SPLUNC1 comes of age? Predicting acute exacerbations in cystic fibrosis

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Measurement of the levels of SPLUNC1 in sputum may be a useful biomarker of cystic fibrosis exacerbations https://bit.ly/2TBfaB9

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Cystic fibrosis (CF) was first recognised as a specific disease in 1938 in an autopsy study of malnourished infants who displayed mucus plugging of glandular ducts [1]. The disease was characterised by malabsorption of fat and protein, steatorrhea, growth failure and pulmonary infection, which was ultimately fatal [1, 2]. Since that time, life expectancy for patients with CF has steadily improved from around 6 months to more than 40 years [3]. In many countries, the number of adults with CF now exceeds the number of children [3, 4]. Initially, improvement in survival occurred without any knowledge of the basic disease defect, using treatments directed at nutritional repletion, relief of airway obstruction, and antibiotic therapy of lung infection [2, 3]. The discovery of the CFTR gene in 1989 marked an important milestone in the history of CF [5] and led directly to the development of an array of targeted therapeutics that have shown great efficacy in modifying the disease [6, 7]. Although therapies have changed, aggressive treatment remains the foundation of clinical care.

As life expectancy increased it was recognised that CF was marked by repeated acute pulmonary exacerbations which were associated with worsening of symptoms, a decline of lung function and decreased survival [3, 8]. Acute exacerbations are episodes of inflammation that are associated with elevations of proinflammatory cytokines and alterations in the protease activity in the lung that directly result in increased lung inflammation coupled with immune cell infiltration [8]. The major causes of acute pulmonary exacerbations are thought to be infections, but a variety of other insults can change the homeostatic balance of the lung environment [8]. Irrespective of their cause, well validated biomarkers of acute exacerbations are limited [9], and there is a pressing need to identify specific parameters that can be used to predict their occurrence and, perhaps, their outcome. Ideally, these should be non-invasive and easily measured in samples, such as sputum. The work of Khanal *et al.* [10] reported in this issue of the *European Respiratory Journal* describes an intriguing study on one such potential marker, SPLUNC1.

SPLUNC1 is a small secretory protein that is highly expressed in the non-ciliated epithelial cells of the upper respiratory tract [11, 12]. Originally known as PLUNC (for palate, lung and nasal clone) [13], human SPLUNC1 is encoded by the *BPIFA1* gene and is the best studied member of a small family of genes that are restricted to mammals [14, 15]. It is 21 years since we cloned human *SPLUNC1* [11] and its true function remains somewhat of an enigma. All BPIF gene family members conserve a structural fold found in the host defence proteins, bactericidal/permeability-increasing protein (BPI) and lipopolysaccharide binding protein (LBP) [14–16]. On the basis of their restricted expression and the structural similarity to BPI and LBP, we hypothesised that these proteins would serve a role in airway host defence [16]. Efforts to understand the role of SPLUNC1 have subsequently focused on this area and the protein has been shown to exhibit pleiotropic functions, including in antimicrobial defence, acting as a surfactant, as an immune modulator, and as a regulator of airway fluid transport (as recently reviewed [17]). In short, SPLUNC1 appears to serve as a homeostatic regulator of airway function and all of these functions could be important in CF pathophysiology.

