The Molecular Era of Surfactant Biology

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Key Words

Lung diseases · Alveolar homeostasis · Surfactant biology

Abstract

Advances in the physiology, biochemistry, molecular and cell biology of the pulmonary surfactant system transformed the clinical care and outcome of preterm infants with respiratory distress syndrome. The molecular era of surfactant biology provided genetic insights into the pathogenesis of pulmonary disorders, previously termed 'idiopathic', that affect newborn infants, children and adults. Knowledge related to the structure and function of the surfactant proteins and their roles in alveolar homeostasis has provided new diagnostic, prognostic and therapeutic tools to advance our understanding of the causes and treatments of acute and chronic lung diseases. Severe lung disease in newborn infants and older patients is caused by mutations in genes regulating alveolar epithelial cells and surfactant homeostasis. Mutations in genes encoding the surfactant proteins, transcription factors critical for alveolar morphogenesis and surfactant clearance, are now known to play important roles in the pathogenesis of chronic lung diseases. Identification of the genes underlying the diseases of alveolar homeostasis is useful for the diagnosis of lung disease before and after birth.

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Introduction

Rapid advances in physiology and biochemistry made in the mid-20th century provided insights into the mechanisms underlying the previously perplexing problem of respiratory distress syndrome (RDS) in preterm infants. Before 1970, RDS was a common, often lethal, pulmonary disorder causing respiratory failure in preterm newborn infants, regardless of the degree of prematurity. Analysis of lung tissues from preterm infants that had died from RDS revealed a paucity of surface-active lipid-rich material needed to reduce surface tension at the air-liquid interface [1]. The ability to measure alveolar blood gases, adjust oxygenation and ventilation with continuous positive airway pressure and the technique of mechanical ventilation brought rapid improvements in the morbidity and mortality associated with RDS in preterm infants in the 1970s. The knowledge that pulmonary surfactant is rich in phosphatidylcholine, particularly disaturated palmitoyl phosphatidylcholine, provided the impetus to understanding its biochemistry and structure. Physiologic studies of animal models of RDS demonstrated the efficacy of replacing pulmonary surfactant by means of the intratracheal delivery of surfactant and surfactant lipid extracts in reversing atelectasis and the pathological changes in pulmonary physiology [2]. Organic solvent

Table 1. Diseases of alveolar homeostasis

	Neonatal respiratory failure	Chronic lung diseases in children	Adult pulmonary disease
Genes	<i>SFTPB</i> <i>ABCA3</i> <i>SFTPC</i> <i>NKx2-1 (TTF-1)</i> <i>FOXM1</i>	<i>SFTPC</i> <i>NKX2-1</i> <i>CSF-R1/2</i>	<i>SFTPC</i> <i>SFTPA</i> <i>TEL, TELC</i> <i>SLC34ac</i>

extracts of bovine and porcine surfactant material were developed that are widely used for the prevention and treatment of RDS in preterm infants [3–5]. These discoveries transformed the care of preterm infants, reducing the morbidity and mortality associated with RDS [6]. The organic solvent extracts of pulmonary surfactant spread rapidly and are stable during repeated dynamic compression of lipid films. The biophysical behaviors of surfactant lipid-rich extracts are highly distinct from those of synthetic phosphatidylcholine-lipid mixtures, indicating that minor components in the organic solvent extracts contribute importantly to the unique surface activity of pulmonary surfactants. Such observations led our laboratory and others to identify two novel surfactant proteins, now termed SP-B and SP-C, which are critical components of surfactant replacement preparations [7, 8]. The small, hydrophobic peptides, SP-B and SP-C, were found to impart surfactant-like properties to purified phospholipid mixtures needed for the reversal of atelectasis in surfactant-deficient lungs [8–10]. Concomitant with the advances in the clinical application of surfactant replacement in the 1980s were rapid advances in molecular biology that enabled their purification, the identification of their amino acid sequences and the preparation of antibodies and molecular probes used to clone the cDNAs and genes encoding surfactant proteins A, B, C and D [11–15 for review] to identify their functions. These molecular tools opened the investigative doors to beginning to understand fundamental questions regarding lung formation, perinatal lung maturation, the structure and function of the surfactant proteins that have been cornerstones for the application of molecular biology in the study of lung biology and the diagnosis and treatment of pulmonary diseases. This new tool-kit was used to (1) quantitate surfactant proteins in pulmonary tissues, bronchoalveolar lavage fluid and serum, (2) identify disease-associated mutations in the genes encoding both the

surfactant proteins and those critical for the synthesis of surfactant that provided insight into the molecular basis of ‘idiopathic’ respiratory failure in full-term infants and other interstitial lung diseases (ILDs) affecting newborn infants and older individuals [16] and (3) produce recombinant surfactant proteins and peptides for the production of synthetic surfactants. This brief review will focus on some of the advances in the application of molecular biology to the pathogenesis, diagnosis and treatment of pulmonary diseases (table 1).

