Airway Epithelial Cells

Current Concepts and Challenges

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The adult human bronchial tree is covered with a continuous layer of epithelial cells that play a critical role in maintaining the conduit for air, and which are central to the defenses of the lung against inhaled environmental concomitants. The epithelial sheet functions as an interdependent unit with the other lung components. Importantly, the structure and/or function of airway epithelium is deranged in major lung disorders, including chronic obstructive pulmonary disease, asthma, and bronchogenic carcinoma. Investigations regarding the airway epithelium have led to many advances over the past few decades, but new developments in genetics and stem cell/progenitor cell biology have opened the door to understanding how the airway epithelium is developed and maintained, and how it responds to environmental stress. This article provides an overview of the current state of knowledge regarding airway epithelial stem/progenitor cells, gene expression, cell–cell interactions, and less frequent cell types, and discusses the challenges for future areas of investigation regarding the airway epithelium in health and disease.

Keywords: airway epithelium; progenitor/stem cells; gene expression; differentiation

The 2^{21} to 2^{23} branches of the adult human airways are covered with a continuous epithelial sheet comprising less than 1% of the total respiratory epithelial surface (1–3). The airway epithelium is pseudostratified in the large airways, becoming columnar and cuboidal in the small airways. The major cell types are ciliated, columnar, undifferentiated, secretory and basal cells (Figure 1). In the normal adult human, these epithelial cell populations vary as a function of airway level, with decreased numbers of cartilage cells and submucosal glands and the emergence of Clara secretory cells as the airways branch from large to small airways. There are a variety of less frequent cell types throughout the airways, of which neuroendocrine cells have attracted the most interest.

The airway epithelium plays a critical role in maintaining the conduit for air to and from the alveoli (4). It is central to the defenses of the lung against pathogens and particulates inhaled from the environment, with the combined function of secretory and ciliated cells maintaining efficient mucociliary clearance, and a variety of other host defense processes (4–6). The epithelial sheet does not function as an independent entity, but rather as an interdependent functional unit with the other epithelial cells, mesenchymal cells and endothelial cells, and the extracellular matrix comprising the bronchial walls (4, 7). Importantly, airway epithelial cells are central to the pathogenesis of major lung disorders, including chronic obstructive pulmonary disease (COPD), asthma, and bronchogenic carcinoma. In these disorders, the function of the airway epithelium is further modified by local inflammatory/immune signals (5, 6).

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Although there have been significant advances over the past few decades in understanding how the human airway epithelium functions in health and disease, new developments in genetics and stem cell/progenitor cell biology have significant implications for understanding how the airway epithelium develops, how it is maintained, and how it responds to environmental stress. In this context, the NHLBI workshop participants focused on four topics relevant to the airway epithelium: (*1*) airway epithelial stem/progenitor cells, (*2*) gene expression, (*3*) cell–cell interactions, and (*4*) less frequent cell types.

AIRWAY EPITHELIAL STEM/PROGENITOR CELLS

The airway epithelium is a dynamic tissue normally undergoing slow, but constant renewal. On the basis of studies in experimental animals and limited studies in humans, the airway epithelium likely turns over every 30 to 50 days (8). If injured, unless the injury is too severe, extensive, or chronic, the airway epithelium responds vigorously to reestablish an epithelial sheet with normal structure and function, with resident cells as the source of the new cell population (8–12). Although there may be some contribution from circulating stem/progenitor cells, most evidence supports the concept that stem/progenitor cells distributed throughout the airway epithelium are the source of the new epithelial cells, and that these stem/progenitor cells have the potential to differentiate to all of the cell types of the normal epithelium (13, 14). At present, there is no definitive understanding of progenitor cell–progeny relationships in the airway epithelium. It is not understood whether rare epithelial stem/progenitor cells residing in specific niches proliferate, migrate, and differentiate in a highly orchestrated process similar to classical models of high turnover tissues, and/or if alternative models of simple duplication of differentiated cells are more applicable (14).

S. Randell (University of North Carolina) discussed basic concepts relevant to airway epithelial stem/progenitor cells and the murine models used to identify them. Notably, new lineage-tracing studies question long-standing principles of stem cell hierarchies even in well-understood rapid turnover tissues. The notion of separate stem cell compartments for the follicular and interfollicular epidermis has been challenged (15), and, in the intestine, evidence suggests that a novel cell type at the tip of the crypts, rather than the well-known cells at the +4 position, are the stem cells (16). In the conditionally proliferative pancreas, cell replacement may occur via duplication of β-cells rather than differentiation from a precursor cell type (17). Advances in the broader stem cell field require constant reevaluation of even the well-known systems, and a major goal is to apply developing concepts to the airways and lung. An up-to-date review of lung stem cell biology is available (18).