SPLUNC1 exhibits a gradient of expression within the airways. Highest expression is seen in the upper respiratory tract, including the nasal epithelium, and levels reduce more distally within the lungs. In non-diseased tissue, SPLUNC1 protein is not readily detected in the more distal airways or within the alveolar tissue, but staining intensity is greatly increased in patients with a number of chronic lung diseases, including COPD and CF [12, 18, 19]. We showed striking levels of protein staining the epithelium and occluded airways of severe CF cases [19]. Multiple studies have identified SPLUNC1 in respiratory secretions from healthy donors and it is one of the most abundant proteins in apical secretions of differentiated airway cells [20–23]. Recent single cell RNAseq data shows that *BPIFA1* is increased in a secretory cell population in patients with CF [24]. These observations contrast with studies that show levels of SPLUNC1 are reduced in CF sputum [25, 26], a finding confirmed by Khanal *et al*. [10]. So why are levels of protein reduced in sputum in CF? Studies have shown that SPLUNC1 is a target of multiple proteases seen in the CF sputum, including neutrophil elastase [25–27]. The proteolytic environment found in CF airway secretions likely mediates its degradation, as it has been shown that exogenous SPLUNC1 is rapidly degraded by CF sputum compared to sputum from healthy controls [25, 26]. *BPIFA1* is abundantly expressed in the airway epithelium, and Khanal *et al.* [10] show that gene expression is down regulated by the proinflammatory mediators, TNF-α and IL-1β. These factors likely combine to reduce levels of SPLUNC1 in the airway surface fluid. Not only are absolute protein levels reduced in CF airways but its function is also impaired. Specifically, the ability of SPLUNC1 to modulate fluid transport and act as an antimicrobial is reduced by the altered pH of the CF airways [28, 29].

The work of Khanal *et al*. [10] presents the first quantitative analysis of SPUNC1 levels in CF sputum (or indeed in any lung disease) and confirms that levels of the protein in sputum are reduced in acute exacerbations. Perhaps more excitingly, their data shows that lower levels of SPLUNC1 in sputum of stable CF patients are associated with an increased risk of an acute exacerbation. Their analysis also included other, better studied, soluble biomarkers of acute exacerbations [30], including IL-6, IL-8 and TNF-α, and showed that SPLUNC1 levels performed better as a marker in their study. As the authors rightly point out, it is important to understand that the current study is limited by its size. Larger studies are needed to confirm these findings and to gain an understanding of how stable levels of SPLUNC1 are in sputum. Aside from suggesting the use of SPLUNC1 levels as a non-invasive tool to aid clinical decision making, the data in this paper also point to the need to further understand the function of the protein in regulating airway homeostasis in general and in CF in particular. If this can be achieved, then SPLUNC1 really will come of age.

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References

1 Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease. *Am J Dis Child* 1938; 56: 344–399.
2 Davis PB. Cystic fibrosis since 1938. *Am J Respir Crit Care Med* 2006; 173: 475–482.
3 Elborn JS. Cystic fibrosis. *Lancet* 2016; 388: 2519–2531.
4 McKone EF, Ariti C, Jackson A, *et al.* Survival estimates in European cystic fibrosis patients and the impact of socioeconomic factors: a retrospective registry cohort study. *Eur Respir J* 2021; 58: 2002288.
5 Riordan JR, Rommens JM, Kerem B, *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; 245: 1066–1073.
6 O'Shea KM, O'Carroll OM, Carroll C, *et al*. Efficacy of elexacaftor/tezacaftor/ivacaftor in patients with cystic fibrosis and advanced lung disease. *Eur Respir J* 2021; 57: 2003079.
7 Mall MA, Mayer-Hamblett N, Rowe SM. Cystic Fibrosis: emergence of highly effective targeted therapeutics and potential clinical implications. *Am J Respir Crit Care Med* 2020; 201: 1193–1208.
8 Stanford GE, Dave K, Simmonds NJ. Pulmonary exacerbations in adults with cystic fibrosis: a grown-up issue in a changing cystic fibrosis landscape. *Chest* 2021; 159: 93–102.
9 McLeod C, Wood J, Schultz A, *et al.* Outcomes and endpoints reported in studies of pulmonary exacerbations in people with cystic fibrosis: a systematic review. *J Cyst Fibros* 2020; 19: 858–867.
10 Khanal S, Webster M, Niu N, *et al.* SPLUNC1: a novel marker of cystic fibrosis exacerbations. *Eur Respir J* 2021; 58: 2000507.
11 Bingle CD, Bingle L. Characterisation of the human plunc gene, a gene product with an upper airways and nasopharyngeal restricted expression pattern. *Biochim Biophys Acta* 2000; 1493: 363–367.
12 Bingle L, Cross SS, High AS, *et al.* SPLUNC1 (PLUNC) is expressed in glandular tissues of the respiratory tract and in lung tumours with a glandular phenotype. *J Pathol* 2005; 205: 491–497.
13 Weston WM, LeClair EE, Trzyna W, *et al.* Differential display identification of plunc, a novel gene expressed in embryonic palate, nasal epithelium, and adult lung. *J Biol Chem* 1999; 274: 13698–13703.

