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Diseases of Pulmonary Surfactant Homeostasis

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Abstract

Advances in physiology and biochemistry have provided fundamental insights into the role of pulmonary surfactant in the pathogenesis and treatment of preterm infants with respiratory distress syndrome. Identification of the surfactant proteins, lipid transporters, and transcriptional networks regulating their expression has provided the tools and insights needed to discern the molecular and cellular processes regulating the production and function of pulmonary surfactant prior to and after birth. Mutations in genes regulating surfactant homeostasis have been associated with severe lung disease in neonates and older infants. Biophysical and transgenic mouse models have provided insight into the mechanisms underlying surfactant protein and alveolar homeostasis. These studies have provided the framework for understanding the structure and function of pulmonary surfactant, which has informed understanding of the pathogenesis of diverse pulmonary disorders previously considered idiopathic. This review considers the pulmonary surfactant system and the genetic causes of acute and chronic lung disease caused by disruption of alveolar homeostasis.

Keywords

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

LUNG MORPHOGENESIS AND FORMATION OF THE GAS-EXCHANGE REGION OF THE LUNG

The development of the vertebrate lung represents the remarkable adaptation to air breathing that is required for gas exchange and cellular respiration after birth. Derived from anterior foregut endodermal precursors in the embryo, the lung buds invade the splanchnic mesenchyme and undergo branching morphogenesis to form the conducting airways that lead to an expansive region of peripheral saccules, the fetal precursors of alveolar structures that form after birth (Figure 1) (1). Branching morphogenesis, mediated by complex

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signaling and transcriptional programs, is accompanied by the proliferation of diverse epithelial and mesenchymal progenitor cells that form the epithelial, stromal, and vascular components of the lung. During the stages of sacculation (before birth) and alveolarization (after birth), an ever-increasing number of peripheral airspaces are formed that are closely associated with an extensive network of microvasculature structures lined by endothelial cells; these structures are closely apposed to the alveolar epithelium to mediate the efficient exchange of carbon dioxide and oxygen (Figure 1).

The transcriptional and signaling processes mediating branching morphogenesis of the lung are centered around paracrine interactions among multiple cell types, which specify cell fate, migration, and proliferation. Specification of pulmonary epithelial cell types and mesenchymal progenitors is established in the ventral foregut before formation of the lung bud (2). Foregut endodermal cells require signaling by fibroblast growth factor (FGF), sonic hedgehog (SHH), bone morphogenetic proteins (BMP), and Wingless-type MMTV integration site (WNT) to initiate and maintain normal branching morphogenesis (Figure 2) (for reviews, see 3, 4). Before implantation, progenitor cells in the anterior–ventral region of the embryo are restricted to endodermal cell fate by expression of Forkhead Box (FOXA) 2 and Sry-related HMG-Box (SOX) 17 (Figure 2). The primordial lung bud is first demarcated by the expression of Nkx2 homeobox 1 [NKX2-1, also known as thyroid transcription factor-1 (TTF-1)], a transcription factor required for formation of the lung (Figure 2) (5). Distinct SOX2-expressing progenitor cells are restricted to conducting airways, as SOX2 is required for differentiation of conducting airway epithelial cells (6, 7). Peripheral respiratory epithelial cells that will form the alveoli express SOX9 and NKX2-1 (Figure 2) (8). As gestation advances, airway progenitor cells further differentiate into ciliated, serous/secretory, goblet, basal, and neuroendocrine cells in the conducting airways. Type I and type II alveolar epithelial cells differentiate in the peripheral lung saccules and alveoli. From a clinical perspective, congenital malformations affecting the respiratory tract are caused by disruption of these critical modulators of lung formation. For example, pulmonary malformations are associated with altered FGF signaling, as seen in the following syndromes: Pfeiffer [Online Mendelian Inheritance in Man (OMIM) database #101600], Apert (OMIM #101200), and Crouzon (OMIM #123500) (9, 10); with altered SHH signaling, as seen in VATER/VACTERL (OMIM #192350) and Pallister–Hall syndromes (OMIM #146510) (11); with mutations in transcriptional mechanisms, such as *SOX2* (OMIM *184429); as seen in anophthalmia–esophageal–genital syndrome (OMIM #206900) (12); and with mutations in *NKX2-1* (OMIM *600635), which cause brain–thyroid–lung syndrome (OMIM #610978) (13, 14). Mutations in *FOXF1* (OMIM *601089), a gene expressed in the developing pulmonary mesenchyme, causes severe congenital lung disease, termed alveolar capillary dysplasia with misalignment of pulmonary veins (OMIM #265380) (15). Application of whole exome and genome sequencing of infants with congenital malformations of the respiratory tract will extend our knowledge of the genetic basis of pulmonary disorders that cause severe lung disease after birth.

TRANSITION TO AIR BREATHING/PULMONARY MATURATION

Many of the signaling and transcriptional mechanisms regulating early lung branching remain highly active in late gestation as the fetal lung undergoes sacculation, and airway

epithelial cells differentiate to produce pulmonary surfactant (16). During the canalicular, saccular, and alveolar periods of lung morphogenesis, pulmonary mesenchymal cells differentiate to form fibroblasts, lipofibroblasts, pericytes, matrix-associated fibroblasts, smooth muscle, and endothelial cells in the distal lung. From the end of the pseudoglandular period to the beginning of the saccular period of development, the number of peripheral lung tubules increases dramatically and then tubules undergo sacculation as the gas exchange region of the lung forms (Figure 1). Mesenchymal components become relatively less abundant, and as the gas exchange region is created before birth, an extensive pulmonary microvascular capillary bed is formed in close apposition to the epithelial cells (Figure 1). Differentiation of the epithelial cells lining the peripheral lung saccules produces large, squamous, type I epithelial cells that comprise the majority of the alveolar surface and mediate gas exchange. Smaller, cuboidal, alveolar type II epithelial cells account for approximately two-thirds of the alveolar epithelium. Type II epithelial cells produce pulmonary surfactant, which is necessary for reducing surface tension at the air–liquid interface after initiation of ventilation at birth. Many of the genes and transcriptional networks critical for early lung formation have important roles in lung sacculation, including signaling by FOXA2, NKX2-1, SOX9, WNT, β-catenin, SHH, and FGF (Figure 2) (16).

THE PULMONARY SURFACTANT SYSTEM

The seminal study by Avery & Mead in 1959 (17) demonstrated that hyaline membrane disease, now termed infantile respiratory distress syndrome (RDS), was caused by a lack of surfactant lipids in the lungs of the preterm infants who died from this common disorder. Advances in physiology, biochemistry, and molecular biology have identified the chemical composition of pulmonary surfactant as well as the genes and cellular processes involved in surfactant lipid and protein homeostasis. These studies have provided molecular and genetic tools that are useful in identifying the genetic defects underlying the pathogenesis of RDS and the genetic causes of respiratory disease in newborns, infants, and children. Pulmonary surfactant is primarily composed of phospholipids that are enriched with phosphatidylcholine (PC) and phosphatidylglycerol (PG); these phospholipids are produced in increasing amounts in late gestation by type II epithelial cells. Surfactant lipids are routed from the endoplasmic reticulum (ER) to the multivesicular body (MVB) and then to the lamellar body (LB), the surfactant storage organelle in type II epithelial cells (Figure 3). Four surfactant proteins (SPs)—SP-A, SP-B, SP-C, and SP-D—have been identified as critical components of alveolar surfactant, each contributing to lung homeostasis via their distinct protein structures and activities (18). Surfactant lipids and proteins are synthesized primarily by type II epithelial cells, and these are not fully differentiated in preterm infants, resulting in insufficient pulmonary surfactant, which contributes to the pathogenesis of RDS. Surfactant proteins are produced by type II epithelial cells in increasing amounts prior to birth. NKX2-1, a nuclear transcription factor critical for lung morphogenesis (5), binds to and regulates the transcription of surfactant-associated genes and is required for normal surfactant synthesis at birth (19, 20). Knowledge of the surfactant proteins and lipids, including the addition of SP-B and SP-C as critical components of the surfactant-replacement preparations used to treat RDS, enabled widespread use of these preparations to prevent and treat RDS. The intratracheal administration of formulations containing

surfactant lipids, SP-B, and SP-C has dramatically improved morbidity and mortality from RDS in preterm infants (21, 22).

