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Alveolar epithelial disintegrity in pulmonary fibrosis

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Kulkarni T, de Andrade J, Zhou Y, Luckhardt T, Thannickal VJ. Alveolar epithelial disintegrity in pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 311: L185–L191, 2016. First published May 27, 2016; doi:10.1152/ajplung.00115.2016.—Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by progressive decline in lung function, resulting in significant morbidity and mortality. Current concepts of the pathogenesis of IPF primarily center on dysregulated epithelial cell repair and altered epithelial-mesenchymal communication and extracellular matrix deposition following chronic exposure to cigarette smoke or environmental toxins. In recent years, increasing attention has been directed toward the role of the intercellular junctional complex in determining the specific properties of epithelia in pulmonary diseases. Additionally, recent genomewide association studies suggest that specific genetic variants predictive of epithelial cell dysfunction may confer susceptibility to the development of sporadic idiopathic pulmonary fibrosis. A number of genetic disorders linked to pulmonary fibrosis and familial interstitial pneumonias are associated with loss of epithelial integrity. However, the potential links between extrapulmonary clinical syndromes associated with defects in epithelial cells and the development of pulmonary fibrosis are not well understood. Here, we report a case of hereditary mucoepithelial dysplasia that presented with pulmonary fibrosis and emphysema on high-resolution computed tomography. This case illustrates a more generalizable concept of epithelial disintegration in the development of fibrotic lung diseases, which is explored in greater detail in this review article.

pulmonary fibrosis; epithelial barrier dysfunction; cell-cell adhesion; epithelial junctional complex

INTERSTITIAL LUNG DISEASES represent a heterogeneous group of diffuse lung diseases characterized by chronic, progressive dyspnea that occurs primarily in older adults. Idiopathic pulmonary fibrosis (IPF) is the most common among the idiopathic interstitial pneumonias (IIPs) and is characterized by usual interstitial pneumonia on high-resolution computed tomography (HRCT) and lung biopsy (45). Two drugs, pirfenidone and nintedanib, were approved by the United States Food and Drug Administration for IPF based on reduction in the rate of lung function decline (24, 46); however, these drugs do not appear to arrest (or reverse) fibrosis. The mechanisms of their antifibrotic actions remain unclear, and treatment efficacy may be influenced by effects on the epithelium and/or mesenchymal cells.

The fibrosing varieties of IIPs are thought to be associated with alveolar epithelial cell (AEC) dysfunction, characterized by progressive loss of the normal alveolar architecture. The current paradigm is that recurrent injury to AECs followed by aberrant repair/regeneration of epithelial barrier, persistence of activated fibroblasts, and alterations in extracellular matrix (ECM) result in progressive pulmonary fibrosis (52, 71). In recent years, increasing attention has been directed toward the role of the intercellular junctional complex in determining the specific properties of epithelia in pulmonary diseases (19, 23, 26, 29, 50, 54). Identification of familial cases of interstitial pneumonias with gene mutations in surfactant protein C (40) or telomerase (3), and clinical syndromes such as Hermansky-Pudlak syndrome (11, 67), further support a critical role for AEC injury in disease pathogenesis. Additionally, recent population studies in patients with IIP demonstrate multiple susceptibility loci that indicate an increasingly important role of specific genetic variants associated with defects in host defense and cell-to-cell adhesion (8, 17, 41).

A broad spectrum of inherited and acquired conditions, including infections or autoimmune diseases, in which essential components of intercellular junctional complexes are missing or structurally altered, can lead to epithelial barrier disintegration. In this report, we describe a case characterized by epithelial cell defects: hereditary mucoepithelial dysplasia (HMD), presenting with features of pulmonary fibrosis. Translating this finding “bedside to bench,” we explore the role of dysfuncion of components of the intercellular junctional complex in the alveolar epithelium in the development of fibrotic lung diseases, in general. Furthermore, we discuss emerging concepts in the involvement of epithelial cell defects and polymorphisms of genes associated with lung epithelium in the pathogenesis of IPF.

