an HA cell-surface receptor, or treatment with anti-CD44 antibodies, also reduced lung fibrosis,

suggesting that the HAS2–CD44 axis is critical for tissue invasion and relentless fibrosis (45). In addition, HA–Toll-like receptor 2 and HA–Toll-like receptor 4 interactions have been shown to provide signals that initiate inflammatory responses, maintain epithelial cell integrity, and promote recovery from lung injury (99), supporting the concept that HA may function as one of a growing number of damage-associated molecular patterns. Interestingly, the chain length of HA is reduced significantly by cigarette smoke–induced free radical damage, reducing the viscosity of aggregated PGs in emphysema (100). Other studies have shown that low molecular weight HA fragments create a proinflammatory state by stimulating macrophages to release chemokines and MMPs (specifically, MMP-12), resulting in chronic inflammation, destruction of the lung parenchyma, and airway wall remodeling in emphysema (101, 102). In a recent study using a mouse model of porcine pancreatic elastase-induced emphysema, the loss of the structural role of PGs related to the change in charge density on the GAGs reduced alveolar stability and led to a more progressive disease phenotype (85). In that study, the changes in mouse lung tissue stiffness and the alveolar shape distortion under the influence of varying tonicity conditions were increased in experimental emphysema, thus suggesting that the loss of PGs affects lung tissue stability and the progression of emphysema.

Mechanical Properties of ECM

There is emerging evidence that sustained matrix stiffness from abnormal ECM deposition and contractile activity of myofibroblasts is critically involved in influencing fibroblast function. The interactions between the ECM and fibroblasts were examined recently by culturing fibroblasts from IPF or normal lung on decellularized ECM from IPF or normal lung (37). Interestingly, diseased ECM had a greater impact on pathological gene expression at the level of translation compared with the cell autonomous changes themselves; in addition, IPF ECM promoted the translational activation of genes encoding ECM proteins in fibroblasts, suggesting a positive feedback loop.

Several pathways have been implicated in myofibroblast activation by stiff matrices. A stiff matrix may regulate myofibroblast differentiation through an extrinsic mechanotransduction pathway, leading to latent TGF-β1 activation by the induction of Thy-1 integrin $\alpha_v\beta_5$ (38). A stiff matrix also activates GTPase RhoA and its effector Rho kinase via induction of megakaryoblastic leukemia factor-1, which has been shown to regulate the expression of fibrotic genes (36). This intrinsic mechanotransduction pathway involving RhoA/ROCK-dependent actin cytoskeletal remodeling and megakaryoblastic leukemia factor-1 activation mediates myofibroblast differentiation *in vitro* and experimental lung fibrosis *in vivo* (103). It is not known whether alterations in ECM stiffness and mechanotransduction influence the function and behavior of fibroblasts in emphysema or, reciprocally, whether cell autonomous (epigenetic) changes in fibroblasts influence ECM production and remodeling. *Ex vivo* cell/tissue culture models that recapitulate the biochemical and biomechanical properties of the ECM are still evolving. Polyacrylamide hydrogel systems with tunable substrate stiffness are well established; however, they fail to reconstruct all the properties of diseased ECM. Development of new tools to simulate three-dimensional models of acellular, normal, and diseased human lung matrices may be vital to understanding the intricate effects of ECM in regulating cellular phenotypes/fates relevant to pulmonary fibrosis and emphysema.

