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Genetic Disorders of Surfactant Homeostasis

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Pulmonary · Surfactant proteins · Lamellar bodies · Respiratory distress syndrome · Hereditary lung disease

Abstract
Adaptation to air breathing at birth requires the precise orchestration of cellular processes to initiate fluid clearance, enhance pulmonary blood flow, and to synthesize and secrete pulmonary surfactant needed to reduce surface tension at the air-liquid interface in the alveoli. Genetic programs regulating the synthesis of the surfactant proteins and lipids required for the production and function of pulmonary surfactant are highly conserved across vertebrates, and include proteins that regulate the synthesis and packaging of pulmonary surfactant proteins and lipids. Surfactant proteins B and C (SP-B and -C) are small, uniquely hydrophobic proteins that play important roles in the stability and spreading of surfactant lipids in the alveolus. Deletion or mutations in SP-B and -C cause acute and chronic lung disease in neonates and infants. SP-B and -C are synthesized and packaged with surfactant phospholipids in lamellar bodies. Normal lamellar body formation requires SP-B and a member of the ATP-binding cassette (ABC) family of ATP-dependent membrane-associated transport proteins, ABCA3. Mutations in ABCA3 cause fatal respiratory disease in newborns and severe chronic lung disease in infancy. Expression of SP-B, -C, and ABCA3 are coregulated during late gestation by transcriptional programs influenced by thyroid transcription factor-1 and forkhead box a2, transcription factors that regulate both differentiation of the respiratory epithelium and transcription of genes required for perinatal adaptation to air breathing.

Introduction

The lung is lined by diverse respiratory epithelial cell types, whose morphology, gene expression, and function vary along the cephalo-caudal axis of the lung and during lung morphogenesis. Cells lining peripheral air saccules undergo dramatic changes in gene expression prior to birth, as surfactant synthesis is induced in type II cells lining these saccules. Later in gestation, and in the early postnatal period, these saccules dilate, the lung parenchyma thins, and the saccules undergo septation as the alveolar-capillary interface is formed. Late in gestation, type II epithelial cells produce increasing amounts of surfactant phospholipids and proteins. Squamous alveolar type I cells, derived from type II cells, come in close apposition to the alveolar capillaries to form an efficient gas exchange surface required for ventilation after birth. Synthesis of pulmonary surfactant, including both lipids and the surfactant proteins B and C (SP-B and -C), increases in late gestation. These lipids and proteins are co-routed to lamellar bodies where they are packaged and stored prior to secretion into the air space. Since the production of pulmonary surfactant is gestational age dependent and is required for lung function at birth, preterm infants of-

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ten develop respiratory distress syndrome (RDS) related to the lack of pulmonary maturity and surfactant production. Lack of SP-B or -C causes severe respiratory disease in transgenic mice [1, 2]. Likewise, mutations in these surfactant proteins can cause respiratory failure at birth [3–5]. In this paper, we review the role of mutations in SP-B and -C, and in the ATP-binding cassette A3 (ABCA3) transport protein, in the pathogenesis of neonatal RDS and chronic lung disease in infancy.

Surfactant Protein B

SP-B is a hydrophobic amphipathic protein of 78 amino acids that is produced from a larger precursor of 381 amino acids and secreted by type II epithelial cells in the lung [for review, see 6, 7]. SP-B is an important component of surfactant replacement preparations used to treat RDS in preterm infants. Deletion of the *Sftpb* gene in transgenic mice causes respiratory failure at birth [1]. SP-B-deficient mice develop all the features of RDS. Reduction of SP-B to 20–30% of normal levels in adult mice also causes respiratory failure related to RDS [8]. Heterozygous SP-B+/– mice are sensitive to lung injury and develop respiratory failure when subjected to hyperoxia [9]. SP-B is required for the synthesis of normal lamellar bodies and the reduction of surface tension at the air-liquid interface. SP-B is required for the formation of lungs and tubular myelin, as well as the proteolytic processing of SP-C. Thus, SP-B-deficient mice and humans are also deficient in SP-C. SP-B, therefore, plays a critical role in surfactant homeostasis required for lung function in the neonatal and adult lung.

