Surfactant protein C mutations and familial pulmonary fibrosis: stuck in a loop on the scenic route

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Dysfunction in protein folding and intracellular processing are important mediators of disease. Detailed characterisation of SP-C deficiency mutations illuminates a pathway to alveolar epithelial cell damage that triggers familial pulmonary fibrosis. https://bit.ly/3KavSgu

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Alveolar lining fluid requires surfactant to prevent alveolar collapse at end expiration. This activity is generated by interactions of phospholipids with the hydrophobic surfactant proteins SP-B and -C [1]. Mutations in SP-B and -C cause familial pulmonary fibrosis [2, 3]. Whilst loss of surfactant activity is associated with respiratory distress syndromes, this does not seem to be the main driver of disease caused by SP-C mutations [4, 5], indicating there is instead a pathogenic gain-of-function. What is going on, and why should we care?

In this issue of the *European Respiratory Journal*, Dickens *et al.* [6] have addressed this for the I73T mutation, which is the commonest pathogenic variant affecting SP-C. Using state-of-the-art methods they have manipulated cellular pathways involved in protein trafficking to compare how the mutant and wild-type proteins are handled. Their findings demonstrate unexpected complexity in how type 2 alveolar epithelial cells (AECs) normally process SP-C, and how the I73T mutation specifically disrupts this to give rise to the distinctive cellular phenotype observed previously [7]. Those more interested in clinical disease than cell trafficking *per se* may care because it precisely defines a highly specific mechanism of type 2 AEC injury which results in pulmonary fibrosis, similar to what is observed with a wide range of other injury stimuli [8]. This improves our ability to interpret disease mechanism data from this model and so identify common downstream pathways for transduction of chronic injury into fibrosis. Ultimately, "splitting" and "lumping", personalisation and generalisation, provide complementary insights and therapeutic possibilities.

Secreted proteins are usually synthesised as polypeptide chains and directly deposited into the cell's endoplasmic reticulum (ER), where they fold into their functional three-dimensional structure. They are transported first through the Golgi compartment and then secretory vesicles that fuse with the cell surface to release the protein cargo extracellularly (figure 1a). Most secreted proteins fold with predominantly hydrophilic residues at the interface with hydrated intra- and extracellular environments. The correct processing of mature SP-C is therefore known to require a number of modifications, owing to its hydrophobicity [2, 3]. First, it is synthesised as the product of the *SFTPC* gene as a transmembrane helix flanked by two more hydrophilic domains within a pro-protein (pro-SP-C). It is joined by a linker to the C-terminal BRICHOS domain that also appears to act as a self-chaperoning unit to limit misfolding. These regions are cleaved prior to storage of mature SP-C within organelles known as lamellar bodies, ready for secretion. The highly ordered packing of SP-B and -C is complemented by import of phospholipids and cholesterol *via* the ATP-binding cassette subfamily A member (ABCA)3 channel (figure 1b). ABCA3 sits within the lamellar body outer membrane and operates under the control of thyroid transcription factor (TTF)-1.

