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Review Article

Pulmonary fibrosis: Cellular and molecular events

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Connective tissue remodeling of the interstitium is an important feature of chronic lung diseases encompassing interstitial inflammatory changes and subsequent pulmonary fibrosis. The early inflammatory phase is usually associated with the release of several cytokines and chemokines by activated resident cells and infiltrating cells which, in turn, help further recruit inflammatory mononuclear cells. Cytokines and growth factors secreted by inflammatory cells and by interstitial cells (fibroblasts and myofibroblasts) play an important role in the fibrogenic phase of pulmonary fibrosis by inducing matrix synthesis. In addition, matrix-degrading enzymes and their inhibitors also contribute to extracellular matrix (ECM) remodeling in pulmonary fibrosis. This review addresses the pathophysiology of wound healing and different phases of pulmonary fibrosis.

Key words: collagen, fibrogenic factors, fibrosis, lung.

Fibrosis is a progressive pathological process involving gradual expansion of the fibrotic mass leading to the destruction of involved tissues and organs. The pathogenesis of fibrotic disorders is mostly similar regardless of the tissue involved. Physiologically, there is a balance between matrix formation and degradation. This balance is impaired in fibrosis. Although the exact molecular mechanism of fibrosis is not yet clear, studies have shown that the proliferation of matrix-producing cells with subsequent overproduction and accumulation of matrix proteins contributes to various human and experimental fibrotic diseases.[1–9] Pulmonary fibrosis is a complex, chronic illness that could develop following a variety of acute and chronic lung diseases. Gradual expansion of the fibrotic mass destroys the air sacs surrounding lung tissues and capillaries, causing permanent loss of function, including oxygen transport. Pulmonary fibrosis is a disease for which there is no known cure in most cases. Fibrotic diseases present major challenges in clinical practice because effective antifibrotic agents are not yet widely available. A detailed and comprehensive review of all aspects of pulmonary fibrosis is beyond the scope of this article. Rather, our review will be limited to the cellular and molecular events of different phases of pulmonary fibrosis. In addition, we will briefly discuss how this information forms the basis for developing new therapies to control the progression of pulmonary fibrosis.

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WOUND HEALING

Wound healing is a complex and dynamic process that comprises an ordered sequence of inflammatory reactions, matrix deposition and resolution. Unlike regular wound healing, no true resolution occurs in pulmonary fibrotic diseases. Instead, fibroblast proliferation and matrix synthesis and accumulation continue, thereby resulting in progressive destruction of the normal lung parenchyma, and eventual impairment of normal pulmonary functions. It is the persistent activation of the genes encoding for matrix proteins that distinguishes controlled wound repair from uncontrolled connective tissue deposition, leading to pathological fibrosis. In pulmonary fibrosis, matrix synthesis often continues despite the apparent resolution of initial triggering and/or inflammatory events.

Wound healing is a consequence of complex interactions among the fibroblasts, cytokines/growth factors, proteases and extracellular matrix (ECM) proteins. Epidermal growth factor (EGF) is mitogenic for epithelial cells and fibroblasts,[10] while platelet-derived growth factor (PDGF) plays a key regulatory role in the proliferation and migration of fibroblasts and myofibroblasts during wound healing.[11] Fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) control endothelial cell function during angiogenesis,[12,13] and transforming growth factor-β (TGF-β) regulates matrix turnover,[14] thus contributing to the healing process.

In contrast to adult wounds, fetal wounds heal without leaving any histological evidence of scarring in early gestation.

The mechanism of scarless repair of fetal wounds is not clear, but intrinsic functions of dermal fibroblasts, ECM components, inflammatory responses, cytokine profiles and intracellular signal transduction during fetal wound healing differ from those of adult wound healing. The accelerated rate of healing, relative lack of an acute inflammatory response and absence of neovascularization distinguish fetal wound healing from adult wound healing. The relatively lower levels of TGF-β and b-FGF are attributed to the lack of inflammatory responses and scarless fetal wound healing.[15] Exogenous addition of TGF-β1 in fetal wounds has been reported to result in healing with scar tissue formation.[16] Lower levels of bone morphogenetic proteins (BMP), members of the TGF-β superfamily and BMP receptors were detected recently in the fetal wound-healing process and are considered to contribute to scarless wound healing in the fetus when compared with adult wound healing.[17] In fetal wounds, there is mainly mononuclear cell infiltration with less activity of polymorphonuclear leukocytes. A low level of interleukin 8 (IL-8) is thought to be responsible for the lack of cellular recruitment and inflammatory responses seen in fetal wound healing, and might eventually contribute to scarless wound healing.[18] Similarly, a lower level of IL-6 expression has been reported in fetal wound healing compared to adult wound healing, while exogenous addition of IL-6 resulted in fetal wound healing with scar tissue.[19] Rapid upregulation of epidermal integrin receptors specific for fibronectin and other wound matrix proteins is noted in human fetal repair, which might allow for the migration of keratinocytes and re-epithelialization, thereby limiting the induction of inflammatory and fibrotic events.[20] Understanding the basic molecular and cellular mechanisms of scarless fetal wound healing and applying this knowledge as a basis for developing new therapies to control or manipulate the adult wounds and progressive fibrotic diseases to a scarless repair should be a great challenge.[21]

