

## Diseases of alveolar homeostasis

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Diseases of alveolar homeostasis cause acute and chronic lung disease. Surfactant homeostasis genes are critical for lung function, and are implicated in both acute and interstitial lung disease in children and adults. Genes involved in the pathological processes of these diseases have been isolated, and provide targets for genetic and cellular therapy for pulmonary disorders. This presentation discusses some of the genes and proteins responsible for surfactant production and function, and the effects of deficiencies in these proteins on pulmonary disease, focusing on three key genes: SP-B, SP-C and ABCA3.

### Components of pulmonary surfactant

Pulmonary surfactant is synthesised in the alveolar type II cells of the lungs, and is stored in lamellar bodies within these cells. Surfactant is required to reduce surface tension at the air-liquid interface of the epithelial cells, thus stabilising the alveoli during respiration. Deficiencies in pulmonary surfactant therefore cause respiratory failure.

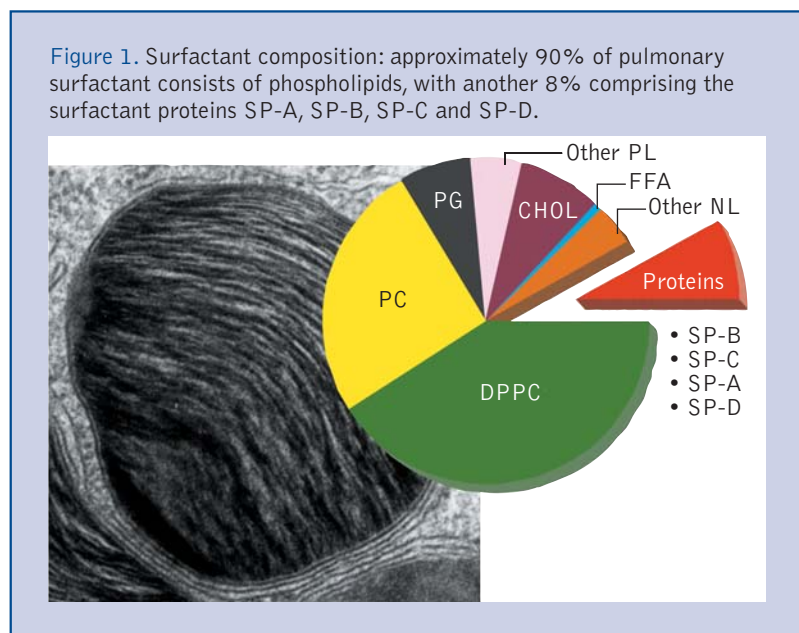
Pulmonary surfactant is 90% phospholipid, with another 8% being surfactant-associated proteins (Figure 1).

There are four proteins which play critical roles in surfactant function, structure and homeostasis: the hydrophobic proteins SP-B and SP-C, and the hydrophilic proteins SP-A and SP-D.

The hydrophilic SP-A and SP-D proteins do not appear to be involved in respiratory function, but are part of the innate immune system, responsible for binding pathogens in the lung. Both proteins influence the ultrastructure of surfactant, but do not contribute substantially to surface tension reduction.

The hydrophobic proteins SP-B and SP-C are involved in the production of tubular myelin, surfactant film formation and the recycling of phospholipids (Figure 2). Both increase the stability, adsorption and spreading of the surfactant phospholipids, and are essential for normal pulmonary homeostasis. Both SP-B and SP-C are stored in lamellar bodies and, along with surfactant lipids, are secreted into the alveolus. SP-B interacts with SP-A to form tubular myelin. The formation of multilayers and monolayers from tubular myelin reduces surface tension at the air-liquid interface.

In addition, ABCA3, the protein encoded by the gene for the ATP-binding cassette transporter A3, is involved in surfactant function and the formation of lamellar bodies, and may play a role in phospholipid transport within the type II cell.



Mutations in SP-B, SP-C and ABCA3 result in disruption of type II epithelial cell processes, leading to altered surfactant structure and function, and in turn respiratory distress and interstitial lung disease. Although they act by distinct mechanisms, SP-B, SP-C and ABCA3 deficiencies all cause respiratory failure and produce similar histopathology.

### SP-B is essential from birth

The hydrophobic protein SP-B is a 79 amino acid peptide produced from a 381 amino acid precursor by proteolytic processing which occurs specifically in pulmonary cells.<sup>[1]</sup> SP-B is selectively expressed in alveolar type II cells, and is required

for ventilation from birth. It is involved in the assembly of lamellar bodies and tubular myelin, and the formation of lipid monolayers and bilayers to maintain alveolar homeostasis during the respiratory cycle.

SP-B deficiency is conferred by autosomal recessive inheritance. Evaluation of the effects of SP-B deficiency in knockout mice has shown that animals homozygous for SP-B deficiency experience RDS at birth and die of irreversible respiratory failure. SP-B-deficient mice are unable to make lamellar bodies in type II cells, and package lipids in multivesicular bodies which cannot form tubular myelin. Heterozygous animals survive but have TTN, and require more time to recoup their lung volumes. These mice are more susceptible to oxygen toxicity and other causes of lung injury.