Autosomal Recessive Inheritance of SFTPB Mutations in Human Infants

More than 75 infants with mutations in *SFTPB* gene have been identified [10]. These mutations lead to the lack of synthesis of proSP-B and SP-B or to the synthesis of mutant proSP-B proteins within the lungs. Infants with hereditary SP-B deficiency are generally full-term and develop respiratory distress within the first few hours of birth. Clinical findings are consistent with those seen in preterm infants with RDS. However, in spite of surfactant treatment, ventilation, and extracorporeal membrane oxygenation, these infants generally die in the first few months after birth from respiratory failure. Lung pathology, observed after biopsy or autopsy, is consistent with congenital alveolar proteinosis, bronchopulmonary dysplasia, and chronic lung injury. Active SP-B peptide is absent in the lung, as assessed by immunostaining for proSP-B and SP-B, in *SFTPB* mutations in which the mRNA is unstable or truncated. In contrast, proSP-B or abnormal SP-B fragments may accumulate in the lungs of patients bearing mutations in which an abnormal proSP-B protein is synthesized. A definitive diagnosis is made from the nucleotide sequence of the mutated SP-B gene or cDNA in affected individuals and/or relatives. More than 25 distinct mutations in the SP-B gene have been identified, and all have been inherited as autosomal recessive genes [10]. The most common allele, the 121ins2, results in an unstable mRNA and lack of synthesis of proSP-B and SP-B [3]. Hereditary SP-B deficiency is generally fatal in early infancy despite intensive therapy. Several surviving infants have received lung transplants [11].

Surfactant Protein C

SP-C is a small, hydrophobic 33- to 34-amino acid polypeptide synthesized and secreted by type II epithelial cells in the lung [for review, see 6, 7]. Like SP-B, SP-C is routed through multivesicular bodies, proteolytically processed, and packaged in the lamellar body, where it is secreted into the airspace with SP-B and phospholipids. SP-C enhances surfactant spreading and stability and is an abundant component of surfactant replacement preparations derived from mammalian tissue. Mature (or active) SP-C is proteolytically cleaved from a larger primary translation product termed proSP-C and is subsequently palmitoylated. Processing of SP-C requires the presence of SP-B. Synthesis of the SP-C mRNA and protein increase prior to birth in preparation for respiration. Deletion of SP-C in SP-C-null mutant (SP-C–/–) mice disturbed surfactant stability and caused severe lung disease in mice but did not cause respiratory failure at birth. Although lamellar body formation occurred in SP-C–/– mice, inflammation, remodeling, and abnormal lipid accumulations were noted within the lungs of SP-C-deficient mice [2]. Likewise, SP-C deficiency was associated with severe interstitial lung disease in a family lacking mature (or active) SP-C [12, 13].

Dominantly Inherited Mutations in the SFTPC Gene

While deficiency of SP-C has been associated with severe lung disease in infancy, most mutations in the *SFTPC* gene are inherited or caused by de novo mutations that are inherited as a dominant gene [5]. Mutated proSP-C proteins have been identified in infants with