Mutations in Genes Encoding ‘Surfactant-Associated’ Proteins Cause Respiratory Failure and Chronic Pulmonary Disease

Mutations in the genes encoding surfactant proteins play a central role in the pathogenesis of respiratory failure in full-term, newborn infants whose clinical courses are unresponsive to intensive care therapies. An ever-enlarging catalogue of mutations and alleles in genes critical for lung formation and function is associated with respiratory disease in infants and older patients [17, 18 for review]. Sequencing of surfactant-related proteins and thyroid transcription factor-1 (TTF-1) genes provided the diagnostic tools to identify lung disease causing alleles and those contributing to disease susceptibility. Genetic causes of respiratory failure are now routinely considered when term infants present with severe, unexplained respiratory failure with signs and symptoms of RDS that are normally associated with lung disease in preterm infants. These infants usually require oxygen, mechanical ventilation and with continued respiratory failure, are often treated with extracorporeal membrane oxygenation but fail to respond to therapy. *SFTPB*, encoding the surfactant peptide SP-B, a small, amphipathic peptide synthesized by type II alveolar epithelial cells [11, 12], was the first gene associated with this disorder [19]. SP-B is present in animal-based surfactant preparations and when added to phospholipids, enhances their surface properties. SP-B is processed from a precursor protein, proSP-B, which is proteolytically processed by several proteases including napsin, pepsinogen and cathepsins as it transits to lamellar bodies in type II epithelial cells in the alveoli. Studies in transgenic mice lacking SP-B demonstrated that it is required for the formation of lamellar bodies, tubular myelin and surface tension lowering in the peripheral lung, resulting in respiratory failure at birth [20]; likewise, the loss of SP-B in the adult mouse causes respiratory failure [21]. Lack of SP-B disrupts the formation of the lamellar

body, blocks the normal processing of proSP-C and impairs surfactant reuptake, causing respiratory failure. A number of mutations have been identified in *SFTPB* that are inherited as autosomal recessive genes [18, 22]. Heterozygosity for alleles causing nonsynonymous changes in the *SFTPB* have been associated with an increased risk for RDS in patients with chronic obstructive pulmonary disease. Infants with signs and symptoms similar to SP-B deficiency may instead have a deficiency of another protein, ABCA3 (ATP-Binding Cassette A3) as the cause of respiratory failure [23, 24]. ABCA3 is a large, transmembrane protein located on the surface of the limiting membranes of lamellar bodies in type II epithelial cells. ABCA3 mediates the transfer of phosphatidylcholine into lamellar bodies. A lack of ABCA3 inhibits surfactant packaging and secretion, causing respiratory failure after birth. ABCA3 is encoded by a relatively large gene and cDNA in which numerous mutations have been identified that cause severe lung disease in newborn infants [24 for review]. Pathological evaluations of tissue from infants with SP-B- and ABCA3-related pulmonary disease are consistent with the pathological descriptions of disorders termed 'chronic pneumonitis of infancy', 'infantile alveolar proteinosis', 'infantile chronic lung disease' or 'non-specific interstitial lung disease', the pathology often being influenced by the age of the infants and the therapies used to treat them [17].

Mutations in *NKX2-1* (Thyroid Transcription Factor-1) Cause Cerebral-Thyroid-Lung Syndrome

The cloning of the surfactant protein genes *SFTPA*, *SFTPB*, *SFTPC*, *SFTPD* and *ABCA3* enabled the identification of the transcription factor TTF-1 (encoded by the *NKX2-1* gene, a homeodomain-containing transcription factor) as a critical regulator of their expression in type II epithelial cells in the developing and mature lung [25]. TTF-1 is expressed in the thyroid, lung and forebrain, where it plays diverse roles in their formation and function. Subsequent studies demonstrated that TTF-1 is required for lung morphogenesis, regulating the differentiation and proliferation of epithelial progenitor cells that form the respiratory epithelium [26]. TTF-1 regulates the expression of many genes associated with the differentiation of type II epithelial cells prior to birth, including *ABCA3* and the genes encoding the surfactant proteins, its activity being required for respiratory adaptation at birth [27]. Mutations in the *NKX2-1* gene were identified in patients with respiratory failure or severe chronic lung

disease that is variably associated with CNS dysfunction (chorea) and hypothyroidism [28, 29]. *NKX2-1*-related disease is generally associated with haploinsufficiency or mutations in *NKX2-1* that are now amenable to genetic diagnosis. A recent study identified an association of a mutation in *NKX2-1* with the pulmonary disorder termed neuroendocrine hyperplasia of infancy [30].