14 Bingle CD, Craven CJ. Meet the relatives: a family of BPI- and LBP-related proteins. *Trends Immunol* 2004; 25: 53–55.
15 Bingle CD, LeClair EE, Havard S, *et al.* Phylogenetic and evolutionary analysis of the PLUNC gene family. *Protein Sci* 2004; 13: 422–430.
16 Bingle CD, Craven CJ. PLUNC: a novel family of candidate host defence proteins expressed in the upper airways and nasopharynx. *Hum Mol Genet* 2002; 11: 937–943.
17 Liu Q, Wang Z, Zhang W. The multifunctional roles of short palate, lung, and nasal epithelium clone 1 in regulating airway surface liquid and participating in airway host defense. *J Interferon Cytokine Res* 2021; 41: 139–148.
18 De Smet EG, Seys LJ, Verhamme FM, *et al.* Association of innate defense proteins BPIFA1 and BPIFB1 with disease severity in COPD. *Int J Chron Obstruct Pulmon Dis* 2017; 13: 11–27.
19 Bingle L, Barnes FA, Cross SS, *et al.* Differential epithelial expression of the putative innate immune molecule SPLUNC1 in cystic fibrosis. *Respir Res* 2007; 8: 79.
20 Casado B, Pannell LK, Iadarola P, *et al.* Identification of human nasal mucous proteins using proteomics. *Proteomics* 2005; 5: 2949–2959.
21 Wu J, Kobayashi M, Sousa EA, *et al.* Differential proteomic analysis of bronchoalveolar lavage fluid in asthmatics following segmental antigen challenge. *Mol Cell Proteomics* 2005: 4: 1251–1264.
22 Campos MA, Abreu AR, Nlend MC, *et al.* Purification and characterization of PLUNC from human tracheobronchial secretions. *Am J Respir Cell Mol Biol* 2004; 30: 184–192.
23 Kesimer M, Kirkham S, Pickles RJ, *et al.* Tracheobronchial air-liquid interface cell culture: a model for innate mucosal defense of the upper airways? *Am J Physiol Lung Cell Mol Physiol* 2009; 296: L92–L100.
24 Carraro G, Langerman J, Sabri S, *et al.* Transcriptional analysis of cystic fibrosis airways at single-cell resolution reveals altered epithelial cell states and composition. *Nat Med* 2021; 27: 806–814.
25 Webster MJ, Reidel B, Tan CD, *et al.* SPLUNC1 degradation by the cystic fibrosis mucosal environment drives airway surface liquid dehydration. *Eur Respir J* 2018; 52: 1800668.
26 Sesma JI, Wu B, Stuhlmiller TJ, *et al.* SPX-101 is stable in and retains function after exposure to cystic fibrosis sputum. *J Cyst Fibros* 2019; 18: 244–250.
27 Jiang D, Wenzel SE, Wu Q, *et al.* Human neutrophil elastase degrades SPLUNC1 and impairs airway epithelial defense against bacteria. *PLoS ONE* 2013; 8: e64689.
28 Garland AL, Walton WG, Coakley RD, *et al.* Molecular basis for pH-dependent mucosal dehydration in cystic fibrosis airways. *Proc Natl Acad Sci USA* 2013; 110: 15973–15978.
29 Ahmad S, Gilmore RC, Alexis NE, *et al.* SPLUNC1 loses its antimicrobial activity in acidic cystic fibrosis airway secretions. *Am J Respir Crit Care Med* 2019; 200: 633–636.
30 Bene Z, Fejes Z, Macek M Jr, *et al.* Laboratory biomarkers for lung disease severity and progression in cystic fibrosis. *Clin Chim Acta* 2020; 508: 277–286.