論文の内容の要旨

論文題目　Region- and Cell-specific Expression of Secretory Mucins MUC5AC and MUC5B in Normal Human Airways
(正常ヒト気道における分泌型ムチン MUC5AC と MUC5B の領域および細胞特異的発現について)

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Background: Mucociliary clearance (MCC) is a critical innate defense system for maintenance of lung health. Mucin secretion into airway lumen is one of the key components of the MCC. Dysregulated mucin secretion can produce MCC dysfunction and worsening of chronic lung disease including cystic fibrosis, chronic bronchitis and asthma. MUC5AC and MUC5B are the predominant gel-forming mucins in the mucus layer of human airways. Each mucin has distinct functions and site-specific expression. However, the regional distribution of expression and cell types that secrete each mucin in normal human airways are not fully understood. This study provides a comprehensive description of secretory mucin expression in the normal human lung essential for understanding how abnormal regulation of secretory mucin expression contributes to the pathogenesis of muco-obstructive lung diseases.

Objectives: To characterize the regional distribution of MUC5AC and MUC5B in normal human airways and assess which cell types produce these mucins, referenced to the club cell secretory protein (CCSP).

Methods: Multiple airway regions from 16 non-smoker lungs without a history of lung disease were studied. MUC5AC, MUC5B, and CCSP expression/co-localization were assessed by RNA in situ hybridization (ISH) and immunohistochemistry in 5 lungs with histologically normal airways. Droplet digital PCR (ddPCR) was performed for absolute quantification of MUC5AC/5B ratios in different airway regions, including large and small airway epithelia. Large airway epithelial (LAE) and small airway epithelial (SAE) cells were cultured in air-liquid interface condition and utilized for MUC5AC and MUC5B protein secretion.

Results: Submucosal glands expressed MUC5B, but not MUC5AC. However, MUC5B was also extensively expressed in superficial epithelia throughout the airways except for terminal bronchioles. Morphometric calculations revealed that the predominant site for MUC5B production in the superficial epithelium was the distal airways, whereas MUC5AC production was concentrated in large, cartilaginous airways. RNA ISH revealed both MUC5AC and MUC5B were co-localized with CCSP-positive secretory cells in proximal superficial epithelia, whereas MUC5B and CCSP-co-positive cells dominated distal regions. Absolute quantification of MUC5AC and MUC5B transcript copy numbers by ddPCR in freshly isolated airway epithelia revealed that MUC5B transcripts were significantly higher than MUC5AC in distal airways. In vitro study, mass spectrometry identified significantly more abundant MUC5B protein than MUC5AC in apical secretions of SAE cells. RNA ISH showed MUC5B mRNA expression in

CCSP mRNA positive non-ciliated secretory cells in both LAE and SAE cells. These ddPCR and in vitro data were consistent with the findings shown in human airway tissues by RNA ISH and immunohistochemistry.

Conclusions: In normal human airways, MUC5B is the dominant secretory mucin in the superficial epithelium as well as submucosal glands, with distal airways being a major site of expression. MUC5B and MUC5AC expression is a property of CCSP positive secretory cells in superficial airway epithelia.