The Normal ASL Peptidome modulates cell function and is altered in CF DISEASE.

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The ASL proteome contains approximately 1,000 proteins, including high molecular weight mucins, globular proteins and short (<10 kDa) peptides. Proteases and anti-proteases present in the ASL regulate the cleavage of proteins and the generation of peptides. Known peptides modify airway physiology, infection and inflammation. However, there is evidence from several studies that there are hundreds of peptides reported in the ASL which have not been identified and/or for which function is currently unassigned. In the CF lung, chronic neutrophilia and infection lead to an increase in free protease levels that induce protein/peptide cleavage. This could impair/alter functionality of peptides in CF ASL.

We set out to firstly define the normal ASL peptidome and to determine its physiological role. We size-fractionated sputum from normal subjects and studied these fractions by LC-MS/MS without conventional tryptic digestion in order to detect endogenously produced peptides. The peptidome from normal sputum was antiproliferative. We identified ~130 endogenous peptides in normal sputum and utilized SPOT synthesis technology to make a library of peptides that included the entire normal peptidome. We then performed high content imaging using 384 well plates as described(Sassano MF et al, PLoS Biology, 16(3):e2003904) in order to determine whether these peptides could affect cell growth. We found that the normal peptidome contained 10 peptides that increased cell growth by 27±1.2% and 11 peptides that inhibited cell growth to 57.3±1.6%. The peptides ranged in size from 6-15 residues and the inhibitory peptides were smaller than the stimulatory peptides, and in both cases were all 5-6 residues long. Using additional screening techniques, we also found peptides that modified intracellular Ca2+ release, CFTR and ANO1 expression. In contrast, analysis of the CF peptidome identified peptides of both mammalian and bacterial origin. Approximately 700 peptides were identified that were common to all CF patients. Only 60 peptides were common to both normal and CF subjects. Using principle component analysis and k-means clustering, we found that the normal and CF peptidomes were p<0.00013 different. The CF peptidome had 3 independent peptides that increased cell growth by 24.8±2.8% compared to control and 10 peptides that inhibited cell growth to 56±2.1% of control. Interestingly, all of the CF inhibitory peptides were derived from *Pseudomonas aeruginosa*.

We suggest that the normal lung peptidome plays a key role in modulating cell function through as-yet undetermined mechanisms. How proteolysis and infection affects the peptidome and its function in CF disease remains to be determined. We speculate that understanding how peptides modify cell growth, intracellular Ca2+ and CFTR/ANO1 could provide novel therapeutics to aid restoration of lung epithelial function in respiratory disease.

Funded by UNC-Chapel Hill and St. George’s, University of London