The airway tree is complex and cell populations vary systematically both by airway generation and by species (2). Despite potential differences between animal models and humans, current concepts in this field come mostly from studies of the rodent airways and lung. Furthermore, the conditional

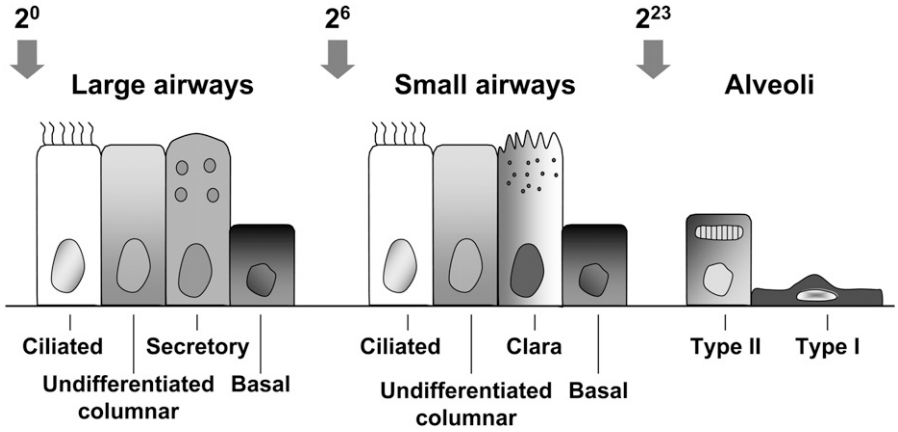

Figure 1. Major cell types of the lung epithelium. In the large airways (2^0 to 2^5 branches), the major cells types are ciliated, undifferentiated columnar, secretory, and basal cells. In the small airways (2^6 to 2^{23} branches), the cell types are similar, with relatively more ciliated cells, and the secretory cells shift to the Clara cell type. After 2^{23} branches, the airway epithelium merges with the alveolar epithelium, with type I and type II cells. Not shown are a variety of less common cell types, such as cartilage cells and mucus glands in the large airways, and neuroendocrine cells.

nature of airway and lung cell proliferation typically requires injury (bleomycin, naphthalene, SO_2, transplantation, etc.) or cell culture testing to experimentally recruit stem and progenitor cells, which introduces uncertainty regarding broader applicability to steady-state renewal and/or other types of repair. Nevertheless, DNA metabolic pulse-labeling studies show that both basal and columnar secretory cell populations of the pseudostratified epithelium divide, albeit relatively infrequently in normal airways (19, 20). Epithelial repair is rapidly induced after injury (10), with both "dedifferentiation" and migration of cells to cover defects, with proliferation and "redifferentiation" ongoing. Despite early data suggesting that only basal or secretory cells were pluripotent, it appears that both basal and columnar cells can reconstitute a full epithelium (21). Thus, there is abundant plasticity within the tissue as indicated by progenitor cells from multiple cell compartments. The rapid migratory and proliferative response of the pseudostratified airway epithelium likely indicates the high priority given to repair of denuding epithelial injuries. Although secretory, nondifferentiated columnar and parabasal cells can proliferate (20), the tentative consensus from DNA label-retention (22), lineage mapping (23, 24), and *in vitro* studies (25) is that a subpopulation of basal cells are most likely the stem cells of the pseudostratified zone.

There are many unanswered questions regarding epithelial stem and progenitor cells in the larger airways. In view of the ongoing revision of basic stem cell hierarchies in general, have the existing studies accurately identified niches in the pseudostratified epithelium? What are the criteria for stem cells of this tissue, and what assays are needed to find them? Do they express unique molecular markers? What are the molecular relationships between stem cells, the matrix, and lamina propria cells? Will a better understanding of cell lineages and regulation of proliferation and differentiation presage novel therapeutic approaches to modulate airway epithelial phenotype? Are the tissue stem cells targets for malignant transformation in bronchogenic carcinoma, and will understanding the stem cells assist in the detection, monitoring, and treatment of this most prevalent cancer?

Depending on species, basal cells disappear as one moves from the trachea toward the alveoli. The epithelium becomes simple columnar and then cuboidal in the small airways, a critical zone for several common lung diseases, such as COPD (26) and bronchiolitis obliterans, a severe complication of both lung and allogenic hematopoietic stem cell transplantation that limits long-term survival (27, 28). A tenable hypothesis is that a relatively limited reservoir of stem cells renders small airways susceptible to injury, phenotypic modulation, and, in some cases, denudation followed by fibrotic obstruction. An insightful murine model of small airway epithelial renewal is administration of naphthalene to mice, which causes death of the majority of the Clara cell population due to selective metabolic activation of the toxin. Ciliated cells shed their cilia and migrate to cover the denuded basement membrane (29–31), but do not appear to enter the cell cycle (32). A small proportion of variant Clara cells residing within neuroepithelial bodies survive, and are the source of cells that proliferate and differentiate to regenerate the epithelium (30), and this unit may function as a stem cell niche. Another proposed airway epithelial stem/progenitor cell niche is located near the junction of the bronchi and alveoli (13, 33). A specific subset of epithelial cells reported to express secretoglobin, family 1A, member 1 (SCGB1a1), surfactant protein (SP)-C, CD-34, and stem cell antigen (Sca)-1 were found to proliferate in response to injury, grow clonally *in vitro*, and differentiate into Clara and alveolar epithelial cells, and were the source of carcinoma in a model of Ras oncogene activation (13).

