



NIH Public Access

Author Manuscript

Annu Rev Med. Author manuscript; available in PMC 2014 August 11.

Published in final edited form as:
Annu Rev Med. 2010 ; 61: 105–119. doi:10.1146/annurev.med.60.041807.123500.

Alveolar Surfactant Homeostasis and the Pathogenesis of Pulmonary Disease

Jeffrey A. Whitsett, Susan E. Wert, and Timothy E. Weaver

Perinatal Institute, Section of Neonatology, Perinatal and Pulmonary Biology, Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Cincinnati, Ohio 45229

Jeffrey A. Whitsett: jeff.whitsett@cchmc.org; Susan E. Wert: susan.wert@cchmc.org; Timothy E. Weaver: tim.weaver@cchmc.org

Abstract

The alveolar region of the lung creates an extensive epithelial surface that mediates the transfer of oxygen and carbon dioxide required for respiration after birth. Maintenance of pulmonary function depends on the function of type II epithelial cells that synthesize and secrete pulmonary surfactant lipids and proteins, reducing the collapsing forces created at the air-liquid interface in the alveoli. Genetic and acquired disorders associated with the surfactant system cause both acute and chronic lung disease. Mutations in the *ABCA3*, *SFTPA*, *SFTPB*, *SFTPC*, *SCL34A2*, and *TERT* genes disrupt type II cell function and/or surfactant homeostasis, causing neonatal respiratory failure and chronic interstitial lung disease. Defects in GM-CSF receptor function disrupt surfactant clearance, causing pulmonary alveolar proteinosis. Abnormalities in the surfactant system and disruption of type II cell homeostasis underlie the pathogenesis of pulmonary disorders previously considered idiopathic, providing the basis for improved diagnosis and therapies of these rare lung diseases.

Keywords

alveolar proteinosis; interstitial lung disease; respiratory distress syndrome; pulmonary fibrosis; pulmonary alveolar microlithiasis

INTRODUCTION

The respiratory tract consists of a remarkable, highly branched, tubular structure that leads to ~300 million alveolar sacs, creating an extensive surface area through which oxygen and carbon dioxide are exchanged in blood within alveolar capillaries. The alveolar surface is lined by type II and type I alveolar epithelial cells that are in direct contact with respiratory gases, creating collapsing forces at the air-liquid interface. To maintain inflation, these surface forces are mitigated by the presence of pulmonary surfactant (a mixture of proteins

Copyright © 2010 by Annual Reviews. All rights reserved

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

and lipids) that is synthesized and secreted onto the alveolar surface by type II epithelial cells. Because pulmonary surfactant reduces surface tension, it is critical for the maintenance of lung volumes during the respiratory cycle. Lack of pulmonary surfactant in preterm infants with respiratory distress syndrome or adults with acute respiratory distress syndrome causes atelectasis leading to respiratory failure.

The type II cell plays a critical role in surfactant production and in repair of the lung following injury, and it is the progenitor cell for type I epithelial cells, which comprise the majority of the gas-exchange region of the alveolus. It is increasingly clear that alterations in genes and processes affecting both type II cell homeostasis and surfactant function underlie the pathogenesis of a number of severe pulmonary diseases affecting infants, children, and adults, which, until very recently, were considered idiopathic.

PULMONARY SURFACTANT

Pulmonary surfactant is a complex mixture of lipids, mostly phosphatidylcholine (PC), and associated proteins. Four of these surfactant proteins, designated SP-A, SP-B, SP-C, and SP-D, play critical roles in various aspects of surfactant structure, function, and metabolism (1). All four are expressed at relatively high levels in type II cells and have distinct structures and functions. Alveolar surfactant takes on discrete physical forms, including the abundant, highly ordered material called tubular myelin as well as multilamellated and smaller, vesicular, protein-lipid structures that can be isolated from the lung. Surfactant forms are determined by the presence or absence of surfactant proteins and lipids, by the physical forces generated during the respiratory cycle, and by selective processes mediating uptake or degradation (Figure 1). Surfactant multilayers, derived from highly lamellated and tubular myelin forms, spread over the surface of the alveolus and reduce surface tension. Surfactant components are recycled by type II cells or catabolized by alveolar macrophages in a highly regulated system that maintains precise levels of pulmonary surfactant throughout life. Both surfactant lipids and proteins are synthesized primarily by type II cells. Surfactant lipids are stored in large, lipid-rich, intracellular organelles, termed lamellar bodies. Lamellar bodies are rich in the surfactant-associated phospholipids, PC and phosphatidylglycerol (PG), and in two low-molecular-weight, hydrophobic surfactant proteins, SP-B and SP-C, that are cosecreted with the surfactant lipids and interact closely with them (2).

SP-B and SP-C alter lipid packing and spreading and enhance the surface tension–lowering activity of the lipids, as well as stabilizing the lipid layers during the respiratory cycle. The surfactant proteins SP-A and SP-D are larger, relatively abundant, oligomeric proteins that are also synthesized and secreted by type II cells. SP-D and SP-A are structurally related members of the collectin family of C-type mammalian lectins that share distinct collagen-like and globular, carbohydrate-binding domains. SP-A is required for the formation of tubular myelin and plays diverse roles in host-defense functions of the lung (3–5). SP-A binds lipopolysaccharides and various microbial pathogens, enhancing their clearance from the lung. Unlike SP-B and SP-C, SP-A does not play a critical role in surface functions, metabolism, or pulmonary surfactant under normal conditions. SP-D, however, influences the structural forms of pulmonary surfactant and is important in the regulation of alveolar surfactant pool sizes and reuptake (6–8). SP-D is also necessary in the suppression of

pulmonary inflammation and in host defense against viral, fungal, and bacterial pathogens (see References 3, 5 for review). Taken together, surfactant lipids and proteins play critical roles in (a) reducing surface tension in the alveolus, as required for ventilation, and (b) modulating various aspects of innate host defense of the lung against diverse pulmonary pathogens.

GENETIC BASIS OF DISORDERS OF THE SURFACTANT SYSTEM

Genetic causes of pulmonary disease related to the pulmonary surfactant system were first recognized in full-term infants who presented with severe respiratory failure and failed medical management, including surfactant replacement (see Reference 9 for review). Two major classes of surfactant system-related disorders have been recognized, those that cause disease by disrupting the functions of proteins critical for surfactant homeostasis and those that cause alveolar cell injury mediated by protein misfolding or toxic gain of function. Genetic disorders of surfactant homeostasis cause severe respiratory failure in the newborn period and interstitial lung disease (ILD) in older infants, children, and adults (Table 1). Histological diagnoses associated with these disorders include congenital alveolar proteinosis, desquamative interstitial pneumonitis, chronic pneumonitis of infancy, nonspecific interstitial pneumonitis, and usual interstitial pneumonitis (9). Diffuse alveolar diseases, including idiopathic pulmonary fibrosis (IPF), pulmonary alveolar proteinosis (PAP) (10), and chronic pulmonary microlithiasis, have been associated, respectively, with mutations in SP-A (11) and the telomerase reverse transcriptase gene (*TERT*) (12, 13), in GM-CSF receptors (14, 15), and in the sodium/phosphate transport protein, SCL34A2 (16–18). Genetic causes, presentations, and courses of these disorders are relatively distinct.

