The Alveolar Epithelium and Pulmonary Fibrosis

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Abstract: Idiopathic interstitial pneumonias (IIPs) are a heterogeneous group of diffuse pulmonary parenchymal diseases that are comprised of seven distinct clinical and pathological entities. Idiopathic pulmonary fibrosis (IPF) and cryptogenic organizing pneumonia (COP) represent two of the most prevalent members of the disease group with major differences in their pathogenesis, clinical course and prognosis. IPF is a refractory and lethal IIP characterized by fibroblast proliferation, deposition of extracellular matrix (ECM) and progressive lung scarring. The incidence of IPF is estimated at 15 to 40 cases per 100,000 per year, and the mean survival from the time of diagnosis is 3 to 5 years regardless of treatment. While its pathogenesis is incompletely understood, the currently accepted paradigm proposes that injury of the alveolar epithelium is followed by a burst of pro-inflammatory and fibroproliferative mediators that invoke responses associated with dysregulated repair of the damaged alveolar epithelium. Recently, there have been studies suggesting that the activation of the alveolar epithelial cells (AECs) may play an active role in the pathogenesis of pulmonary fibrosis. Here, we review the advances in recent studies on the role of the alveolar epithelium in pulmonary fibrosis.

AECS: A BRIEF OVERVIEW OF STRUCTURE AND PHYSIOLOGICAL FUNCTIONS

Two types of AECs populate the alveolar epithelium in normal adult lungs, alveolar epithelial type I (AT I) and alveolar epithelial type II (AT II) cells. AT I pneumocytes comprise 40% of the AECs and cover 90% of the internal surface area of the lung. AT I cells are highly attenuated and form an interface with pulmonary capillaries that are intimately involved in gas exchange. These cells also take part in peptide and amino acid transportation across the lung by pinocytosis and transporter-mediated transport [1]. Recently, AT I cells have been shown to be also important in the transportation of water and sodium across the lung [2, 3].

AT II cells, on the other hand, AT II cells make up 16% of the parenchymal cells in the lung and account for only 5% of the alveolar surface. These cells are cuboidal cells that are situated between AT I cells and they contain characteristic lamellar bodies and apical microvilli. AT II cells synthesize, store and secrete the pulmonary surfactant (PS), which consists of lipids and their associated proteins and whose function is to reduce the surface tension of the lung alveolus and prevent it from collapse or overdistension with respiration. AT II cells also regulate alveolar fluid balance in normal lungs and during the resolution of pulmonary edema [4] and the pathogenesis of acute lung injury with pulmonary edema is associated with an insufficient number of AT II cells and transportation of water into the lung alveolus. Recently, AT II cells have been shown to synthesize and secrete immunomodulatory proteins important for host defense, including surfactant proteins A (SP-A) and D (SP-D) [5]. SP-A can combine with lipid A in the lipopolysaccharide on the surface of bacterium and it also regulates the activities of pulmonary alveolar macrophages and modulates phagocytosis [6].

It has been long assumed that AT I cells are terminally differentiated cells and cannot self-renew or self-repair when they are injured. AT II cells, on the other hand, are capable of self-renewal and differentiation and may function as stem cells to regenerate AT I cells [7, 8]. Damaged AT I cells may also be replaced by bone marrow derived stem cells that can differentiate into AT I cells [9], suggesting that alveolar progenitor cells may not only be derived from local sources of self-renewing progenitor cells but also from extrapulmonary reparative progenitor subpopulations. This concept of exogenous progenitor cells for replenishing damaged AT I cells is also supported by the findings of Abe *et al.* who demonstrated that bone marrow derived stem cells could differentiate into AT I cells [10].

AT I CELLS ARE INVOLVED IN PULMONARY FIBROSIS

The lung is an organ situated in the body that provides an interface between the host's internal milieu and the external environment. AT I cells, which cover most of the surface of the lung, form tight intercellular junctions and deny access of fibrogenic particles like silica to interstitial macrophages and fibroblasts in the lung. Injury of the AECs exposes the ECM to fibrogenic particles and annuls the protection of the pulmonary integrity by AT I cells. It also initiates the process of hemorrhage and extravasation of plasma into the lung tissue, resulting in the activation of both the intrinsic and extrinsic coagulation pathway and subsequent fibrin deposition. AT I cells, fibroblasts and alveolar macrophages express tissue factors, urokinase type plasminogen activators (uPA) and plasminogen activator inhibitors (PAI), and regulate the activity of fibrinolysis and the clearance of fibrin in the lung alveolus [11]. Injury of the AECs compromises the ability of AT I cells to clear redundant fibrin in the lung alveolus and impairs their capability to endocytoze fibrin deposits.