Mutations in *FOXF1* Cause Alveolar Capillary Dysplasia

Alveolar capillary dysplasia (ACD) is a rare cause of respiratory failure in newborn infants [31]. Infants usually present soon after birth with severe pulmonary hypertension, cyanosis and respiratory failure that is refractory to ventilatory support and does not resolve after extracorporeal membrane oxygenation. Histopathological studies demonstrate abnormal alveolar formation, a paucity of alveolar capillaries and, frequently, a misalignment of pulmonary arteries and veins. Haploinsufficiency of *Foxm1* in transgenic mice caused respiratory failure at birth, associated with striking abnormalities in the formation of the pulmonary vasculature with features similar to those in infants with ACD [32]. Genetic studies on patients with ACD have identified mutations in the human gene [33]. Mutations in *FOXF1* are now recognized as the most common genetic cause of the disorder in newborn infants. *Foxf1*, expressed in mesenchymal cells in the early embryo, plays an important role in the formation of other organs including the heart and gastrointestinal tract. Infants with ACD and mutations in *Foxf1* frequently have malformations of other organs [31]. The diagnosis of ACD can be made by identification of mutations in *FOXF1*, useful for the prenatal and postnatal diagnosis of this severe respiratory disease. In the postnatal mouse lung, *Foxf1* plays a role in the regulation of lung inflammation; this supports its potential role in the pathogenesis of lung disease beyond the newborn period [34].

Mutations in *SFTPC* and Infantile Chronic Lung Diseases

While neonatal respiratory distress has been associated with mutations of *SFTPC* in a small number of infants, patients with *SFTPC*-related disease usually present after the newborn period in infancy [17, 18 for review]. Like SP-B, the active SP-C peptide is synthesized, processed and secreted by alveolar type II epithelial cells [35]. SP-C

is present in lamellar bodies, tubular myelin and animal-based surfactant replacement preparations used clinically. SP-C plays an important role in enhancing the rapid spreading and stability of surfactant lipids in the alveolus. Transgenic *Sftpc*^{-/-} mice, lacking SP-C, survive postnatally but develop severe ILD after birth and after lung injury caused by viral and bacterial pathogens and endotoxin [36–38]. Consistent with these findings, infants with mutations in *SFTPC* generally present with chronic ILD that is often exacerbated by viral infection. *SFTPC*-associated ILD is caused by dominantly inherited mutations that cause misfolding of the ‘BRICHOS’ domain of proSP-C [39, 40]. Misfolded proSP-C accumulates intracellularly, causing injury to type II epithelial cells [41] which, in turn, causes lung inflammation and remodeling. In some mutations, misfolded proSP-C induces the endoplasmic-reticulum stress response resulting in cytotoxicity, inflammation, remodeling and fibrosis that is associated with ILD [41, 42]. *SFTPC*-related lung disease is variably penetrant [43], being influenced by both environmental and other genetic modifiers. *SFTPC* gene-related disorders may present early in the neonatal period, during childhood or adulthood, although it is rare as a cause of chronic lung disease in adults [17, 44]. Findings from patients with mutations in *SFTPC* were the first to highlight the role of the misfolded protein/endoplasmic reticulum stress response in the pathogenesis of pulmonary fibrosis, ‘non-specific interstitial pneumonitis’, idiopathic pulmonary fibrosis and other disorders broadly classified as ILD.

