HK J Paediatr (new series) 2001;**6**:96-99

Functions of Surfactant Proteins in Vivo Discerned by Gene Targeting in Transgenic Mice

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Abstract Mammalian pulmonary surfactants contain four distinct surfactant proteins, termed surfactant proteins A, B, C, and D (SP-A, SP-B, SP-C, and SP-D), that play unique roles in host defense, surfactant phospholipid homeostasis, and surfactant function. While in vitro studies supported overlapping roles for the surfactant proteins, unique functions for each surfactant protein were identified by studies in transgenic mice in which the surfactant proteins were deleted by gene targeting. Findings in transgenic mice lacking SP-A and SP-D demonstrated that each plays distinct roles in host defense, and in the regulation of surfactant structure and metabolism. Targeting of SP-B gene demonstrated its critical role in lung function after birth; mutations in the mouse or human SP-B gene caused respiratory failure in the immediate postnatal period. Likewise, mutations in the SP-C gene altered pulmonary function in mice. Thus, surfactant proteins play distinct and important roles in pulmonary function and host defense.

Key words Pulmonary surfactant protein

Composition of Pulmonary Surfactant

The critical role of the reduction of surface tension at the air-liquid interface in the pathophysiology of respiratory distress syndrome and the transition to air breathing after birth was recognized more than 40 years ago.[1] Surfactant phospholipids play a critical role in reducing surface tension in the alveolus, and the lack of surfactant causes respiratory distress syndrome (RDS) in preterm infants. In subsequent years intense research activities were focused to determine the composition and function of pulmonary surfactants.[2] While consisting predominantly of phospholipids, mammalian surfactant also contain neutral lipids, fatty acids, and proteins. Four distinct surfactant proteins, now termed SP-A, SP-B, SP-C, and SP-D, were identified in pulmonary surfactants. In the human lung, surfactant proteins are synthesized and secreted by respiratory epithelial cells, including Type II epithelial cells in the alveolus, and subsets of non-ciliated secretory cells in tracheal glands and conducting airways.[3-6] The structures of surfactant proteins were further elucidated by cDNA cloning, nucleotide and polypeptide sequencing that provided their primary structures. More recently, the role of surfactant proteins in lung function was determined by gene targeting in transgenic mice.

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Received November 2, 2000

Gene Targeting of the Pulmonary Collectins: SP-A and SP-D

SP-A and SP-D are members of a family of calcium-dependent collagenous lectins, encoded on a shared locus on human chromosome 10.[7] SP-A and SP-D share considerable structural homology, each containing conserved carboxy-terminal globular lectin-like domains (carbohydrate recognition domains or CRDs), and triple helical, collagenous regions that are bridged by amino terminal cysteines, that form the larger oligomers present in the airway. Under non-reducing conditions, SP-A migrates primarily as an octadecamer, while SP-D forms dodecamers. SP-A is expressed primarily in the lung being synthesized by serous cells in conducting airways, alveolar Type II cells, and in serous cells of tracheal-bronchial glands. In contrast, SP-D is expressed in numerous tissues, including the lung. In pulmonary tissues, SP-D is found in a distribution similar to that of SP-A, primarily being synthesized by epithelial cells in tracheal-bronchial glands

and in alveolar Type II cells. SP-A and SP-D share structures with other polypeptides in the collectin family, including conglutinin, CL-43, and the mannose binding lectin (MBL), a serum protein implicated in host defense. From shared structures and their ability to bind complex carbohydrates on the surfaces of microbes, it has been proposed that the collectins are a family of polypeptides involved in the binding and clearance of various bacterial, viral and fungal pathogens, serving important roles in innate defense.