SURFACTANT METABOLISM

Surfactant lipids are synthesized by type II epithelial cells in the ER. Metabolic substrates for lipogenesis are derived from precursors taken up from the circulation, from de novo synthesis of lipids, and from reuptake of lipids by type II epithelial cells. Within type II epithelial cells, PC is transferred to LBs in a process requiring adenosine triphosphate (ATP)-binding cassette transporter A3 (ABCA3), a membrane-spanning transporter of the ATP-binding cassette family of proteins that is located on the limiting membrane of the LB. Surfactant precursor proteins pro-SP-B and pro-SP-C are processed proteolytically by napsin, cathepsin, and pepsinogen during trafficking to MVBs and LBs. The active hydrophobic proteins SP-B and SP-C are assembled with surfactant phospholipids into bilayer membranes that are stored in LBs (for a review, see 18). The contents of LBs are secreted into the airway via a process stimulated by catecholamines, purinoreceptor agonists, and cell stretch. Control of secretory processes requires GPR116, an orphan G protein–coupled receptor located on respiratory epithelial cells, which modulates the secretory process (23, 24).Genetic deletion of *Gpr116* in mice causes excess secretion of surfactant with accumulation in the airspaces after birth. After secretion, LBs unwind and interact with SP-A and SP-D to produce tubular myelin and multilayered surface films that spread over the alveolus and reduce surface tension. SP-A and SP-B are required for formation of tubular myelin (25, 26) and have important roles in innate host defense in the alveolus. SP-D regulates extracellular forms of surfactant and has an important role in controlling the size of the surfactant lipid pool (27, 28). SP-D and SP-A are members of the collectin family of host defense proteins that bind pulmonary microbial pathogens and products, including viruses, fungi, and bacteria, and serve as critical innate host defense proteins with anti-inflammatory properties (29–31). Pulmonary surfactant is recycled, catabolized or reutilized actively by alveolar type II epithelial cells in a process influenced by surfactant proteins. Alveolar macrophages play a critical part in surfactant uptake and degradation in a process that depends upon signaling by granulocyte macrophage colony–stimulating factor (GM-CSF) or CSF2 (for a review, see 32). Autoantibodies against GM-CSF or mutations in GM-CSF receptors cause the disorder known as pulmonary alveolar proteinosis (PAP). Figure 3 provides an integrated schematic of important aspects of the processes critical for surfactant homeostasis in the alveolus.

UNIQUE BIOPHYSICAL ACTIVITY OF PULMONARY SURFACTANT

The close apposition of alveolar type I epithelial cells to underlying capillary endothelial cells forms a highly diffusible air–blood barrier across which gas exchange occurs. The stabilization of alveolar structure during breathing-induced expansion and contraction is achieved by the formation and maintenance of a phospholipid-rich film (pulmonary surfactant) that spreads over the thin liquid layer (the aqueous hypophase) that covers the alveolar epithelial cell surface (Figure 3) (33). The unique biophysical properties of surfactant prevent alveolar collapse (atelectasis) at low lung volumes by reducing surface tension, which is generated by the aqueous hypophase, to very low levels (<2 mN/m).

During alveolar expansion, surface tension increases (to a maximum of 20–25 mN/m), stabilizing the alveolus at higher lung volumes. The unique biophysical properties of the surface film are directly related to the incorporation of dipalmitoyl phosphotidylcholine (DPPC), a saturated phospholipid that allows acyl chains to be very tightly packed as the film is compressed during exhalation. Incorporation of cholesterol and phospholipids with the unsaturated acyl chains helps to maintain the fluidity of the surface film at body temperature. SP-B and SP-C facilitate remodeling of newly secreted surfactant membranes by promoting the incorporation and spreading of lipids as the surface film expands during inhalation. Neonatal lethality in knockout mice indicates that SP-B is indispensable for this process (25). SP-C deficiency is not lethal and the function of SP-C is less well understood, but it is likely required for optimal function of the surface film. Lipid–protein complexes are removed from the surface film during compression and are degraded by alveolar macrophages or are recycled in type II epithelial cells; the recycling process depends at least partly on SP-D, which enhances uptake of surfactant lipids by type II epithelial cells (34). Overall, maintenance of the surface film is a highly dynamic process that requires integration of pathways involved in synthesis and assembly, secretion, recycling, and degradation of surfactant. Dysregulation can lead to alterations in the size, composition, or both of the alveolar surfactant pool, resulting in PAP (surfactant accumulation) or RDS (surfactant insufficiency). Thus, sensing of the size or composition, or both, of the alveolar pool is essential for pathway integration and maintenance of alveolar homeostasis. Recent evidence has suggested that GPR116 may be a component of the sensing machinery (23, 24), but knowledge about the signaling pathway that couples this receptor to surfactant metabolism is lacking.

GENETIC DISORDERS OF SURFACTANT HOMEOSTASIS AND ALVEOLAR DEVELOPMENT

The identification of the genes and cellular processes involved in maturation and function of the pulmonary surfactant system, as well as in alveolar development, has provided genetic tools that are useful for diagnosing rare lung diseases presenting in newborns, infants, and children.

Abnormalities in Surfactant Packaging, Function, and Degradation

Pathological and genetic analyses of full-term infants with refractory respiratory failure after birth have identified the role of mutations in the genes encoding SP-B (*SFTPB*), SP-C (*SFTPC*), and ABCA3 (*ABCA3*) in the pathogenesis of respiratory failure and diffuse, chronic interstitial lung disease (ILD) in newborn infants and children (35–37). Classification of the histopathology associated with these disorders (Table 1) includes neonatal or congenital PAP, infantile desquamative interstitial pneumonia (DIP), chronic pneumonitis of infancy, nonspecific interstitial pneumonia (NSIP), and usual interstitial pneumonia (UIP) (in older children and adolescents). The histopathology of these disorders often overlaps and depends on the nature of the mutations, the age of the child, and the clinical therapy provided. Mutations in *SFTPB* and *ABCA3* are inherited as autosomal recessive genes, usually presenting in full-term newborn infants as cyanosis, grunting, retractions, and atelectasis. Pulmonary disease associated with these mutations is

unremitting despite the use of intensive care and surfactant replacement; this contrasts with RDS that is associated with prematurity in newborns and responds to surfactant replacement. Mutations in the *SFTPC* gene are inherited as autosomal dominant disorders that are associated with chronic ILD in older infants, children, and adults; infants and children with chronic ILD often present with symptoms of diffuse lung disease including tachypnea, retractions, hypoxemia, and digital clubbing. Mutations in the genes encoding receptors for GM-CSF (*CSF2RA*, *CSF2RB*) are inherited as autosomal recessive genes and are associated with progressive dyspnea and congenital PAP. These disorders are classified as surfactant metabolism dysfunctions, pulmonary (SMDP) types 1, 2, 3, and 4, and are summarized in Table 2.

Mutations in *SFTPB* associated with fatal lung disease in newborn infants

The surfactant protein SP-B is encoded by *SFTPB* on human chromosome 2p11.2 (OMIM *178640), which produces a 381-amino-acid preproprotein that is expressed and processed by type II epithelial cells. Mutations in *SFTPB* are associated with a complete loss of SP-B protein as well as with secondary effects on the processing of pro-SP-C to its mature peptide that result in the accumulation of partially processed, inactive pro-SP-C in the alveoli (35, 38, 39). SP-B is processed proteolytically to the active, 79-amino-acid, amphipathic peptide that is closely associated with lipids in LBs and alveolar surfactant (for a review, see 40). Studies in *Sftpb*$^{-/-}$ mice have demonstrated that the effects of SP-B are specific to the lung, with newborn mice dying from atelectasis and respiratory failure at the time of birth in the absence of SP-B (25). SP-B has critical roles in the intracellular packaging and processing of surfactant lipids and proteins. SP-B is required for the formation of LBs, proteolytic processing of pro-SP-C, formation of tubular myelin, and generation of the surface-active films required to reduce surface tension (18; for a review, see 40). Infants who are deficient in SP-B may have a family history of perinatal respiratory failure in siblings because the mutations are inherited as autosomal recessive alleles. A number of distinct *SFTPB* mutations have been identified in infants with neonatal respiratory failure, enabling both prenatal and postnatal genetic diagnoses (39, 41). Rarely, less severe mutations in SP-B production are consistent with the diagnosis of chronic lung disease in infants (42). *SFTPB*-related lung disease (SMDP1 #265120) is generally fatal in the first months of life, although some infants have been supported by lung transplantation (43, 44). Surfactant replacement and other ventilatory support are not effective therapies for this disorder. Histopathological diagnoses described in SP-B deficiency are associated primarily with neonatal PAP or infantile DIP. Histological findings include (*a*) variable amounts of amorphous, eosinophilic and/or periodic acid–Schiff (PAS)-positive, lipoproteinaceous material in the alveolar spaces; (*b*) foamy alveolar macrophages; (*c*) alveolar epithelial hyperplasia with prominent alveolar type II cells; and (*d*) thickened interstitial septa (Figure 4).