PULMONARY FIBROSIS

Approximately five million people are affected by pulmonary fibrosis worldwide and, of these, 200 000 suffer from this disease in the USA, and more than 40 000 die annually. In 1994, a population-based registry in Bernalillo County, New Mexico, USA, reported that the prevalence of all interstitial lung diseases was 80.9/100 000 among men and 67.2/100 000 among women.[22] Pulmonary fibrosis**,** a potentially fatal disease, results from acute and chronic interstitial lung diseases. The excessive accumulation of matrix proteins, mainly produced by fibroblasts and myofibroblasts, is responsible for the derangement of alveolar walls, loss of elasticity and development of rigid lung. The progression of pulmonary fibrosis results in the widening of interstitial matrix, eventual compression and destruction of normal lung parenchyma, with resultant damage to the capillaries leading to ventilatory insufficiency. The level of disability experienced by a person is directly related to the extent of tissue scarring. The process and progression of pulmonary fibrosis can be broadly divided into three phases and/or events: initial triggering events, inflammatory events and fibrotic events. These phases sometimes overlap. We will briefly discuss here the pathological relevance of these stages in pulmonary fibrosis.

Initial triggering events

Irreversible end-stage organ failure due to fibrotic diseases is a major cause of morbidity and mortality, and treating these progressive fatal diseases is a major challenge in modern medicine. In order to treat or control the progression of these diseases, the ideal approach would be to identify the initial triggering factors of a particular fibrotic disease and control, eradicate or eliminate such factors as early as possible. However, the primary causes of fibrosis are diverse and include toxic vapors, inorganic dusts, drugs and radiation that can induce pulmonary fibrosis, while the etiology of pulmonary fibrosis is yet to be identified in a significant number of cases. The incidence of approximately 7–10 cases of idiopathic pulmonary fibrosis per 100 000 people per year, with 50–70% mortality at 5 years after diagnosis suggests a fatal outcome of this condition.[23] Physical or chemical injuries and immunological disorders can lead to cutaneous fibrosis such as keloids, hypertrophic scars and scleroderma. Alcohol and viral infections are major causes of liver fibrosis,[24,25] while glomerulonephritis, diabetic mellitus and hypertension are major causes of renal scarring.[26–29] Diffuse cardiac fibrosis is one of the major complications of hypertension[30] usually associated with progressive heart failure. A number of drugs such as bleomycin, cisplatin, cyclosporine and gentamicin can also induce fibrosis of the lung and kidney.[31–35] Although pulmonary fibrosis is triggered by diverse known and yet to be identified factors, including drugs and exposure to inorganic dusts or radiation, not all exposed individuals develop fibrosis, suggesting a possible genetic predisposition.[36] Identifying a genetically susceptible population by genetic profiling might allow for early detection and intervention. Moreover, a recent classification divided interstitial pneumonias into seven disease entities[37] of which usual interstitial pneumonia (UIP) and acute interstitial pneumonia (AIP) have poor prognostic outcomes, while other types of pneumonias have relatively better prognoses and reversibility. The molecular mechanisms of such variability in the outcome of different forms of interstitial pneumonias are not yet known, and the role of genetic background in determining the outcome needs to be further explored. In addition, whether the response of resident cells to exogenous stimuli differ in various forms of interstitial pneumonias, for example, the recruit-

ment of inflammatory cells and subsequent inflammatory responses, might help us to understand the variability in the prognosis of various forms of interstitial pneumonias. In this respect, it is well known that different responses by adult and fetal dermal fibroblasts against a similar type of injury result in healing with scarring in adults and healing without scarring in fetuses.[21,38]

Interstitial lung diseases are usually associated with recurrent episodes of inflammation with gradual widening of the interstitium as a result of the accumulation of matrix proteins. The etiology of interstitial lung diseases is diverse, ranging from occupational or environmental exposure to drugs and radiation and, in a significant number of cases, the cause is yet to be determined. Table 1 provides a partial list of the common agents/factors that are associated with interstitial lung diseases. The nature of the initial insult to the lung is not always known. A number of causative agents, factors or disorders that are known to cause interstitial lung diseases might act as initial triggering factors for the activation or injury of alveolar epithelial cells, leading to inflammatory reactions in the interstitium. The induction of various mitogenic and fibrogenic factors by inflammatory cells and resident cells then acts on interstitial cells causing their proliferation, differentiation and production of collagen or scar tissue in response to this damage (Fig. 1).

Table 1 List of common causes of interstitial lung diseases. Some additional factors/disorders that might play a role in the induction and propagation of interstitial lung diseases are not included in order to keep the list simple and limited

Occupational and environmental exposures
Asbestosis
Bird breeder's lung
Coal worker's pneumoconiosis
Detergent worker's lung
Farmer's lung
Inorganic dusts
Malt worker's lung
Organic dusts
Silicosis
Talc pneumoconiosis
Primary and systemic diseases
AIDS
Adult Respiratory Distress Syndrome
Amyloidosis
Bone marrow transplantation
Eosinophilic granuloma
Malignancy
Sarcoidosis
Tuberculosis
Lymphoma
Connective tissue or collagen vascular diseases
Ankylosing spondylitis
Mixed connective tissue disease
Polymyositis-dermatomyositis
Rheumatoid arthritis
Scleroderma
Sjögren's syndrome
Systemic lupus erythematosus
Systemic sclerosis
Drug-induced
Amiodarone
Azathioprine
Bleomycin
Busulfan
Cocaine
Cyclophosphamide
Furantoin
Gold
Methotrexate
Mitomycin C
Nitiofurantoin
Penicillamine
Phenytoin
Propranolol
Sulfasalazine
Tocainide
Radiation-induced
Idiopathic pulmonary fibrosis