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The amount of tissue factor, which binds to and activates factor VII and initiates the activation of downstream coagulation cascade, increases during lung injury, resulting in enhanced coagulation in the lung. There is also disequilibrium between uPA and PAI in the lung. uPA and its receptor, uPAR, combine to increase fibrinolysis. Additionally, the activities of uPA in AT I cells have been shown to be regulated at the posttranscriptional level. Shetty *et al.* found that a urokinase mRNA binding protein binds to a 66 nucleotide region in the mRNA and destabilize it [12], suggesting that the level of uPA is tightly regulated in the normal lung. Platelet activation and degranulation also occur during coagulation, leading to the release of cytokines that can activate leukocytes, endothelial cells, macrophages, fibroblasts and AECs. Recent evidence also suggests that efficient repair of damaged alveolar epithelium depends on the attainment of a fibrinolytic optimum in the pulmonary microenvironment [13]. Therefore, dysregulated coagulation and fibrinolysis upon injury to the AECs and compromised repair capability of AT I cells may lead to multiple cycles of coagulation and fibrinolysis, which may aggravate alveolar injury and induce pulmonary fibrosis.

THE ROLE OF AT II CELLS IN PULMONARY FIBROSIS

Unlike AT I cells, which are terminally differentiated and incapable of self-renewal or repair, AT II cells are able to self-renew, repopulate and repair damaged alveolar epithelium [14]. AT II cells have been shown to interact with fibroblasts and ECM in the lung [15, 16]. Their foot processes pass through the basement membrane and are in contact with fibroblasts in the pulmonary interstitium, an observation that has been confirmed by electron microscopy, suggesting that direct contact between lung fibroblasts and AT II cells may serve as a conduit for signal conduction among the cells, thereby coordinating the activities of AT II cells and fibroblasts [17]. When the alveolar epithelium is damaged, AT II cells start proliferating and transdifferentiate into AT I cells to reestablish a functional alveolar epithelium. Fibroblasts that are in contact with proliferating AT II cells also become activated and start proliferating to participate in the repair process. Unrestrained proliferation of fibroblasts in the lung interstitium following lung injury would contribute to the development of pulmonary fibrosis.

The basement membrane is a complex structure that contains type IV collagen, laminin, fibronectin, entactin and heparin sulfate–chondroitin proteoglycans and it plays a dynamic role in maintaining the integrity and differentiation of the alveolar epithelium. AT II cells synthesize some of the essential components of the basement membrane such as fibronectin, laminin and type IV collagen that are important for maintaining the integrity of the alveolar epithelium and for regulating the migration, differentiation and adhesion of fibroblasts and other types of cells. Injury of AT II cells compromises their production of the components of the basement membrane, resulting in the loss of the integrity of the basement membrane. Fibroblasts and myofibroblasts can migrate into the alveolar space through the partially disrupted and denuded basement membrane, which promotes the development of pulmonary fibrosis.

AT II CELLS AND THE INFLAMMATORY REACTION

Lung injury that triggers pulmonary fibrosis is followed by inflammatory events where damaged alveolar epithelium and other resident cells release inflammatory mediators that promote the recruitment of inflammatory cells. Pulmonary fibrosis is a progressive and irreversible disease process and is believed to be due to repeated inflammations, resulting in an excessive proliferation of fibroblasts and deposition of ECM. Sime *et al.* used active TGF-β transgene to induce lung fibrosis in rats and found that inflammation was present and peaked before maximal fibrosis [18]. SP-A, which is produced by AT II cells, promotes the alveolar macrophages to produce various inflammatory mediators and cytokines that reinforce the inflammatory reaction. Immunohistochemical studies further reveal that most of AT II cells express intercellular adhesion molecule-1 (ICAM-1) and a minority of them express integrin-β_1 subunit-α_4 (CD49α) and vitronectin receptor subunit-α_5 (CD51) in normal lung. ICAM-1 binds to lymphocyte function-associated antigen-1 (LFA-1) on lymphocytes during lung injury to promote the inflammatory reaction by recruiting leucocytes into the lung interstitium [19]. CD44 isoforms are also expressed in AT II cells. Kasper and colleagues have shown that, in normal lung tissue, CD44s is expressed on the cell surface of epithelial cells, which, however, is located in the cytoplasm of epithelial cells in pulmonary fibrosis [20]. The CD44v variants, CD44v6 and CD44v9 and CD44s are located at the basolateral aspect of AT II cells, suggesting that these proteins may be involved in epithelial cells-fibroblast interaction during lung repair [21] CD44s also likely regulates the synthesis and degradation of ECM [22].