Mutations in genes encoding SP-C (*SFTPC*), telomerase (*TERT*), and, most recently, SP-A (*SFTPA*) have been linked to familial interstitial pneumonia. These loci do not account for all familial forms of the disease; ILD is a significant component of other inherited disorders, such as Hermansky-Pudlak syndrome. Familial interstitial pneumonia accounts for ~2% of all interstitial pneumonias and is characterized by autosomal dominant inheritance with incomplete penetrance and early onset of disease relative to idiopathic forms (19).

HEREDITARY SURFACTANT PROTEIN B (SP-B) DISORDERS

Hereditary SP-B deficiency (OMIM #265120) is a relatively rare autosomal recessive disorder with a carrier rate estimated to be less than 1:2000 (20).

Clinical Presentation

Hereditary SP-B deficiency usually presents in full-term infants with unexplained respiratory distress and findings typical of respiratory distress syndrome in preterm infants, the latter lacking surfactant on a maturational basis (21). Infants may have a family history of unexplained neonatal death or consanguinity. Cyanosis and respiratory distress are usually noted in the first hours after birth, followed by respiratory failure that is refractory to surfactant replacement, ventilation, and other supportive care. Initial chest X-rays show diffuse opacification consistent with atelectasis. Respiratory failure is progressive, generally

leading to death within weeks to months of age despite assisted ventilation, oxygen, or extracorporeal membrane oxygenation.

Pathogenesis and Diagnosis

The *SFTPB* gene is located on chromosome 2 (OMIM #178640). Hereditary SP-B deficiency has been linked to a number of distinct mutations in the *SFTPB* gene. These include deletion, termination, missense, and substitution mutations that interfere with the synthesis of proSP-B or produce an aberrant proSP-B protein that is not fully processed, leading to a loss of the active, mature SP-B peptide. Mature SP-B is a 79-amino-acid, amphipathic peptide that interacts closely with the surface of phosphatidylcholine-rich membranes in surfactant. The active peptide is produced by proteolytic processing of a 381-amino-acid proprotein during trafficking from the endoplasmic reticulum (ER) to multivesicular and lamellar bodies (2). A relatively common insertional mutation, 121ins2, causes the synthesis of an unstable mRNA with loss of synthesis of both proSP-B and SP-B peptides (9, 21). Neither proSP-B nor SP-B is detected in the lungs of patients bearing the 121ins2 mutation. In contrast, other *SFTPB* mutations result in the production of an incompletely processed proSP-B protein that is detected within the type II cells.

In most forms of hereditary SP-B deficiency, the active SP-B peptide is absent in the surfactant isolated from alveolar wash or in the alveoli as assessed by immunohistochemistry (22). Consistent with the importance of SP-B in surfactant function, surfactant activity is deficient in material isolated from patients with this disorder. Histological findings in hereditary SP-B deficiency include (a) the accumulation of eosinophilic or periodic acid-Schiff (PAS)-positive, lipoproteinaceous material in the alveolar spaces, which often contain foamy alveolar macrophages; (b) hyperplastic alveolar epithelia with prominent type II cells; and (c) thickening of the alveolar septa, which is caused by proliferation of interstitial fibroblasts (Figure 2). At the ultrastructural level, type II cells lack lamellar bodies and contain large, atypical, multivesicular bodies, likely representing the failure of phospholipid membrane fusion during the packaging of surfactant lipids (Figure 2). Tubular myelin is absent, and abnormally processed proSP-C and SP-A accumulate with lipids in the alveolar spaces, providing a basis for the pathological diagnosis of congenital alveolar proteinosis. The alveoli are generally remodeled and lined by cuboidal type II cells, although this varies depending on the age of the infant and supportive care. Inflammation is also variable, generally consisting of foamy alveolar macrophages. Physiological, biochemical, and histological findings in lungs of patients with hereditary SP-B deficiency are consistent with those seen in *Sftpb*^{-/-} gene targeted mice, supporting the concept that the pathogenesis of lung dysfunction in this disorder is related primarily to the lack of SP-B in type II cells and in the alveolus (23).

The diagnosis of hereditary SP-B deficiency, when suspected clinically and pathologically, is confirmed by the identification of mutations in the *SFTPB* gene. Because SP-B is required for both intracellular and extracellular aspects of surfactant homeostasis, the disease has not been treated successfully with surfactant replacement and supportive care. Survival has been extended by lung transplantation in a small number of infants with hereditary SP-B deficiency (24).

HEREDITARY ATP-BINDING CASSETTE A3 (ABCA3) DISORDERS

Mutations in the ABCA3 lipid transport protein cause a relatively rare autosomal recessive disorder that is presently the most common genetic cause of respiratory failure in full-term infants.

Clinical Presentation

Most infants with mutations in the ATP-binding cassette, subfamily A, member 3 (or *ABCA3*) gene (OMIM #610921) present with severe respiratory distress and cyanosis requiring ventilatory support in the immediate newborn period. The clinical findings are consistent with surfactant deficiency (25). Radiographic findings demonstrate diffuse alveolar disease and atelectasis. Respiratory failure occurs despite surfactant replacement and ventilatory support, and most infants die in the first months of life.

Pathogenesis and Diagnosis

The *ABCA3* gene is located on chromosome 16 (OMIM #601615). *ABCA3* deficiency is inherited as an autosomal recessive mutation, and >150 distinct mutations have been identified in association with severe neonatal lung disease. The vast majority of *ABCA3* mutations cause neonatal lung disease and death within the first months of life. Less frequently, milder *ABCA3* mutations (for example, E292V) are associated with chronic lung disease with features of ILD, desquamative interstitial pneumonitis, and nonspecific interstitial pneumonitis presenting in childhood (26, 27). The *ABCA3* gene encodes a large, multiple-membrane-spanning polypeptide that is a member of the family of ABC transporters, which includes the cystic fibrosis transmembrane conductance regulator (CFTR) and the multiple drug resistant transporter (MDR). Although the *ABCA3* protein is expressed in many tissues, it is expressed highly in type II cells, where it is located at the limiting membranes of lamellar bodies (28, 29).

ABCA3 mutations are associated with histological features of congenital alveolar proteinosis (25, 27). The respiratory epithelium undergoes cuboidal hyperplasia with remodeling and loss of alveoli, as well as accumulation of lipid-rich macrophages in the airspaces (Figure 2). Surfactant from patients with *ABCA3*-related lung disease is deficient in both PG and PC and has little surface activity (30). *ABCA3* is located at the limiting membrane of lamellar bodies in alveolar type II cells, where it plays an important role in lipid transport, including both cholesterol and PC (29, 31). At the ultrastructural level, normal lamellar bodies are lacking in lungs of patients with *ABCA3*-related disease (25, 27). The presence of small intracellular organelles containing electron-dense material, likely representing lamellar bodies that lack appropriate lipid constituents, is useful in the tentative diagnosis of *ABCA3*-related disease (Figure 2). In general, the surfactant proteins A, B, C, and D are detected by immunohistochemistry in lung tissue from patients with lung disease caused by mutations in *ABCA3*. As in hereditary SP-B deficiency, the diagnosis of *ABCA3*-related disease is made by clinical and pathological findings supported by histological and ultrastructural studies. Definitive diagnosis is made by the identification of mutations in the *ABCA3* gene locus. *ABCA3*-related lung disease is severe and usually results in death in the

first months of life. Life has been extended by lung transplantation in a number of patients with *ABCA3* mutations (27, 30).