***SFTPA* and Telomerase in the Pathogenesis of ILD in Adults**

Human SP-A is encoded by two *SFTPA* genes, *SFTPA1* and *SFTPA2*. SP-A is synthesized and secreted by airway epithelial cells where it is required for formation of tubular myelin. SP-A plays diverse roles in the innate immune defenses of the lung [14, 45]. In contrast to SP-B and ABCA3, SP-A is not required for perinatal survival of *Sftpa*^{-/-} mice [46]. Mutations in *SFTPA* are associated with ILD in adults and with susceptibility to pulmonary adenocarcinoma [47 for review]. SP-A consists of a glycosylated C-terminal lectin domain and an NH₂-terminal collagenous domain that forms trimeric complexes that further oligomerize to form multimers in the airway. SP-A plays a critical role in the structure of surfactant lipid particles, but does not play a major role in the regulation of surfactant pool sizes. SP-A binds to endotoxin and viral, bacterial and fungal pathogens in the lung, enhancing their opso-

nization and clearance. Like *SFTPC*, some mutations in *SFTPA* cause misfolded protein responses and epithelial cell injury. Recent studies support the concept that mutations in SP-A activate TGF- β signaling that may be involved in the pathogenesis of alveolar lung disease and lung cancer [47, 48]. The concept that recurrent epithelial injury is involved in the pathogenesis of ILD is supported by studies demonstrating that mutations in telomerase-associated genes (*TEL* and *TELC*) are associated with chronic pulmonary disease in adults [49–51 for review].

Molecular Diagnostics for Pulmonary Diseases

Identification of the genes and molecular pathways regulating surfactant homeostasis in type II epithelial cells provided the reagents for the development of clinical tests with genetics, immunochemistry or immunoassay that are useful for diagnosis and prognosis of acute and chronic lung diseases. Purification of the surfactant proteins from lung tissue or after the expression of recombinant surfactant proteins or the synthesis of surfactant protein peptides enabled the development of useful antibodies for the establishment of immunohistochemistry and immunoassays (ELISAs) that are now widely used for the identification of lung cells for the study of lung development and disease. These markers have served as useful indicators of the course of various pulmonary disorders [52–56]. Serum ELISAs for SP-D and SP-A are useful for the prognosis and treatment of patients with various interstitial lung disorders, including sarcoidosis, idiopathic pulmonary fibrosis and pulmonary alveolar proteinosis (PAP) [57]. Likewise, genetic testing has been useful for the identification of ‘disorders of surfactant homeostasis’ in the newborn period. The considerable allelic heterogeneity in genes encoding the surfactant proteins has enabled assessment of the risk or susceptibility of various surfactant protein gene alleles to lung disease. The molecular identification of mutations in the surfactant protein genes and TTF-1 are now useful tests for prenatal and postnatal diagnosis and the prognosis of disorders of surfactant homeostasis.

The Role of GM-CSF Signaling in the Pathogenesis of Pulmonary Alveolar Proteinosis

Studies in granulocyte macrophage colony-stimulating factor (GM-CSF) and GM-CSF receptor-deficient mice identified the important role of GM-CSF signaling

in the regulation of surfactant protein and lipid clearance from the lung [58 for review]. Subsequent clinical studies demonstrated that the common form of PAP in adults was caused by anti-GM-CSF antibodies that block GM-CSF signaling in alveolar macrophages [59], which is required for the catabolism of pulmonary surfactant proteins. Recent genetic studies in children with early-onset PAP with clinical features similar to those associated with adult autoimmune PAP, demonstrated the autosomal recessive inheritance of mutations in the GM-CSF receptors, *CSF2RB* and *CSF2RA* [60–62]. The identification of the critical role of GM-CSF signaling in the regulation of surfactant catabolism has provided novel assays for the diagnoses of both forms of PAP and has enabled new therapies based on treatment with GM-CSF [63] or the correction of GM-CSF receptor-signaling defects that are being actively studied at present [64–66].

Synthesis of Synthetic Surfactant Proteins and Peptides

Elucidation of the genes, cDNAs and amino acid sequences of the surfactant proteins has provided information that is useful for the engineering of recombinant or synthetic surfactant proteins and peptides. Peptides modeled from the structures of SP-B and SP-C are now produced and recombined with surfactant-like lipids to produce synthetic surfactant mixtures. Novel peptides have been synthesized that confer excellent surfactant-like properties to lipid mixtures. SP-B- and SP-C-like peptide mimics are being actively studied for the treatment of pulmonary diseases [67–70]. KL4, an amphipathic peptide with features of SP-B, was recently approved for the treat-

ment of RDS in the USA [69]. Likewise, mixtures of SP-B- and SP-C-like peptides have been tested in animal models where they have been found to be effective in enhancing lung function. Synthetic peptide-lipid mixtures have excellent biophysical properties and may be useful for future therapies for the treatment or prevention of RDS and other lung disorders [71, 72].

Summary

The molecular era of surfactant biology has provided new insights into alveolar homeostasis useful for the diagnosis, prognosis and therapy of lung disorders previously termed ‘idiopathic’. The postgenome era of whole genomic and exomic sequencing will likely uncover mutations causing or contributing to inherited and acquired lung diseases that will provide a conceptual framework for the prevention and treatment of pulmonary diseases in patients of all ages in the future.