Cultured AT II cells and rat AT II cells exposed to silica expressed chemotactic factors for neutrophils such as macrophage inflammatory protein-2 (MIP-2), which recruit heterophile granulocytes that produce collagenase, elastase and other types of proteases, which can degrade ECM proteins [23, 24]. AT II cells are the main site of metabolism for arachidonic acids in the lung, which causes the release of lipid mediators of inflammation such as leukotrienes (LT) and prostaglandins (PG). Injury to AT II cells in pulmonary fibrosis causes a massive release of prostaglandins E_2 (PGE_2), which leads to increased vascular permeability in the lung and, as a consequence, alveolar proteinosis. This pathological change in which large amounts of plasma proteins permeate the alveolar space is positively correlated with pulmonary fibrosis [25]. In addition, AT II cells also synthesize interferon-like proteins, complements C_2, C_3, C_4, C_5 and factor B, which are also involved in inflammatory reaction.

GROWTH FACTORS AND LUNG FIBROSIS

Growth factors like transforming growth factor β (TGF-β) and insulin-like growth factor 1 (IGF-1) and others promote the proliferation of lung fibroblasts, induce chemotaxis of fibroblasts and regulate the synthesis of collagens, which may be associated with the development of pulmonary fibrosis. TGF-β consists of a highly homologous group of multifunctional regulatory peptides that are differentially expressed and involved in the control of cell growth, differentiation, morphogenesis and ECM remodeling and is the most potent and efficacious cytokine in promoting fibrosis. TGF-

β1 promotes fibroblasts and AT II cells to synthesize ECM proteins such as collagen type I, III, IV and fibronectin. Sime *et al*. showed that TGF-β transgeene promoted lung fibrosis in rats [18] and similar findings were also obtained in mice that overexpression of TGF-β promoted lung fibrosis [26]. TGF-β1 is upregulated in various human and experimental fibrotic diseases including pulmonary fibrosis [27], and inhibition of its bioactivity suppresses matrix production and could modulate the fibrotic process [28, 29], suggesting that TGF-β1 may represent an ideal target for developing targeted therapies.

IPF is characterized by the proliferation of macrophages and AT II cells and deposition of ECM and immune complexes. These cells express increased mRNA and protein levels of platelet-derived growth factor (PDGF), IGF-1 and their corresponding receptors [30, 31]. The release of PDGF, TGF-β1, tumor necrosis factor (TNF)-α by these and other cells activate resident inflammatory cells that intensify both the inflammatory and fibrotic events in the lung. PDGF regulates TGF-β1 and TNF-α and actively contributes to pulmonary fibrosis. Noticeably, increased expression of PDGF has been observed in IPF [32]. PDGF and IGF-1 induce the proliferation of fibroblasts and stimulate collagen synthesis. PDGF was shown to effectively induce the proliferation of fibroblasts at very low doses (pg to ng/ml) and was chemotactic for fibroblasts *in vitro* [33]. TNF-α saw its increased expression in AT II cells in lung specimens from patients with cryptogenic fibrosing alveolitis [34]. In addition, TGF-β upregulated the levels of interleukin (IL)-1 and IL-8 in AT II cells from patients with pulmonary fibrosis and *in vitro* [35]. TNF-α and IL-1 stimulate the production of collagen type I and III, and fibronectin [36, 37]. Sakai and colleagues detected the presence of hepatocyte growth factors (HGF) in bronchoalveolar lavage fluid from patients with IPF and showed that HGFs were produced by pulmonary macrophages and AT II cells [38]. HGF has been shown to inhibit the progression of bleomycin-induced pulmonary fibrosis in mice, which may be due to inhibition by HGF of TGF-β signaling through induction of Smad-7, an inhibitor of TGF-β signaling [39]. These findings taken together suggest that multiple cellular factors are involved in the process of lung fibrosis.