HEREDITARY SP-C DISORDERS

SP-C is a small, extremely hydrophobic peptide synthesized and secreted with surfactant lipid into the alveoli by type II alveolar cells. SP-C is produced from a precursor (proSP-C) encoded by the *SFTPC* gene. Mutations in *SFTPC* have been recently linked to familial and sporadic ILD (OMIM #610913).

Clinical Presentation

The index case was an infant who developed respiratory symptoms at six weeks of age and was diagnosed subsequently with interstitial pneumonitis (32). DNA sequence analysis of the *SFTPC* gene identified a mutation in a donor splice site (c.460 + 1G → A) of one allele that resulted in deletion of exon 4 and consequent loss of 37 amino acids in the C-terminal peptide of the proprotein (referred to as SP-C ^{exon4}). A separate *SFTPC* mutation (exon 5+128T→A) that results in substitution of glutamine for leucine (L188Q) was recently identified in two independent kindreds (33, 34). Family members exhibited variable onset of disease, from infancy to adulthood, with autosomal dominant inheritance and incomplete penetrance.

Pathogenesis and Diagnosis

The *SFTPC* gene is located in chromosome 8 (OMIM #178620). To date, approximately 40 missense, splice, or frameshift *SFTPC* mutations have been associated with familial or sporadic ILD. In general, *SFTPC* mutations are located in the brichos domain of proSPC, causing misfolding or aberrant processing of the precursor. Histological features of lung disease caused by mutations in the *SFTPC* gene include (a) diffuse alveolar damage of varying severity, (b) interstitial thickening with mild lymphocytic inflammation, (c) thickening of the alveolar septa, (d) accumulation of foamy alveolar macrophages and variable amounts of eosinophilic alveolar proteinosis material, and (e) regenerating alveolar epithelium lined by hyperplastic type II cells (Figure 2).

Mutations that alter the ability of a protein to fold correctly result in retention of the unfolded/ misfolded protein in the ER(35, 36). Accumulation of unfolded or misfolded protein in the ER, a condition known as ER stress, induces an ER-to-nucleus signal pathway called the unfolded protein response, which increases transcription of chaperones that promote protein folding. In addition, translation of mRNA is attenuated, effectively slowing entry of newly synthesized protein into the ER and increasing the ratio of chaperone to unfolded protein. Proteins that fail to fold under these conditions are diverted to the ER-associated degradation (ERAD) pathway, in which misfolded protein is translocated to the cytosol for degradation by 26S proteasome. Failure to eliminate rapidly the terminally misfolded proteins can lead to formation of cytotoxic aggregates and/or trigger cell death pathways. Both SP-C ^{exon4} and SP-CL188Q proproteins cause ER stress and readily aggregate when the ERAD pathway is compromised (37, 38).

Targeted expression of SP-C exon⁴ in type II cells of transgenic mice resulted in dose-dependent cytotoxicity leading to disruption of lung morphogenesis and respiratory failure at birth (39). Importantly, lung dysmorphogenesis occurred in the presence of two wild-type *Sftpc* alleles, consistent with a toxic gain-of-function effect. This phenotype was not due to degradation and loss of mature SP-C peptide in the airspaces, since perinatal lung development and function were normal in *Sftpc*^{-/-} mice (40). Collectively, these results suggest that certain *SFTPC* mutations lead to irreversible misfolding of the SP-C proprotein and accumulation of misfolded protein that, in turn, leads to cell injury and death. This hypothesis is supported by the results of experiments confirming the cytotoxicity of mutant SP-C protein in cell culture (41, 42).

The cytotoxic effect of mutant SP-C fits well with the emerging concept that epithelial cell injury and aberrant re-epithelialization are critical steps in the pathogenesis of ILD (43). What remains unclear is why the onset and severity of disease are so variable within a kindred. For example, in the L188Q kindred, the age of onset ranged from 4 months to 57 years, with histopathological findings ranging from non-specific interstitial pneumonitis in infants to usual interstitial pneumonitis in older patients (33). Phenotypic variability may be due in part to the influence of other genes, which render some patients more susceptible or resistant to disease, and in part to environmental stresses that may trigger or exacerbate disease. Interestingly, cells that stably expressed SP-C exon⁴ in culture adapted to chronic ER stress imposed by the mutant protein and grew normally; however, adaptation was accompanied by greatly increased susceptibility to viral-induced cell death (42). These findings provide a potential explanation for delayed onset of disease but have yet to be translated to an animal model.

Two recent studies of IPF patients support a role for ER stress in pathogenesis. One study detected markers of ER stress and apoptosis in alveolar epithelial cells of IPF patients but not in those of patients with chronic obstructive pulmonary disease (44). The other study confirmed evidence of both ER stress and herpesvirus infection (45). Thus, ER stress may be a molecular pathway common to both familial and idiopathic forms of ILD. The precise roles of ER stress and environmental stress in the initiation and/or progression of ILD remain to be defined.

HEREDITARY SP-A DISORDERS

A recent study reported an association between two heterozygous mutations in the *SFTPA2* gene (OMIM #178642) and familial pulmonary fibrosis that segregated with lung cancer (11). The results of preliminary studies suggested that the mutations caused misfolding and trapping of SP-A in the ER that could lead to loss of protein in the airspaces. Since *Sftpa*^{-/-} mice have normal lung structure and function and do not develop ILD (46), it is possible that *SFTPA2* mutations exert a toxic gain-of-function effect similar to *SFTPC* mutations. It remains unclear, however, if mutant SP-A actually induces ER stress, is degraded by ERAD, or forms cytotoxic aggregates.

PULMONARY ALVEOLAR PROTEINOSIS

Pulmonary alveolar proteinosis (PAP) (OMIM #610910) is a rare disorder in which the alveoli become filled with surfactant lipids and proteins leading to progressive respiratory insufficiency (10, 47, 48).

Clinical Presentation

PAP is rarely diagnosed in childhood; it more commonly presents in adulthood in both acquired and secondary forms. Patients usually present with progressive dyspnea, exercise intolerance, and clubbing.