Inflammatory events

The triggering events are usually followed by inflammatory events where damaged alveolar epithelial cells and other resident cells release inflammatory mediators to promote the recruitment of inflammatory cells. Accumulation of neutrophils, eosinophils, lymphocytes, mast cells, monocytes and alveolar macrophages is a consistent histological feature of the early stage of various pulmonary fibrotic diseases. Some of these infiltrating cells are also active and proliferating. For example, high numbers of activated alveolar macrophages have been detected in patients with pulmonary fibrosis.[39] These macrophages are the source of high levels of IL-8 in patients with cryptogenic fibrosing alveolitis,[40] and IL-8 is an important neutrophil chemoattractant that mediates neutrophil trafficking into the lower respiratory tract.[41,42] Moreover, macrophages secrete PDGF, and TGF-β1 can directly activate fibroblasts and myofibroblasts to produce various matrix proteins.[43,44] Increased accumulation and local proliferation of macrophages are important events during chronic inflammation and pulmonary fibrosis. Macrophage colony-stimulating factor (m-CSF), also known as CSF-1, regulates the survival, proliferation and differentiation of mononuclear phagocytes. Numerous studies have shown a close association between the expression of m-CSF and local proliferation of macrophages in experimental and human renal and ocular diseases.[45–47] Similarly, increased numbers of alveolar macrophages, due to local proliferation, suggested their involvement in the chronic inflammatory process of pulmonary fibrosis, as m-CSF and granulocyte macrophage (GM)-CSF produced by lung fibroblasts are thought to regulate local proliferation of alveolar macrophages in chronic inflammatory/fibrogenic lung disorders.[48]

Increased numbers of both $CD4^+$ and $CD8^+$ T cells have been detected in diseases associated with pulmonary fibrosis,[49] and counts of T-cell subpopulations are good predictors of outcome in pulmonary fibrosis.[50] The role of T-cell subsets in the pathogenesis of fibrotic lung diseases is an area of active investigation. Recently, endothelin 1 (ET-1) was found to be involved in the recruitment of T cells. In ET-1 transgenic mice, the accumulation of infiltrating mononuclear cells in the lungs, with a preponderance of $CD4^+$ cells, has been reported.[51] These transgenic mice eventually develop fibrotic lesions in various organs including the lungs, suggesting a potential role for ET-1 in the recruitment of inflammatory cells. In addition, IL-4 is known to activate mononuclear cells as well as fibroblasts, and has a stimulatory effect on collagen synthesis, which is important in the pathogenesis of pulmonary fibrosis. In the murine model of bleomycin-induced pulmonary fibrosis, increased expression of IL-4 messenger ribonucleic acid in the lung occurs on days 3 and 14 after induction of lung injury, decreasing towards the control level after day 21, as demonstrated by *in situ* hybridization and by immunohistochemistry, and IL-4-expressing cells were identified as mononuclear cells and macrophages localized to areas of active fibrosis.[52] Further, IL-4 might play an important role in pulmonary fibrosis by amplifying the inflammatory responses and by stimulating collagen synthesis in fibroblasts, thereby contributing to the progression of fibrosis and end-stage lung disease. Therefore, the release of certain chemokines (e.g. regulated upon activation, normal T cell expressed, and secreted (RANTES), monocyte chemoattractant protein 1 (MCP-1)), cytokines (e.g. IL-1, IL-4 and IL-8), and growth factors (e.g. PDGF, TGF-β1, tumor necrosis factor (TNF)-α) by inflammatory cells and activated resident cells could intensify both the inflammatory and fibrotic events in the lung.[43,44,53] In addition, reactive oxygen species (ROS) play an important role in the pathogenesis of interstitial lung diseases as the anti-oxidant defense system is thought to be impaired during fibrogenesis. Alveolar macrophages isolated from patients with idiopathic pulmonary fibrosis and experimental fibrotic models generate ROS, and arachidonic acid metabolites. The ROS have also been implicated in mediating fibroblast proliferation.[54] Furthermore, high levels of myeloperoxidase and low levels of glutathione, an important antioxidant, have been detected in the alveolar epithelial lining fluid of patients with idiopathic pulmonary fibrosis,[55,56] suggesting the role of oxidant-mediated injury in pulmonary fibrosis.

Fibrotic events

Excessive synthesis of collagen is a common pathological feature of most fibrotic diseases. The synthesis and systemic accumulation of matrix proteins are essential for normal tissue development, homeostasis and wound repair. Physiologically, a balance exists between matrix formation and degradation. This balance is disrupted in fibrotic diseases, usually due to the increased production and decreased degradation of matrix proteins, as seen in pulmonary fibrosis, systemic sclerosis, keloids, cirrhosis of the liver and renal fibrosis.[1–9] In fibrotic diseases, the normal architecture of the tissues is replaced by fibrotic tissue, resulting in failure to perform their physiological functions. In pulmonary fibrosis, excessive accumulation of interstitial collagens destroys lung architecture, and subsequently leads to respiratory failure. It has been suggested that the injured epithelium in idiopathic pulmonary fibrosis releases a number of cytokines and growth factors, which control fibroblast proliferation, differentiation, chemotaxis and matrix production. Type II alveolar epithelial cells are one of the main sources of these fibrogenic factors. In the fibrotic phase, the activated resident and transformed interstitial matrix-producing cells synthesize an excessive amount of matrix proteins, which are deposited in the interstitial space, and gradually develop to form irreversible pulmonary fibrosis. Accumulation of various types of collagens, such as type I, type III and type VI collagens, has been described in fibrotic lesions of the lung.[57–59] A similar expression and deposition of collagens has been noted in various other fibrotic diseases, including idiopathic pulmonary fibrosis, a poorly understood disorder of unknown cause. Clinically, it is characterized by progressive reduction in the functional capacity of the lung that results in disruption of gas exchange between alveoli and capillaries. The three major histopathological features of this disease are the influx of inflammatory cells, proliferation of type II pneumocytes and the accumulation of collagens. The altered composition of matrix proteins in the fibrosing tissues or organs might activate surrounding cells to produce factors that might influence both inflammatory and fibrotic events. Moreover, ECM-derived signals might exert chemotactic effects on a number of cells, including fibroblasts, that are involved in transcriptional activation of activator protein-1 (AP-1) and nuclear factor κB (NF-κB) to produce inflammatory cytokines such as IL-1 and TNF-α, and thereby exacerbate the fibroproliferative process.[60,61] Recently, Selman *et al.* proposed the concept of inflammation-independent pulmonary fibrosis, and emphasized the role of alveolar epithelial injury and formation of fibroblast-myofibroblast foci as a basis for fibrogenesis.[62]

