Putting Mucins on the Map

Airway mucus has a central role in the pathogenesis of many major lung diseases (1). Excessive mucus production is the hallmark of chronic bronchitis in chronic obstructive pulmonary disease and is associated with reduced lung function (2) and poor clinical outcomes. Mucus plugging is a major contributor to airway obstruction in fatal asthma. Mucoviscidosis, a synonym for cystic fibrosis, describes a change in the properties of mucus that results in increased susceptibility to infection and progressive loss of lung function in this disease. Understanding the regulation and functional properties of mucus promises to open new avenues to treatment of many individuals with lung disease (3).

Airway mucus is a heterogeneous mixture of water, ions, and organic macromolecules. Mucins are large, heavily glycosylated, gel-forming proteins that represent approximately 1.5% of the dry weight of normal airway mucus. Two homologous mucin genes, *MUC5AC* and *MUC5B,* play prominent roles in the lung. These genes arose from a common ancestor but have evolved to have different expression patterns and distinct functions (4). Much of our knowledge about mucins comes from mouse studies. In mice, *Muc5b* expression begins during embryonic development and continues postnatally (5). MUC5B protein is found in secretory cells within large (cartilaginous) airways and, at lower concentrations, in intrapulmonary axial airways, but not in bronchioles. MUC5B is also produced in submucosal glands, which in mice are limited to the proximal trachea. Deletion of *Muc5b* resulted in impaired mucociliary clearance and airway infections (6). *Muc5ac* is transiently expressed during embryonic lung development, but little if any MUC5AC is found in postnatal lungs unless mice are infected or challenged with allergen. In response to these stimuli, proximal airway secretory cells produce and secrete large amounts of MUC5AC. Deleting *Muc5ac* reduced airway reactivity in an asthma model (7), whereas transgenic overexpression of *Muc5ac* provided protection against respiratory viral infection (8).

Mouse and human airways differ in several respects, and it is important to understand how insights from mouse studies apply to humans. In contrast to mice, humans have increased airway diameters, submucosal glands in more distal airways, and easily detectable *MUC5AC* expression, even in the absence of apparent infection or allergy. In this issue of the *Journal*, Okuda and colleagues (pp. 715–727) systematically map the regional distribution of MUC5AC and MUC5B along the proximal–distal axis of the normal human lung (9). A strength of this work is that multiple methods were used to measure mucins: mucin mRNAs were localized and quantified using *in situ* hybridization and digital droplet polymerase chain reaction, and quantitative immunohistochemistry was used to localize and quantify mucin proteins within epithelial cells. As reported previously, *MUC5B* was expressed in gland cells, and *MUC5AC* was not. In the superficial epithelium of the trachea and proximal and segmental bronchi, both mucins were expressed at roughly similar levels, as determined by measurements of *MUC5B* and *MUC5AC* mRNAs and the volume of intracellular MUC5B and MUC5AC protein staining. In distal bronchi and bronchioles, *MUC5B* and especially *MUC5AC* expression decreased, resulting in an increased *MUC5B:MUC5AC* ratio. Neither mucin was detected in terminal bronchioles. Because distal bronchioles have a much larger surface area than more proximal airways, the authors estimate that most airway *MUC5B* expression is accounted for by distal bronchioles. In contrast, most *MUC5AC* expression was attributed to larger airways (bronchi).

Okuda and colleagues also highlight secretory cell heterogeneity by using *in situ* hybridization to show that mucin-producing gland cells are $MUC5B^{+}$ $MUC5AC^{-}$ $CCSP^{-}$, whereas superficial airway cells are typically $MUC5B^{+}$ $MUC5AC^{-}$ $CCSP^{+}$ (distal bronchioles) or $MUC5B^{+}$ $MUC5AC^{+}$ $CCSP^{+}$ (proximal bronchi). As noted by the authors, the nomenclature used for airway secretory cells, which includes club cells, goblet cells, mucous cells, serous cells, and indeterminate cells, is based principally on histologic criteria that fail to capture the heterogeneity revealed by recent studies. In the absence of a clear consensus about what these names signify, we suggest that investigators use molecular markers for classifying secretory cells. The application of single-cell RNA sequencing to airway epithelial cells (10, 11) is revealing airway epithelial cell transcriptomes at unprecedented resolution and will help us to better understand heterogeneity in secretory cells. These methods will need to be combined with other methods that provide spatial information to gain a deeper understanding of how mucin-producing cells and other cells that secrete macromolecules, ions, and water vary regionally in the lung.

Two limitations of the study deserve mention. First, many of the observations were based on a subset of histologically normal-appearing lungs from only 5 subjects, a sample size too small to define the range of normal in human populations. Nonetheless, the methods used here and the resulting insights into typical patterns of mucin expression are a useful basis for designing future studies dependent on measuring mucins in normal and diseased lungs. A second limitation is that the methods used here measure mucin RNA and stored protein-stained volumes, and not mucin protein secreted from different portions of the airway. The results of this study suggest that the MUC5B:MUC5AC protein ratio within airway mucus is higher in the distal lung (where very little MUC5AC is produced). However, this may be offset by secretion of substantial amounts of MUC5B-rich mucus from glands in the proximal airway.

Many important questions remain to be addressed. Understanding how expression of mucins and other genes are spatially regulated within the normal airway epithelium, and how

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genetic and environmental factors that affect gene regulation affect disease risk, remains a major challenge. The discovery that a regulatory variant affecting *MUC5B* expression in distal airways is associated with a very large increase in risk of developing pulmonary fibrosis is a compelling early step toward this goal (12). It will also be critical to identify disease-associated changes in mucin gene expression in different regions of the lung and to understand how these affect mucus function. Recent studies show that differences in mucus composition are associated with dramatic differences in mucus organization and function. For example, MUC5B and MUC5AC are found within distinct domains of mucus plugs in fatal asthma, and the MUC5AC-rich domains play a unique role in mucostasis by tethering to the epithelium (13). In pigs, MUC5B from submucosal gland ducts formed strands composed of multiple MUC5B filaments, whereas MUC5AC emerged from superficial secretory cells as wispy threads or sheets, and it seems likely that these distinct structures contribute differently to mucociliary transport (14). Understanding how regional and disease-associated differences in mucins and other mucus components affect host defense and lung function is likely to be a long but rewarding journey. Okuda and colleagues have provided a map that will help us find our way. ■

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Can a Physiologic Insight "Resuscitate" Research in Cardiopulmonary Resuscitation?

Anyone who has ever performed successful cardiopulmonary resuscitation (CPR) knows instantly that they have done something truly incredible. The significance of the act and the elemental feeling of usefulness is the same for all members of the extended healthcare team or the public who have just saved a life. CPR is also unique in that its core principles are well understood by many, or at least that's what we think. It consists of clearing a patient's airway, rhythmically pumping the thorax to circulate blood around the body, providing some ventilation to replace spontaneous breathing, and if a shockable arrhythmia is present, performing defibrillation.

Initial research into CPR yielded major gains. Campaigns to convert bystanders into competent responders have been success stories of major proportions, leading to marked improvements in rates of survival with good neurological outcome (1–3). However, recent clinical research into CPR has not improved its effectiveness. Large randomized controlled trials have tested the optimal types

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