Mutations in *SFTPC* associated with interstitial lung disease

SFTPC is located on chromosome 8p21.3 (OMIM *178620) and encodes the preprotein pro-SP-C that is expressed selectively in alveolar type II epithelial cells in the lung (for a review, see 18). Like SP-B, SP-C is produced by proteolytic processing of its 197-amino-acid precursor during transit from the ER to MVBs and LBs, where it is stored with phospholipids. The active SP-C peptide of 35 amino acids consists of a highly hydrophobic

α helix that spans lipid membranes and contributes to the spread of surfactant in the alveoli. The preprotein contains a C-terminal BRICHOS domain that is involved in the proper folding and trafficking to the secretory pathway (45, 46). While SP-C is not required for survival of newborn *Sftpc*$^{-/-}$ mice, a lack of SP-C causes progressive lung inflammation, emphysema, and remodeling in some mouse strains as well as marked susceptibility to infection, inflammation, and fibrosis, indicating its role in innate immune responses in this animal model (47–49). Acute and chronic lung disease are associated with mutations in *SFTPC*, which cause missense mutations in the BRICHOS domain and result in the misfolding, misrouting, and/or misprocessing of pro-SP-C that then accumulates within type II alveolar cells, causing cell toxicity (45, 50–52).Cellular responses to the misfolded pro-SP-C products include ER stress, the activation of reactive oxygen species, autophagy, and injury related to the accumulation of misfolded protein in abnormal cellular compartments. *SFTPC*-related disease (SMDP2 #610913) is typically inherited as an autosomal dominant disease with variable penetrance (41, 53–55), although sporadic mutations also have been identified (41, 54). Infants with *SFTPC* mutations usually exhibit features of chronic pneumonitis of infancy, including diffuse alveolar injury with regenerating alveolar epithelia lined by hyperplastic type II epithelial cells, variable amounts of alveolar proteinosis material containing cholesterol clefts, foamy macrophages, and interstitial thickening with lymphocytic inflammation and muscularization of the alveolar walls (Figure 4) (35, 36, 56). Patients with SPC-related disease who present later in childhood exhibit chronic interstitial lung disease, usually classified as NSIP or UIP (36, 37); older adults often present with ILD or pulmonary fibrosis (55). Variability and presentation are influenced by the mutations as well as by environmental factors including viral infections (57, 58). The age at presentation and severity vary greatly within the same kindred (55). Definitive diagnosis is made by identifying mutations in the *SFTPC* gene. The observation that cell injury is caused by the misfolding of pro-SP-C provided critical insight into the role that injury to alveolar epithelial cells has in the pathogenesis of ILD (for a review, see 51). Although mutations in *SFTPC* are not a common cause of ILD in adults (59), 25% of patients in a Dutch cohort with familial pulmonary fibrosis carried *SFTPC* mutations (60). Both pharmaceutical and genetic approaches to inhibit the production or misfolding of the mutant protein are being studied. Hydroxychloroquine, as well as lung transplantation, have been used for therapy (37, 58, 61).

Mutations in *ABCA3* associated with acute and chronic lung disease

Mutations in *ABCA3* (chromosome 16p13.3; OMIM #601615) are the most common cause of hereditary respiratory failure in newborns. ABCA3 is a large membrane-spanning protein sharing structural similarities with the cystic fibrosis transmembrane conductance regulator (CFTR). Mutations have been identified throughout the *ABCA3* gene that cause abnormal processing, misrouting, or impaired lipid transport (62–65) as well as secondary effects on processing SP-B and SP-C (66, 67). Lung histology is consistent with congenital PAP or infantile DIP (Figure 4) (35, 68, 69). Ultrastructural studies of type II cells from these patients demonstrate the absence of normal LBs and the presence of electron-dense inclusions within small vesicular structures, both of which are characteristic of the disorder (35, 68, 70). While ABCA3 is expressed in various tissues, abnormalities are found only in the lung. Pulmonary disease associated with ABCA3 deficiency is generally fatal (68, 69),

although a number of infants have been treated by lung transplantation (43). Prenatal and postnatal diagnoses are best made by identifying mutant *ABCA3* alleles. Diseases related to *ABCA3* and *SFTPB* are similar in onset and clinical course and are generally associated with severe respiratory failure, although several *ABCA3* mutations have been associated with chronic ILD in older infants, children, and adolescents (67, 71–73). Histopathological findings vary depending upon the age of the patient and the mutation and often overlap with those found in deficiencies of SP-B and SP-C. Biopsy specimens obtained from young infants are associated with PAP and DIP; those obtained from older infants are associated with DIP and NSIP; and those from older children are associated with NSIP, which often exhibits additional focal proteinosis, cholesterol clefts, and thickened alveolar septa. Clinical symptoms in older infants and children include cough, tachypnea, dyspnea, exercise intolerance, digital clubbing, failure to thrive, and hypoxemia. Treatment modalities include systemic corticosteroids, hydroxychloroquine, and lung transplantation.

Role of GM-CSF signaling defects in pulmonary alveolar proteinosis

Although surfactant catabolism by alveolar macrophages represents a relatively small contribution to surfactant recycling and metabolism, studies in transgenic mice in which GM-CSF (*Csf*) and GM-CSF receptors (*Csf2ra* and *Csf2rb*) were mutated or deleted, found that the mice developed a syndrome virtually identical to that associated with idiopathic PAP (iPAP) in adult patients (74, 75). Analysis of surfactant catabolism in mouse models has demonstrated that GM-CSF signaling activated PU.1 and was required not only for the maturation of alveolar macrophages but also for their function, including their ability to catabolize surfactant lipids and proteins (76). Defects in surfactant catabolism related to a lack of GM-CSF signaling result in the accumulation of surfactant in the alveolus. Most patients with iPAP present with progressive dyspnea and radiographic findings termed crazy paving, which are caused by heterogeneous alveolar infiltrates resulting from the accumulation of surfactant. The seminal findings by Kitamura et al. (77) demonstrated that most patients with adult iPAP produced neutralizing antibodies against GM-CSF (*CSF2*; chromosome 5q31.1; OMIM #13906), defining the disorder as an acquired, autoimmune disease (OMIM #610910). Pathological findings consist of heterogeneous accumulation of PAS-positive diastase-resistant surfactant material in the peripheral lung parenchyma associated with foamy macrophages that are rich in neutral lipids. In general, alveolar structures are preserved in iPAP (Figure 4). Subsequently, both pediatric and adult onset of PAP have been associated with mutations in the GM-CSF receptors *CSF2RA* (chromosome Xp22.33; OMIM #306250) (78–80) or *CSF2RB* (chromosome 22q12.3; OMIM #138981) (81–83). Patients with these mutations develop congenital PAP with clinical and histopathological findings identical to those of acquired (autoimmune) iPAP. Congenital PAP is inherited as an autosomal recessive disorder (OMIM #300770 and #614370). *CSF2RA* is located on the X chromosome's pseudoautosomal region 1. Sequential bronchoalveolar lavage has been utilized to treat acquired autoimmune iPAP for many years (84). The recognition that defective GM-CSF signaling underlies the disorder has provided new diagnostic approaches for measuring GM-CSF autoantibodies and other serum markers of the disease (e.g., SP-D and KL6). Functional assays have been developed to assess the function of GM-CSF-dependent macrophages. The use of inhaled GM-CSF and rituximab, the latter used to inhibit production of autoantibodies, has been actively studied for

treatment of iPAP (85–88). Similarly, novel treatments based on restoring GM-CSF receptor activity using gene or cell transfer are being actively studied in animal models for the potential development of new therapeutic strategies to treat congenital PAP (89, 90).

