Airway Epithelial Stem Cells and the Pathophysiology of Chronic Obstructive Pulmonary Disease

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Characteristic pathologic changes in chronic obstructive pulmonary disease (COPD) include an increased fractional volume of bronchiolar epithelial cells, fibrous thickening of the airway wall, and luminal inflammatory mucus exudates, which are positively correlated with airflow limitation and disease severity. The mechanisms driving general epithelial expansion, mucous secretory cell hyperplasia, and mucus accumulation must relate to the effects of initial toxic exposures on patterns of epithelial stem and progenitor cell proliferation and differentiation, eventually resulting in a self-perpetuating, and difficult to reverse, cycle of injury and repair. In this review, current concepts in stem cell biology and progenitor–progeny relationships related to COPD are discussed, focusing on the factors, pathways, and mechanisms leading to mucous secretory cell hyperplasia and mucus accumulation in the airways. A better understanding of alterations in airway epithelial phenotype in COPD will provide a logical basis for novel therapeutic approaches.

Keywords: epithelium; hyperplasia; metaplasia; mucus hypersecretion; stem cells

The human and societal toll of chronic obstructive pulmonary disease (COPD) is considerable, and there is a clear need for better prevention and early detection/intervention, including more specific and effective therapies for all stages of the disease. COPD is characterized by, and defined as, sustained and largely irreversible airflow limitation on forced exhalation, associated with known risk factors and excluding other specific causes (1, 2). The key pathologic changes underlying the physiologic hallmarks are loss of lung elasticity and small airway tethers due to emphysema, thickening of the small airway wall to reduce caliber, and luminal obstruction with inflammatory mucoid secretions (3–5). The airway epithelium is a primary interface with the outside world and is a target of the toxic particles and gases from tobacco smoke and other environmental agents that are the main cause of COPD. As indicated by changes in gene expression, airway epithelial cells respond dynamically to the inciting stimuli (6) and are the focus of viral (7) and bacterial (8–10) infections that exacerbate COPD and accelerate deterioration of lung function. The characteristic pathologic changes in the airway epithelium (Figures 1 and 2) are integral to the initiation and progression of COPD. Generalized epithelial hyperplasia, mucous secretory cell hyperplasia, squamous metaplasia, and mucus accumulation must result from disruptions in normal cell and tissue dynamics caused by both the initial stimuli and the spiral of infectious complications. Locations of stem cells, patterns of cell migration/differentiation, and the regulatory mecha-

nisms governing tissue dynamics are relatively well understood in some organs, but not in the lungs. However, some progress has been made in recent years. The main goal of this article is to concisely review current concepts in stem cell biology applicable to our understanding of progenitor–progeny relationships in the airway epithelium, focusing on cellular mechanisms leading to mucous secretory cell hyperplasia and mucus accumulation. Additional specific issues in lung stem cell biology highly relevant to COPD, but not necessarily focused on the airway epithelium, are also addressed. Of interest, the proceedings of a joint NHLBI/Cystic Fibrosis Foundation workshop on adult lung stem cells (11) and an incisive and comprehensive review of lung epithelial stem cells (12) have been published recently.

MODELS OF CELL REPLACEMENT AND APPLICABILITY TO THE LUNGS

Historically, the short-term administration of the radioactive DNA precursor ³H thymidine to experimental animals followed by autoradiography of tissue sections (“pulse labeling” to mark actively dividing cells) revealed three major categories of organs with different levels of cell proliferation in the mature adult (13). Bone marrow, skin, and gut proliferated continuously, whereas organs such as the liver and kidney had slower, but inducible, levels of cell growth after injury, and the central nervous system was viewed as static. In the 1960s, Blenkinsopp examined the available data in lung and also performed experiments in rats, indicating a relatively slow epithelial cell turnover, estimated to be approximately 100 d in the normal rodent tracheobronchial tree (14). Numerous studies since then have confirmed relatively slow rates of cell turnover in normal lungs, but proliferation was highly induced after injury (15–20).