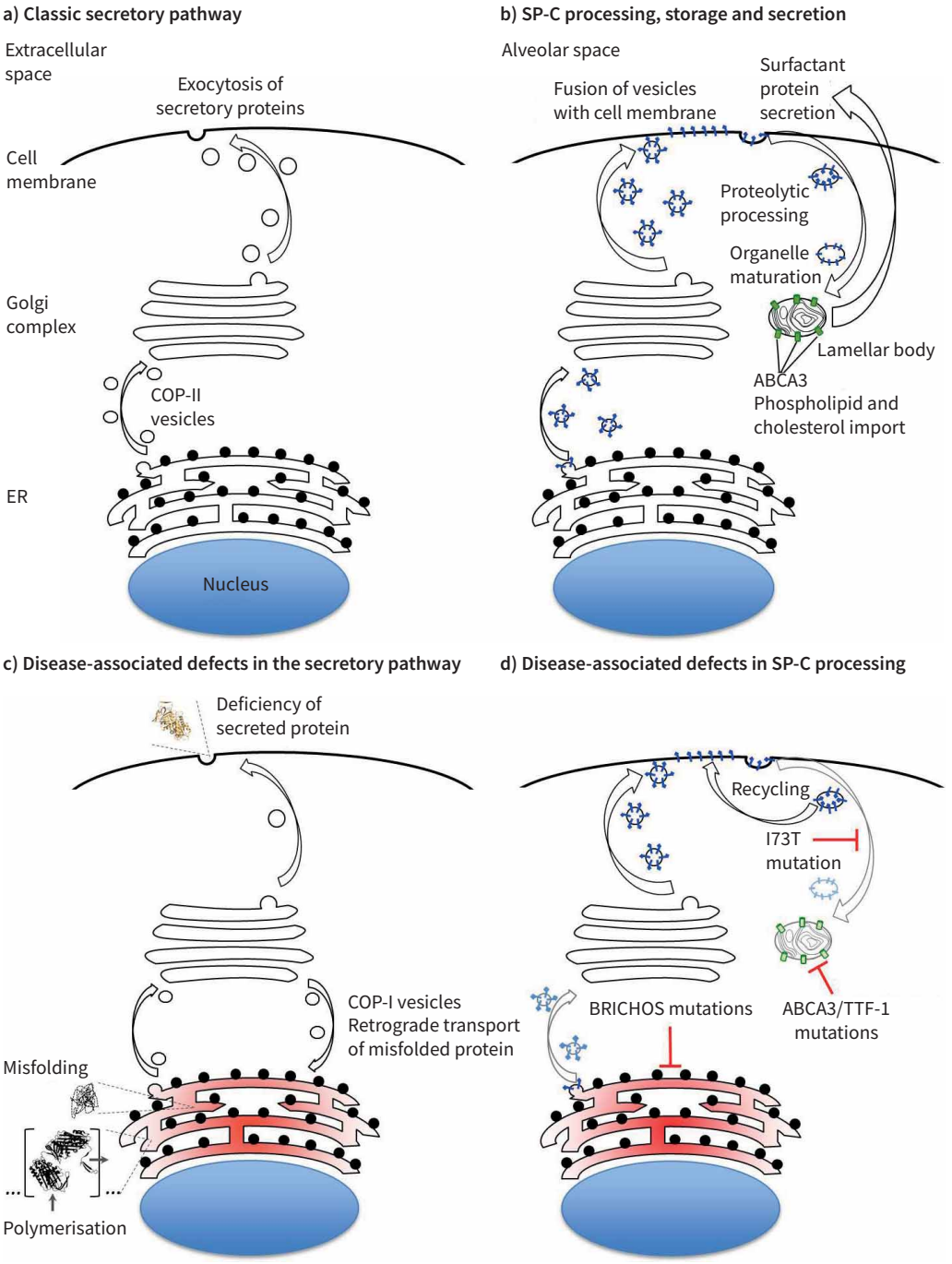

FIGURE 1 Protein handling and respiratory disease. a) In the classic secretory pathway mRNA transcripts include a signal sequence that directs protein translation from the ribosome (filled circles) into the endoplasmic reticulum (ER), where the protein folds into its functional three-dimensional structure. The folded protein is then packaged within a vesicle surrounded by a COP-II membrane protein lattice and transported into the Golgi complex, where further post-translational processing can occur, and packaged into secretory vesicles. These fuse with the cell membrane to release the protein into the extracellular space. Cell membrane proteins are similarly transported to the cell surface embedded within vesicle membranes, and retained. b) Whilst surfactant protein (SP)-C is a secreted protein, the findings of Dickens *et al.* [6] indicate that in type 2 alveolar epithelial cells (AECs) the SP-C precursor protein pro-SP-C (blue) initially adopts the latter course and is then re-internalised through endocytosis. Subsequent proteolysis of pro-SP-C generates mature SP-C. Endosomes containing SP-C develop into lamellar bodies where surfactant proteins and lipids are packed within spiral and parallel membrane arrangements, ready for secretion. Lipid import is enabled through the ATP-binding cassette subfamily A member (ABCA)3 channel (green) in the limiting membrane. c) Cellular

physiology appears very sensitive to dysfunction within protein folding, processing and trafficking pathways. Such events mediate a wide range of disease, illustrated here for α_1-antitrypsin (A1AT) deficiency. A1AT polymerisation is shown by A1AT molecules in a basic dimer linkage; arrows indicate further linkage events elongating polymer chains. The spectrum of consequences of protein misfolding within the ER, including aggregation and ER stress, is indicated by red shading. d) Mutations affecting SP-C synthesis, trafficking and storage demonstrate distinct cellular phenotypes but result in overlapping lung disease phenotypes, suggesting common downstream pathways in type 2 AECs. TTF-1: thyroid transcription factor 1.

Pathogenic mutations are found both within the BRICHOS domain of pro-SP-C and outside it, as is the case for the I73T variant which occurs within the linker region. Despite the clinical coherence of the broad disease phenotype, given the relatively small number of cases described, mutation site heterogeneity appears echoed by phenotypic heterogeneity at the cell level. In general, mutations that affect secretory proteins post-translationally within the cell do so during folding within the ER. Misfolded proteins are recognised and degraded by quality control mechanisms but, as these become saturated, ER stress ensues (figure 1c). A number of mutations have been associated with ER accumulation and/or stress, consistent with misfolding of pro-SP-C; however, the I73T variant does not do so [9–13]. Instead, it has been associated with apparent mistrafficking to the cell surface, as well as localisation to an intracellular compartment [7]. Interactions of misfolded proteins with endomembrane compartments have been proposed to mediate further trafficking consequences of misfolding and potentially provide further proteostatic quality control beyond the ER [14, 15]. Given the existing framework for understanding these processes, it seemed most likely that a trafficking error was occurring after the Golgi, causing misdirection to these locations. Dickens *et al.* [6] provide multiple lines of evidence indicating that the phenotype instead arises because, astonishingly, wild-type pro-SP-C is normally trafficked *via* the cell surface before re-entering the cell through endocytosis *en route* to lamellar bodies (figure 1b). The mutation blocks a pro-SP-C cleavage event following endocytosis, causing the protein to accumulate within early endosomes (figure 1d). The data further indicate this incompletely processed protein is recycled back to the cell membrane, rather than accumulating there simply because downstream traffic is blocked.

