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The Molecular Era of Surfactant Biology

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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 diseases of alveolar homeostasis is useful for the diagnosis of lung disease before and after birth.

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 dying 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 (CPAP) and mechanical ventilation brought rapid improvements in the morbidity and mortality associated with RDS in preterm infants in the 1970s. Knowledge that pulmonary surfactant was highly enriched in phosphatidylcholine and particularly in disaturated palmitoyl phosphatidylcholine provided the impetus to understand its biochemistry and structure. Physiologic studies in animal models demonstrated the efficacy of replacing pulmonary surfactant by intratracheal delivery of surfactant and surfactant lipid extracts in reversing atelectasis and the pathological changes in pulmonary physiology in animal models of RDS [2]. Organic

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solvent 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 were stable during repeated dynamic compression of lipid films. The biophysical behaviors of surfactant lipid rich extracts were highly distinct from those of synthetic phosphatidylcholine-lipid mixtures, indicating that minor components in the organic solvent extracts contributed importantly to the unique surface activity of pulmonary surfactants. These observations led our laboratory and others to identify two novel surfactant proteins, now termed SP-B and SP-C, that 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 reversal of atelectasis in surfactant deficient lungs [8–10]. Concomitant with advances in the clinical application of surfactant replacement in the 1980s were rapid advances in molecular biology that enabled their purification, identification of their amino acid sequences, preparation of antibodies and molecular probes that were 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 begin to understand fundamental questions regarding lung formation, perinatal lung maturation, the structure and functions 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 synthesis of surfactant that provided insight into the molecular basis of “idiopathic” respiratory failure in full-term infants and other interstitial lung diseases affecting newborn infants and older individuals [16] and 3) produce recombinant surfactant proteins and peptides for 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.

Mutations in Genes Encoding “Surfactant Associated” Proteins Cause Respiratory Failure and Chronic Pulmonary Disease

Mutations in the genes encoding surfactant proteins play central roles in 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 and 18 for review]. Sequencing of surfactant related proteins and 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 (ECMO) 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, that 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 formation of lamellar bodies, tubular myelin and surface tension lowering in peripheral lung, resulting in respiratory failure at birth [20]; likewise, loss of SP-B in the adult mouse causes respiratory failure [21]. Lack of SP-B disrupts lamellar body formation, blocks 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 non-synonymous changes in the *SFTPB* have been associated with increased risk for RDS in patients with chronic obstructive pulmonary disease (COPD). 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 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 transfer of phosphatidylcholine into lamellar bodies. 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 “nonspecific interstitial lung disease,” the pathology often influenced by age and therapies used to treat the infants [17].

Mutations in *NKX2-1* (Thyroid Transcription Factor-1, TTF-1) Caused 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 thyroid, lung and forebrain where it plays diverse roles in their formation and function. Subsequent studies demonstrated that TTF-1 was required for lung morphogenesis, regulating differentiation and proliferation of epithelial progenitor cells that form the respiratory epithelium [26]. TTF-1 regulates expression of many genes associated with 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* Caused 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 ECMO. Histopathological studies demonstrate abnormal alveolar formation, paucity of alveolar capillaries and frequently misalignment of pulmonary arteries and veins. Haploinsufficiency of Foxm1 in transgenic mice caused respiratory failure at birth, associated with striking abnormalities in formation of the pulmonary vasculature with features similar to those in infants with ACD [32]. Genetic studies in patients with ACD 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 important roles in the formation of other organs including the heart and gastrointestinal tract. Infants with ACD and mutations in Foxf1 frequently have malformations in 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 regulation of lung inflammation supporting 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 and 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 rapid spreading and stability of surfactant lipids in the alveolus. Transgenic *Sftpc*$^{-/-}$ mice, lacking SP-C, survive postnatally but develop severe interstitial lung disease after birth and after lung injury caused by viral, bacterial pathogens and endotoxin [36–38]. Consistent with these findings, infants with mutations in *SFTPC* generally present with chronic interstitial lung disease that is often exacerbated by viral infection. *SFTPC* associated interstitial lung disease 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] that 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 interstitial lung disease [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 a rare case 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/ER stress response in the pathogenesis of pulmonary fibrosis “non-specific interstitial pneumonitis” (NSIP), idiopathic pulmonary fibrosis (IPF) and other disorders broadly classified as interstitial lung disease (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 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 regulation of surfactant pool sizes. SP-A binds to endotoxin, viral, bacterial and fungal pathogens in the lung, enhancing their opsonization 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-ß 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*, *TELC*) are associated with chronic pulmonary disease in adults [49–51 for review].

Molecular Diagnostics for Pulmonary Diseases

Identification of the genes and molecular pathway regulating surfactant homeostasis in type II epithelial cells provided the reagents for development of clinical tests by genetic, immunochemical 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 expression of recombinant surfactant proteins or synthesis of surfactant protein peptides enabled the development of useful antibodies for establishment of immunohistochemistry and immunoassays (ELISAs) that are now widely used for identification of lung cells for 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 [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 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 prognosis of disorders of surfactant homeostasis.

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

Studies in GM-CSF and GM-CSF receptor deficient mice identified the important role of GMCSF signaling in 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] that is required for 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 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 regulation of surfactant catabolism has provided novel assays for the diagnoses of both forms of PAP and enabled new therapy based on treatment with GM-CSF [63] or 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 provided information useful for the engineering of recombinant or synthetic surfactant proteins and peptides. Peptides modeled from the structures of SP-B and SP-C were 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 treatment of pulmonary diseases [67–70]. KL4, an amphipathic peptide with features of SP-B, was recently approved for treatment of RDS in the US [69]. Likewise, mixtures of SP-B and SP-C-like peptides have been tested in animal models where they have been effective in enhancing lung function. Synthetic peptide-lipid mixtures have excellent biophysical properties and may be useful for future therapies for 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 diagnosis, prognosis and therapy of lung disorders previously termed “idiopathic.” The post-genome era of whole genomic and exomic sequencing will likely uncover mutations causing or contributing to inherited and acquired lung diseases that will provide the conceptual framework for prevention and treatment of pulmonary diseases in patients of all ages in the future.

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Table 1

DISEASES OF ALVEOLAR HOMEOSTASIS

	Neonatal Respiratory Failure	**Chronic Lung Diseases in Children**	**Adult Pulmonary Disease**
GENES:	*SFTPB*	*SFTPC*	*SFTPC*
	ABCA3	*NKX2-1*	*SFTPA*
	SFTPC	*CSF*-R1/2	*TEL, TELC*
	NKx2-1 (TTF-1)		*SLC34ac*
	FOXM1		