Our understanding of a stem cell hierarchy derives mainly from studies of continuously proliferating tissues. Many of the studies constituting the knowledge base of mammalian stem cell biology were performed in mice, and it is widely assumed that fundamental properties will be similar in humans. However, the cell type composition of the epithelium is dissimilar at certain airway levels in mice and humans (21), and there are likely important distinctions between the two species. In the classical model, adult stem cells reside in specialized niches from which they are minimally recruited to maintain tissue homeostasis, a strategy to conserve stem cells for the life of the organism. A “transiently amplifying” downstream progenitor compartment is responsible for the bulk of tissue proliferation. The property of infrequent cycling serves as the basis for detection of stem cells. In this technique, DNA precursors are administered for long periods to label stem cells, followed by a chase to “flush the label” out of the transient amplifying cells. The remaining “label-retaining cells” are believed to represent the stem cell compartment. Differentiated cells with minimal growth potential are ultimately generated from stem and transient amplifying cells through a series of tightly regulated temporal and spatial commitment steps. An overview of this model, with its applicability to lung and implications for repair after injury, has been given previously (21). The molecular regulation of stem cell fate

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Figure 1. Representative images of normal and chronic obstructive pulmonary disease (COPD) bronchial epithelia. Note the characteristic marked expansion of mucous secretory cells in the COPD bronchus (blue-stained material in the center panels). Patchy conversion to a stratified squamous epithelium devoid of mucous secretory cells is also commonly observed (right panels). All photomicrographs are at the same magnification (originally 400 $\times$). Formalin-fixed, paraffin-embedded section. AB-PAS = alcian blue–periodic acid Schiff's stain; H&E = hematoxylin and eosin.

decisions has historically been investigated in model invertebrate species, but considerable advances in recent years have revealed key mechanisms governing stem cell dynamics in mammalian blood, gut, and skin (recently reviewed in Reference 22). The Wnt, bone morphogenetic protein, and Notch signaling pathways, and their downstream effectors, are key regulators of the stem cell niche.

As opposed to well-accepted stem cell hierarchies in continuously proliferating tissues, progenitor–progeny relationships in complex organs such as the breast, kidney, liver, pancreas, prostate, and lung, which normally have low, but conditionally active, rates of cell turnover, remain less certain. It is not clear whether these organs have analogous hierarchic cell lineage systems that are quiescent until injury or if they operate by other paradigms. The distinction is critical for regenerative medicine. For example, adult tissue stem cells may serve as a “guilt free” source of therapeutic progenitors, avoiding the controversies inherent to embryonic stem cells. To address this issue in the endocrine pancreas, a prime target for cell therapy, Dor and colleagues applied modern cell lineage tracing techniques involving permanent, heritable labeling of specific cell types (23). The results strongly suggest that adult pancreatic beta cells are maintained

by “simple duplication” of a large number of equipotent, differentiated beta cells rather than a small stem cell reserve. Thus, adult beta cells likely originate from other differentiated beta cells, which are destroyed in patients with type I diabetes. Supplies of transplantable cadaveric organ donor beta cells are limited, and allografts require harmful immunosuppression. Thus, beta cells generated from embryonic stem cells created by somatic cell nuclear transfer (24) will likely be the best option for cell therapy of type I diabetes (25). A more comprehensive understanding of cell lineage characteristics and dynamics in the airway and alveolar epithelium and in the mesenchymal cellular compartments of the lung will provide a logical basis for pharmacotherapy directed at the characteristic airway and parenchymal changes in COPD and for regenerative therapy of emphysema.

STEM CELL AGING AND COPD

Decreased lung elastic recoil, reduced indices of forced expiration, and emphysema-like lung histology inevitably occur during aging, even in nonsmokers (26), and cellular aging may contribute to the development and progression of COPD. It is widely assumed that aging may limit the ability of tissues to repair, or

Figure 2. Representative images of normal and COPD bronchiolar epithelia. Note the absence of stored mucosubstances in the normal bronchiole (left panels). Emphysematous parenchyma, prominent accumulation of luminal mucus (blue-stained material) and lymphoid nodules are visible in the COPD specimen (center panels). Higher magnification reveals blue-stained, metaplastic mucous secretory cells adjacent to the mucus plug in the COPD bronchiolar epithelium (lower right panel). Photomicrograph original magnification as indicated. Formalin-fixed, paraffin-embedded section.

conversely, that deficits in mechanisms controlling cell proliferation may underlie the increased incidence of cancer in the elderly (reviewed in Reference 27). Cell autonomous changes in hematopoietic stem cells in old mice are likely associated with immune decline and the development of leukemia (28), and non-cell autonomous effects of the aging environment surrounding progenitor cells determines the ability of muscle to repair (29). Fibroblasts explanted from emphysematous lungs exhibit markers of cellular senescence and do not grow as well as fibroblasts from normal lungs (30, 31). It is important to determine if there are similar age-related changes in lung alveolar epithelial and endothelial cells because apoptosis in these compartments is likely critical in development of emphysema (32). Furthermore, polymorphisms in genes related to cellular aging may contribute to COPD susceptibility.