Role of *NKX2-1* in the Pathogenesis of Brain-Lung-Thyroid Syndrome

Analysis of the functions of NKX2-1 (chromosome 14q13.3;OMIM#600635) in transgenic mouse models has demonstrated its critical role in initiating lung morphogenesis and perinatal lung maturation (5, 20). NKX2-1 is a transcriptional regulator of many of the genes involved in surfactant function and lung maturation (19). NKX2-1 is a highly conserved member of a larger family of homeodomain-containing transcription factors involved in organogenesis and cell differentiation in various organs. *NKX2-1*, encoding TTF-1, is selectively expressed throughout development in forebrain, thyroid, and lung (91). Mutations in *NKX2-1* cause a variably penetrant disorder that affects the central nervous system of newborns—usually manifesting as a choreiform movement disorder, thyroid agenesis, or hypoplasia with associated hypothyroidism—and produces both acute and chronic respiratory disease (OMIM #610878) (13, 14). *NKX2-1* is located on chromosome 14q13.3 near *NKX2–8* and *PAX9*, genes that are also expressed in the respiratory epithelium. Haploinsufficient mutations in *NKX2-1* are associated with pulmonary disease in infants and with variable inhibitory effects on the expression of target genes (e.g., the surfactant proteins) (13, 92, 93). The clinical presentation of *NKX2-1*-related pulmonary disease is also variable, ranging from neonatal RDS with and without pulmonary hypertension to ILD in older children and pulmonary fibrosis in adults (13, 14, 92–96). Recurrent respiratory infections are frequently encountered (14). Clinically, the diagnosis is supported by congenital hypothyroidism together with lung disease in a term infant. The pulmonary histopathology is heterogeneous, both among and within individual cases (13, 14, 92). In some cases, histological features are consistent with those seen in the genetic surfactant disorders and include thickening of the alveolar septa, hyperplasia of alveolar type II epithelial cells, and accumulation of foamy alveolar macrophages (Figure 4). In other cases, there are clear growth abnormalities with alveolar simplification and cyst formation. Homogeneous regions of alveolar septal thickening with fibrosis and chronic interstitial inflammation, resembling NSIP, have also been found in several cases (Figure 4). Genetic analysis of the *NKX2-1* locus is useful for diagnosis by identifying point mutations and deletions in the gene. Recently, an infant who presented with features consistent with brain-lung-thyroid disorder and an intact *NKX2-1* coding region was found to have a deletion in a noncoding segment of DNA located in a potential regulatory region of the *NKX2-1* locus, demonstrating that alterations in noncoding regulatory regions of the *NKX2-1* gene may also be responsible for this syndrome (13, 14, 97).

Role of *FOXF1* in the Pathogenesis of Alveolar Capillary Dysplasia

FOXF1 (chromosome 16q24.1, OMIM #601089) is a member of the forkhead ortholog family of transcription factors that is expressed in the embryonic splanchnic mesenchyme. Deletion of *Foxf1* in the mouse has been shown to cause severe pulmonary vascular and gastrointestinal malformations that are lethal at birth (98). Subsequently, mutations in *FOXF1* have been identified as the most common genetic cause of alveolar capillary dysplasia with misalignment of the pulmonary veins (ACD/MPV) (15). These infants

generally present soon after birth as full-term infants with severe pulmonary hypertension and respiratory failure. ACD/MPV is suspected when severe cyanosis is refractory to supportive and pharmacological therapies in the absence of structural heart disease, with or without a family history of respiratory failure in the immediate postnatal period. Patients with ACD/MPV often have abnormalities in other organs, including gastrointestinal, genitourinary, and cardiovascular malformations (15, 99–103). Infants with ACD/MPV are often placed on extracorporeal membrane oxygenation but fail to respond to intensive care. More than 30 *FOXF1* mutations have been identified in patients with ACD/MPV (104). Most are sporadic mutations, although several have been inherited as autosomal recessive disorders with maternal inheritance consistent with paternal imprinting, thus enabling prenatal and postnatal genetic diagnoses to be made (104). Pulmonary pathology in ACD/MPV is characterized by abnormalities in the growth and development of the respiratory parenchyma, which result in immature lobular development with reduced alveolar and capillary formation as well as immature thickened alveolar septa or mesenchyme. Overall, these features imply severe growth arrest of the distal lung and suggest that pulmonary immaturity, with reduced levels of surfactant and poor oxygen diffusion, causes respiratory distress and failure in these cases. In addition, the pulmonary veins are thin-walled, dilated, congested, and mislocated or malpositioned within the pulmonary segments. Instead of coursing independently through the parenchyma, the pulmonary veins are found adjacent to the pulmonary arteries as part of the bronchoarterial compartment (Figure 5). The arteries and arterioles are muscularized and can be immunostained with antibodies to α smooth muscle actin (Figure 5). As indicated above, both the alveolar compartment and the capillary bed are malformed and reduced. The capillaries are dilated and are generally located in abnormally thickened interstitial regions of the distal lung instead of being in close proximity to the alveolar epithelium (Figure 5).

GENETIC DISORDERS ASSOCIATED WITH ALVEOLAR CELL INJURY: ADULT ONSET OF INTERSTITIAL LUNG DISEASE

Interstitial pulmonary fibrosis (IPF) is a progressive disease that involves chronic injury to the alveolar parenchyma, resulting in the replacement of normal lung tissue with excess connective tissue, and limiting oxygen diffusion across the alveolar–capillary membranes. Recently, genetic disorders that compromise alveolar cell function or cause cell injury have been associated with familial IPF (Table 3).

Mutations in *SFTPA* Associated with Familial Pulmonary Fibrosis

SP-A is encoded by two genes, *SFTPA1* and *SFTPA2*, located near *SFTPD* (105) on chromosome 10q22.3 (OMIM #178630 and *178642, respectively). Heterozygous mutations in *SFTPA2* have recently been recognized as a rare cause of adult-onset IPF (OMIM #178500) and lung cancer in older individuals (106). Mutations in *SFTPA2* likely disrupt normal routing and processing of the protein, supporting the concept that, like mutations in *SFTPC*, SP-A-related disease results from misfolding of the protein in alveolar type II epithelial cells. The mutant proteins are retained in the ER, which leads to activation of the unfolded protein response and ER-associated degradation pathways, chronic ER stress, and cell injury (107). Recent studies have supported the role of mutant forms of both *SFTPA2*

and *SFTPA1* in the activation of latent transforming growth factor β (TGF-β), which results in a profibrotic response (108, 109).

Mutations in the Phosphate Transporter *SLC34A2* Associated with Pulmonary Microlithiasis

Pulmonary microlithiasis (OMIM #265100) is a rare disorder in which patients present with dyspnea, interstitial lung disease, and the characteristic micronodular calcifications seen on radiographs and computed tomography (CT) scans (for a review, see 110). It is an autosomal recessive disorder that has been mapped to locus 4p15.31-p15.2, which contains the *SLC34A2* gene (OMIM *604217); allelic variants include both deletions and point mutations in this gene (111, 112). Although most patients present in middle age, this diagnosis has been made in infants. SLC34A is a sodium-dependent phosphate transporter expressed at high levels in alveolar type II epithelial cells. The disease is common in patients of Chinese, Turkish, and Japanese descent (113). The clinical course is variable but often results in slow deterioration of the lung, and ends in severe pulmonary fibrosis, respiratory insufficiency, and cardiac failure. Many patients are asymptomatic. The disease is usually detected on chest X-rays during routine examination or by family history. Histopathological findings include PAS-positive intraalveolar microliths that consist of concentric lamellae arranged around a central nucleus that has an amorphous or granular appearance. These structures are composed primarily of calcium phosphate, which accumulates in the alveolus. The microliths gradually grow from smaller to larger structures that eventually fill the entire alveolar space, resulting in injury to the alveolar walls, and are replaced by fibrotic tissue. Recent anecdotal reports support the use of disodium etidronate to treat this rare cause of interstitial lung disease, although lung transplantation has been beneficial (110, 114).

Mutations in Telomerases Associated with Familial Pulmonary Fibrosis

Severe, progressive, lung disease with pathological diagnoses of adult-onset ILD and pulmonary fibrosis are associated with mutations in two essential components of the telomerase enzyme complex: telomerase reverse transcriptase or *TERT* (chromosome 5p15.33; OMIM +187270; #614743), and telomerase RNA component or *TERC* (chromosome 3q26.2; OMIM *602322; #614743), which regulates the maintenance of telomeres during cell proliferation (115–117). Diagnosis of familial pulmonary fibrosis was initially recognized by associating mutations in the *TERT* or *TERC* genes with severe lung disease in patients with dyskeratosis congenita, further highlighting the importance of epithelial cell injury and repair in the pathogenesis of chronic alveolar diseases (118–124). Inheritance is primarily autosomal dominant, with incomplete penetrance. Patients with these mutations have shortened telomeres and variable penetrance of pulmonary fibrosis, liver fibrosis, and bone marrow failure. Pulmonary fibrosis associated with mutations in *TERT* is diagnosed primarily in older adults and correlates with a history of smoking. It is a progressive, lethal disease with a mean life expectancy of 3 years after diagnosis (125). Radiographic and histological findings are consistent with UIP. Heterozygous mutations in *TERT* have been found in 8–15% of patients with IPF of adult onset; less than 1–3% had heterozygous mutations in *TERC* (117). Altogether, autosomal dominantmutations in *TERT*, *TERC*, *SFTPC*, and *SFTPA2* account for only 15–20% of familial IPF cases, indicating novel genes are involved that have not yet been identified (126, 127).