MATRIX REMODELING DURING FIBROSIS

The imbalance between the production of newly synthesized matrix proteins and their inadequate removal, degradation or clearance results in excessive accumulation of matrix pro-

teins, and the net effect is the alteration of the structure and function of the involved tissues and organs. The generation of ECM is predominantly achieved through the production of collagenous and non-collagenous proteins, whereas degradation of ECM is predominantly achieved by various proteolytic enzymes, such as matrix metalloproteinases (MMP) and a disintegrin and metalloproteinase domain (ADAM). In addition, tissue inhibitors of metalloproteinases (TIMP) also play an active role in matrix remodeling by neutralizing MMP activities. The increased expression of MMP-1, -2 and -9 has been detected during the process of pulmonary fibrosis.[63] Increased levels of MMP are also associated with increased levels of their inhibitory enzymes, TIMP-1 and -2.[64] The expression of TIMP (1 and 2) could neutralize the collagenolytic effects of MMP in diseases associated with pulmonary fibrosis, and the end result is matrix accumulation. In another study, Selman *et al*. reported a wider distribution of TIMP-1, -2, -3 and -4 compared with the collagenases (MMP-1 and MMP-8) in the lung parenchyma during fibrogenesis in idiopathic pulmonary fibrosis, again suggesting less collagenolytic activity during the development of pulmonary fibrosis. In the same study, excessive MMP-2 and MMP-9 production was detected in the fibrotic lung and was suggested to play a role in the disruption of basement membrane, and enhancing fibroblast invasion to the alveolar spaces.[65]

Little is known about ADAM[66] functions during matrix remodeling, but these proteases are considered to contribute to fibrogenesis. Recently, a subgroup of ADAM has been identified, designated ADAM-TS (thrombospondin motif), that unlike typical membrane-anchored ADAM, lack a transmembrane domain and a cytoplasmic domain at the C terminus. Instead, these metalloproteinases contain a variable number of thrombospondin type-1 domains.[67] Recent studies have suggested a pathological role for ADAM-TS1 in experimental liver fibrosis in cirrhotic rats.[68]

In order to understand the matrix remodeling in pulmonary fibrosis, it is important to determine the specific numbers of MMP, ADAM, ADAM-TS and TIMP, and their roles in the fibrotic process. If precise numbers of involved molecules in pulmonary fibrosis can be defined, hypothetically, restoration of their balance by biochemical manipulations should alter the fibrotic process. Increased activity of MMP-2 and MMP-9 in bronchoalveolar lavage fluid (BALF) has been shown to be associated with the severity of bleomycin-induced pulmonary fibrosis, while treatment with batimastat, a synthetic inhibitor of MMP, resulted in a significant improvement of fibrosis in mice. Batimastat treatment was effective in reducing MMP-2 and MMP-9 activities as well as the TIMP-1 level in BALF.[69] These results suggest that restoring the balance of enzymes involved in matrix remodeling might be useful in preventing the progression of fibrosis, and could theoretically form a basis for new therapies.

FACTORS REGULATING FIBROSIS

Complex networks of cellular and molecular interactions regulate the fibrotic process. Mediators such as cytokines, chemokines and growth factors released by resident cells or infiltrating inflammatory cells usually contribute to ECM remodeling. Changes in the microenvironments due to the altered composition of matrix proteins affect cellular functions, and could further augment the fibrotic process. For example, proteolytic digestion of ECM proteins gives rise to fragments of matrix molecules that might interfere with the intercellular signaling events of matrix receptors. Fragments of type II collagen and its synthetic peptides could increase the level of MMP-3. Previous studies showed that a type I collagen-rich microenvironment activates hepatic stellate cells, which are the main matrix-producing cells.[70] These activated cells express higher levels of collagen receptors, such as $\alpha1\beta1$ and $\alpha2\beta1$ integrins,[71] and some of these integrins are related to intercellular signaling events that regulate proliferation, migration and adhesion of matrix-producing cells, and thus contribute to the fibrotic process. Integrin $\beta6$ knockout mice are protected from bleomycin-induced pulmonary fibrosis, possibly by modulating the bioactivities of TGF-$\beta1$.[72] Moreover, various 'profibrotic' factors, including IL-4, PDGF, TGF-$\beta1$, connective tissue growth factor (CTGF), ET-1, TNF-α, heat shock protein 47 (HSP47), insulin-like growth factor (IGF) and its binding proteins, have been suggested to mediate different events of human and experimental fibrosis. Of these, TGF-$\beta1$ is a well-studied molecule.