Somatic mutations induced by tobacco smoke carcinogens and methylation-induced silencing of tumor suppressor genes cause lung cancer, a significant comorbid risk in COPD. During aging, the gene hypermethylated in cancer (*HIC1*) becomes methylated and silenced, which results in up-regulation of the stress-controlling protein SIRT1, in turn attenuating p53 function and allowing damaged cells to resist apoptosis (33). This is one example of a molecular mechanism that the organism may use to preserve aging cells, but which may also promote cancer. A greater appreciation of cell dynamics in young versus old lungs and mechanisms controlling altered behavior of aging lung cells may help to identify cells at greatest risk for transformation; assist in the detection, monitoring, and treatment of lung cancer; and will likely improve our understanding of the mechanisms causing emphysema-like changes in the aging lung.

REVOLUTIONS IN STEM CELL BIOLOGY

There have been significant advances and controversies in stem cell biology in recent years, some of which are highly relevant to COPD. The ability of circulating progenitors to home to the lung and adopt parenchymal cell fates is highly controversial and has been reviewed in detail previously (21) and updated more recently (34). There is hope that bone marrow-derived cells can be harvested, perhaps expanded and/or manipulated *ex vivo*, and delivered to the lung to rebuild missing or destroyed lung tissue. However, the conversion of bone marrow-derived cells to airway and alveolar epithelium appears to occur rarely, and significant increases in efficiency will be necessary for this approach to cell therapy to be relevant to the epithelium. The formation of gastrointestinal tract epithelial cancers by bone marrow-derived cells (35) is even more controversial, and it is unknown if a similar phenomenon is relevant to lung. Nevertheless, inflammation and stromal cells play critical roles in the cellular cross-talk that generates and regulates epithelial cancers (reviewed in Reference 36), which is one example of the contribution of circulating cells to lung tissue reactions important in COPD; two others are discussed below.

The discovery of putative circulating endothelial progenitor cells (EPCs) (37) has revised concepts of postnatal vascular homeostasis and angiogenesis and has suggested novel cellular therapies for cardiovascular diseases (reviewed in References 38 and 39). Failure to increase numbers of circulating EPCs in pneumonia (40) and acute lung injury (41) portends a poor outcome, and circulating EPC numbers appear to be reduced in people with severe restrictive or obstructive lung disease (42). There are many questions regarding EPCs in COPD: Do reduced circulating EPCs play a role in the loss of capillaries and the development of pulmonary hypertension in emphysema, and can administration of EPCs help to remodel the vascular bed toward normalcy? The airway microvasculature plays a key role

in asthmatic airway wall remodeling (reviewed in Reference 43), and one can envision EPCs similarly contributing to the thickening of the small airway wall that is characteristic of COPD. Postnatal vascular remodeling is much more complex than just recruitment of EPCs (see References 44 and 45). Recent compelling studies show that hematopoietic, non-EPC mononuclear cells attracted by vascular endothelial growth factor and then kept in place by stromal-derived factor-1 (SDF-1) are critical to support the growth of local vascular cells during angiogenesis, and that signals generated by ephrin-B2 are key to the positioning of mural vascular cells, such as pericytes and smooth muscle cells (46–48). Thus, coordinated interactions among EPCs, non-EPC circulating mononuclear cells, and local vascular progenitors regulate normal vascular homeostasis and remodeling events that are undoubtedly important in the development and progression of COPD.

Fibrocytes are another type of circulating CD45⁺ mononuclear cell, which homes to diseased lungs where it appears to interact with local cells, express collagen, and contribute to fibrosis (49–51) (recently reviewed in Reference 52). Distinctions between fibrocytes and the non-EPC bone marrow-derived mononuclear cells that contribute to angiogenesis, as discussed above, are not totally clear and require further study. Analogous to EPCs and the microvasculature in the asthmatic airway, fibrocytes likely contribute to small airway wall thickening in COPD and may also support the paradoxical patchy parenchymal fibrosis sometimes seen in emphysema. The potential therapeutic use of exogenous stem cells illustrates a potential two-edged sword in lung regenerative medicine. The notion that circulating cells can home to and protect the lung suggests the potential future use of fibrocytes, or other progenitors, to rebuild emphysematous lung parenchyma. However, devising a strategy to accurately reconstruct the complex cell and matrix architecture of alveolar septae, while avoiding deleterious fibrosis, is a major challenge to the field.