SUMMARY

Knowledge regarding the cellular and molecular mechanisms controlling the pulmonary surfactant system has enabled recognition and diagnosis of hereditary disorders underlying lung diseases previously described as idiopathic. Insights regarding the pathogenesis of PAP have led to new diagnostic and therapeutic advancements that are leading to treatment of both acquired and hereditary PAP. Although diagnostic criteria for hereditary disorders that disrupt surfactant function and cause lung remodeling are now well established, a deeper understanding of the biological processes leading to pulmonary cell injury and tissue remodeling will be required to enable new therapies for these life-threatening diseases in the future.

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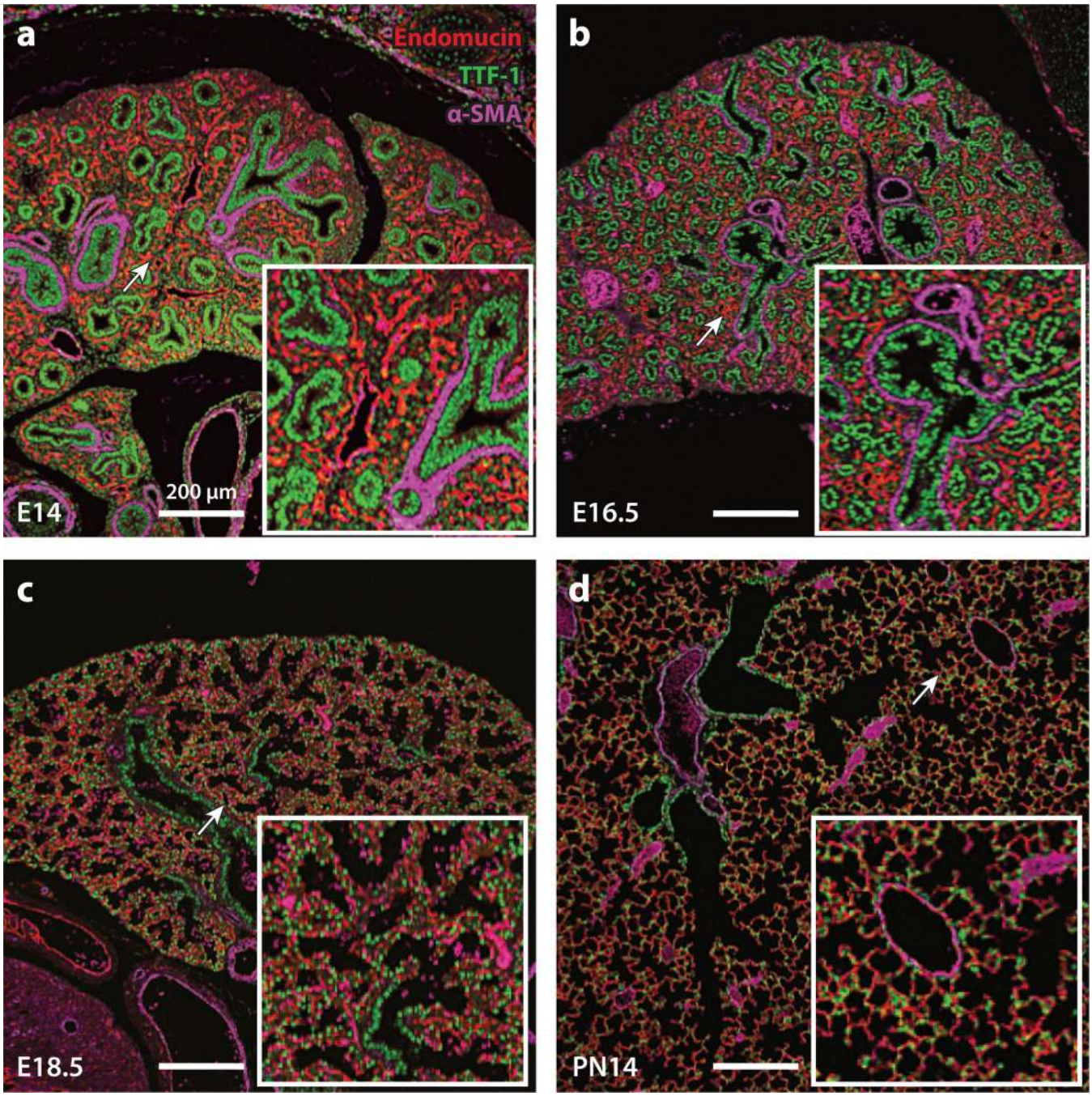

Figure 1.

Stages of lung morphogenesis. Confocal microscopy was used to image lung architecture in the mouse from (*a*) day E14 (pseudoglandular) to (*b*) E16.5 (canalicular), (*c*) E18.5 (saccular), and (*d*) PN14 (alveolar period). TTF-1 (*green*) staining identifies epithelial cells; α-SMA (*purple*) identifies smooth muscle cells. Endomucin (*red*) marks pulmonary endothelial cells. Arrows indicate the regions of enlargement illustrated in panel insets. Scale bar = 200 μm for all panels. Immunofluorescent images courtesy of Dr. John Shannon, Department of Pediatrics, Cincinnati Children's Hospital Medical Center and the University

of Cincinnati, Cincinnati, Ohio. Abbreviations: α-SMA, α smooth muscle actin; E, embryonic; PN, postnatal; TTF-1, thyroid transcription factor-1.