Transforming growth factor-β1

Transforming growth factor-β comprises a highly homologous family of multifunctional regulatory peptides that are differentially expressed and involved in the control of cell growth and differentiation, morphogenesis and matrix remodeling. At present, three isoforms of TGF-$\beta1$ have been characterized in mammals. The biologically active molecule is a 25-kDa homodimer of two 12.5-kDa disulfide-linked monomers.[73] Recent studies suggest that different isoforms of TGF-$\beta1$ might play different roles during wound healing. Both TGF-$\beta1$ and TGF-$\beta2$ contribute to scar tissue formation, while TGF-$\beta3$ might actually have anti-scarring effects.[74] Transforming growth factor-$\beta1$ is consistently found to be upregulated in various human and experimental fibrotic diseases, and blocking its bioactivity could suppress matrix production and modulate the fibrotic process.[75] In addition, it does not only increase the transcription of collagens, but also decreases its degradation by inhibiting collagenase activity through increased production of matrix metalloproteinase inhibitors such as TIMP and plasminogen activator inhibitor. Increased expression of TGF-$\beta1$ has been detected in lung tissues

obtained from patients with pulmonary fibrosis.[76] The fibrogenic role of TGF-β1 is confirmed in animal models of pulmonary fibrosis, and the blockade of the biological activities of TGF-β1 has significantly decreased interstitial accumulation of collagens in these models.[77,78] When intracellular SMAD signaling cascade of TGF-β1 was genetically manipulated in mice, a similar attenuation of pulmonary fibrosis was identified in experimental models of pulmonary fibrosis.[79,80] Existing information suggests a fibrogenic role for TGF-β1 in pulmonary fibrosis. However, TGF-β1 does not only induce matrix proteins during wound healing and fibrogenesis, but it also has other important effects, for example, it suppresses the growth of epithelial cells, inhibits keratinocyte proliferation, enhances neovascularization, acts as a chemoattractant for monocytes and fibroblasts, and is involved in immunosuppression.[81,82] Although blocking the expression of TGF-β has some negative effects on the fibrotic process in experimental models, such blockage disrupts other non-fibrotic physiological functions. For example, spontaneous development of necroinflammatory hepatitis has been reported in mice deficient in TGF-β1.[83] Hypothetically, identifying the fibrosis-specific factors and their blockage will be ideal for developing future therapies. Connective tissue growth factor is a TGF-β-inducible gene, which is an important downstream mediator of TGF-β1, and is known to promote the synthesis and secretion of collagens and other matrix proteins. In addition, CTGF could be potentially useful as an important fibrosis-specific gene.

Connective tissue growth factor

Connective tissue growth factor is a heparin-binding 38-kDa cysteine-rich peptide, and was first isolated from human endothelial cells.[84] Subsequent studies have shown that CTGF promotes the proliferation of fibroblasts and collagen synthesis.[85] The bioactivity of CTGF is partly controlled by TGF-β1, and a brief exposure of fibroblasts to TGF-β1 resulted in a high level of CTGF expression.[86] The identification of a novel TGF-β-responsive element in the CTGF promoter has established that induction by TGF-β1 has a direct effect on the transcriptional activation of the CTGF gene. The induction of CTGF by TGF-β1 was identified as one of the downstream regulators for TGF-β1 during wound healing. It is abundantly present in fibroproliferative disorders such as idiopathic pulmonary fibrosis, diabetic nephropathy and proliferative glomerulonephritis.[87–89] In the lungs, both fibroblasts and bronchial epithelial cells are the main source of CTGF.[90] A preliminary trial of interferon (IFN)-γ cotherapy in idiopathic pulmonary fibrosis (IPF) patients showed clinical improvement that correlated with inhibition of CTGF expression,[91] suggesting that the modulation of CTGF expression might be a potentially useful therapy against pulmonary fibrosis.

Endothelin-1

Endothelin-1 is a powerful vasoconstrictor agent released by endothelial cells. Human ET-1, derived from a 212-amino acid precursor, preproET-1, is rapidly cleared from the circulation but has a long local effect on vascular tone. It is ubiquitously expressed and involved in diverse cellular functions, including cell proliferation and matrix production. It elicits physiological responses by stimulating different ET receptor subtypes such as ET_A and ET_B.[92,93] Endothelial cells, epithelial cells, alveolar macrophages, polymorphonuclear leukocytes and fibroblasts in the lung are known to secrete ET-1. The increased production of ET-1 has been reported to contribute to the fibrotic process in patients with idiopathic pulmonary fibrosis by promoting growth and differentiation of epithelial cells and fibroblasts, and by inducing the synthesis of collagen.[94] In addition, expression of endothelin-converting enzyme-1 has been reported to correlate with the fibrogenic activities of the lung.[95,96] Compared with control subjects, a fivefold increase in ET-1 levels was detected in BALF obtained from patients with pulmonary fibrosis associated with systemic sclerosis.[97] Furthermore, ET-1 transgenic mice showed features of chronic inflammation and pulmonary fibrosis with accumulation of mononuclear cells and deposition of ECM proteins in the perivascular and peribronchial spaces.[51,98] Recent studies showed that ET-1 can stimulate the proliferation of normal airway epithelial cells, and that such action can be blocked by phosphoramidon.[99] Modulating the biological activities of ET-1 by blocking its receptors also resulted in the inhibition of fibrosis in a rat model of bleomycin-induced pulmonary fibrosis.[100]