EPITHELIAL CELL APOPTOSIS IS INVOLVED IN LUNG FIBROSIS

The alveolar epithelium is not only the primary site of lung damage but also the primary site of a series of inflammatory events that are critical to the development of pulmonary fibrosis including the release of inflammatory mediators. Alterations in the structure and function of lung epithelial cells may affect the expression or production of important molecules that modulate the lung's response to injury. Epithelial cells in IPF secrete a number of molecules, such as growth factors and their receptors, proteases, surfactant proteins, adhesion molecules and ECM proteins that regulate the inflammatory and fibrotic response within the lung. Prominent injury of AECs is a characteristic feature of IPF. Although AT I pneumocytes comprise 40% of the AECl population and cover over 90% of the alveolar surface in the normal lung, they are markedly decreased in numbers in the area of severe inflammation following extensive injury and cell death in the lung tissue from patients with IPF. AT II cells are capable of self-renewal and repair and rapidly proliferate following epithelial cell injury. In the most severely damaged alveolar epithelium, the denuded basement membrane is covered by proliferating AT II cells, and there is a dearth of both AT I and II cells with massive infiltration by fibroblasts and myofibroblasts [40].

Recent findings in tissue specimens from patients with IPF revealed that AT II cells undergo severe endoplasmic stress and show evidence of apoptotic death [41, 42]. Bleomycin rapidly produces extensive DNA damage in the lung and electron microscopy shows the characteristic features of apoptosis in bronchiolar and alveolar epithelial cells [43], suggesting that DNA damage and apoptosis of epithelial cells may be associated with pulmonary fibrosis. DNA damage and apoptosis were also observed in bronchiolar and alveolar epithelial cells in IPF using an in situ DNA nick-end labeling method and electron microscopy [44-46]. DNA damage and apoptosis in lung epithelial cells have also been reported for acute lung injury and diffuse alveolar damage [47, 48]. Increased expression of proapoptotic proteins and decreased expression of antiapoptotic proteins have also been observed in IPF [49, 50]. One of the intracellular events required for apoptotic cell death in several systems, including the Fas-FasL pathway, is the activation of caspases. The tripeptide benzyloxycarbonyl-Val-Ala-Asp fluoromethylketone (Z-VAD.fmk), a broad-spectrum caspase inhibitor, inhibited the intracellular activation of caspase-like proteases *in vivo*, and protected mice against LPS-induced acute lung injury [51, 52]. It also attenuated bleomycin-induced pulmonary fibrosis in mice [53, 54]. Although the precise mechanisms whereby epithelial cell apoptosis leads to pulmonary fibrosis remain to be examined, epithelial cell apoptosis probably has an important role in the pathogenesis of lung injury and fibrosis (Fig. **1**). Studies of human IPF show high rates of apoptosis and increased levels of pro-apoptosis markers in AECs; however, adjacent fibroblasts/myofibroblasts are relatively resistant to apoptosis [49, 55]. The mechanisms of their resistance to apoptosis remain to be elucidated.

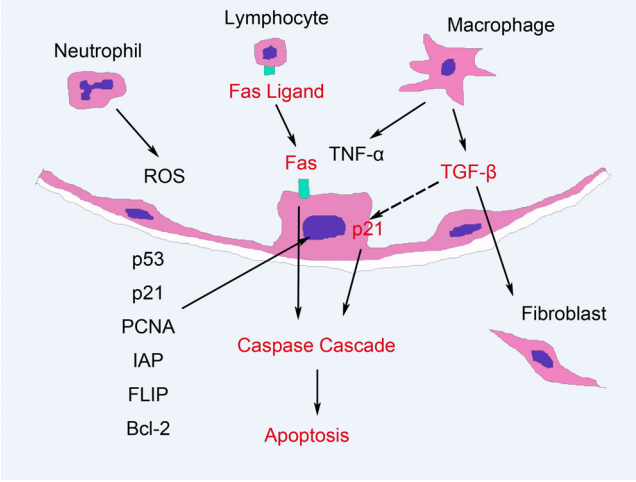

Fig. (1). Apoptosis of alveolar epithelial cells in idiopathic pulmonary fibrosis.