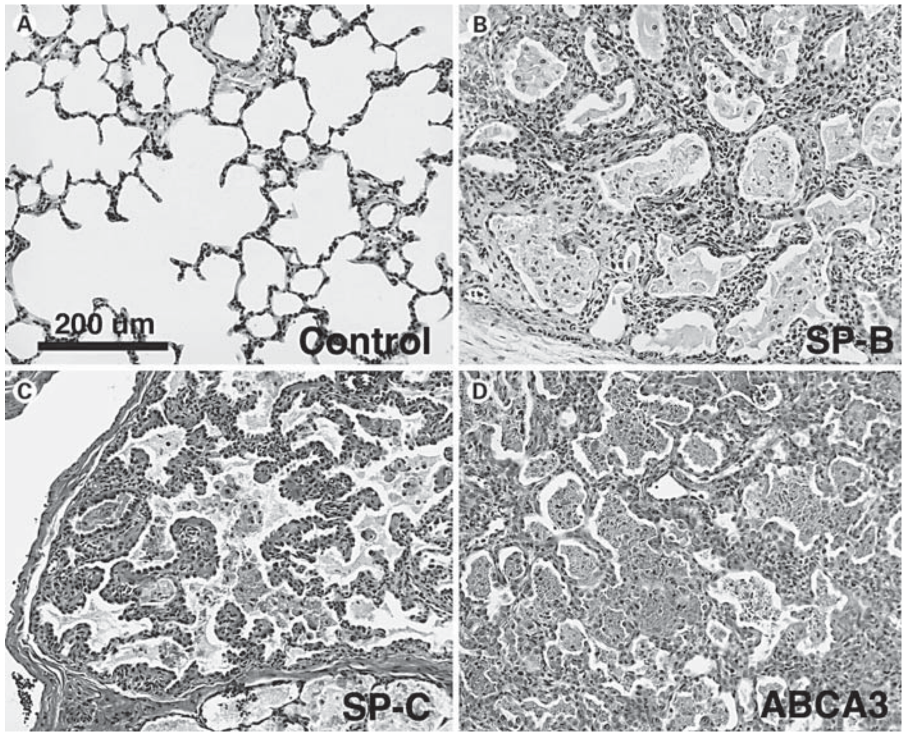

Fig. 1. Histopathology of surfactant abnormalities found in the lungs of human patients. **A** Normal pediatric lung. **B** Compound heterozygous allelic mutations in the *SFTPB* gene (121ins2/282–2delA). **C** A single allelic mutation in the *SFTPC* gene (P115L). **D** Homozygous allelic mutations in the *ABCA3* gene (4909+G→ A/4909+G→A). Common histopathological findings in infants with mutations in the *SFTPB*, *SFTPC,* or *ABCA3* genes (panels **B**, **C**, and **D**, respectively) include varying degrees of interstitial thickening and muscularization of the alveolar septa, remodeling of the alveolar epithelium with type II cell hyperplasia, and accumulation of eosinophilic, granular material mixed with alveolar macrophages in the airspaces. All tissue sections were stained with hematoxylin and eosin. All panels are shown at the same magnification. Bar = 200 µm.

RDS, severe chronic lung disease in infancy (chronic pneumonitis of infancy), and in nonspecific interstitial pneumonitis, interstitial pulmonary fibrosis, and usual interstitial fibrosis in older individuals [5, 12–14]. The pathology and age at disease presentation is highly variable, and the pathological findings are influenced by age, clinical course, and antecedent infections. Therefore, individuals from families bearing the same mutation may have highly variable age of onset and severity of lung disease. This suggests that environmental, as well as genetic, factors may play an important role in the pathogenesis of lung disease caused by *SFTPC* mutations. More than 25 mutations in the *SFTPC* gene have been identified, which are associated with severe lung disease. A definitive diagnosis is made from the nucleotide sequence of the SP-C cDNA or genes.

ABCA3 Transporter and Interstitial Lung Disease

SP-B and -C mutations account for a relatively small percentage of full-term babies who suffer severe respiratory failure in the newborn period. Recently, mutations in the ABCA3 transport protein were associated with respiratory failure in full-term infants [15] and inherited as an autosomal recessive allele. The ABCA3 protein is a member of a large family of ATP-dependent transporters, such as cystic fibrosis transmembrane conductance regulator and multiple-drug-resistant protein that are known to transport a variety of ligands, ions, proteins, peptides, carbohydrates, and lipids. In yeast, related proteins are known to transport phospholipids. The ABCA3 transport protein was identified at the limiting membrane of lamellar bodies in type II epithelial cells [16] supporting its potential role in lamellar body formation or function. The ABCA3 transporter is a large, 1,704-amino acid protein that is expressed in epithelial cells of the lung. Infants with ABCA3 transport mutations generally develop severe respiratory failure in the first 24 h of life. Chest X-rays are consistent with surfactant deficiency, displaying ground glass appearance and diffuse airspace abnormalities. Respiratory failure is refractory to surfactant replacement and mechanical ventilation. Lung pathology is consistent with pulmonary alveolar proteinosis and desquamating interstitial pneumonitis. There is accumulation of lipids, proteins, and macrophages within the airspaces, and the presence of a reactive cuboidal hyperplastic epithelium lining peripheral lung saccules is commonly observed. Thickened interstitium and abnormal remodeling ac-

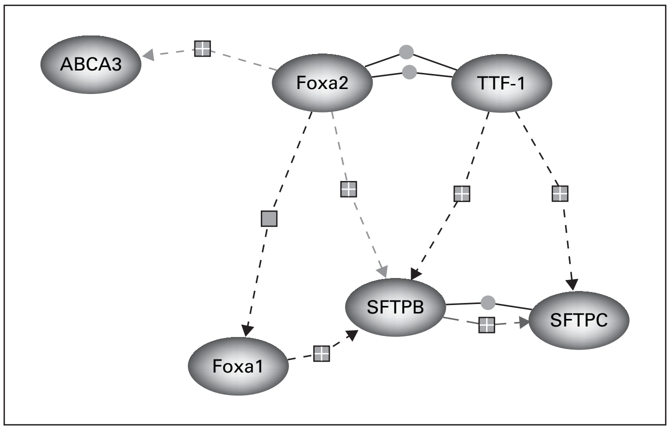

Fig. 2. Pathway interaction analysis. Functional associations and regulation of SP-B, -C, and ABCA3 were analyzed using Pathway Assist (Ariadne Genomics). Ovals indicate individual proteins. Each line indicates a regulatory relationship based upon the literature references (a solid line linking two nodes with a dot in between represents binding, a dashed line with a box in between represents expression). Regulatory relationships are denoted by arrows and signs within the box (plus sign indicates positive regulation, lack of sign indicates that regulation is unknown).