Tumor necrosis factor-α

A potent pro-inflammatory cytokine, TNF-α, is secreted as a 26-kDa membrane-bound glycopeptide and is cleaved by TNF-α converting enzyme to release a 17-kDa monomeric peptide.[101] Both free and membrane-bound forms are biologically active. In addition, TNF-α exerts its biological effects through two TNF-α receptors: TNFR1 (55 kDa) and TNFR2 (75 kDa),[102] and appears to exert a wide range of functions, including recruitment of inflammatory cells by inducing chemoattractant molecules, helping in mesenchymal cell proliferation, and regulating apoptosis and collagen synthesis in fibrotic lung diseases. Th exposure of C57BL/6 silica-sensitive mice to silica resulted in induction of expression of TNF-α and its receptors (both TNFR1 and TNFR2) in lung tissues, while no such changes were noted in the silica-resistant C3H/HeJ mice.[103,104] Furthermore, genetically manipulated mice overexpressing TNF-α on a lung-specific promoter showed features of lung fibrosis,[105] while TNFR knockout mice showed resistance to bleomycin-induced

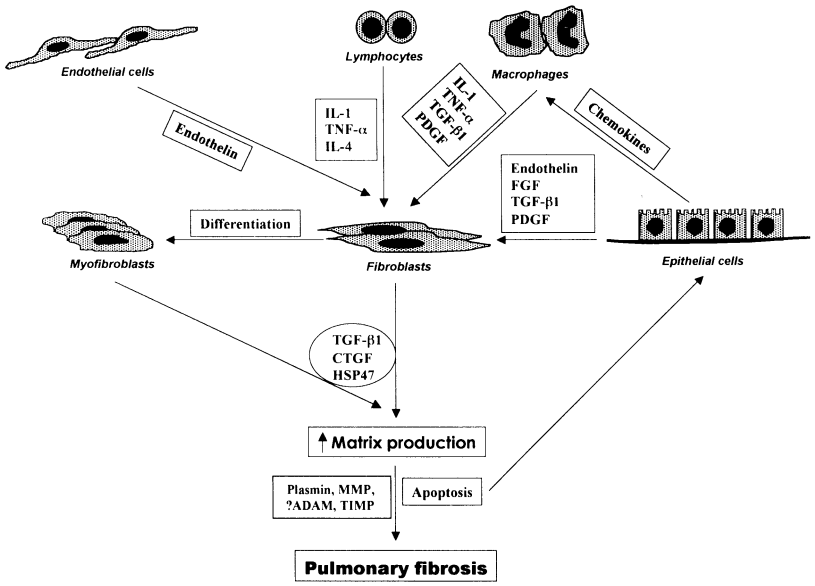

Figure 1 Possible molecular interactions during the process of pulmonary fibrosis. Some other factors that might play significant roles in the interstitial fibrotic process are not included in order to keep the diagram as simple as possible. ADAM, a disintegrin and a metalloproteinase; CTGF, connective tissue growth factor; FGF, fibroblast growth factor; HSP47, heat shock protein 47; IL-1, interleukin-1; IL-4, interleukin-4; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; TGF-β1, transforming growth factor beta1; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor alpha.

fibrosis.[106] This evidence suggests an important role for the TNF-α system in the development of pulmonary fibrosis.

Heat shock protein 47

Heat shock protein 47 is a molecular chaperone for the biosynthesis of procollagens,[107] and is thought to contribute to the fibrotic process in the lungs, liver, skin and kidney by regulating increased synthesis of procollagen.[108–112] For example, a high expression of HSP47 was found in the lungs of rats with bleomycin-induced pulmonary fibrosis. A coordinated upregulation of HSP47 with increased interstitial accumulation of collagens is also seen in human pulmonary fibrotic diseases (Fig. 2).[113] Fibroblasts and myofibroblasts are the main source of HSP47 in the fibrotic lungs (Fig. 3).[112,113] Moreover, preliminary studies have suggested that blocking and/or modulating the expression of HSP47 have negative effects on the progression of renal fibrosis.[114] In summary, there is circumstantial evidence for the role of HSP47 in lung fibrosis, and further studies should determine whether HSP47 could be a future therapeutic target for developing an antifibrotic agent to control pulmonary fibrosis.

Other factors

In addition to the previously mentioned factors, FGF, TGF-α, PDGF, IL-4, hepatocyte growth factor and IGF-1 have been reported to play some roles in pulmonary fibrosis. Of these, PDGF seems to actively contribute to pulmonary fibrosis, possibly by regulating a number of fibrogenic mediators including TNF-α, TGF-β, IL-1 and FGF. Compared to the low levels of expression of both PDGF and the PDGF receptor in normal adult lungs in alveolar macrophages, increased expression was noted in idiopathic pulmonary fibrosis.[115] Furthermore, the overexpression of PDGF-B gene produces histopathological features of fibrosis in experimental animals.[116] In addition, IGF-1 can also induce fibroblast proliferation and collagen synthesis, and the upregulation of its expression and its receptor have been reported in the early stages of pulmonary fibrosis.[117]

Figure 1 summarizes the roles of some of the important molecules and their contribution to the initiation and progression of the pulmonary fibrotic process.