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References

- 1 Avery ME, Mead J: Surface properties in relation to atelectasis and hyaline membrane disease. *Am J Dis Child* 1959;97:517–523.
- 2 Enhorning G, Robertson B: Lung expansion in the premature rabbit fetus after tracheal deposition of surfactant. *Pediatrics* 1972;50:58–66.
- 3 Fujiwara T, Maeta H, Chida S, Morita T, Watabe Y, Abe T: Artificial surfactant therapy in hyaline-membrane disease. *Lancet* 1980;1: 55–59.
- 4 Yu S, Harding PG, Smith N, Possmayer F: Bovine pulmonary surfactant: chemical composition and physical properties. *Lipids* 1983;18: 522–529.
- 5 Egberts J, de Winter JP, Sedin G, de Kleine MJ, Broberger U, van Bel F, Curstedt T, Robertson B: Comparison of prophylaxis and rescue treatment with Curosurf in neonates less than 30 weeks’ gestation: a randomized trial. *Pediatrics* 1993;92:768–774.
- 6 Stevens TP, Harrington EW, Blennow M, Soll RF: Early surfactant administration with brief ventilation vs. selective surfactant and continued mechanical ventilation for preterm infants with or at risk for respiratory distress syndrome. *Cochrane Database Syst Rev* 2007; 4:CD003063.
- 7 Whitsett JA, Hull WM, Ohning B, Ross G, Weaver TE: Immunologic identification of a pulmonary surfactant-associated protein of molecular weight = 6000 daltons. *Pediatr Res* 1986;20:744–749.
- 8 Whitsett JA, Ohning BL, Ross G, Meuth J, Weaver T, Holm BA, Shapiro DL, Notter RH: Hydrophobic surfactant-associated protein in whole lung surfactant and its importance for biophysical activity in lung surfactant extracts used for replacement therapy. *Pediatr Res* 1986;20:460–467.
- 9 Notter RH, Shapiro DL, Ohning B, Whitsett JA: Biophysical activity of synthetic phospholipids combined with purified lung surfactant 6000 dalton apoprotein. *Chem Phys Lipids* 1987;44:1–17.