Role of SP-A in Surfactant Structure and Host Defense

The mouse SP-A gene was targeted by homologous recombination, producing heterozygous and homozygous SP-A deficient mice.[8] SP-A deficient mice survive and breed normally under vivarium conditions. Careful analysis of pulmonary surfactant function and structure revealed that SP-A was not critical to pulmonary surfactant activity, lung function, surfactant phospholipid composition, secretion or reuptake. However, SP-A mice lack tubular myelin, the uniquely organized form of phospholipids in the airspace. The alveolar surfactant in the SP-A knockout (-/-) mouse consisted of poorly organized vesicles and membrane sheets.[8,9] Surfactant from SP-A (-/-) mice was less active than normal in reducing surface tension at low concentrations but functioned normally under physiological conditions. Furthermore, surfactant function in SP-A (-/-) mice was not altered after exercise, hyperoxia or lung injury.[10]

Role of SP-A in Host Defense

In spite of relatively normal lung function, SP-A deficient mice were highly susceptible to various bacterial and viral pathogens in vivo.[11-13] Because of the importance of group B streptococcal infection in neonates, and the low levels of SP-A seen in the lungs and amniotic fluid from preterm infants, susceptibility of the SP-A (-/-) mice to group B streptococcal infection was assessed. Bacteria were killed less efficiently in SP-A (-/-) mice.[11] Likewise, SP-A (-/-) mice were susceptible to *H. influenza*, *E. coli*, and *P. aeruginosa*. Pulmonary inflammation and expression of proinflammatory cytokines was increased during pulmonary infection of SP-A (-/-) mice. Neutrophilic infiltrates were also more severe and resolved more slowly in the absence of SP-A.[14] Increased susceptibility to inflammation and infection by influenza, RSV, and adenovirus were observed in the SP-A (-/-) mice.[12,13] Defects in binding, opsonization, and killing by alveolar macrophage was observed in cells from the SP-A (-/-) mice. SP-A plays a critical role in innate host defense, enhancing binding, opsonization and oxidative killing of respiratory pathogens, and modifying inflammatory response after infection. SP-A is decreased in lung of preterm infants, infants with bronchopulmonary dysplasia (BPD), viral and bacterial pneumonia[15] supporting its potential role in the pathogenesis of acute and chronic lung disease.

SP-D Regulates Surfactant Lipid Homeostasis and Host Defense

SP-D was deleted by gene targeting in transgenic mice.[16,17] SP-D (-/-) mice survive and breed normally under laboratory conditions. However, unlike SP-A (-/-) mice, markedly increased surfactant phospholipid pools were noted in lungs of SP-D (-/-) mice. Alveolar phospholipid content was increased three to five fold in SP-D (-/-) mice from birth to adulthood. The structure of surfactant phospholipids in SP-D (-/-) mice was abnormal, with decreased proportions of tubular myelin observed by electronmicroscopy.[16] Abnormalities in surfactant lipid pools, synthesis, secretion, reuptake, and catabolism were also observed in the SP-D (-/-) mice. Furthermore, dramatic changes in lung structure were consistently observed in the SP-D (-/-) mice from three weeks of age and thereafter. Histologic lesions in the SP-D (-/-) consisted of pulmonary infiltrates with enlarged, foamy macrophages, increased numbers of lymphocytes, emphysema and mild pulmonary fibrosis.[18] Increased oxidant production and matrix metalloproteinase expression (MMP-2, MMP-9 and MME) were observed in the lungs of SP-D (-/-) mice.

SP-D (-/-) Mice are Susceptible to Viral but Not Bacterial Infection

While SP-D (-/-) mice were not highly susceptible to bacterial infections by GBS, E. coli, H. influenzae, or P. aeruginosa, the mice were highly susceptible to infection by both respiratory syncytial virus and influenza A virus. Pulmonary infiltrates and expression of proinflammatory cytokines were increased and viral clearance was markedly decreased in SP-D (-/-) mice compared to wild type controls. Thus, SP-D plays an important role in viral clearance and pulmonary inflammation after infection. SP-D also plays an unanticipated role in the regulation of alveolar macrophage function, regulating production of reactive oxygen species, metalloproteinase or surfactant phospholipid homeostasis (Table 1).