MODULATION OF FIBROSIS

Identification of the factors responsible for the initiation of fibroproliferative diseases and their elimination or inhibition is not always practical and easy because of the diversity of primary causes of fibrotic diseases. Thus, the next rationale for therapy is a blockade of the molecules responsible for the early events of inflammation and subsequent fibrosis. Specific treatments for fibrotic diseases are not yet available, and are limited to the restriction of inflammatory events occurring in the lungs with the expectation that prevention of inflammation might delay the progression of fibrotic events. Corticosteroids are usually administered in an attempt to control the inflammation in fibrotic lungs; however, the beneficial effects of these agents are not well established, and the issue of long-term side-effects is a major drawback.

Preliminary studies designed to specifically block the fibrogenic factors have provided encouraging results that could

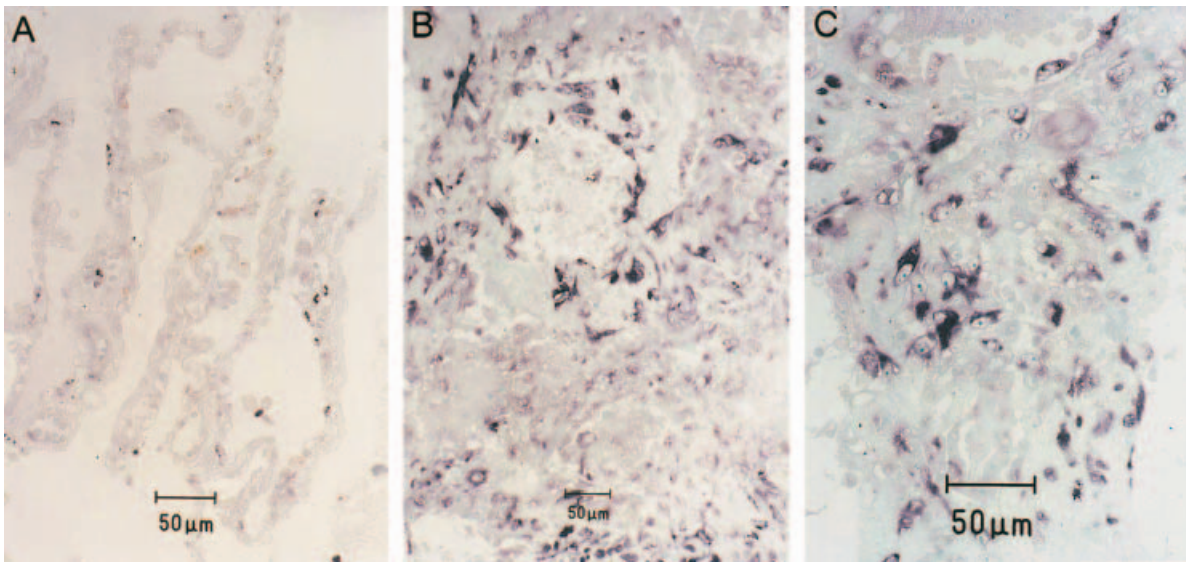

Figure 2 Expression of heat shock protein 47 (HSP47) in control (**a**) and fibrotic lung (**b**,**c**) tissues. Note an increased number of HSP47-expressing cells in the fibrotic lung sections.

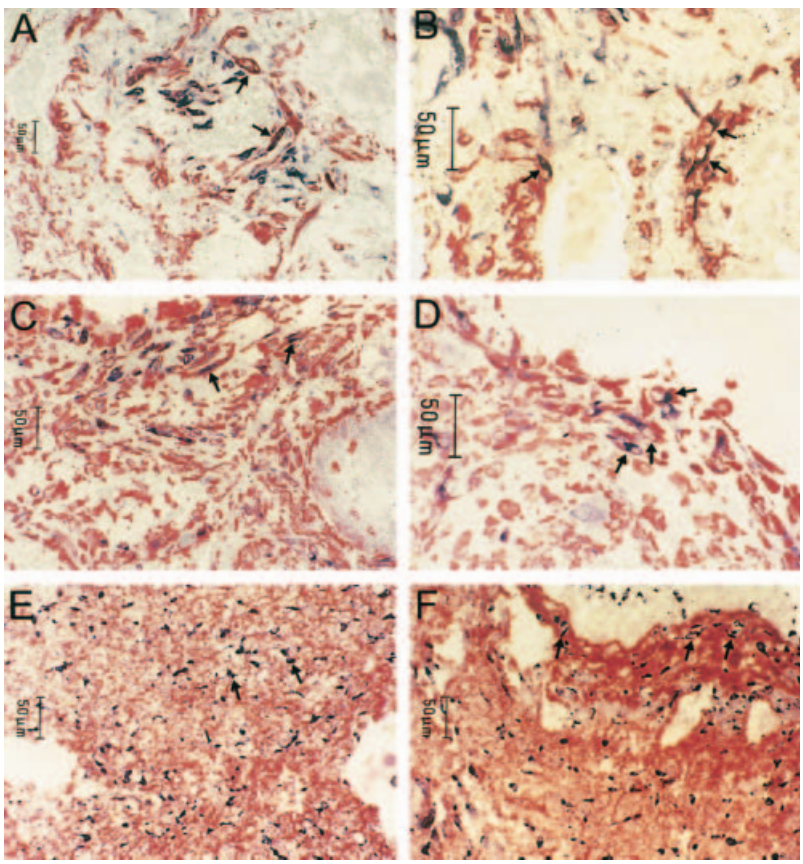

Figure 3 Double staining of heat shock protein 47 (HSP47) (black) with α-smooth muscle actin (red), vimentin (red) and type III collagen (red). Note the coexpression of HSP47 and α-smooth muscle actin (arrows) in the fibrotic lung sections (**a**,**b**), suggesting that some of the HSP47-expressing cells are myofibroblasts. Similarly, the coexpression of HSP47 and vimentin (arrows) is detected in the fibrotic lung sections (**c**,**d**), suggesting that some of the HSP47-expressing cells are interstitial fibroblasts. The colocalization of HSP47 (arrows) with an increased deposition of type III collagen has been detected in the fibrotic lung sections (**e**,**f**).