ADULT LUNG EPITHELIAL STEM CELLS

The subject of lung epithelial stem cells has been comprehensively reviewed elsewhere (12, 21, 34), and is only reiterated here, focusing on newer data related to mechanisms of goblet cell hyperplasia and metaplasia. A depiction of putative lung epithelial stem cell compartments is given in Figure 3. There is likely strong evolutionary pressure to preserve a patent airway by efficiently repairing denuding epithelial injuries. Basbaum and Jany introduced the concept of plasticity in the airway epithelium, meaning that cells can adopt alternative fates after injury (53). The term “transdifferentiation” was coined to describe the generation of one differentiated cell from another without intervening cell division. This term is also used to connote the formation of organ-specific cell types by stem cells from another organ—for example, airway epithelium from bone marrow (<http://www.isscr.org/glossary/index.htm#Transdifferentiation>). DNA metabolic pulse-labeling studies showed that both basal and columnar secretory cell types in the pseudostratified airway epithelium divide (15, 17). *In vivo* and *in vitro* studies show that epithelial repair is rapid and dynamic, involving epithelial dedifferentiation, cytoskeletal rearrangement, migration, and redifferentiation (e.g., see References 54–56). Indeed, both basal and columnar cell types reconstituted a complete epithelium in an *in vivo* model of rat tracheas denuded of their own cells and implanted in immune compatible hosts (57, 58). However, colony formation on plastic dishes (59) and lineage tracing studies (60), as well as more recent analysis of clonal growth (61) and genetic lineage mapping (62, 63), suggest that mouse tracheal basal cells

have enhanced ability to form large differentiated epithelial colonies. Furthermore, mouse tracheal basal cells are the majority of “label-retaining cells” produced by long-term DNA labeling followed by a chase period, which, as discussed above, is believed to represent the infrequent cycling property of stem cells (64). Thus, the current consensus is that many cells can contribute to repair of injury, but that basal cells likely represent a stem cell compartment in the adult pseudostratified epithelium.

The precise location depends on the species, but as one proceeds distally down the airways, basal cells disappear and the pseudostratified epithelium transitions to a simple columnar, and then cuboidal, morphology, composed of Clara, ciliated, and neuroendocrine cells (65, 66). Secretoglobin (SCGB) 1a1, also known as CC10 or CCSP, is a member of the SCGB family that has been widely used as a marker for Clara cells. Several different SCGBs are also expressed in the airway epithelium (67). SCGB1a1 is expressed in human upper airway secretory cells that do not appear to proliferate (68). There have been many studies of SCGB1a1 expression in relationship to cell lineage in mice, and the definition of the Clara cell has become confusing. If Clara cells are defined as domed, nonciliated cells of the simple bronchiolar epithelium as originally described, then SCGB1a1-expressing secretory cells in the pseudostratified portions of the airway are distinct from bronchiolar Clara cells. However, Clara cells are commonly defined based on SCGB1a1 expression, in which case they likely represent a spectrum of cells, present in different airway levels, with different potentials and behaviors.

After injury by oxidant gases that damage ciliated cells, surviving bronchiolar Clara cells proliferate to restore the bronchiolar epithelium (18, 69), but this observation, in itself, does not provide evidence for a stem cell hierarchy within the Clara cell population. When naphthalene is administered to mice, almost all Clara cells are killed due to selective metabolic activation of the toxin. Ciliated cells shed their cilia and cover the denuded bronchiolar basement membrane, and the few surviving Clara cells then proliferate (70). The naphthalene-resistant progenitor cells represent a subset of Clara cells residing within neuroepithelial bodies, and label retention studies suggest that this unit constitutes a stem cell niche (71). After naphthalene injury, pulmonary neuroendocrine cells proliferate (72), but they are apparently a distinct lineage system not requiring nor generating Clara cells (73). A second epithelial stem cell niche has been identified in the zone where airways terminate and form alveoli (74, 75). Specific cells in this zone coexpressed SCGB1a1, the type II cell marker surfactant protein (SP)-C, cluster of differentiation (CD)34, and stem cell antigen-1 (Sca-1) (75). The putative “mouse bronchioalveolar stem cells” proliferated in response to naphthalene or bleomycin injury, and when purified cells were cultured appropriately, they demonstrated a high clonal growth capacity and differentiation potential to form both Clara cells and distal lung epithelium composed of cells expressing type I or type II cell markers (75). Furthermore, the cells expanded when an active K-ras oncogene was induced *in vitro* and appeared to generate adenocarcinoma when expressing an active K-ras oncogene *in vivo* (75). It is important to determine if a similar multipotential cell exists in the human bronchiolar-alveolar duct junction zone. These advances point the way for additional studies needed to elucidate steady-state and repairing cell lineages and the regulation of cell dynamics in both the airway and alveolar epithelium in mice and humans.