Table 1 Functions of the surfactant collectins in vivo

SP-A	SP-D
Tubular myelin	Phospholipid metabolism
Bacterial host defense	Alveolar macrophage function
LPS binding	1. oxidant production
Innate defense: bacterial	2. metalloproteinase activity
viral	Innate defense: viral, other
Inflammation ↓	Anti-emphysema
Alveolar macrophage function	

Role of Hydrophobic Surfactant Proteins SP-B and SP-C

SP-B and SP-C are relatively abundant, extremely hydrophobic proteins that are strongly associated with surfactant lipids in the airspace. Both proteins are translated as larger preproproteins that are proteolytically processed and modified to generate the active peptides associated with surfactant phospholipids in the airway.[19] The active proteins of SP-B and SP-C are synthesized and secreted by Type II epithelial cells in the lung. While SP-C is expressed only in Type II epithelial cells, proSP-B is expressed in both serous cells in the conducting airways and in alveolar Type II cells in the lung. However, the active SP-B peptide is selectively produced by the alveolar Type II cell.[20] SP-B and SP-C are routed to and stored in lamellar bodies, and secreted into the air space, where they are detected in association with tubular myelin.[21] Both SP-B and SP-C are present as major protein components in mammalian based surfactant protein preparations used for therapy of RDS, including Survanta®, Curosurf®, Infrasurf®, and Alveofact®.[22] Addition of SP-B or SP-C to mixtures of phospholipids enhances the speed of spreading and stability during compression, consistent with function of mammalian surfactants.

Mutations in the SP-B Gene Cause Respiratory Failure in Newborn Mice and Infants

Targeted disruption of the mouse SP-B gene caused respiratory failure at birth.[23] While lung size and structure were normal, SP-B deficient mice did not inflate their lungs postnatally. Pressure volume curves demonstrated marked surfactant dysfunction in newborn SP-B (-/-) mice. Surfactant from SP-B (-/-) mice did not contain tubular myelin, and did not reduce surface tension. Furthermore, lamellar bodies were lacking from SP-B (-/-) mice and large multivesicular bodies accumulated in Type II cells. Furthermore, the proSP-C was not proteolytically processed to the active SP-C peptide, and the alveolar spaces of SP-B deficient mice filled with proSP-C. To date, more than 190 infants with hereditary SP-B deficiency related to mutations in the SP-B gene have been identified.[24] Most of the infants die from RDS or chronic lung disease in the neonatal period, in spite of intensive therapy. Replacement surfactant has not been a useful therapy for SP-B deficiency, since the disorder is characterized by marked dysfunction of alveolar Type II cells, and misrouting and processing of both lipids and proteins. More than 15 distinct mutations have been identified, and most result in respiratory failure.[24] Less severe SP-B mutations have been identified and associated with chronic lung disease in infancy.[25]

Mutation of the SP-C Gene Alters Surfactant Function

While data is preliminary at present, deficiency of SP-C has been associated with inherited chronic lung disease in several families.[26] Likewise, altered surfactant activity and lung function was noted in SP-C gene targeted mice.[27] Surfactant from SP-C (-/-) mice was less stable than controls, and lung function was abnormal during ventilation at low lung volumes. Recently, decreased SP-C in pulmonary tissues and mutations in the SP-C gene have been associated with chronic lung disease in infants.[28] These studies support studies demonstrating the efficacy of surfactant preparations containing recombinant SP-C and phospholipids.[29]

Summary

Gene targeting and experience with human infants with lung disease caused by abnormalities in surfactant proteins have revealed distinct functions of each surfactant protein in host defense, surfactant function, and surfactant homeostasis. As such, these proteins play important modulatory roles in maintaining surfactant function and structure. Surfactant proteins, SP-A and SP-D are critical modulators of host defense and inflammatory responses following various lung infections. Elucidation of the unique roles of each of the surfactant proteins has been facilitated by gene targeting in transgenic mice. It is hoped that improved diagnosis and therapies for pulmonary

disease in infants and adults will result from the elucidation of the roles of the surfactant proteins in lung function and host defense.

Acknowledgements

This summary was presented at the Department of Pediatrics, University of Hong Kong, March, 2000, and represents collaborations with numerous co-workers at Children's Hospital Medical Center, Cincinnati, Ohio.

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