- 10 Rider ED, Ikegami M, Whitsett JA, Hull W, Absolom D, Jobe AH: Treatment responses to surfactants containing natural surfactant proteins in preterm rabbits. *Am Rev Respir Dis* 1993;147:669–676.
- 11 Whitsett JA, Wert SE, Weaver TE: Alveolar surfactant homeostasis and the pathogenesis of pulmonary disease. *Annu Rev Med* 2010; 61:105–119.
- 12 Whitsett JA, Weaver TE: Hydrophobic surfactant proteins in lung function and disease. *N Engl J Med* 2002;347:2141–2148.
- 13 McCormack FX, Whitsett JA: The pulmonary collectins, SP-A and SP-D, orchestrate innate immunity in the lung. *J Clin Invest* 2002;109: 707–712.
- 14 Arika S, Nishitani C, Kuroki Y: Diverse functions of pulmonary collectins in host defense of the lung. *J Biomed Biotechnol* 2012;2012: 532071.
- 15 Perez-Gil J, Weaver TE: Pulmonary surfactant pathophysiology: current models and open questions. *Physiology* 2010;25:132–141.
- 16 Curstedt T, Calkovska A, Johansson J: New generation synthetic surfactants. *Neonatology* 2013;103:327–330.
- 17 Kurland G, Deterding RR, Hagood JS, Young LR, Brody AS, Castile RG, Dell S, et al; American Thoracic Society Committee on Childhood Interstitial Lung Disease and the Child Research Network: An official American Thoracic Society clinical practice guideline: classification, evaluation, and management of childhood interstitial lung disease in infancy. *Am J Respir Crit Care Med* 2013;188:376–394.
- 18 Wert SE, Whitsett JA, Noguee LM: Genetic disorders of surfactant dysfunction. *Pediatr Dev Pathol* 2009;12:253–274.
- 19 Noguee LM, Garnier G, Dietz HC, Singer L, Murphy AM, deMello DE, Colten HR: A mutation in the surfactant protein B gene responsible for fatal neonatal respiratory disease in multiple kindreds. *J Clin Invest* 1994;93: 1860–1863.
- 20 Clark JC, Wert SE, Bachurski CJ, Stahlman MT, Stripp BR, Weaver TE, Whitsett JA: 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.
- 21 Melton KR, Nesselin LL, Ikegami M, Tiche-lar JW, Clark JC, Whitsett JA, Weaver TE: SP-B deficiency causes respiratory failure in adult mice. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L543–L549.
- 22 Noguee LM, Wert SE, Proffitt SA, Hull WM, Whitsett JA: Allelic heterogeneity in hereditary surfactant protein B (SP-B) deficiency. *Am J Respir Crit Care Med* 2000;161:973–981.
- 23 Shulenin S, Noguee LM, Annilo T, Wert SE, Whitsett JA, Dean M: ABCA3 gene mutations in newborns with fatal surfactant deficiency. *N Engl J Med* 2004;350:1296–1303.
- 24 Hayes D Jr, Lloyd EA, Fitch JA, Bush A: ABCA3 transporter deficiency. *Am J Respir Crit Care Med* 2012;186:807.
- 25 Bohinski RJ, Di Lauro R, Whitsett JA: The lung-specific surfactant protein B gene promoter is a target for thyroid transcription factor 1 and hepatocyte nuclear factor 3, indicating common factors for organ-specific gene expression along the foregut axis. *Mol Cell Biol* 1994;14:5671–5681.
- 26 Kimura S, Hara Y, Pineau T, Fernandez-Salguero P, Fox CH, Ward JM, Gonzalez FJ: The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. *Genes Dev* 1996;10:60–69.
- 27 DeFelice M, Silberschmidt D, DiLauro R, Xu Y, Wert SE, Weaver TE, Bachurski CJ, Clark JC, Whitsett JA: TTF-1 phosphorylation is required for peripheral lung morphogenesis, perinatal survival, and tissue-specific gene expression. *J Biol Chem* 2003;278:35574–35583.
- 28 Guillot L, Carre A, Szinnai G, Castanet M, Tron E, Jaubert F, Broutin I, Counil F, Feldmann D, Clement A, Polak M, Epauld R: NKX2-1 mutations leading to surfactant protein promoter dysregulation cause interstitial lung disease in 'Brain-Lung-Thyroid Syndrome'. *Hum Mutat* 2010;31:E1146–E1162.
- 29 Hamvas A, Deterding RR, Wert SE, White FV, Dishop MK, Alfano DN, Halbower AC, et al: Heterogeneous pulmonary phenotypes associated with mutations in the thyroid transcription factor gene NKX2-1. *Chest* 2013; 144:794–804.