Case Report

A 39-yr-old Caucasian male was evaluated for progressively worsening dyspnea on exertion and persistent nonproductive cough for over 5 years. He has had alopecia, skin scaling, and visual disturbances since 8 years of age. He denied any other rashes, joint pains, or mucosal lesions. He was a lifelong nonsmoker without identifiable environmental exposures. He has a family history of HMD confirmed by pathology reports in a sibling (sister) who died at age 12 of pulmonary complications. His father also died at an early age (24 yr) of an unknown lung disease. With this family history and characteristic clinical presentation during childhood, he was given the diagnosis of HMD prior to his presentation to our institution. Physical
exam at presentation to our clinic was remarkable for fine crackles and scant expiratory wheezes bilaterally, digital clubbing, and skin scaling of the arms. Pulmonary function tests (PFTs) revealed severe obstruction [forced expiratory volume in 1 s (FEV1) = 1.57 liters (37%); forced vital capacity (FVC) = 3.36 liters (59%); FEV1/FVC ratio = 47%], air trapping [total lung capacity (TLC) = 6.45 liters (81%); residual volume (RV) = 4.70 liters (61%)]; and moderately reduced diffusion [carbon monoxide diffusion capacity (DLCO) = 13.45 (43%)]. During the 6-min walk test, he was able to walk 351 m without oxygen desaturation. HRCT scans showed predominantly reticulations with honeycombing and traction bronchiectasis in a peripheral and basilar distribution with coexistent paraseptal emphysema (Fig. 1). Complete blood count and routine chemistry panels were normal. Tests for specific antibodies including antinuclear factor (ANA), anti-SS-A and SS-B antibodies, antineutrophil cytoplasmic antibodies (ANCA), anti-DNA antibodies, anti-Jo-1, anti-Scl-70, and anticyclic citrullinated peptide ( CCP) antibodies were all negative. Over the course of 4 years of follow-up in our clinic, his PFTs showed a decline in FVC to 2.40 liters (43%), TLC 4.82 liters (61%), and DLCO 9.50 liters (35%). He has been treated with inhaled corticosteroids and bronchodilators.

Pathogenesis of Hereditary Mucoepithelial Dysplasia

HMD is a dyshesive, dyskeratotic epithelial syndrome caused by an abnormality in desmosomes and gap junctions (GJs) with autosomal dominant inheritance (64). It can present with phenotypic variants involving mucosae, skin, hair, lungs, and eyes. Patients have severe airflow obstruction with combined interstitial fibrosis and emphysema; lung involvement commonly presents with spontaneous pneumothorax from rupture of giant bullae (4, 32, 63, 64). There are fewer reports of an association with pulmonary fibrosis, perhaps related to availability of HRCT until more recently. A 1979 report indicated the presence of fibrosis with thickened septa and small cysts throughout the lung parenchyma on postmortem examination of lung tissue section in a patient with HMD (63); in this report, histopathology of oral and vaginal mucosa demonstrated dyshesive epithelium with lack of maturation and atrophy, dyskeratosis, and unusual cytoplasmic inclusions. Ultrastructural studies showed a paucity of desmosomes and the presence of perinuclear filamentous inclusions resembling internalized GJ and desmosome material in dyskeratotic cells (63). A more recent report of mucosal biopsies of eight patients with HMD indicated the presence of numerous cytoplasmic vacuoles and the filament bundles interspersed between these vacuoles expressing keratin; however, the expression of junctional and cytoskeletal proteins was normal (4). These findings suggest that this disease likely represents a defect in the development of cytoskeletal components and/or assembly of intercellular GJs. The desmosomal cadherin gene cluster in chromosome 18q12.1 including desmoglein and desmocollin was excluded as possible candidate genes in the haplotype analysis of one family (4). However, the specific genetic mutation(s) involved in HMD is yet to be identified.