Radiologic studies demonstrate diffuse alveolar opacities, termed “crazy-paving,” but lack the fibrosis or remodeling associated with ILD (49). Alveolar wash or histologic assessment of lung biopsy demonstrates the accumulation of surfactant lipids and proteins and foamy alveolar macrophages in the lung. Surfactant lipids and proteins, including SP-A, SP-B, SP-C, and SP-D, accumulate in large quantities, and the material isolated from PAP patients maintains its surface-active properties. Thus, lung function and structure are generally well preserved. The pathological findings in acquired PAP are distinct from those in genetic pulmonary disorders related to SP-B, SP-C, or ABCA3, wherein surfactant is dysfunctional with resultant respiratory failure, pulmonary fibrosis and remodeling.

Pathogenesis and Diagnosis: Role of GM-CSF in Acquired PAP

The critical role of GM-CSF signaling in the pathogenesis of PAP was first recognized from studies in transgenic mice lacking GM-CSF (50, 51) or the common β -chain of the GM-CSF receptor (52). Both of these mouse models developed severe PAP with features virtually identical to those in human patients with acquired PAP. The mouse models demonstrated that the disorder was related to the inability of alveolar macrophages to clear or metabolize surfactant lipids and proteins. Replacement of GM-CSF in the lung rapidly restored alveolar macrophage differentiation and surfactant clearance, correcting the PAP (53, 54). The recognition that most adult patients with acquired PAP have high circulating levels of anti-GM-CSF autoantibodies provided a critical link between the mouse studies and the clinical disorder (55). Thus, it has been established that GM-CSF plays a critical, nonredundant role in the regulation of differentiated functions of the alveolar macrophage in the lung. Its absence results in failure to degrade surfactant components, leading to the progressive accumulation of normal lipids and proteins in the lung (56, 57). Because alveoli are well preserved in PAP, lung function can be restored by physical removal of the excess surfactant using alveolar wash or by treatment with GM-CSF using lung aerosol or systemic administration.

Role of GM-CSF Receptors in Hereditary PAP

GM-CSF activity requires signaling via the dimerization of its receptors, consisting of an α -chain and common β -chain that activate JAK/STAT5 signaling to enhance macrophage differentiation and function. Dysfunction of the common β -chain of the GM-CSF receptor (OMIM #300770) has been associated with PAP (58). Recently, mutations in the α -chain of the GM-CSF receptor (OMIM #306250) were implicated in the pathogenesis of PAP (14,

15). A number of patients with PAP related to mutations in the α -chain of the GM-CSF receptor have now been identified, usually presenting with signs and symptoms in childhood. Clinical and histopathological features are similar to those of acquired PAP, although these patients usually present at earlier ages (Figure 2).

The diagnosis of hereditary PAP is supported by clinical, pathological, and radiological findings. The definitive diagnosis can now be made by the identification of mutations in the genes encoding the GM-CSF receptors. Since some of the GM-CSF α -chain mutations retain residual activity, it is expected that some patients will respond to treatment with additional GM-CSF. PAP caused by mutation in the common β -chain in mice was rescued by bone marrow transplantation (59), providing support for the feasibility of future cell-based therapies for refractory PAP in patients with defects in GM-CSF signaling.

HEREDITARY PULMONARY ALVEOLAR MICROLITHIASIS

Hereditary pulmonary alveolar microlithiasis (*SCL34A2*; OMIM #265100) is a rare autosomal disorder causing diffuse alveolar disease in which calcified, crystalline microliths are observed throughout the airspaces. The disease is transmitted as an autosomal recessive disorder, generally presenting in middle age with progressive respiratory deterioration. Dense, diffuse, reticulonodular densities are seen on CT scanning (60). More than 600 cases of pulmonary alveolar microlithiasis have been reported (61), and recent genome-wide studies demonstrate its linkage to a sodium-phosphate cotransport protein, *SCL34A2* (OMIM #604217). *SCL34A2* is a sodium-dependent phosphate transporter that is highly expressed in type II cells in the lung (62, 63). The precise pathogenesis of this disorder, leading to the accumulation of calcium phosphate crystals in the alveolus, has been proposed to be related to the degradation of surfactant phospholipids and the release of phosphate into the alveolus, which, in turn, must be cleared from the lung by transport via *SCL34A2*. A number of mutations have been identified in the *SCL34A2* gene, including missense, frameshift, gene termination, amino acid substitutions, and promoter deletions (16–18). Although the disorder generally presents with pulmonary symptoms, testicular microlithiasis, a cause of male sterility, has also been associated with mutations in *SCL34A2* (16). The diagnosis of pulmonary microlithiasis is made by clinical and radiologic criteria, and the definitive diagnosis can now be made by the identification of mutations in the *SCL34A2* gene.

PULMONARY FIBROSIS ASSOCIATED WITH DEFECTS IN TELOMERASE

Heterozygous mutations in genes encoding two essential components of telomerase, TERT (OMIM #187270) and telomerase RNA component (TERC, OMIM #602322), are also associated with pulmonary fibrosis (12, 13). Telomerase activity is elevated in proliferating cells, and loss of activity results in telomere shortening that can trigger cell death pathways. Telomere shortening has also been detected in IPF patients without mutations in telomerase (64). Thus, it is likely that pathogenesis is related to telomere shortening rather than to mutations in the telomerase genes. The current view is that telomere shortening leads to loss of epithelial cells during a critical window of reepithelialization that, in turn, drives a fibrotic response (Figure 1).

Acknowledgments

The authors acknowledge the secretarial support of Ann Maher, as well as ongoing collaborations with Dr. Lawrence Nogee (Johns Hopkins Hospital) and Dr. Bruce Trapnell (Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine). Authors are supported by NHLBI-HL085610, HL061646, and HL090156.

Glossary

PC	phosphatidylcholine
PG	phosphatidylglycerol
ILD	interstitial lung disease
IPF	idiopathic pulmonary fibrosis
PAP	pulmonary alveolar proteinosis