companies this disorder, and the pathology is dependent on clinical course and therapies required to support the infant. Mutations have been identified throughout the *ABCA3* gene. The cellular basis for type II cell dysfunction and the lack of surfactant in patients with ABCA3-related disease remains unknown. Electron microscopy of lung tissue from infants with ABCA3 transport mutations shows characteristic abnormalities in lamellar body structure [15]. Lamellar bodies in ABCA3-related lung disease are small and contain densely stained inclusions. Tubular myelin is absent and the airspaces are filled with macrophages, as well as lipid and proteinaceous debris. It has been proposed that defects in the ABCA3 transporter cause abnormalities in surfactant packaging, secretion, and function that lead to respiratory failure. Although the prevalence of ABCA3 transport mutations is not entirely clear at present, this disorder is more common than hereditary SP-B and -C deficiency, and may also contribute to familial interstitial lung disease in childhood and adults.

Taken together, abnormalities in surfactant homeostasis that are caused by mutations in the SP-B, -C, and ABCA3 genes disrupt type II epithelial cell function, surfactant production, and homeostasis, which result in similar clinical and pathological findings (fig. 1).

Transcriptional Programs Regulate Surfactant Homeostasis prior to Birth

Late in gestation, the mammalian lung undergoes dramatic changes in morphology, epithelial cell differentiation, and gene expression. The proteins and processes necessary for adaptation to air breathing are induced prior to birth. A number of critical processes are required for this adaptation including clearance of lung fluid, maintenance of appropriate airway hydration, mucociliary clearance, innate host defense to maintain sterility, and surfactant production to reduce surface tension at the air-liquid interface. Since ventilation is not required in utero, processes mediating formation of the embryonic lung are likely to be highly distinct from those required for adaptation to air breathing. The applications of mRNA microarray analyses and gene targeting have led to the identification of a number of genes which regulate or influence respiratory adaptation. Since cell differentiation and functional maturation are often mediated by nuclear transcription factors that govern gene expression, we have begun to explore the genetic networks underlying lung epithelial cell differentiation and adaptation at birth. The promoters, which direct expression of the surfactant proteins and ABCA3, share elements consistent with their activation by transcription factors, including thyroid transcription factor-1 (TTF-1) and the forkhead box a (Foxa) family of the same transcription factors (fig. 2). Targeted deletion of TTF-1 in the mouse prevents formation of peripheral air saccules and differentiation of alveolar type II and type I cells [17]. To identify genes regulated by TTF-1, a phosphorylation mutant was generated in transgenic mice by genetic 'knockin' or replacement of the TTF-1 gene [18]. Although this phosphorylated mutant TTF-1 supported formation of the peripheral lung tissue, thereby rescuing lung morphogenesis, the mice died at birth from respiratory failure. Microarray analysis demonstrated a reduction in the mRNAs encoding transcription of a number of potential direct targets of TTF-1, including the surfactant proteins, a number of host defense, and fluid transport proteins that are likely to play a role in type II epithelial cell function and neonatal adaptation. Since TTF-1 expression overlaps with that of Foxa2 and Foxa1, these two genes were deleted from the respiratory epithelium by a combination of gene targeting and/or conditional gene deletion to generate mice lacking Foxa2 in the respiratory epithelium or both Foxa2 and Foxa1 [19–21]. Deletion of Foxa2 resulted in the birth of pups who developed respiratory distress in the first 24 h with signs of pulmonary immaturity, lack of lamellar body formation, and surfactant deficiency [20]. Phospholipid synthesis and SP-A, -B, and -C were decreased in the Foxa2-deleted mice. Lung pathology demonstrated atelectasis, hyaline membranes, and pulmonary immaturity. Microarray analysis identified decreased SP-B, -C, and ABCA3 mRNAs, demonstrating the critical role of Foxa2 gene expression for production of these proteins which are critical for surfactant homeostasis. Thus, transcriptional programs dependent upon TTF-1 and Foxa2 play important roles in the regulation of genes that determine respiratory epithelial maturation and surfactant homeostasis in the perinatal period. These same transcriptional regulators are likely to play roles in repair of the respiratory epithelium following injury and to influence the pathogenesis of lung disease in the postnatal period.

In summary, it is increasingly clear that pulmonary homeostasis, both at birth and later in life, depends on the precise regulation of synthesis of the SP-B and -C, as well as the formation of lamellar bodies, which together, play critical roles in the establishment and maintenance of alveolar homeostasis. It is highly probable that other genes regulating surfactant homeostasis will be associated with lung disease in the future. Understanding the genetic networks regulating alveolar homeostasis will contribute to determination of the pathogenesis of both acute and chronic human lung disease.

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