Epithelial Barrier Composition and Dynamics in Pulmonary Fibrosis

Persistent exposure to cigarette smoke or environmental toxins results in AEC injury with associated basement membrane damage. This may lead to dysregulated repair/regeneration and altered epithelial-mesenchymal communication with subsequent progressive fibrosis (13, 25). Cell-cell adhesion is critical for maintaining the integrity of alveolar epithelium, thus maintaining its protective barrier function against toxic agents or pathogens and allowing interepithelial transfer of molecules and signals. Cell adhesion molecules (CAMs), a group of specialized proteins, are found on epithelial cell surfaces and mediate the adhesive cell-cell interactions between adjacent epithelial cells. The intercellular junction complexes at cell-cell contact sites of epithelial cells are comprised of the tight junction (TJ), adherens junction (AJ), GJ, and desmosomes (Fig. 2).

Epithelial barrier dysfunction due to repetitive tissue injury leads to host responses involving a myriad of interactions among various cells and soluble factors that are orchestrated to restore normal lung structure and function. Few studies have examined the potential role of intercellular junctional complex proteins in maintenance of the epithelial barrier integrity and development of pulmonary fibrosis following loss of this barrier function. In this section, we will briefly review the role of each component of the alveolar epithelial intercellular junctional complex.

Tight junctions. TJ form the apical component of the junction complex and are essential for innate immunity, as well as cellular differentiation and proliferation. They comprise the membrane proteins (claudins and occludins) in addition to scaffolding proteins known as zona occludens (ZO-1, ZO-2, and ZO-3) (48). Claudins, particularly claudin 18, are the major proteins contributing to the epithelial barrier function of TJs in the lungs and maintain alveolar fluid homeostasis (33, 47).
Disruption of TJJs can result in increased paracellular permeability, thus permitting entry of antigens, toxins, and protein-rich fluid into alveolar spaces. Reduced expression of Claudins, particularly Claudin-18, along with lower levels of mRNA encoding TJ proteins was reported in an experimental bleomycin-induced lung injury model (42). Differential Claudin and cadherin expression in hyperplastic AECs compared with normal AECs during an aberrant repair process suggests focal changes in permeability of this barrier (19, 29). Although the lower expression of Claudins could simply be due to epithelial cell death from bleomycin exposure, the structural disruption of TJJs in fibrotic lesions suggests that bleomycin injury causes alveolar barrier dysfunction by other possible mechanisms. Transforming growth factor-β1 (TGF-β1), a well-established profibrotic cytokine, has been shown to cause disruption of TJJs in human alveolar epithelial cells and induce epithelial-to-mesenchymal transition (EMT) (42). Additionally, TGF-β1-induced TJ disruption was augmented in a bleomycin injury model of phosphatase and tensin homolog (pten)-null mice (38). In this study, pten-null mice demonstrated disassembly of TJJs of AECs and exacerbated lung fibrosis following injury. Furthermore, this study also demonstrated decreased PTEN expression in AECs of human IPF lungs. Taken together, these studies suggest that structural disruption of TJJs with subsequent loss of alveolar epithelial integrity play an important role in the development of pulmonary fibrosis.

In addition to preservation of barrier function, adequate expression of Claudins may be essential to restore alveolar epithelial barrier during normal injury-repair responses. Increased expression of both TJ and AJ proteins was demonstrated in regenerative alveolar epithelium (29). However, expression of Claudin-1, Claudin-3, and Claudin-4 in fibrotic lung was shown to be similar to or even lower than that measured in the healthy controls (29). Interestingly, Claudin knockout mice demonstrated impaired alveologenesis and alveolar barrier dysfunction (28). It is possible that the diminished capacity of epithelial cells to produce Claudin could lead to incomplete repair and differentiation of epithelial cells, thus resulting in hyperplastic type II AECs seen in pulmonary fibrosis.