LITERATURE CITED

1. Perez-Gil J. Structure of pulmonary surfactant membranes and films: the role of proteins and lipid-protein interactions. *Biochim. Biophys. Acta.* 2008; 1778:1676–1695. [PubMed: 18515069]
2. Whitsett JA, Weaver TE. Mechanisms of disease: hydrophobic surfactant proteins in lung function and disease. *N. Engl. J. Med.* 2002; 347:2141–2148. [PubMed: 12501227]
3. Kingma PS, Whitsett JA. In defense of the lung: surfactant protein A and surfactant protein D. *Curr. Opin. Pharmacol.* 2006; 6:277–283. [PubMed: 16580255]
4. Korfhagen TR, Bruno MD, Ross GF, et al. Altered surfactant function and structure in SP-A gene targeted mice. *Proc. Natl. Acad. Sci. USA.* 1996; 93:9594–9599. [PubMed: 8790375]
5. Wright JR. Immunoregulatory functions of surfactant proteins. *Nat. Rev. Immunol.* 2005; 5:58–68. [PubMed: 15630429]
6. Korfhagen TR, Sheftelyevich V, Burhans MS, et al. Surfactant protein-D regulates surfactant phospholipid homeostasis in vivo. *J. Biol. Chem.* 1998; 273:28,438–28,443. [PubMed: 9417042]
7. Ikegami M, Whitsett JA, Jobe A, et al. Surfactant metabolism in SP-D gene-targeted mice. *Am. J. Physiol.* 2000; 279:L468–L476.
8. Ikegami M, Na CL, Korfhagen TR, Whitsett JA. Surfactant protein D influences surfactant ultrastructure and uptake by alveolar type II cells. *Am. J. Physiol.* 2005; 288:L552–L561.
9. Nogee LM. Alterations in SP-B and SP-C expression in neonatal lung disease. *Annu. Rev. Physiol.* 2004; 66:601–623. [PubMed: 14977415]
10. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. *N. Engl. J. Med.* 2003; 349:2527–2539. [PubMed: 14695413]
11. Wang Y, Kuan PJ, Xing C, et al. Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis and lung cancer. *Am. J. Hum. Genet.* 2009; 84:52–59. [PubMed: 19100526]
12. Tsakiri KD, Cronkhite JT, Kuan PJ, et al. Adult-onset pulmonary fibrosis caused by mutations in telomerase. *Proc. Natl. Acad. Sci. USA.* 2007; 104:7552–7557. [PubMed: 17460043]
13. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 2007; 356:1317–1326. [PubMed: 17392301]
14. Martinez-Moczygemba M, Doan ML, Elidemir O, et al. Pulmonary alveolar proteinosis caused by deletion of the GM-CSFR α gene in the X chromosome pseudoautosomal region 1. *J. Exp. Med.* 2008; 205:2711–2716. [PubMed: 18955567]
15. Suzuki T, Sakagami T, Rubin BK, et al. Familial pulmonary alveolar proteinosis caused by mutations in CSF2RA. *J. Exp. Med.* 2008; 205:2703–2710. [PubMed: 18955570]
16. Corut A, Senyigit A, Ugur SA, et al. Mutations in SLC34A2 cause pulmonary alveolar microlithiasis and are possibly associated with testicular microlithiasis. *Am. J. Hum. Genet.* 2006; 79:650–656. [PubMed: 16960801]

17. Huqun IS, Miyazawa H, Ishii K, et al. Mutations in the SLC34A2 gene are associated with pulmonary alveolar microlithiasis. *Am. J. Respir. Crit. Care Med.* 2007; 175:263–268. [PubMed: 17095743]
18. Ishihara Y, Hagiwara K, Zen K, et al. A case of pulmonary alveolar microlithiasis with an intragenetic deletion in SLC34A2 detected by a genome-wide SNP study. *Thorax.* 2009; 64:365–367. [PubMed: 19329736]
19. Marshall RP, Puddicombe A, Cookson WO, Laurent GJ. Adult familial cryptogenic fibrosing alveolitis in the United Kingdom. *Thorax.* 2000; 55:143–146. [PubMed: 10639533]
20. Cole FS, Hamvas A, Rubinstein P, et al. Population-based estimates of surfactant protein B deficiency. *Pediatrics.* 2000; 105:538–541. [PubMed: 10699106]
21. Nogee LM, Garnier G, Dietz HC, et al. A mutation in the surfactant protein B gene responsible for fatal neonatal respiratory disease in multiple kindreds. *J. Clin. Invest.* 1994; 93:1860–1863. [PubMed: 8163685]
22. Nogee LM, Wert SE, Proffit SA, et al. Allelic heterogeneity in hereditary surfactant protein B (SP-B) deficiency. *Am. J. Respir. Crit. Care Med.* 2000; 161:973–981. [PubMed: 10712351]
23. Clark JC, Wert SE, Bachurski CJ, et al. Targeted disruption of the surfactant protein B gene disrupts surfactant homeostasis, causing respiratory failure in newborn mice. *Proc. Natl. Acad. Sci. USA.* 1995; 92:7794–7798. [PubMed: 7644495]
24. Hamvas A, Nogee LM, Mallory GB, et al. Lung transplantation for treatment of infants with surfactant protein B deficiency. *J. Pediatr.* 1997; 130:231–239. [PubMed: 9042125]
25. Shulenin S, Nogee LM, Annilo T, et al. ABCA3 gene mutation in newborns with fatal surfactant deficiency. *N. Engl. J. Med.* 2004; 350:1296–1303. [PubMed: 15044640]
26. Bullard JE, Wert SE, Whitsett JA, et al. ABCA3 mutations associated with pediatric interstitial lung disease. *Am. J. Respir. Crit. Care Med.* 2005; 172:1026–1031. [PubMed: 15976379]
27. Doan ML, Guillerman RP, Dishop MK, et al. Clinical, radiological and pathological features of ABCA3 mutations in children. *Thorax.* 2008; 63:366–373. [PubMed: 18024538]
28. Stahlman MT, Besnard V, Wert SE, et al. Expression of ABCA3 in developing lung and other tissues. *J. Histochem. Cytochem.* 2007; 55:71–83. [PubMed: 16982851]
29. Mulugeta S, Gray JM, Notarfrancesco KL, et al. Identification of LBM180, a lamellar body limiting membrane protein of alveolar type II cells, as the ABC transporter protein ABCA3. *J. Biol. Chem.* 2002; 277:22147–22155. [PubMed: 11940594]
30. Garmany TH, Moxley MA, White FV, et al. Surfactant composition and function in patients with ABCA3 mutations. *Pediatr. Res.* 2006; 59:801–805. [PubMed: 16641205]
31. Cheong N, Madesh M, Gonzales LW, et al. Functional and trafficking defects in ABCA3 mutants associated with respiratory distress syndrome. *J. Biol. Chem.* 2006; 281:9791–9800. [PubMed: 16415354]
32. Nogee LM, Dunbar AE, Wert SE, et al. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N. Engl. J. Med.* 2001; 344:573–579. [PubMed: 11207353]
33. Thomas AQ, Lane K, Phillips J 3rd, et al. Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. *Am. J. Respir. Crit. Care Med.* 2002; 165:1322–1328. [PubMed: 11991887]
34. Chibbar R, Shih F, Baga M, et al. Nonspecific interstitial pneumonia and usual interstitial pneumonia with mutation in surfactant protein C in familial pulmonary fibrosis. *Mod. Pathol.* 2004; 17:973–980. [PubMed: 15133475]
35. Vembar SS, Brodsky JL. One step at a time: endoplasmic reticulum-associated degradation. *Nat. Rev. Mol. Cell. Biol.* 2008; 9:944–957. [PubMed: 19002207]
36. Nakatsukasa K, Brodsky JL. The recognition and retrotranslocation of misfolded proteins from the endoplasmic reticulum. *Traffic.* 2008; 9:861–870. [PubMed: 18315532]
37. Wang WJ, Mulugeta S, Russo SJ, Beers MF. Deletion of exon 4 from human surfactant protein C results in aggresome formation and generation of a dominant negative. *J. Cell Sci.* 2003; 116:683–692. [PubMed: 12538769]
38. Nerelius C, Martin E, Peng S, et al. Mutations linked to interstitial lung disease can abrogate antiamyloid function of prosurfactant protein C. *Biochem. J.* 2008; 416:201–209. [PubMed: 18643778]