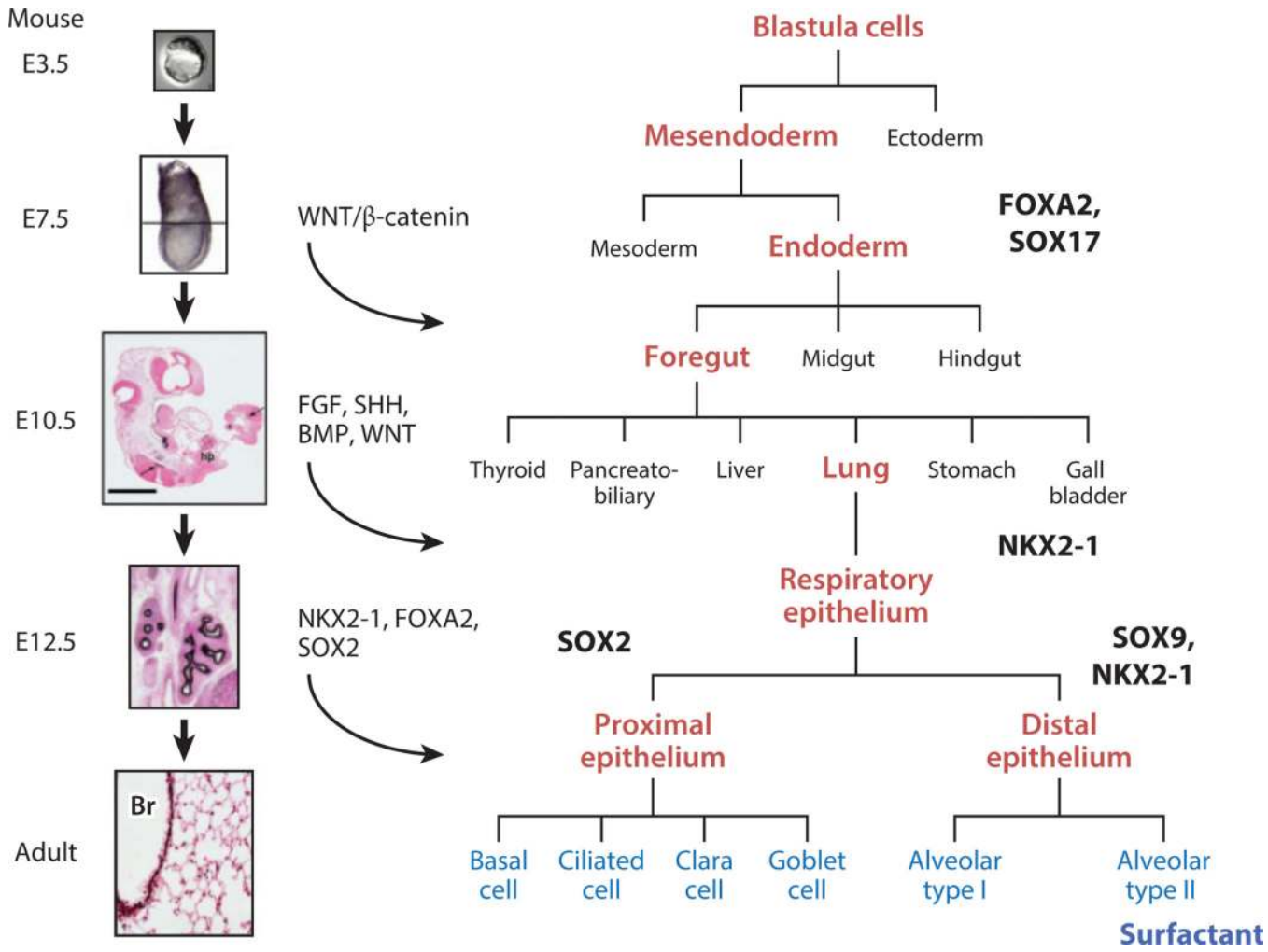

Figure 2.

Blueprint for specification and differentiation of the respiratory epithelium in the mouse, from day E3.5 to adult. The lungs are derived from progenitor cells located in the ventral region of the embryonic foregut. Endoderm is specified from neuroectoderm and mesoderm prior to the implantation of the embryo. This process is mediated in part by the expression of SOX17 and FOXA2, transcription factors critical for the formation of endoderm. Under the influence of WNT, SHH, FGF, and BMP signaling, the lung buds are distinguished from the gut tube and marked by the expression of NKX2-1 (thyroid transcription factor-1). The esophagus separates from the trachea, the latter marked by expression of SOX2, which is required for the formation and differentiation of airways and airway epithelial cells. Peripheral lung progenitors, expressing both NKX2-1 and SOX9, produce alveolar type I and type II epithelial cells. Surfactant proteins and lipids are produced after the differentiation of type II epithelial cells, which in preparation for birth are also under the control of NKX2-1, FOXA2, and associated transcription factors. Mutations in the genes encoding the transcription factors SOX2 and NKX2-1 cause pulmonary malformations; NKX2-1 regulates genes that are critical for alveolar homeostasis after birth. Abbreviations: Br, bronchiole; E, embryonic.

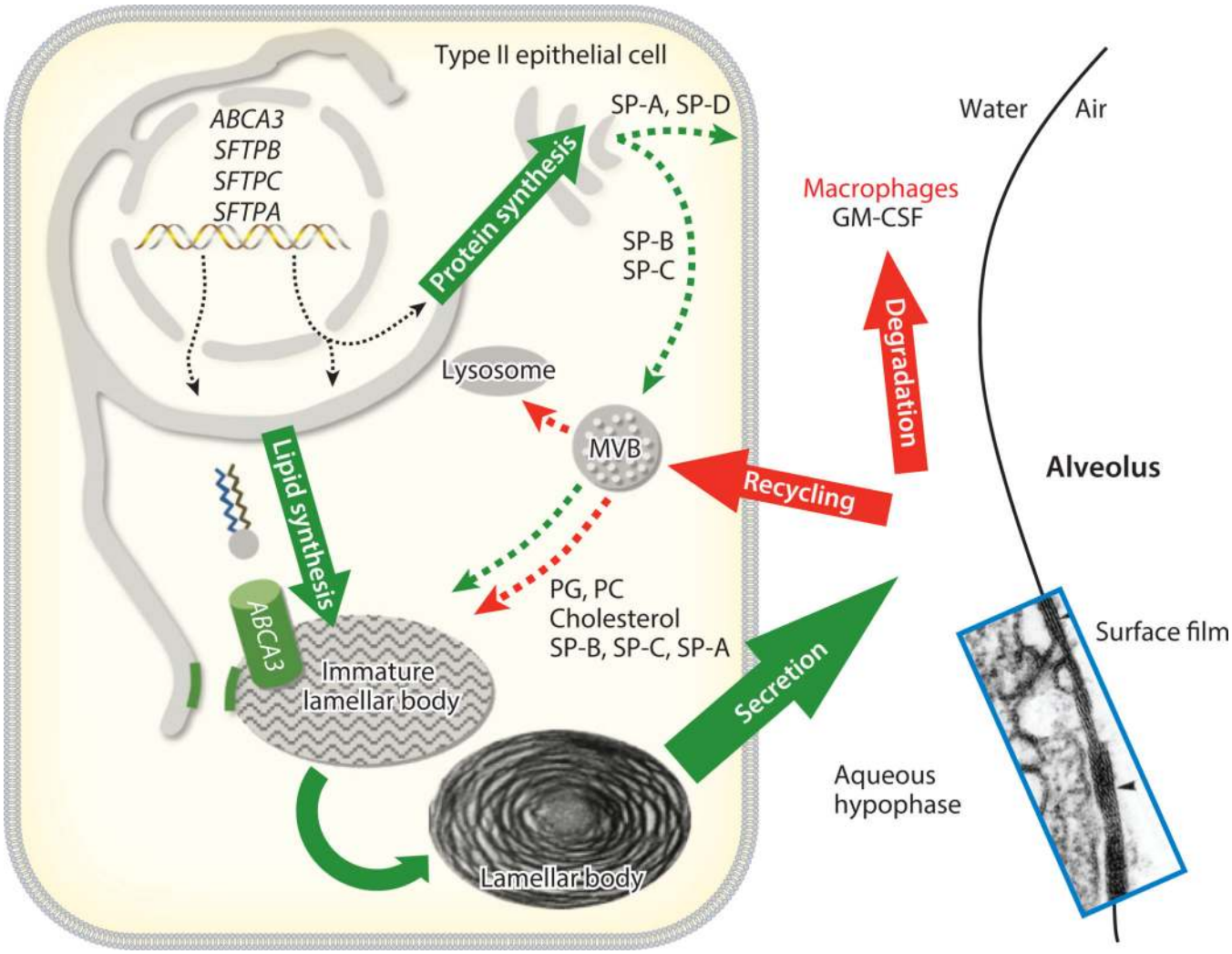

Figure 3.

Biosynthesis of surfactant likely involves distinct pathways for surfactant proteins and lipids. SP-B and SP-C are trafficked from the endoplasmic reticulum to lamellar bodies via the Golgi complex and MVB; in contrast, surfactant phospholipids are likely directly transported from the endoplasmic reticulum to specific lipid importers (ABCA3) in the lamellar body–limiting membrane. Surfactant proteins and lipids are assembled into bilayer membranes that are secreted into the alveolar airspace, where they form a surface film at the air–liquid interface. Cyclical expansion and compression of the bioactive film results in the incorporation (*large green arrow*) and loss (*red arrows*) of lipids and proteins from the multilayered surface film. Surfactant components removed from the film are degraded in alveolar macrophages or are taken up by type II epithelial cells for recycling or degradation in the lysosome (*red arrows*). The MVB plays a key part in the integration of pathways for surfactant synthesis, recycling, and degradation. Abbreviations: ABCA3, ATP-binding cassette transporter A3; GM-CSF, granulocyte macrophage colony–stimulating factor; MVB, multivesicular body; PC, phosphatidylcholine; PG, phosphatidylglycerol; SP/SFTP, surfactant protein.