Adherens junctions. Located more basal to TJJs, AJs consist of cadherin and the nectin family CAMs; they are linked to the actin cytoskeleton through the binding proteins catenins and afadin, respectively. AJs function to stabilize cell-cell adhesion and regulate actin cytoskeletal organization, intracellular signaling, and gene transcription. Epithelial cadherin (E-cadherin) is a calcium-dependent CAM with pivotal roles in epithelial cell behavior (20), and it is often used as a marker of epithelial cell expression. Expression of E-cadherin is reduced in lung sections of patients with IPF; cytoplasmic localization of this protein during EMT is associated with disruption of epithelial barrier function and increased cell migration (20, 61). Cigarette smoke impairs the proteins, ZO-1, ZO-2, and E-cadherin in human bronchial epithelial cells, thus resulting in altered permeability of the epithelial barrier and, potentially, mesenchymal differentiation of epithelial cells (49, 69). Thoracic radiation and bleomycin-induced lung injury can also decrease E-cadherin and aquaporin-5 expression in AECs, increasing plasma/water permeability into alveolar spaces (2, 9). Immunohistochemistry of lung tissue in aquaporin-5 knockout mice demonstrated fibrosis with increased deposition of type I collagen in alveolar walls (9). Although these changes in permeability and increased alveolar fluid accumulation are typically associated with acute lung injury, persistent injury to the epithelial barrier may lead to a cascade of reactions with release of soluble factors that promote myofibroblast differentiation and ECM deposition.

α3β1 Integrin is a laminin receptor that promotes cell-cell communications through its interactions with the E-cadherin/β-catenin complex (34, 59). It was recently reported that α3β1 integrin interacts with E-cadherin and the TGF-β receptor to form a trimolecular complex that triggers phosphorylation of β-catenin at Y654 in AECs (23). Phosphorylated β-catenin subsequently interacts with phosphorylated Smad2, a TGF-β receptor-regulated effector protein, to induce fibrogenic gene expression. This study suggests a role for integrations between α3β1, E-cadherin, and β-catenin signaling in the development of lung fibrosis. Additionally, increased expression of cadherin-11 (CDH11) in IPF patients and animal models of lung fibrosis have been reported (50); treatment with CDH11-blocking antibody or genetic deletion of CDH11 protects mice against bleomycin injury-induced lung fibrosis. These data support a pivotal role of CDH11 in pulmonary fibrosis. Given the fact that multiple cell populations including AECs, fibroblasts, and macrophages express CDH11 in pulmonary fibrosis, it is likely that CDH11 regulates multiple steps in the fibrotic process.
**Gap junctions.** GJs are essential for intercellular communication and secretion of surfactant necessary for barrier function. They consist of an array of transmembrane channels composed of connexins (Cx) that connect to similar structures in the adjacent cells. Differential expression of various connexins in the lung, especially Cx43 and Cx46, has an important role in the regulation of normal lung homeostasis and remodeling in response to epithelial cell injury (1, 31). Fibroblasts from patients with IPF demonstrate significant reduction in Cx43 mRNA with alteration to GJ intercellular communication compared with fibroblasts from normal subjects (53). Additionally, mice deficient in vascular endothelial cell-specific Cx43 and Cx40 develop spontaneous fibrosis with fibroblast accumulation and aberrant alveolar remodeling (26). Further studies are required to determine whether abnormalities in alveolar epithelial cell-specific connexins and epithelial cell GJs predispose to lung fibrosis.

**Hemidesmosomes.** Hemidesmosomes are specialized multi-protein transmembrane complexes that facilitate the binding of keratin intermediate filament (IF) in epithelial cells to the underlying basement membrane and ECM. This binding is essential in maintenance of integrity and mechanical stability of the lung. The hemidesmosomes are formed by integrin α6β4, laminin 5, and tetraspanin CD151 (37, 56). Tetraspanins belong to a family of proteins that form multimolecular complexes with a variety of other proteins including integrins.