possibly form the basis for future developments of new therapeutic agents. Beneficial effects after blocking PDGF, IL-1, TNF-α and IL-1 receptor have already been reported in pulmonary fibrotic diseases. Ziesche *et al.*[91] reported the results of an open clinical trial study of 18 subjects where nine were treated with low-dose prednisolone (7.5 mg/day) and were compared with another group of nine patients treated with IFN-γ (200 μg, three times daily) and prednisolone (7.5 mg/week). After 12 months of treatment, total lung capacity decreased by 4% in the control group but increased

by 9% in the IFN-γ treated group. The resting partial pressure of arterial oxygen (PaO_2) also decreased in the control group, but increased in the IFN-γ treated group, with comparable improvements in postexercise PaO_2.

Both *in vivo* and *in vitro* studies have documented that relaxin blocks TGF-β1 bioactivities and modulates collagen metabolism.[118] Relaxin also suppresses TGF-β1-induced type I and type III collagens by lung fibroblasts. Furthermore, relaxin reduced collagen accumulation in bleomycin-induced pulmonary fibrosis.[118] Although blocking the biological activities of individual factors or cytokines has some beneficial effect on the course of pulmonary fibrosis, in reality, several cytokines or growth factors exert their functions simultaneously during the development of pulmonary fibrosis. Therefore, strategies to block the action of multiple cytokines simultaneously or inhibit the actions of such factors by targeting common pathways might have a better chance of being therapeutically effective.

Recently, pirfenidone (5-methyl-1-phenyl-2-(1H)-pyridone) has been successfully used as an antifibrotic agent in experimental pulmonary fibrosis, renal fibrosis and sclerosing peritonitis.[119–123] Pirfenidone ameliorated bleomycin-induced pulmonary fibrosis in hamsters[124] and is thought to act by suppressing the TGF-β gene at a transcriptional level.[125] In addition, pirfenidone has shown encouraging results in human fibrotic diseases, particularly in terminally ill patients with advanced idiopathic pulmonary fibrosis.[126] Based on published studies, pirfenidone appears to be a promising new antifibrotic drug.[127]

The angiotensin-converting enzyme inhibitor, captopril, has been shown to reduce fibroblast proliferation and pulmonary fibrosis in irradiated rats.[128,129] Angiotensin II stimulates matrix production by fibroblasts, and angiotensin II receptor blockers have been reported to inhibit pulmonary fibrosis resulting from irradiation and bleomycin treatment. Because these drugs are already used for treating various other human diseases and can be administered orally, further evaluation will determine the beneficial effects of these drugs in the treatment of pulmonary fibrosis.

Recently, blocking specific post-translational enzymes of collagen synthesis has been attempted to reduce collagen accumulation in fibrotic diseases. Prolyl 4-hydroxylase is involved in the synthesis of collagens by catalyzing the formation of 4-hydroxyproline, which is essential for the assembly of triple-helical structures. Modulating the activity of this enzyme resulted in reduced collagen accumulation.[130,131] Likewise, HSP47 is a collagen-specific molecular chaperone and helps in procollagen synthesis.[107] The upregulation of HSP47 is closely associated with collagen accumulation in various human and experimental fibrotic diseases.[108,111,132–136] The modulation of HSP47 expression resulted in less accumulation of collagen in renal scarring models.[114] Therefore, it is likely that specific inhibitors of post-translational enzymes of collagen synthesis might have potential antiscarring effects.

Initial phases of fibrosis encompass inflammatory events, including proliferation of matrix-producing cells and inflammatory infiltrates. In the late stages, most of these proliferating and infiltrating cells are cleared or removed (possibly by apoptosis), leaving mostly an acellular fibrotic mass. The increased rate of apoptosis has been demonstrated during the development of renal scarring in both human and experimental animals.[31,137–139] A similar increase has been detected in pulmonary fibrosis, and suppression of apoptosis by caspase inhibitors could attenuate fibrotic process in the lung,[140] suggesting that modulation of apoptosis might have potential therapeutic implication in fibrosis.

CONCLUSIONS

Pulmonary fibrosis is a progressive disorder in which the air sacs or 'alveoli' of the lungs are progressively replaced by fibrous tissues. The scar tissue interferes with oxygen transfer from the lungs to the bloodstream, resulting in breathlessness and eventual respiratory failure. Significant progress has been made in our understanding and identification of the molecules involved in pulmonary fibrosis. However, further studies are required in order to determine how these molecules are regulated and interact with each other, in order to develop effective strategies for specific intervention or modification of pulmonary fibrosis.

In this brief review, we discussed the pathomechanisms of pulmonary fibrosis as well as their relevance to other fibrotic diseases. We believe that this approach might have wide implications for fibrogenesis in general, and might help us to understand some important unresolved issues of pulmonary fibrosis. We realize that scarring of the lung is a multistep and multifactorial phenomenon. To that end, we have tried to present basic information of a limited number of essential molecules that could be important, significant and might play central roles in matrix remodeling in pulmonary fibrosis.

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