MOLECULAR AND CELLULAR MECHANISMS DRIVING MUCOUS SECRETORY CELL HYPERPLASIA/METAPLASIA

The molecular mechanisms controlling the initial establishment and maintenance of cell type distributions at different levels in

the normal adult airway epithelium are undoubtedly complex and not well understood. However, it is well known that diverse stimuli increase the numbers of mucous secretory cells in locations where they normally exist (hyperplasia) or induce them in locations where they are normally absent (metaplasia). Gland hypertrophy, mucous secretory cell hyperplasia in the bronchi, and metaplasia in the bronchioles are prominent features of COPD (76). The stimuli increasing the numbers of mucous secretory cells include allergic sensitization, bacterial products, chemical irritants, chemokines, cytokines, growth factors, oxidants, the protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA), proteases, and viral infection, variably operating through the Jak/Stat, mitogen-activated protein kinase, nuclear factor- κ B, and PI3 kinase/Akt, transforming growth factor- β pathways, among others (for reviews, see References 77–80). Differentiation of mucous secretory cells likely represents a complex series of coordinated events. Per cell increases in mucin mRNA result from transcriptional activation of mucin genes and/or message stabilization, whereas mucin glycoprotein production requires creation of the characteristic synthetic and secretory apparatus. Once differentiated, mucous secretory cells with few granules can divide, whereas cells with large numbers of granules appear to divide infrequently (16). The life cycle of mucous secretory cells is not fully appreciated and it is unclear if engorged secretory cells degranulate, divide, and then reaccumulate granules. Increased percentages of secretory cells may result from selective suppression of apoptosis (81). Finally, the regulation of mucin glycoprotein flux through the synthetic and secretory apparatus will determine mucin production. Differences in these processes likely exist in airway zones where increases represent either hyperplasia or metaplasia; thus, the generation of mucous secretory cells in the pseudostratified epithelium may be different than in the simple bronchiolar epithelium. A better understanding of each step in the mucous secretory cell life cycle in different airway zones theoretically provides an opportunity to intervene to decrease mucin production.

Colony-forming (60, 61, 63) and label retention (64) studies suggest a general hierarchy for the pseudostratified epithelium as illustrated in Figure 4. However, as discussed above, wound repair (55) and cell isolation studies (57) indicate that all cell types can be generated from columnar cells of the pseudostratified epithelium via an intermediate, poorly differentiated cell type (*downward pointing, red arrows* in Figure 4). Whether “de-differentiated” transiently amplifying progenitors can achieve “stemness” equivalent to the normal putative basal stem cell subpopulation requires further study.

As illustrated in Figure 5, the hierarchy in the simple epithelium of the bronchioles includes an apparently independent cycle among the pulmonary neuroendocrine cell population, likely involving the hedgehog signaling pathway (82). It is not clear at this time if there is a small population of stem or progenitor neuroendocrine cells or if they are maintained by larger numbers of differentiated cells, as reported for beta cells in pancreatic islets (23). Variant Clara cells in the bronchioles are likely the progenitors of other Clara and ciliated cells (71) and the SCGB1a1/SP-C/Sca-1/CD34-expressing cell of the bronchiolar-alveolar junction appears to regenerate both the bronchiole and the proximal alveolar region (74, 75). In the distal alveoli, there is evidence for subpopulations of type II cells with different growth potentials (83) (see Reference 84), but again, it is also possible that a model similar to the pancreatic islets applies, and that a broad population of differentiated cells may maintain the distal lung epithelium.