39. Bridges JP, Wert SE, Nogee LM, Weaver TE. Expression of a human surfactant protein C mutation associated with interstitial lung disease disrupts lung development in transgenic mice. *J. Biol. Chem.* 2003; 278:52739–52746. [PubMed: 14525980]
40. Glasser SW, Burhans MS, Korfhagen TR, et al. Altered stability of pulmonary surfactant in SP-C-deficient mice. *Proc. Natl. Acad. Sci. USA.* 2001; 98:6366–6371. [PubMed: 11344267]
41. Mulugeta S, Maguire JA, Newitt JL, et al. Misfolded BRICHOS SP-C mutant proteins induce apoptosis via caspase-4- and cytochrome c-related mechanisms. *Am. J. Physiol.* 2007; 293:L720–L729.
42. Bridges JP, Xu Y, Na CL, et al. Adaptation and increased susceptibility to infection associated with constitutive expression of misfolded SP-C. *J. Cell Biol.* 2006; 172:395–407. [PubMed: 16449190]
43. Behr J, Thannickal VJ. Update in diffuse parenchymal lung disease 2009. *Am. J. Respir. Crit. Care Med.* 2008; 179:439–444. [PubMed: 19264979]
44. Korfei M, Ruppert C, Mahavadi P, et al. Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 2008; 178:838–846. [PubMed: 18635891]
45. Lawson WE, Crossno PF, Polosukhin VV, et al. Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. *Am. J. Physiol.* 2008; 294:L1119–L1126.
46. Ikegami M, Korfhagen TR, Whitsett JA, et al. Characteristics of surfactant from SP-A-deficient mice. *Am. J. Physiol.* 1998; 275:L247–L254. [PubMed: 9700084]
47. Seymour JF, Presneill JJ. Pulmonary alveolar proteinosis: progress in the first 44 years. *Am. J. Respir. Crit. Care Med.* 2002; 166:215–235. [PubMed: 12119235]
48. Inoue Y, Trapnell BC, Tazawa R, et al. Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan. *Am. J. Respir. Crit. Care Med.* 2008; 177:752–762. [PubMed: 18202348]
49. Frazier AA, Franks TJ, Cooke EO, et al. From the archives of the AFIP: pulmonary alveolar proteinosis. *Radiographics.* 2008; 28:883–899. [PubMed: 18480490]
50. Dranoff G, Crawford AD, Sadelain M, et al. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science.* 1994; 264:713–716. [PubMed: 8171324]
51. Stanley E, Lieschke GJ, Grail D, et al. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc. Natl. Acad. Sci. USA.* 1994; 91:5592–5596. [PubMed: 8202532]
52. Robb L, Drinkwater CC, Metcalf D, et al. Hematopoietic and lung abnormalities in mice with a null mutation of the common beta subunit of the receptors for granulocyte-macrophage colony-stimulating factor and interleukins 3 and 5. *Proc. Natl. Acad. Sci. USA.* 1995; 92:9565–9569. [PubMed: 7568173]
53. Reed JA, Ikegami M, Cianciolo ER, et al. Aerosolized GM-CSF ameliorates pulmonary alveolar proteinosis in GM-CSF-deficient mice. *Am. J. Physiol.* 1999; 276:L556–L563. [PubMed: 10198353]
54. Zsengeller ZK, Reed JA, Bachurski CJ, et al. Adenovirus-mediated granulocyte-macrophage colony-stimulating factor improves lung pathology of pulmonary alveolar proteinosis in granulocyte-macrophage colony-stimulating factor-deficient mice. *Hum. Gene Ther.* 1998; 9:2101–2109. [PubMed: 9759936]
55. Uchida K, Beck DC, Yamamoto T, et al. GM-CSF autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. *N. Engl. J. Med.* 2007; 356:567–579. [PubMed: 17287477]
56. Shibata Y, Berclaz PY, Chroneos ZC, et al. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. *Immunity.* 2001; 15:557–567. [PubMed: 11672538]
57. Ikegami M, Ueda T, Hull W, et al. Surfactant metabolism in transgenic mice after granulocyte-macrophage-colony stimulating factor ablation. *Am. J. Physiol.* 1996; 14:L650–L658. [PubMed: 8928826]

58. Dirkson U, Nishinakamura R, Groneck P, et al. Human pulmonary alveolar proteinosis associated with a defect in GM-CSF/IL-3/IL-5 receptor common β chain expression. *J. Clin. Invest.* 1997; 100:2211–2217. [PubMed: 9410898]
59. Cooke KR, Nishinakamura R, Martin TR, et al. Persistence of pulmonary pathology and abnormal lung function in IL-3/GM-CSF/IL-5 β c receptor-deficient mice despite correction of alveolar proteinosis after BMT. *Bone Marrow Transplant.* 1997; 20:657–662. [PubMed: 9383229]
60. Chung MJ, Lee KS, Franquet T, et al. Metabolic lung disease: imaging and histopathologic findings. *Eur. J. Radiol.* 2005; 54:233–245. [PubMed: 15837404]
61. Mariotta S, Ricci A, Papale M, et al. Pulmonary alveolar microlithiasis: report on 576 cases published in the literature. *Sarcoidosis Vasc. Diffuse Lung Dis.* 2004; 21:173–181. [PubMed: 15554073]
62. Hashimoto M, Wang DY, Kamo T, et al. Isolation and localization of type IIb Na/Pi cotransporter in the developing rat lung. *Am. J. Pathol.* 2000; 157:21–27. [PubMed: 10880371]
63. Traebert M, Hattenhauer O, Murer H, et al. Expression of type II Na-P(i) cotransporter in alveolar type II cells. *Am. J. Physiol.* 1999; 277:L868–L873. [PubMed: 10564169]
64. Cronkhite JT, Xing C, Raghu G, et al. Telomere shortening in familial and sporadic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 2008; 178:729–37. [PubMed: 18635888]

RELATED RESOURCES

Laboratories in the United States offering clinical testing for mutations in the *ABCA3*, *SFTPB* and *SFTPC* genes include Ambry Genetics Corp. (<http://www.ambrygen.com>) and the DNA Diagnostic Lab at Johns Hopkins Hospital (<http://www.hopkinsmedicine.org/dnadiagnostic>).

Children's Interstitial Lung Disease (chILD) Foundation (<http://www.childfoundation.us>) is a nonprofit organization that provides support, education, and hope to families with children affected by pediatric interstitial lung disease and raises funds for scientific research.

Langston C, Patterson K, Dishop MK, et al. A protocol for the handling of tissue obtained by operative lung biopsy: recommendations of the ChILD pathology co-operative group. *Pediatr. Dev. Pathol.* 2006; 9(3):173–180. [PubMed: 16944976]

Deutsch GH, Young LR, Deterding RR, et al. Diffuse lung disease in young children: application of a novel classification scheme. *Am. J. Respir. Crit. Care Med.* 2007; 176:1120–1128. [PubMed: 17885266]

Faro A, Hamvas A. Lung transplantation for inherited disorders of surfactant metabolism. *NeoReviews.* 2008; 9:e468–e476.