- 30 Young LR, Deutsch GH, Bokulic RE, Brody AS, Noguee LM: A mutation in TTF1/NKX2.1 is associated with familial neuroendocrine cell hyperplasia of infancy. *Chest* 2013;144: 1199–1206.
- 31 Alameh J, Bachiri A, Devisme L, Truffert P, Rakza TR, Riou Y, Manouvrier S, Lequien P, Storme L: Alveolar capillary dysplasia: a cause of persistent pulmonary hypertension of the newborn. *Eur J Pediatr* 2002;161:262–266.
- 32 Kalinichenko VV, Lim L, Stolz DB, Shin B, Rausa FM, Clark J, Whitsett JA, Watkins SC, Costa RH: Defects in pulmonary vasculature and perinatal lung hemorrhage in mice heterozygous null for the Forkhead Box f1 transcription factor. *Dev Biol* 2001;235:489–506.
- 33 Stankiewicz P, Sen P, Bhatt SS, Storer M, Xia Z, Beijani BA, Ou Z, et al: Genomic and genic deletions of the FOX gene cluster on 16q24.1 and inactivating mutations of FOXF1 cause alveolar capillary dysplasia and other malformations. *Am J Hum Genet* 2009;84:780–791.
- 34 Kalin TV, Meliton L, Meliton AY, Zhu X, Whitsett JA, Kalinichenko VV: Pulmonary mastocytosis and enhanced lung inflammation in mice heterozygous null for the FOXF1 gene. *Am J Respir Cell Mol Biol* 2008;39:390–399.
- 35 Vorbroker DK, Voorhout WF, Weaver TE, Whitsett JA: Posttranslational processing of surfactant protein C in rat type II cells. *Am J Physiol* 1995;269:L727–L733.
- 36 Glasser SW, Detmer EA, Ikegami M, Na CL, Stahlman MT, Whitsett JA: Pneumonitis and emphysema in sp-C gene targeted mice. *J Biol Chem* 2003;278:14291–14298.
- 37 Glasser SW, Senft AP, Whitsett JA, Maxfield MD, Ross GF, Richardson TR, Prows DR, Xu Y, Korfhagen TR: Macrophage dysfunction and susceptibility to pulmonary *Pseudomonas aeruginosa* infection in surfactant protein C-deficient mice. *J Immunol* 2008;181:621–628.
- 38 Glasser SW, Maxfield MD, Ruetschilling TL, Akinbi HT, Baatz JE, Kitzmiller JA, Page K, Xu Y, Bao EL, Korfhagen TR: Persistence of LPS-induced lung inflammation in surfactant protein-C-deficient mice. *Am J Respir Cell Mol Biol* 2013;49:845–854.
- 39 Willander H, Askarieh G, Landreh M, Westermarck P, Nordling K, Keranen H, Hermanson E, et al: High-resolution structure of a BRICHOS domain and its implications for anti-amyloid chaperone activity on lung surfactant protein C. *Proc Natl Acad Sci USA* 2012;109:2325–2329.
- 40 Noguee LM, Dunbar AE, 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA: A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med* 2001;344:573–579.
- 41 Bridges JP, Xu Y, Na, CL, Wong HR, Weaver TE: Adaptation and increased susceptibility to infection associated with constitutive expression of misfolded SP-C. *J Cell Biol* 2006; 172:395–407.
- 42 Woischnik M, Sparr C, Kern S, Thurm T, Hector A, Hartl D, Liebis G, et al: A non-BRICHOS surfactant protein c mutation disrupts epithelial cell function and intercellular signaling. *BMC Cell Biol* 2010;11:88.
- 43 Thomas AQ, Lane K, Phillips J 3rd, Prince M, Markin C, Speer M, Schwartz DA, 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.
- 44 Lawson WE, Grant SW, Ambrosini V, Ambrosini V, Womble KE, Dawson EP, Lane KB, et al: Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. *Thorax* 2004;59:977–980.
- 45 Kingma PS, Whitsett JA: In defense of the lung: surfactant protein A and surfactant protein D. *Curr Opin Pharmacol* 2006;6:277–283.
- 46 Korfhagen TR, Bruno MD, Ross GF, Huelsman KM, Ikegami M, Jobe AH, Wert SE, et al: Altered surfactant function and structure in SP-A gene targeted mice. *Proc Natl Acad Sci USA* 1996;93:9594–9599.
- 47 Garcia CK: Idiopathic pulmonary fibrosis: update on genetic discoveries. *Proc Am Thorac Soc* 2011;8:158–162.
- 48 Maitra M, Dey M, Yuan WC, Nathanielsz PW, Garcia CK: Lung fibrosis-associated surfactant protein A1 and C variants induce latent transforming growth factor beta1 secretion in lung epithelial cells. *J Biol Chem* 2013; 288:27159–27171.