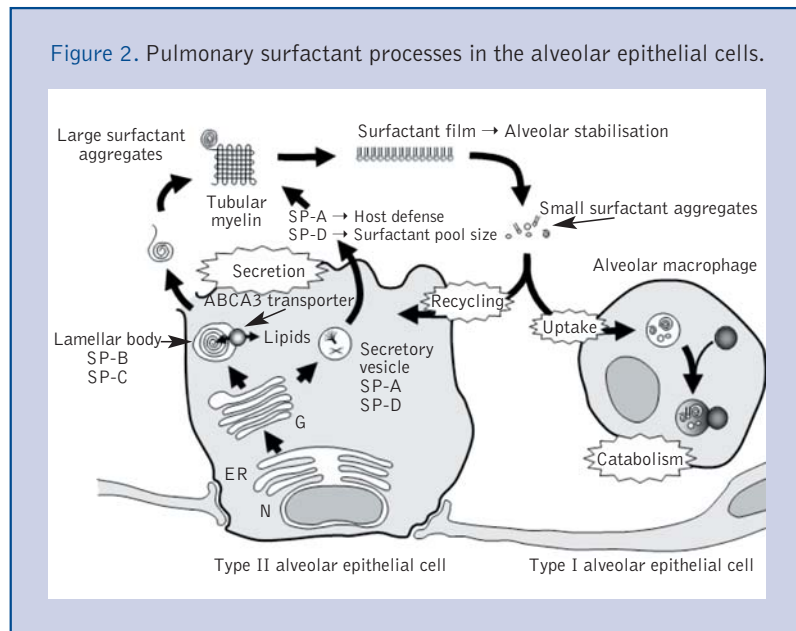
In humans, genetic SP-B deficiency manifests as perinatal RDS. Death generally occurs from severe lung disease within 3–6 months of birth. These infants have no lamellar bodies, tubular myelin or surfactant function, and the response to surfactant replacement therapy is unsustainable.

There are more than 15 distinct mutations which cause SP-B deficiency in humans, the most common being the 121 ins (null) mutation.<sup>[2]</sup> Most mutations in the SP-B gene cause truncation, leading to a lack of SP-B protein; however, some mutations lead to the production of a misfolded protein which can be detected by immunohistochemistry. SP-B deficiency can be diagnosed by sequencing of the cDNA or genes, assessment of the protein secreted into the air space, or by immunohistochemistry. SP-B-deficient individuals also misprocess the pro-SP-C precursor protein and therefore lack the SP-C protein. In addition, the misprocessed pro-SP-C is secreted into the alveolar air space where it accumulates, causing further damage to respiratory function.

### SP-C deficiency has multiple phenotypes

SP-C is a 34 amino acid protein formed from a 197 amino acid precursor, pro-SP-C, which is encoded by a single, relatively small gene on chromosome 8.<sup>[1,3]</sup> The SP-C protein disrupts packing and allows for the rapid spreading of lipids over the alveolar surface, with the consequent reduction of surface tension. At birth, SP-C deficiency is not as serious as SP-B deficiency, as this protein does not appear to be essential for the initiation of respiration. Most patients with mutations in the SP-C gene have a misfolded form of the protein, which produces a form of CLD caused by intrinsic epithelial cell abnormalities, rather than extrinsic inflammation. The misfolded protein accumulates and causes injury of type II epithelial cells. In addition, SP-C mutations may cause aggregation of misfolded precursor forms, which also leads to injury and inflammation of the lungs.

Hereditary SP-C deficiency follows a dominant inheritance pattern. Penetration of the phenotype is variable and



may manifest as lung disease in infants, RDS in neonates, ARDS and interstitial lung disease or idiopathic pulmonary fibrosis in adults and children. The effects of SP-C deficiency often present at times of infection, and it is likely that viral infection induces further misfolding of the protein, increasing the cellular stress and the severity of interstitial lung disease.

As is the case with SP-B, hereditary SP-C deficiency is caused by a number of different mutations. Due to the relatively small size of the SP-C gene, mutations can be diagnosed by gene sequencing.

### **ABCA3 is required for lamellar body formation**

The ABCA3 protein is required for alveolar homeostasis. It is essential for lamellar body formation and surfactant function. Its structural similarity to ABCA1 and ABCA4 suggests that ABCA3 is also likely to be involved in phospholipid transport.

Virtually all patients with ABCA3 mutations lack normal lamellar bodies in type II cells.<sup>[4]</sup> As with SP-B, mutations in the ABCA3 gene cause fatal surfactant deficiency in newborns, and may also be involved in other pulmonary disorders.

More than 75 different mutations have been identified in patients with ABCA3 deficiency. ABCA3 mutations are characterised by abnormal lamellar body structure, with patients having multiple small lamellar bodies with eccentrically placed, dense protein cores. Thus, electron microscopy can be used to diagnose ABCA3 mutation-associated lung disease.

### **Other genes yet to be identified**

The cellular disorders of alveolar homeostasis discussed in this presentation produce acute and chronic lung disease, and are caused by deficiencies in SP-B, SP-C and ABCA3. However, it is likely that additional genes in similar pathways, that are involved in the processing and trafficking of proteins and lipids in alveolar type II cells, may be implicated in the pathogenesis of acute and interstitial lung diseases in the future.

### **References**

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