THE EPITHELIAL MESENCHYMAL TRANSITION AND LUNG FIBROSIS

Although the exact mechanisms for the pathogenesis of pulmonary fibrosis remain unknown, the long prevailing inflammatory fibrosis hypothesis asserts that chronic inflammation plays an essential role in pulmonary fibrosis [56]. It holds the view that the loss of AT I pneumocytes and the proliferation of AT II cells are the results of lung injuries and the subsequent unrelenting inflammatory process, leading to the development of pulmonary fibrosis. However, many IPF patients show little evidence of ongoing inflammation, and treatment focused on inhibition of inflammation with steroids and/or immunosuppressive drugs has had little impact on the disease [57].

An alternative hypothesis has been proposed that IPF likely results from an aberrant activation of AVEs after injury that provoke the migration, proliferation, and abnormal activation of mesenchymal cells, leading to the exaggerated accumulation of ECM with an irreversible destruction of the pulmonary parenchyma [58-60]. Witschi *et al*. suggested that pulmonary fibrosis may result from impeded restoration of the damaged alveolar epithelium [61]. In IPF, the alveolar epithelium shows a marked loss of or damage to AT I cells and hyperplasia of AT II cells. Injury to the AECs and the denudement of the basement membrane initiates the attempt to restore the damaged alveolar epithelium by AV II cells, whose capacity to restore damaged AT I cells, however, is seriously compromised, leading to inadequate reepithelialization of the damaged alveolar epithelium and unregulated behaviors of fibroblasts/myofibroblasts. Earlier electron microscopic studies showed a loss of AECs and the presence of widely scattered loose aggregated interstitial fibroblasts [62, 63]. These so-called fibroblastic foci, which are characteristic features in the lung parenchyma of patients with IPF, comprise aggregates of mesenchymal cells that underlie sites of unresolved microscopic epithelial injury and which are associated with the progression of pulmonary fibrosis.

Several potential cellular sources of myofibroblasts in IPF have been suggested, including resident fibroblasts, circulating fibroblasts, and circulating progenitor cells. Recently, the possibility that AECs may transdifferentiate into fibroblasts/ myofibroblasts has gained attention [58, 64]. The epithelial mesenchymal transition, which occurs widely under both physiological and pathological conditions, is important for cellular differentiation by enabling the development of the mesoderm from the epithelium during development or a disease process. Willis and colleagues have shown that AECs may serve as a novel source of myofibroblasts in IPF through the epithelial mesenchymal transition under stimulation by TGF-β [65, 66] and this finding has also been shown by others [67, 68]. The epithelial mesenchymal transition and the interaction between AECs and mesenchymal cells may play an important role in the pathogenesis of fibrosis (Fig. **2**).

In summary, pulmonary fibrosis remains a devastating disease process whose mechanisms of pathogenesis remains to be elucidated. Existing knowledge suggests that there is interplay between multiple types of cells and diverse cellular factors. The AECs, which become activated by the disease process, may play dual roles in pulmonary fibrosis by participating in the repair process to restore the integrity of the damaged alveolar epithelium and by involving in the pathological process of fibrosis through the production of inflammatory cytokines and probably through transdifferentiation into myofibroblasts by the epithelial mesenchymal transition. Further defining the role of the AECs in pulmonary fibrosis is not only important for understanding the pathogenesis of pulmonary fibrosis but may also lead to the development of novel potential therapeutic targets.

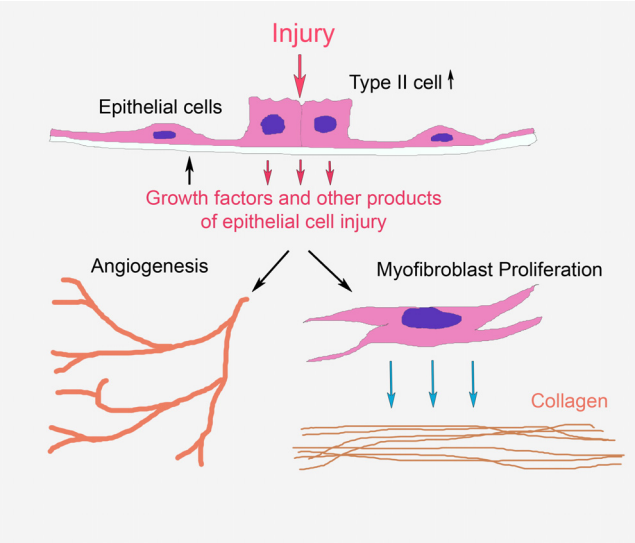

Fig. (2). Interaction between alveolar epithelial cells and mesenchymal cells.

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