- 49 Iniesta P, Gonzalez-Quevedo R, Moran A, Garcia-Aranda C, de Juan C, Sanchez-Permaute A, Torres A, Diaz-Rubio E, Balibrea JL, Benito M: Relationship between 3p deletions and telomerase activity in non-small-cell lung cancer: prognostic implications. *Br J Cancer* 2004;90:1983–1988.
- 50 Devine MS, Garcia CK: Genetic interstitial lung disease. *Clin Chest Med* 2012;33:95–110.
- 51 Brennan P, Hainaut P, Boffetta P: Genetics of lung-cancer susceptibility. *Lancet Oncol* 2011;12:399–408.
- 52 Todd DA, Marsh MJ, George A, Henderson NG, Barr H, Sebastian S, Clark GT, Koster G, Clark HW, Postle AD: Surfactant phospholipids, surfactant proteins, and inflammatory markers during acute lung injury in children. *Pediatr Crit Care Med* 2010;11:82–91.
- 53 Ichiyasu H, Ichikado K, Yamashita A, Iyonaga K, Sakamoto O, Suga M, Kohrogi H: Pneumocyte biomarkers KL-6 and surfactant protein D reflect the distinct findings of high-resolution computed tomography in nonspecific interstitial pneumonia. *Respiration* 2012;83:190–197.
- 54 Zhang Y, Kaminski N: Biomarkers in idiopathic pulmonary fibrosis. *Curr Opin Pulm Med* 2012;18:441–446.
- 55 Ishikawa T, Kubota T, Abe H, Hirose K, Nagashima A, Togashi T, Seki K, Honma T, Yoshida T, Kamimura T: Surfactant protein-D is more useful than Krebs von den Lungen 6 as a marker for the early diagnosis of interstitial pneumonitis during pegylated interferon treatment for chronic hepatitis C. *Hepatogastroenterology* 2012;59:2260–2263.
- 56 Bowler RP: Surfactant protein D as a biomarker for chronic obstructive pulmonary disease. *COPD* 2012;9:651–653.
- 57 Honda Y, Kuroki Y, Matsuura E, Nagae H, Takahashi H, Akino T, Abe S: Pulmonary surfactant protein D in sera and bronchoalveolar lavage fluids. *Am J Respir Crit Care Med* 1995;152:1860–1866.
- 58 Trapnell BC, Whitsett JA, Nakata K: Pulmonary alveolar proteinosis. *N Engl J Med* 2003;349:2527–2539.
- 59 Kitamura T, Tanaka N, Watanabe J, Uchida, Kanegaski S, Yamada Y, Nakata K: Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. *J Exp Med* 1999;190:875–880.
- 60 Suzuki T, Maranda B, Sakagami T, Catellier P, Couture CY, Carey BC, Chalk C, Trapnell BC: Hereditary pulmonary alveolar proteinosis caused by recessive CSF2RB mutations. *Eur Respir J* 2011;37:201–204.
- 61 Suzuki T, Sakagami T, Rubin BK, Nogee LM, Wood RE, Zimmerman SL, Smolarek T, et al: Familial pulmonary alveolar proteinosis caused by mutations in CSF2RA. *J Exp Med* 2008;205:2703–2710.
- 62 Tanaka T, Motoi N, Tsuchihashi Y, Tazawa R, Kaneko C, Nei T, Yamamoto T, et al: Adult-onset hereditary pulmonary alveolar proteinosis caused by a single-base deletion in CSF2RB. *J Med Genet* 2011;48:205–209.
- 63 Uchida K, Nakata K, Carey B, Chalk C, Suzuki T, Sakagami T, Koch DE, et al: Standardized serum GM-CSF autoantibody testing for the routine clinical diagnosis of autoimmune pulmonary alveolar proteinosis. *J Immunol Methods* 2014;402:57–70.
- 64 Suzuki T, Mayhew C, Sallese A, Chalk C, Carey BC, Malik P, Wood RE, Trapnell BC: Use of induced pluripotent stem cells to recapitulate pulmonary alveolar proteinosis pathogenesis. *Am J Respir Crit Care Med* 2014;189:183–193.
- 65 Luisetti M, Kadja Z, Mariani F, Rodi G, Campo I, Trapnell BC: Therapy options in pulmonary alveolar proteinosis. *Ther Adv Respir Dis* 2010;4:239–248.
- 66 Suzuki T, Sakagami T, Young LR, Carey BC, Wood RE, Luisetti M, Wert SE, et al: Hereditary pulmonary alveolar proteinosis: pathogenesis, presentation, diagnosis, and therapy. *Am J Respir Crit Care Med* 2010;182:1292–1304.
- 67 Sarin VK, Gupta S, Leung TK, Taylor VE, Ohning BL, Whitsett JA, Fox JL: Biophysical and biological activity of a synthetic 8.7-kDa hydrophobic pulmonary surfactant protein SP-B. *Proc Natl Acad Sci USA* 1990;87:2633–2637.
- 68 Baatz JE, Sarin V, Absolom DR, Baxter C, Whitsett JA: Effects of surfactant-associated protein SP-B synthetic analogs on the structure and surface activity of model membrane bilayers. *Chem Phys Lipids* 1991;60:163–178.
- 69 Cochrane CG, Revak SD, Merritt TA, Heldt GP, Hallman M, Cunningham MD, Easa D, Pramanik A, Edwards DK, Alberts MS: The efficacy and safety of KL4-surfactant in preterm infants with respiratory distress syndrome. *Am J Respir Crit Care Med* 1996;153:404–410.
- 70 Cochrane CG: Surfactant protein B and mimic peptides in the function of pulmonary surfactant. *FEBS Lett* 1998;430:424;disc 425.
- 71 Speer CP, Sweet DG, Halliday HL: Surfactant therapy: past, present and future. *Early Hum Dev* 2013;89:S22–S24.
- 72 Seehase M, Collins JJ, Kuypers E, Jellema RK, Ophelders DR, Ospina OL, Perez-Gil J, Bianco F, Garzia R, Razzetti R, Kramer BW: New surfactant with SP-B and C analogs gives survival benefit after inactivation in preterm lambs. *PLoS One* 2012;7:e47631.