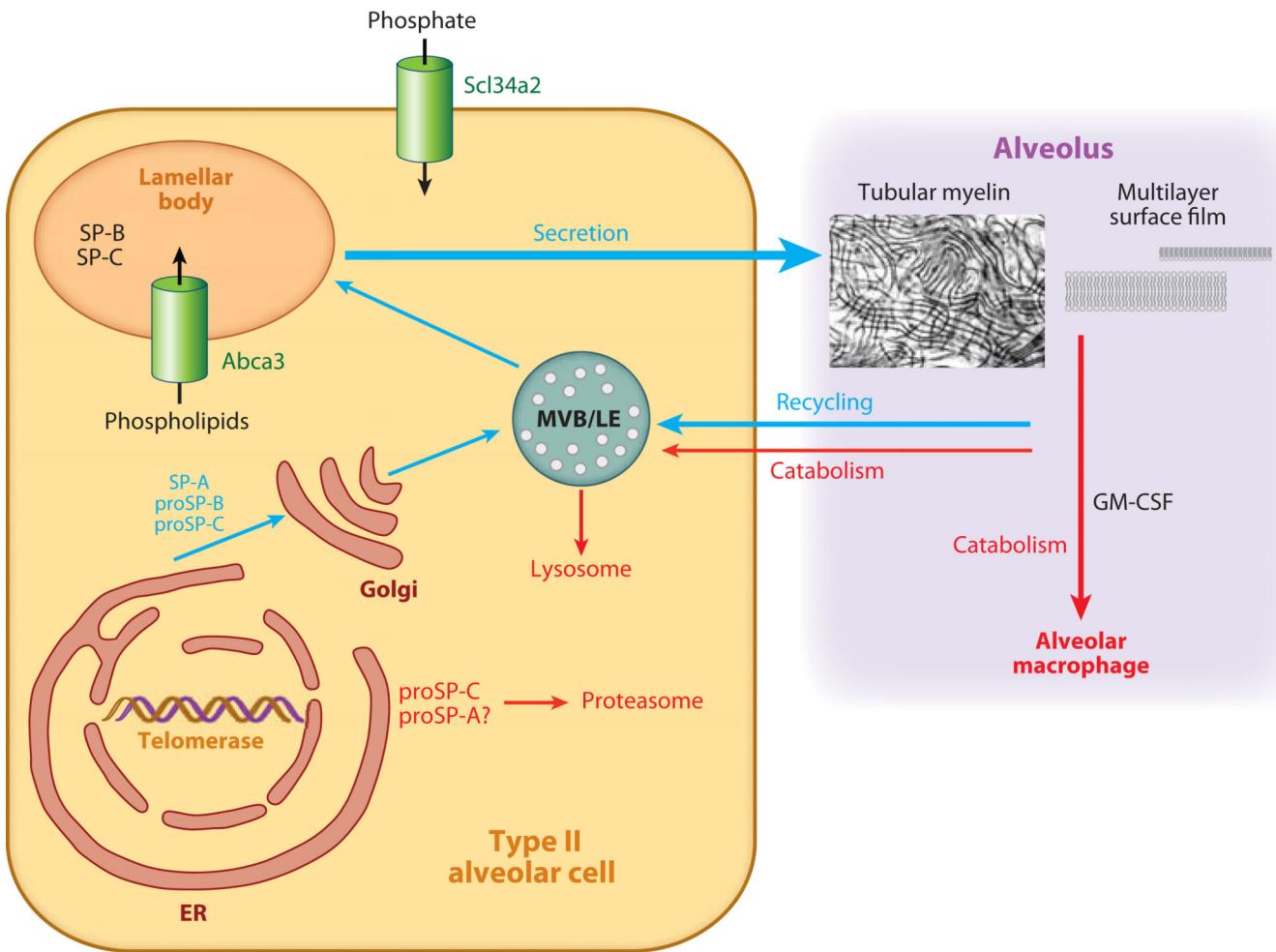
Gower WA, Wert SE, Nogee LM. Inherited surfactant disorders. *NeoReviews.* 2009; 9:e459–e467.

SUMMARY POINTS

1. Pulmonary surfactant is produced by alveolar type II cells and is required for lung function after birth.
2. Pulmonary surfactant is composed of lipids and four lipid-associated proteins, SP-A, SP-B, SP-C, and SP-D, that regulate surfactant function, structure, metabolism, and innate host defense.
3. Mutations in the genes encoding SP-A, SP-B, and SP-C are the cause of acute respiratory distress syndrome or interstitial lung disease in neonates and older individuals.
4. Mutations in *ABCA3*, a gene required for surfactant lipid packaging in type II cells, cause acute respiratory distress syndrome and, less commonly, interstitial lung disease.
5. Mutations in genes regulating aspects of surfactant catabolism cause diffuse alveolar disease, including alveolar pulmonary microlithiasis (*SCL34A2*) and pulmonary alveolar proteinosis (GM-CSF receptor).
6. Protein misfolding associated with SP-C-related lung disease causes type II cell injury.
7. The definitive diagnosis of a number of lung diseases can now be made by genetic analysis.
8. Defects in GM-CSF signaling caused by autoantibodies against GM-CSF or by mutations in GM-CSF receptors disrupt alveolar macrophage function, leading to pulmonary alveolar proteinosis.

FUTURE ISSUES

1. Identification of genetic modifiers influencing the pathogenesis of surfactant and type II cell-related disorders.
2. Elucidation of the role of protein misfolding in interstitial lung disease.
3. Identification of additional genes influencing surfactant homeostasis and interstitial lung disease.
4. Development of new therapies for disorders of surfactant homeostasis and type II cell injury.

**Figure 1.**

Surfactant metabolism. The contents of the lamellar body, including surfactant proteins and lipids, are secreted into the alveoli, where they form a phospholipid-rich film that is essential for preventing alveolar collapse. Approximately half of the alveolar surfactant pool is cleared through a GM-CSF-dependent alveolar macrophage pathway. Most of the remaining surfactant is taken up by the type II epithelial cell and recycled to the lamellar body, via the multivesicular body/late endosome (MVB/LE), for resecretion, while a portion is degraded in lysosomes. Biosynthesis of surfactant proteins and lipids is integrated with the recycling/degradation pathways to maintain intracellular and alveolar surfactant pool size. Mutations resulting in decreased GM-CSF (granulocyte macrophage colony-stimulating factor) signaling lead to increased alveolar surfactant pool size. Mutations in genes encoding SP-B and ABCA3 lead to altered lamellar body structure and loss of surfactant function. Mutations in genes encoding SP-C and possibly SP-A lead to misfolded protein that is degraded by the proteasome; failure to clear misfolded protein causes cytotoxicity and, ultimately, interstitial lung disease (ILD). Mutations in the gene encoding telomerase are also associated with cell death and ILD. Mutations in the gene *SCL34A2* disrupt phosphate transport and are associated with formation of microliths in the alveolar airspaces.