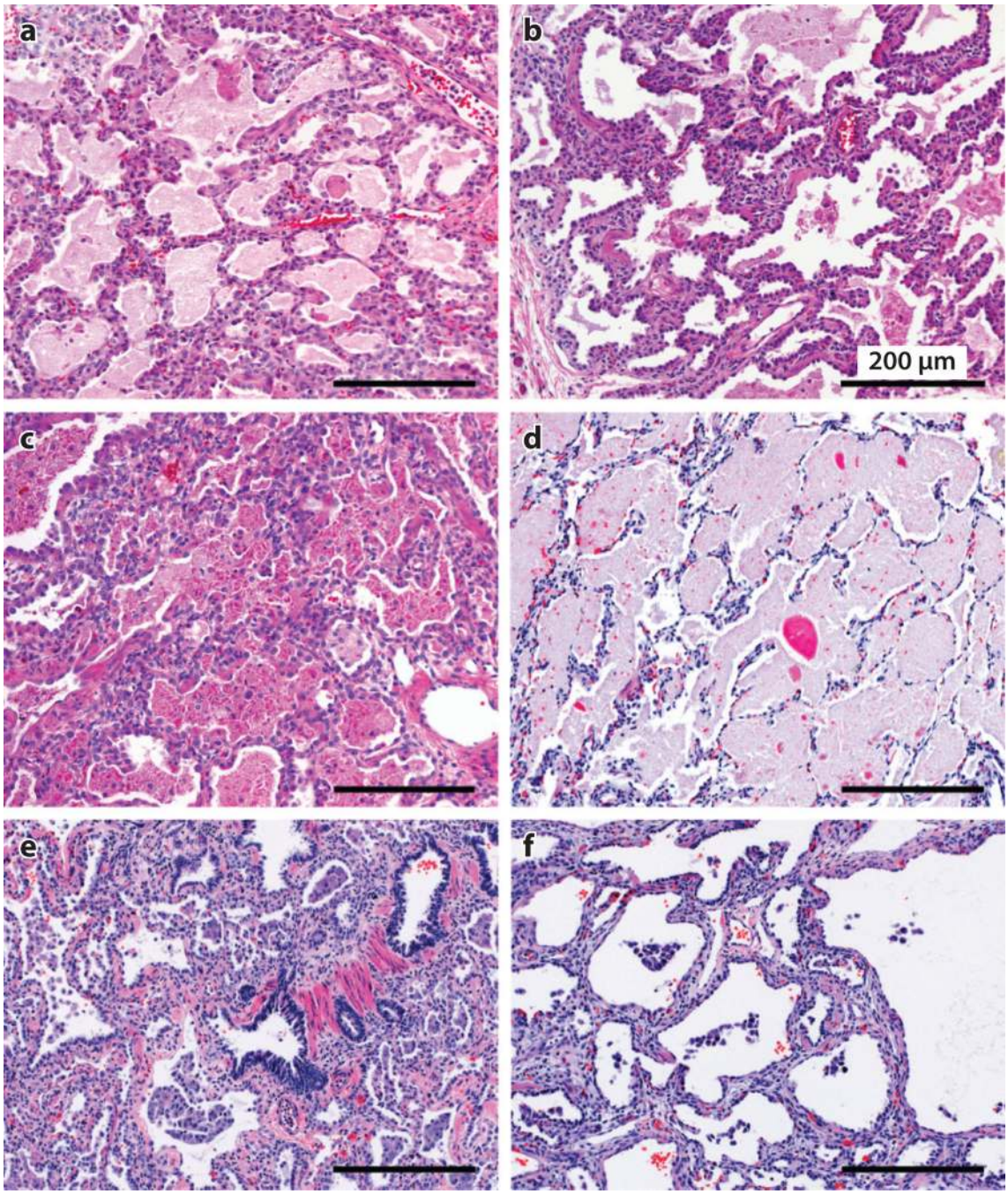

Figure 4.

Histopathology of genetic disorders of surfactant homeostasis. (*a*) Histopathological specimen from the lung of a neonate with a lethal *SFTPB* mutation, demonstrating the typical pattern of pulmonary alveolar proteinosis composed of intraalveolar, foamy, eosinophilic, lipoproteinaceous material and thickened alveolar septa. (*b*) Histopathological specimen from the lung of an infant with a *SFTPC* mutation and chronic pneumonitis of infancy, showing muscularization of the thickened alveolar septa; patchy, intraalveolar, granular, proteinosis material; and alveolar type II cell hyperplasia. (*c*) Histopathological

specimen from the lung of a neonate with a lethal ATP-binding cassette transporter A3 (*ABCA3*) mutation, demonstrating granular, eosinophilic, alveolar proteinosis material mixed with macrophages; thickened alveolar septa; and alveolar type II cell hyperplasia. (*d*) Histopathological specimen from the lung of a child with a mutation in the αchain of the granulocyte macrophage colony–stimulating factor (GM-CSF) receptor (*CSF2RA*), demonstrating both foamy and globular intraalveolar pulmonary alveolar proteinosis but with alveolar septa that appear thin and more normal. (*e*) Histopathological specimen from an infant with an NKX2-1 mutation, demonstrating features consistent with the genetic surfactant disorders including thickened, muscularized alveolar septa; intraalveolar accumulation of foamy macrophages (as seen in desquamative interstitial pneumonia); and hyperplastic alveolar type II cells. (*f*) Histopathological specimen from an infant with an *NKX2-1* (*TTF-1*) mutation, demonstrating a mixed phenotype consisting of a diffuse alveolar growth abnormality that is superimposed on chronic interstitial inflammation and fibrosis and that resembles nonspecific interstitial pneumonia. All specimens are stained with hematoxylin and eosin; scale bars = 200 μm for all panels.

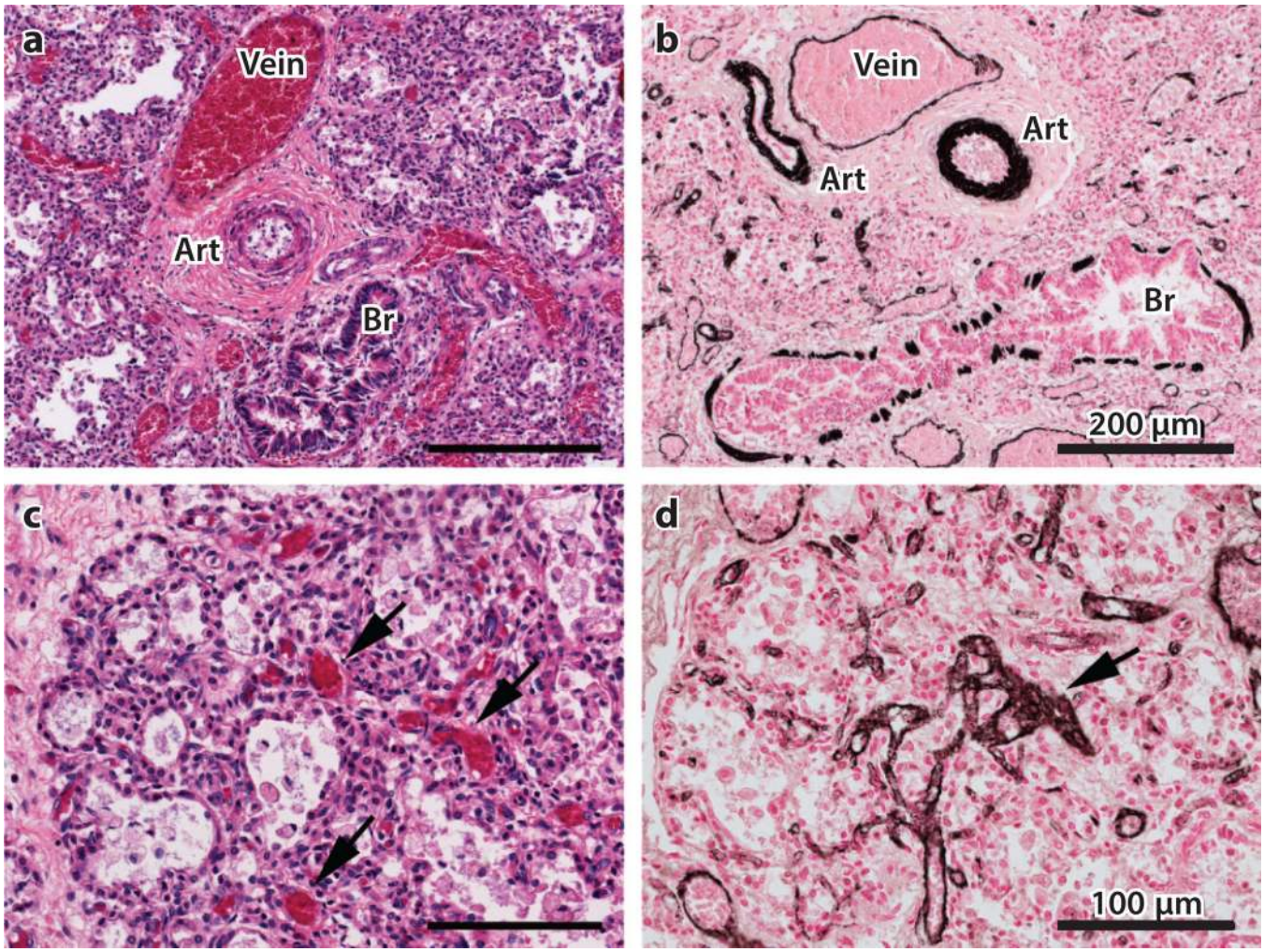

Figure 5.