Tetraspanin CD151 is predominantly expressed in the basolateral surface of epithelial cells and is crucial for the maintenance of epithelial integrity via adhesion of the basal surface of AECs to basement membrane. Deletion of CD151 in AECs results in alterations to the cell structure and degradation of the epithelial integrity due to impaired adhesion to basement membrane (54). In this study, CD151 knockout mice were shown to spontaneously develop age-related pulmonary fibrosis; AECs from these mice exhibit fibroblast-like changes through up-regulation of TGF-β1 signaling and augmented phosphorylated Smad2. Furthermore, decreased CD151 expression was observed in AECs from patients with IPF (54), supporting a role for loss of CD151 and epithelial integrity in at least a subset of IPF patients. Interestingly, tetraspanin CD151-integrin α3β1 association has been shown to be functionally important for α3β1-integrin-mediated cell migration and matrix remodeling (21). Further studies to understand the role of tetraspanin CD151-integrin interactions and their effects on TGF-β1 in the development of pulmonary fibrosis are warranted.

**Genetic Variants Affecting Epithelial Cell Integrity**

Over the past decade, there has been remarkable progress in the understanding of IPF pathogenesis. In addition to acquired defects in epithelial barrier integrity, there have been recent advances in identifying polymorphisms of genes associated with lung epithelium and their association with higher risk of IPF. Population studies initially implicated the role of specific genetic variants including MUC5B, TERT, TERC, SFTPC, and SFTPA2 in the development of IPF and other fibrosing IIPs (8, 17, 41). Additional genetic variants including desmoplakin (DSP) and dipeptidyl peptidase 9 (DPP9) genes associated with cell-to-cell adhesion were recently identified in patients with fibrotic IIP (8).

Desmosomes are intercellular junctions located in the basolateral membranes of epithelial cells. There is calcium-dependent transmembrane interaction between the extracellular domains of the desmosomal cadherins between adjacent cells. The cadherin cytoplasmic tails then associate with the linker proteins plakoglobin and plakophilins. Linkage of this desmosomal assembly to the cytoskeleton is mediated through a series of interactions between binding proteins, desmoplakin, and linker proteins (7). Thus desmosomes primarily provide mechanical support for maintenance of tissue architecture by tethering the keratin IF network to the plasma membrane (10). This is particularly important in maintaining the integrity of tissues that experience mechanical stress such as peripheral portions of the lungs, myocardium, skin, bladder, and gastrointestinal mucosa.

Mutations in expression of DSP gene that encodes for desmoplakin have been associated with several skin diseases including keratoderma, alopecia, and severe acantholytic epidermolysis bullosa (18, 60). More importantly, DSP mutations that affect exon 24 encoding the COOH-terminal domain have been associated with cardiac interstitial fibrosis and arrhythmogenic right ventricular dysplasia cardiomyopathy (35, 66). This was shown to be due to disruption of mechanical linkage between cells and modifications to cell-cell adhesion proteins. The minor allele of variant rs2076295 in intron 5 has been associated with decreased whole lung DSP expression and higher risk of IPF (8, 36). The differential expression of DSP in association with this variant suggests that disruption of desmosomal integrity with resultant impairment of cell-cell adhesion and aberrant epithelial barrier injury-repair response may participate in the development of IPF.

DSP has been shown to inhibit Wnt/β-catenin signaling pathway through regulation of plakoglobin, β-catenin, and matrix metalloproteinase 14 in a lung cancer model (65).
Aberrant activation of Wnt/β-catenin signaling pathway has been implicated in the development of pulmonary fibrosis (6) and is, thus, one potential mechanism for a profibrotic role of DSP in IPF. Interestingly, increased DSP gene expression was demonstrated in lung tissue of IPF patients without the DSP gene variant rs2076295 (36). This increase could be the consequence of epithelial cell injury-repair response to maintain epithelial barrier integrity in response to persistent epithelial injury. Desmosomes have intra- and extracellular components, and changes to any part of this structure could lead to alterations in yet-undetermined cell signaling pathways.