Further studies are clearly needed to better define the spectrum of "stemness" within the small airway epithelial cell population, to elucidate additional molecular markers for stem/progenitor cells, to understand the molecular pathways regulating their behavior, and to extend observations from murine models to humans. A key question is whether therapies directed at enhancing repair via modulation of stem and progenitor cell regulatory pathways will be a reasonable approach to limit injury and/or to preserve small airway function, and conversely to inhibit growth and treat cancers that originate in this zone.

J. Engelhardt (University of Iowa) focused on the putative niches for stem/progenitor cells in the lung. Several regions of the airways likely provide specialized microenvironments that regulate stem/progenitor cell behavior in the setting of airway injury, repair, and maintenance. As in other organs, it is likely that niche microenvironments in the lung can control intrinsic stem/progenitor cell properties through cell–cell contact with supporting cells, paracrine and endocrine signaling from either local or distant sources, and/or neural input (34, 35). However, unlike other organs such as the skin and gut for which stem cell compartments are better characterized (36, 37), the unique functional anatomy of the lung must support a number of trophic units that span great distances. Each of these trophic units contains a unique composition of cell types that serve a unique function in the context of lung innate immunity and gas exchange. Given the vast distances at the cellular level between each of these trophic units—tracheobronchial, bronchiolar, and alveolar—it is currently believed that multiple adult stem cell niches would be required to support each of these unique cellular compartments (38).

Submucosal glands have been suggested to serve as a stem cell niche of the proximal cartilaginous airways, on the basis of studies demonstrating that bromodeoxyuridine (5-bromo-2-deoxyuridine) label–retaining cells, characterized by a slow-cycling stem cell phenotype, reside in gland ducts of mouse trachea after injury (22, 39). As discussed by S. Randell, putative stem/progenitor cells in the bronchioles have been associated with neuroepithelial bodies (30, 33). In this region, a subpopulation of naphthalene-resistant Clara cells (termed a variant Clara cell or Clara^{v}) that express the Clara cell secretory protein (CCSP) have been suggested to function as the stem cells of the distal airways. In bronchioles, these cells appear to reside at airway branch points. Pulmonary neuroendocrine cells that reside within neuroendocrine bodies are believed to provide extrinsic components important for the stem cell niche and maintenance of Clara^{v} cells. Recently, an additional potential niche has been identified at the bronchioalveolar duct junctions and contains a second type of Clara^{v} that expresses both CCSP and SP-C (an alveolar type II cell protein) (13). In contrast to the bronchiolar airway, little is known about the phenotype of glandular progenitor cells in the proximal mouse airways. This region of the proximal airway differs between mouse and humans in that submucosal glands are limited to the proximal trachea in mouse but are found throughout the cartilaginous airways (i.e., tracheobronchial) in humans. Therefore, the tracheobronchial progenitor/stem cell niche may be quantitatively and qualitatively different between mice and humans.

It has become increasingly recognized that dysfunction in the microenvironment of a stem cell niche may contribute to the emergence of disease (35). For example, in cystic fibrosis (CF), significant airway glandular remodeling occurs in response to lung infection, with alterations including glandular hypertrophy (expansion of gland mass in existing glands) and metaplasia (replacement of one cell phenotype with another; e.g., serous to mucous cell) (40). Such changes suggest that glandular progenitor/stem cells have altered biology as a result of lung injury and infection. In support of this hypothesis, one study indicates that submucosal gland hyperplasia (the formation of new glands) may occur in the tracheas of CF mice (41), suggesting that the number of proximal airway stem cell niches may be increased in CF mice. Given the potential importance of submucosal glands as stem cell niches for the proximal airway, studies evaluating functional components involved in the establishment of this niche will lead to a better appreciation of changing airway niche characteristics in the setting of disease. Relatively little is known about the signals and cell types involved in airway progenitor/stem cell niche function and whether they are conserved in nonrodent species.