Mucous secretory cell hyperplasia in the upper airway and metaplasia in the bronchioles can be generated by at least two cellular mechanisms. In the allergen-challenged mouse, mucous

Figure 3. A graphic representation of putative stem cell niches in the airway epithelium. (1) Basal cells in the gland duct; (2) surface basal cells typically present in the intercartilaginous zone; (3) variant Clara cells associated with pulmonary neuroendocrine cell (PNEC) bodies; (4) variant Clara cells present at the bronchiolar-alveolar duct junction. See text for further explanation.

Figure 4. A graphic representation of the theoretical stem cell hierarchy in the pseudostratified airway epithelium. In this model, a subset of basal cells within defined niches represents a stem cell compartment that gives rise to transiently amplifying cells with basal, parabasal, and intermediate cell morphologies that generate differentiated cells (straight black arrows). The thickness and size of the circular arrows are representative of growth capacity. The red arrows indicate the presence of plasticity in the airway epithelium. Differentiated cells are capable of "dedifferentiation" (downward-pointing arrows) and "redifferentiation" during injury and repair. Also, phenotypic conversion (SCGB1a1-expressing to mucous secretory) and "transdifferentiation" (ciliated to mucous secretory) may occur under appropriate stimuli.

secretory cells in the trachea and upper generations of the bronchi appear to derive mainly from Clara cells (85, 86), whereas in the airways of mice exposed to Sendai virus, ciliated cells can transdifferentiate into mucous secretory cells (87) by a two-step process regulated by activation of epidermal growth factor receptor family members and interleukin 13 (88). The relative importance of conversion of SCGB1a1-positive cells to mucous secretory cells versus transdifferentiation of ciliated cells to mucous secretory cells in the human large and small airways in relationship to COPD remains to be determined.

DEFECTIVE MUCUS TRANSPORT AND LUMINAL OCCLUSION

Luminal accumulation of mucus likely contributes to airflow obstruction and serves as a nidus for chronic bacterial infection in later stages of COPD. The amount of mucus in the airway must represent the balance among the number of mucous secretory cells, their synthetic and secretory activity, and the ability to clear the airway of secretions. Although routine formalin-fixed histologic sections of the airway usually demonstrate "splitting" between the luminal mucus plugs and the epithelial surface, this is likely to be an artifact due to variable shrinkage of the mucus and the airway wall during processing and sectioning. Frozen sections of the COPD airway avoid this artifact and frequently demonstrate mucus adhesion to the airway wall (Figure 6). The efficiency of mucociliary and cough clearance is likely influenced by many factors in COPD, including the mass of secreted mucus, altered content of DNA and actin as well as other molecules, altered airflow due to loss of elasticity, physical distortion of the airway wall due to loss of tethers, abnormal ciliated cell activity, and changes in ion and water transport resulting in relative surface dehydration (reviewed in Reference 89). Therapies directed at improving mucus clearance may slow the progression of COPD by decreasing the frequency and impact of acute exacerbations.

CONCLUSIONS

Airway wall thickening due to fibrosis, epithelial hyperplasia, and luminal obstruction with inflammatory mucus exudates are

Figure 5. A graphic representation of the theoretical stem cell hierarchy in the bronchiolar epithelium. The thickness and size of the *circular arrows* are representative of growth capacity and differentiation potential. Pulmonary neuroendocrine cells (PNEC) are believed to represent a lineage independent of other epithelial cell types in the adult. Variant Clara cells associated with PNEC bodies can multiply and regenerate ciliated and Clara cells of the bronchiolar epithelium and variant Clara cells present near the bronchiolar–alveolar junction appear to be able to generate both bronchiolar and alveolar epithelium, as well as adenocarcinoma (not illustrated).

correlated with the severity of COPD. An improved understanding of airway stem cell biology, including progenitor–progeny relationships and molecular regulation of cell dynamics leading to mucous secretory cell hyperplasia/metaplasia and airway wall fibrosis, will provide a logical basis for novel therapies directed the characteristic underlying pathology. As well as physically obstructing airflow, luminal mucus likely contributes to chronic bacterial infection associated with more advanced disease, and strategies to improve mucus clearance may also be beneficial.

Figure 6. Representative images of COPD bronchiolar epithelia and luminal mucus in frozen sections. Note the abundance of mucous secretory cells and that luminal mucus is closely apposed to the apical epithelial surface (*blue areas in the lower image*). The epithelial basement membrane appears as a *bright pink line* in both the *upper and lower panels*. Both photomicrographs are at the same magnification (originally 400 $\times$).

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