DPP9 is an enzyme ubiquitously expressed by epithelial cells and fibroblasts and is necessary for intracellular signaling, cell adhesion, and migration (68). DPP9 gene silencing or enzyme inhibition has been shown to suppress the adhesion-signaling pathway through decreased phosphorylation of focal adhesion kinase and paxillin (68). Thus alterations in this gene may result in impaired cell movement during repair and lead to aberrant healing. Taken together, these findings suggest that genetic variations in the expression of DSP and DPP9 contribute to biochemical and biomechanical modifications that alter cell-cell adhesion in the lung. The presence of these genetic variations may increase epithelial cell susceptibility to barrier disintegrity in response to persistent injury, thus resulting in pulmonary fibrosis.

Epithelial-Mesenchymal Cross Talk

Epithelial and mesenchymal cell interactions are critical in the process of lung development, homeostasis during adulthood, and injury-repair responses (5). Loss of epithelial barrier integrity and the altered alveolar microenvironment disrupts the tightly orchestrated temporal and spatial regulation of epithelial-mesenchymal cross talk (52). Injured alveolar epithelial cells and macrophages release/activate profibrotic mediators, in particular TGF-β1 (22, 30). TGF-β1, activated by AEC-integrin-mediated process (39) or by biomechanical signals (62), induces myofibroblast differentiation and activation. Recent studies indicate that myofibroblasts in IPF acquire a senescent and apoptosis-resistant phenotype (15). These mesenchymal cells secrete additional paracrine factors, such as hydrogen peroxide (16, 55), angiotensin-II (43, 57), Fas-ligand (12, 58), and TGF-β1 (14, 51), that may potentiate or perpetuate injury-repair responses of AECs. In turn, the accumulation of a highly cross-linked and stiff matrix may perpetuate mesenchymal activation and progressive fibrosis (27, 70) (Fig. 3).

Future Directions

In summary, there is increasing evidence indicating a role for developmental and acquired defects in epithelial cell-cell adhesion in the pathogenesis of fibrotic lung diseases. In this review, we highlight a unique case of a young man with a known genetic defect that results in loss of cell-cell adhesion that resulted in early and severe fibrosis of the lungs. We posit that developmental or acquired dysfunction of epithelial intercellular junctional complexes may have pivotal role in the pathogenesis of pulmonary fibrosis. Whether changes in the expression of specific intercellular junctional proteins are causally involved in the development of pulmonary fibrosis is yet to be elucidated. Persistent alveolar epithelial cell injury from chronic exposure to cigarette smoke or environmental toxins could alter the expression of proteins critical in maintenance of cell-cell adhesion and alveolar epithelial integrity. Although data exploring the molecular mechanisms associated with these alterations and development of IPF are limited, emerging evidence suggests that failure of normal alveolar injury-repair responses culminate in perpetuating cycles of myofibroblast activation and ECM deposition. Recent progress in identifying genetic variants, specifically the genes associated with alterations in cell-cell adhesion, could transform our current understanding of the pathogenesis of IPF.

An integrated approach including genotyping, environmental risk factor assessment, and proteomic analyses of epithelial cell junctional proteins is needed to develop personalized approaches to diagnosis and treatment of patients with IPF. It is not known whether defects in cell-cell adhesion in distal airway/bronchiolar epithelia contribute to disease pathogenesis; for example, the previously reported association between a MUC5B promoter polymorphism and development of IPF with improved survival (44) suggests loss of epithelial homeostasis in the distal airway. Further investigation of specific mechanisms involved in epithelial disintegrity would be invaluable in providing genetic and molecular targets for the development of interventions that prevent/ameliorate disease progression in IPF.

REFERENCES

Perspectives

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CELL-CELL ADHESION DEFECTS IN PULMONARY FIBROSIS