Biosynthetic pathways are shown in blue and catabolic pathways are colored red; the width of the arrow indicates the relative contribution of each pathway to metabolism.

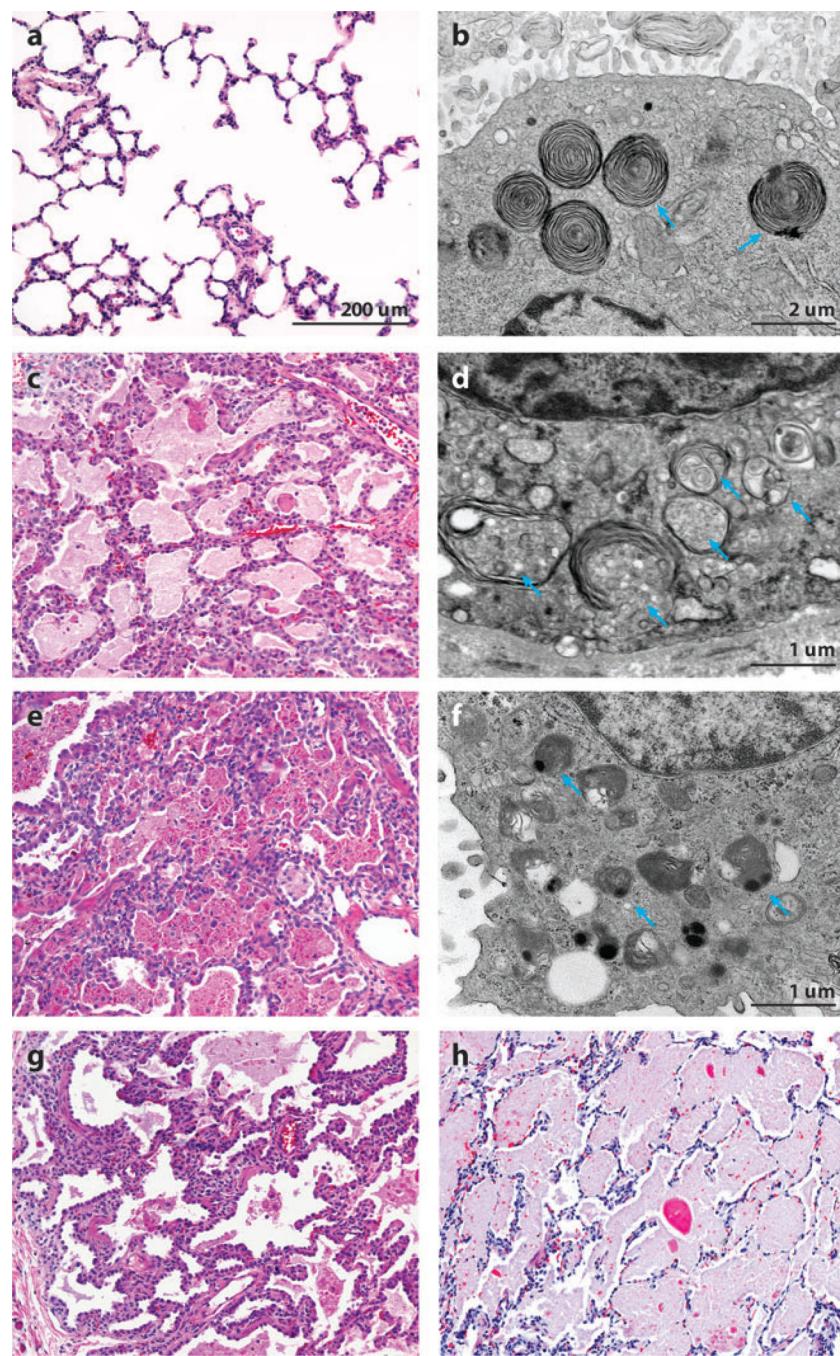


Figure 2.

Histopathology and ultrastructural features of genetic disorders of surfactant homeostasis.
(a) Normal pediatric lung, demonstrating multiple alveoli with thin alveolar septa and normal airspaces. (b) Electron micrograph (EM) of a typical alveolar type II cell from a normal pediatric lung, demonstrating well-developed lamellar bodies (blue arrows). (c) Lung from a neonate with a lethal *SFTPB* mutation, demonstrating the typical pattern of congenital alveolar proteinosis (CAP) with thickened alveolar septa. The alveolar proteinosis material is composed of foamy, eosinophilic, lipoproteinaceous secretions rich in

SP-A, SP-D, and partially processed proSP-C, as well as surfactant phospholipids. (d) EM of an alveolar type II cell from a neonate with a lethal *SFTPB* mutation, demonstrating disorganized multivesicular bodies (*blue arrows*) in lieu of well-developed, organized lamellar bodies. (e) Lung from a neonate with a lethal *ABCA3* mutation, exhibiting granular, eosinophilic, alveolar proteinosis material mixed with macrophages characteristic of CAP. The alveolar proteinosis material is rich in surfactant phospholipids, SP-A, SP-C, SP-B, and proSP-B, but not proSP-C. (f) EM of an alveolar type II cell from a neonate with a lethal *ABCA3* mutation, demonstrating abnormally small lamellar bodies (*blue arrows*) with eccentrically placed, electron-dense inclusions and tightly packed phospholipid lamellae. (g) Lung from an infant with an *SFTPC* mutation, demonstrating chronic interstitial lung disease (chronic pneumonitis of infancy) with thickening of alveolar septa and alveolar proteinosis material in the airspaces. (h) Lung from a child with a mutation in the alpha chain of the GM-CSF receptor, demonstrating foamy alveolar proteinosis material [pulmonary alveolar proteinosis (PAP) is discussed in text] but normal-appearing, thin alveolar septa. H&E stains, original magnification = 10 \times for panels a, c, e, g, and h. EM, original magnification = 15,000 \times for panel b and 30,000 \times for panels d and f.

Table 1

Surfactant disorders causing acute and interstitial lung disease

Genes	Functions	Inheritance	Presentation	Age at presentation
<i>ABCA3</i>	Lipid transport	AR	RDS	Neonates
	Lamellar body, surfactant packaging/function		ILD	Children
<i>SFTPB</i>	Lamellar body, surfactant packaging/function	AR	RDS	Neonates
<i>SFTPC</i>	Surfactant function	AD	ILD > RDS	Infants, children, adults
<i>SFTPA</i>	Tubular myelin, host defense	AD	ILD	Adults
<i>TERT/TERC</i>	Cell homeostasis	AR	ILD	Adults
<i>SCL34A2</i>	Type II cell, Na/PO ₄ -transporter	AR	Pulmonary alveolar microlithiasis	Adults
<i>GMCSFRα</i>	Alveolar macrophage function	AR	PAP	Children
<i>GM-CSF</i>	Alveolar macrophage function	Acquired autoimmune	PAP	Adults

Abbreviations: RDS, respiratory distress syndrome; ILD, interstitial lung disease; PAP, pulmonary alveolar proteinosis; AR, autosomal recessive; AD, autosomal dominant.