The ENaC-targeted Therapeutic Peptide SPX-101 is Resistant to Proteolytic Degradation in Diseased Sputum

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Introduction

Pulmonary diseases such as chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and non-CF bronchiectasis (nCFBE) are characterized by mucous dehydration and decreased mucociliary clearance. The epithelial sodium channel (ENaC) provides the primary driving force for water absorption across airway epithelia. ENaC surface density is negatively regulated by short palate lung and nasal clone 1 (SPLUNC1), a protein secreted by airway epithelia. SPLUNC1 is among the most-abundant proteins in airway surface liquid (ASL), but SPLUNC1 is reported to be reduced or absent in sputum from individuals with COPD and CF. This suggests the excessive airway dehydration characteristic of these diseases could be led by the loss of SPLUNC1-mediated ENaC inhibition. We have developed an optimized therapeutic peptide, SPX-101, that replaces the ENaC internalization function of SPLUNC1. In animal models of CF, SPX-101 prolongs survival and enhances mucociliary clearance. The objective of this study was to determine the abundance of SPLUNC1 in sputum derived from individuals with COPD, CF, and nCFBE as compared to healthy individuals, and to determine the stability and functionality of SPLUNC1 and SPX-101 after exposure to diseased sputum.

Results

SPX-101 induces internalization of ENaC subunits and durably reduces amiloride-sensitive current

SPX-101, but not S18, is stable in CF sputum and neutrophil elastase

Figure 3. Stability of recombinant SPLUNC1 in healthy sputum (n=6) or CF sputum (n=15) was determined by co-incubation and western blot analysis. Representative blots are shown in A and C and quantification of data in B and D. The ability of protease inhibitors to prevent degradation in CF samples is shown E and quantified in F (data are representative of 11 sputum samples). All graphs depict mean ± SEM. ** indicates p<0.01 versus sputum alone. (G) Degradation of recombinant SPLUNC1 by proteases was determined by co-incubation for 15-240 minutes and western blot analysis. Data are representative of at least three experiments per enzyme.

Conclusions

- SPLUNC1 is degraded by multiple proteases present in CF sputum
- SPLX-101 is stable in CF sputum and resistant to cleavage by neutrophil elastase
- SPLX-101 exposed to CF sputum maintains the ability to internalize ENaC, increase ASL height, and increase survival of iENaC transgenic mice.

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