The bulk of the mechanistic experiments were conducted in HeLa cells which are arguably agnostic, rather than partially suited, to the study aims. The findings were validated in transgenic mouse and iPSC-derived lung organoid models. These could be used to identify readouts of pro-fibrotic behaviour and so link the cellular fate of SP-C with organ pathology, although this study did not attempt to do so. Nevertheless, the concept that post-translational mishandling of protein may be causal or closely linked to lung disease is well established [16, 17]. The disease phenotype is determined by the balance between loss- and gain-of-function consequences. The latter may include general fibrotic responses to persistent intracellular congestion.

Cystic fibrosis was an exemplar for the promise of gene therapies, and recent work has renewed the impetus [18]. In the meantime, however, major therapeutic advances have arisen through the development of compounds to rescue misfolding of the CFTR protein within the ER and stabilise its active conformation within the cell membrane [19–21]. ER stress is now known to mediate many chronic diseases and, in interstitial lung diseases, it correlates with severity of fibrosis rather than disease subtype [22, 23]. ER stress increases production of mucin 5B, particularly in carriers of a MUC5B allele that is the major genetic risk factor for sporadic IPF (and increasingly recognised in other fibrosing interstitial lung diseases (ILDs)) [24, 25]. Positive feedback from mucin 5B production is observed within this process. This could also relate to high requirements for chaperoning of disulfide bond formation in mucin 5B. Aberrant folding and polymerisation underlies both liver and lung disease manifestations in α_1-antitrypsin (A1AT) deficiency (figure 1c) [26, 27]. Grossly misfolded A1AT is targeted for ER-associated degradation, and if this is overloaded (*e.g.* in the $\text{Null}_{\text{HongKong}}$ variant) ER stress ensues. More subtle misfolding allows linkage of A1AT molecules into ordered aggregates (polymers) which accumulate within the ER, trigger a specific ER overload response and affect ER behaviour [28, 29].

In the case of hydrophobic surfactant proteins, the familial pulmonary fibrosis phenotype results from mutations affecting various points along the SP-C processing pathway (figure 1d). These affect not only pro-protein folding within the ER and subsequent trafficking *via* the cell membrane, but also lamellar body packing as indicated by pathogenic mutations affecting TTF-1 [30] and ABCA3 [31, 32]. Pathways from specific *SFTPC* gene mutations to specific ILD sub-phenotypic presentations cannot currently be excluded and would be of great interest mechanistically. However, in terms of ILD subtype overall, familial pulmonary fibrosis tends to present heterogeneously in individuals sharing the same mutation. *SFTPC*

mutations also demonstrate this phenomenon [33]. Correlations between genotype and age of onset indicate that within the BRICHOS domain, mutations of cysteine residues required to stabilise its structure through disulfide bond formation are associated with younger presentation (neonates to young adults). BRICHOS mutations adjacent to such residues (*e.g.* L188Q) seem to have a more variable and likely protracted course and has been reported in kindreds with ages ranging from childhood up to the sixth decade [33]. The I73T mutation itself has been associated with chronic, waxing–waning or indolent courses in children and can present later in adulthood. Such time-course variability is clinically important to characterise. Mechanistically it may reflect severity of the molecular insult and the capacity of cells to mitigate it, as seen in other protein misfolding disorders [34, 35], rather than distinct downstream pathogenic pathways. However, the general association appears to be between abnormalities of folding and trafficking within type 2 AECs and lung parenchymal fibrosis. A degree of further convergence is suggested by the association of familial pulmonary fibrosis with mutations in SP-B. However, there are differences in the specific clinical presentations described for this, and loss-of-function mechanisms may play a greater role.

The breadth of these examples, and the range of responses, attest to the critical importance of protein folding and handling in cellular homeostasis and suggest such events may mediate a far wider range of diseases than is currently appreciated. Improved understanding of both specific and generic pathways triggered by these events opens up new therapeutic strategies. “Upstream” approaches may manipulate protein degradation, folding, trafficking, processing and conformational behaviour, whilst targeting “downstream” transduction into common pathogenic pathways may benefit wider disease groupings.

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