TGF-β1 in Fibrosis and Emphysema

Although the biochemical and biomechanical properties of ECM contribute to myofibroblast differentiation and survival, the best recognized soluble mediator in myofibroblast activation and tissue fibrosis is TGF-β1. However, the role of TGF-β1 in emphysema development/progression is limited. TGF-β1 is a 75-kD polypeptide synthesized as a large latent precursor molecule bound to the latency-associated peptide by various cells and is incorporated into the ECM (104). Latent TGF-β1 may be activated by both protease-dependent and protease-independent mechanisms (104). Integrin $\alpha_v\beta_6$–mediated activation of latent TGF-β1 is the most recognized and studied mechanism in pulmonary fibrosis (105). Other mechanisms of TGF-β1 activation involving plasmin, MMPs, thrombospondin, and extracellular acidification have also been described (104), although their relative roles in different modes of lung injury repair are not well defined. In addition, ECM proteins, VCAN, and ED-A–FN can potentially activate latent TGF-β1 (41, 88); whether these are related to mechanosensing in specific cell types warrant further investigation (106). Activated TGF-β1 binds to the TGF-β receptor (TGF-βR) complex composed of dimers of the transmembrane serine/threonine kinase receptors- type I and II TGF-βRs (107, 108). This leads to transphosphorylation of the kinase domain of TGF-βRI by TGF-βRII, inducing the phosphorylation and activation of the intracellular receptor-regulated R-Smad (Smad2 and Smad3) proteins in fibroblasts (107). The R-Smads then interact with Smad4 and translocate to the nucleus to activate transcription of the genes involved in fibroblast differentiation and matrix synthesis. In addition to its fundamental role in myofibroblast differentiation, TGF-β1 promotes myofibroblast survival through the activation and coordination of two major prosurvival pathways, focal adhesion kinase and protein kinase B (109). These pathways may contribute to the prolonged survival of myofibroblasts in fibrotic tissues.

Some studies in human subjects suggest that, in contrast to the profibrotic effects of TGF-β1 signaling in pulmonary fibrosis, TGF-β1 may play a protective role in emphysema. Among several polymorphisms for TGF-β1, SNP T+869C (leucine to proline exchange caused by a transition of nucleotide T to C at position 869) and T allele of SNP rs1800469, both of which are associated with higher TGF-β1 production, are more prevalent in control smokers than in patients with emphysema (110, 111), suggesting that TGF-β1 may be protective. TGF-β1 normally suppresses the expression of MMPs and increases TIMP expression in regulated repair processes and is important for fibroblast-mediated ECM synthesis and contractility (112, 113). In addition, despite adequate levels, it is possible that reduced fibroblast responsiveness to TGF-β1 may influence fibroblast function and ECM maintenance (26, 47, 49). Animal models in which

TGF-β1 signaling has been disrupted support this apparent dichotomy in TGF-β signaling. Mice deficient in the β6 subunit of integrin $\alpha_v\beta_6$ are unable to activate latent TGF-β1; they are protected from bleomycin-induced lung fibrosis and develop age-dependent emphysema (105, 114). Similarly, mice deficient in Smad3, a TGF-βR–regulated effector protein, are protected from bleomycin and TGF-β1–mediated fibrosis (115), but these mice also develop abnormal alveolarization followed by progressive emphysema and alveolar wall destruction (116). These studies support a role for the TGF-β1/Smad pathway in susceptibility to, and potentially progression of, lung fibrosis and emphysema.

Conclusions

In summary, despite common risk factors (e.g., cigarette smoke, aging) and similar fates of susceptible cell populations (e.g., alveolar epithelial cell injury/apoptosis), the distinct tissue remodeling responses seen in lung fibrosis and emphysema are not well understood. In this review, we posited the idea that differences in mesenchymal cell fate/function and ECM dynamics may account for phenotypic expression of fibrosis versus emphysema. Given the multitude of functions ascribed to the ECM and their proteolytic products, dysregulation of the inflammatory and/or repair after diverse forms of lung injury may result in distinct clinical syndromes. Alterations to any of the critical steps in the injury-repair response with aberrant cross-talk between reparative cells and the ECM could result in nonresolving, perpetuating cycles of inflammation and repair. Critical pathways involving TGF-β1/Smad, integrins, PGs and their receptors, glycoproteins, and the biomechanical properties of the ECM have been identified as regulating fibroblast function and fate, and new pathways undoubtedly remain to be discovered.

Halting the molecular events propagating feed-forward loops that contribute to disease progression in pulmonary fibrosis and emphysema may be clinically as important as the development of strategies to reverse the disease process. However, the potential off-target effects associated with the disruption of key injury-repair response pathways need to be taken into consideration. As we gain an improved understanding of the role of the lesser-known components of the ECM and decipher the complexities of the cell–matrix interactions in these diseases, the future development of novel matrix-directed therapies are anticipated. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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