Histopathology of alveolar capillary dysplasia with misalignment of the pulmonary veins. Histopathological specimen from the lung of a neonate with a lethal *FOXF1* mutation. (*a*) The pulmonary veins are large, dilated, congested vessels that are mislocated and lie adjacent to the pulmonary artery in the bronchoarterial compartment. (*b*) The pulmonary medial wall of the arteries is hypertrophic with increased immunostaining for α smooth muscle actin. (*c*) The pulmonary lobes and segments are hypoplastic, with abnormal formation of the parenchyma. There are decreased numbers of acinar and alveolar structures, with thickened interacinar and alveolar septa. The capillary bed is abnormal and has a decreased number of capillaries; these are disorganized and located primarily in the center of the thickened interstitial compartment (*arrows*). (*d*) Immunostaining for von Willebrand factor, a marker of endothelial cells, highlights the abnormal capillary network (*arrows*). Specimens in panels *a* and *c* were stained with hematoxylin and eosin; panels *b* and *d* were stained with immunoperoxidase and nuclear fast red counterstain. Scale bars for panels *a* and *b* = 200 μm; scale bars for panels *c* and *d* = 100 μm. Abbreviations: Art, artery; Br, bronchiole.

Table 1

Histopathology of genetic lung disease

Histopathology	Age groups	Characteristics	Associated genetic mutations or acquired disorder
Growth abnormalities	Newborns, infants, and children	Alveolar simplification with poorly septated airspaces; malformed lobules	*FOXF1*, *NKX2-1*
Pulmonary alveolar proteinosis, neonatal onset	Newborns and infants	Intraalveolar accumulation of granular, eosinophilic, or PAS-positive lipoproteinaceous material, with or without accumulation of large, foamy alveolar macrophages hyperplastic alveolar type 2 cells, and septal thickening	*SFTPB*, *SFTPC*, *ABCA3*, *NKX2-1*
Pulmonary alveolar proteinosis, pediatric and adult onset	Children and adults	Intraalveolar accumulation of granular, eosinophilic, or PAS-positive lipoproteinaceous material, with or without accumulation of large, foamy alveolar macrophages	*CSF2RA*, *CSF2RB*, anti-CSF2
Chronic pneumonitis of infancy	Newborns and infants	Septal thickening with mild lymphocytic inflammation and muscularization of the alveolar septa; intraalveolar accumulation of foamy macrophages; focal proteinosis and cholesterol clefts; hyperplastic type 2 cells	*SFTPC*
Desquamative interstitial pneumonia, infantile	Newborns and infants	Intraalveolar accumulation of alveolar macrophages, with or without hyperplasia of alveolar type 2 cells	*ABCA3*, *SFTPB*, *SFTPC*, *NKX2-1*
Nonspecific interstitial pneumonia	Children and adults	Interstitial pneumonitis with diffuse lymphocytic infiltrates and varying degrees of fibrosis	*SFTPC*, *ABCA3*, *NKX2-1*
Usual interstitial pneumonia	Children and adults	Alternating areas of normal lung with fibroblastic foci; progressing to dense fibrosis, with remodeling and scarring of the lung in adults	*SFTPC*, *ABCA3*, *SFTPA2*, *TERT*, *TERC*

Abbreviations: PAS, periodic acid–Schiff.

Table 2

Comparison of genetic disorders affecting surfactant metabolism

Gene	*SFTPB*	*SFTPC*	*ABCA3*	*CSF2RA* and *CSF2RB*
Chromosome	2p11	8p21	16p13	Xp22 and 22q12
Protein	SP-B	SP-C	ABCA3	CSF2RA and CSF2RB
Phenotype	Surfactant dysfunction 1	Surfactant dysfunction 2	Surfactant dysfunction 3	Surfactant dysfunctions 4 and 5
Inheritance	Autosomal recessive	Autosomal dominant or sporadic, with variable penetrance	Autosomal recessive	Autosomal recessive
Mechanism	Loss of function	Dominant negative or toxic gain of function	Loss of function	Loss of function
Pathogenesis	Lack of SP-B	Lack of mature SP-C; misfolded protein with ER stress	Defective phospholipid transport into LBs	Defective GM-CSF signaling
Age of onset	Neonatal	Infancy to adult	Neonatal > childhood	Childhood or neonatal > childhood > adult
Clinical syndrome	RDS	ILD > RDS	RDS > ILD	Respiratory distress and/or insufficiency; dyspnea; tachypnea
Outcome	Neonatal lethal	Highly variable	Lethal if neonatal; variable severity in childhood	Variable severity
Histopathology	PAP > DIP	Depends on age and mutation Infants: CPI > PAP or DIP Children/adults: NSIP, UIP	Depends on age and mutation Infants: PAP, DIP Children/adolescents: NSIP, UIP	PAP
LB phenotypes	Abnormal: large MVBs with no normal LBs	Variable: normal LBs and/or ↑ in large fusing LBs	Variable: small, dense LBs > normal LBs	ND[a]

Abbreviations: ABCA3, ATP-binding cassette transporter A3; CPI, chronic pneumonitis of infancy; CSF2R, granulocyte-macrophage colony–stimulating factor receptor; DIP, desquamative interstitial pneumonia; ER, endoplasmic reticulum; ILD, interstitial lung disease; LB, lamellar body; MVB, multivesicular body; NSIP, nonspecific interstitial pneumonia; PAP, pulmonary alveolar proteinosis; RDS, respiratory distress syndrome; SP/SFTP, surfactant protein; UIP, usual interstitial pneumonia.

[a]ND, not determined for type II cells; large, foamy alveolar macrophages filled with surfactant material or large vacuoles containing neutral lipids, or both, were observed with electron microscopy.

Table 3

Comparison of genetic disorders affecting growth, patterning, and fibrotic remodeling of the lungs

Gene	*NKX2-1*	*FOXF1*	*SFTPA2*	*SLC34A2*	*TERC* and *TERT*
Chromosome	14q13	16q24	10q22	4p15	3q26 and 5p15
Protein	NKX2-1 (TTF-1)	FOXF1	SP-A	SLC34A2	TERC and TERT
Phenotype	Brain-thyroid-lung syndrome	ACD/MPV	IPF	IPF	IPF
Inheritance	Sporadic or autosomal dominant with variable penetrance	Sporadic or autosomal dominant → maternal inheritance with paternal imprinting	Autosomal dominant	Autosomal recessive	Autosomal dominant with incomplete penetrance
Mechanism	Unknown	Unknown	Loss of function	Loss of function	Loss of function
Pathogenesis	Unknown	Unknown	Misfolded protein with ER stress	Reduced phosphate clearance	Telomere shortening
Age of onset	Neonatal > childhood	Neonatal	Adults	Adults	Adults
Clinical Syndrome	RDS > ILD	RDS	Familial IPF; lung cancer	Familial IPF	Familial IPF
Outcome	Lethal if neonatal; variable severity in childhood	Lethal if neonatal	Survival into adulthood	Survival into adulthood	Survival into adulthood
Histopathology	Growth disorder with alveolar simplification and lobular remodeling; PAP, DIP, NSIP	ACD/MPV; growth disorder with impaired lobe formation	UIP, pulmonary adenocarcinoma	Pulmonary microlithiasis	UIP

Abbreviations: ACD/MPV, alveolar capillary dysplasia with misalignment of pulmonary veins; DIP, desquamative interstitial pneumonia; ER, endoplasmic reticulum; FOX, forkhead box; ILD, interstitial lung disease; IPF, interstitial pulmonary fibrosis; NKX2-1, Nkx2 homeobox 1; NSIP, nonspecific interstitial pneumonia; PAP, pulmonary alveolar proteinosis; RDS, respiratory distress syndrome; SP/SFTP, surfactant proteins; TERC, telomerase RNA compartment; TERT, telomerase reverse transcriptase; TTF-1, thyroid transcription factor-1; UIP, usual interstitial pneumonia.