Characterization of progenitor/stem cell niches in the lung has been heavily dependent on the localization of a nucleotide label-retention phenotype and a limited number of genetic mouse strains with gene-specific promoters and gene products to manipulate airway epithelial cell *in vivo*. Label-retention is only one suggestive phenotype of an adult stem cell population and caution must be used when drawing conclusions about potential stem cell phenotypes without functional lineage analysis (16). For example, after napthelene labeling in the mouse, pulmonary endocrine cells expand and retain nucleotide label. However, on the basis of genetic studies using thymidine kinase ablation of CCSP-expressing Clara cells, pulmonary endocrine cells are not capable of regenerating a ciliated airway epithelium and therefore are not stem cells in the distal airways of the mouse (30). We currently lack good markers for the diversity of cell phenotypes in the lung, which are needed for more definitive characterization of adult stem cell niches and their phenotypes.

BIOLOGIC PHENOTYPING OF THE HUMAN AIRWAY EPITHELIUM

R. Crystal (Weill Cornell Medical College) concentrated on understanding the human airway epithelium at the genetic level. The human airway epithelium is accessible to sampling in humans using fiberoptic bronchoscopy and brushing. With current technology, pure populations of large and small airway epithelium can be repetitively sampled in normal individuals and individuals with lung disease (12, 42–44), easily obtaining 5×10^6 epithelial cells. Using current sophisticated 'omics technologies, the opportunity is ripe to characterize the program of all 25,000 genes of the human airway epithelium at the DNA, mRNA, protein, and metabolic levels in their baseline state, under conditions of environmental stress and in disease states.

Routine bronchoscopy with brushing permits repetitive sampling of both large and small airway epithelium in amounts adequate for application of microarray assessment of expression of all human genes. Although air–liquid interface cultures, the *in vitro* gold-standard culture representative of the human airway epithelium, are useful for a variety of biologic studies, microarray comparison of mRNA expression patterns of air–liquid interface cultures of human large airway epithelium to that of freshly isolated brushed samples of large airway epithelium of normal individuals shows differences in the expression levels of hundreds of genes, with the freshly isolated epithelium expressing more immune response and mucin-related genes, and the cultures expressing more oxidant-related, growth factor, cell cycle, and adhesion genes.

Strikingly, the small airway epithelium of cigarette smokers who are phenotypically normal (no symptoms, normal lung function, normal imaging) has approximately 300 genes significantly up- or down-regulated compared with normal nonsmokers (44). This is the biologic "smoking phenotype." Interestingly, there is considerable variability in the airway epithelium in response to smoking, suggesting a genetic component in the response to smoking (42, 45). Furthermore, normal smokers can be ranked based on their average levels of gene expression in their small airway epithelium as "high responders" and "low responders," independent of age, sex, ancestry, or extent of smoking (i.e., humans vary in terms of the gene expression responses of the airway epithelium to environmental stress) (46). There is also evidence that ancestral background plays a role in the gene expression responses of the airway epithelium, with those of African descent having identifiable differences in their gene expression patterns compared with those of European descent, and specific single nucleotide polymorphisms dictating differences in airway epithelial gene expression (47). The frontier for applying 'omics technologies to phenotyping the human airway epithelium is to characterize each of the multiple cell types that comprise the large and small airway epithelium, and to apply 'omics technologies to define the airway epithelial stem cell populations, and the myriad of signals that control how the airway epithelial population is maintained in health and disease. Using 'omics technologies at the DNA, mRNA, protein, and metabolic levels, it should be possible to develop different phenotypes of the human large and small airway epithelium in health, under environmental stress, and in disease states. A major challenge in understanding the human airway epithelium is to define how genomic variation modulates gene expression, and therefore the phenotype of the cells comprising the epithelium, and how this genomic variation defines risk for the major human disorders of the airways, including COPD, asthma, and bronchogenic carcinoma.

CELL–CELL INTERACTIONS

J. Voynow (Duke University) focused her remarks on mucous and ciliated cells and their interactions. Mucociliary clearance has an important innate immune function in healthy airways, with contributions by the ciliated cells in the conducting airway epithelium and the secretory cells, including goblet cells and the mucous and serous cells in the submucosal glands. Coordinated ciliary motion clears mucus-bound particles from the airways. Mucus binds infectious agents and particulates, but also has antioxidant, antiprotease, and antimicrobial properties. These functional properties are due to proteins (lysozyme, lactoferrin, defensins, secretory leukocyte peptidase inhibitor [SPLI], lactoperoxidase) secreted by serous cells of the submucosal glands (48), as well as CCSP from Clara cells, and SP-A and SP-D from alveolar type II cells. However, the functional properties of the major macromolecular constituent of mucus, the mucin glycoproteins, are far less well established.

The functional contributions of mucins are speculated to be related to the hundreds of diverse *O*-glycosidic carbohydrate structures, which are covalently bound to serine and threonine residues in mucin protein backbones. These are predicted to be important for lectin recognition by bacteria and leukocytes. Another characteristic motif of secreted mucins, cysteine-rich domains at the carboxyl- and amino-termini, are the sites for oligomerization of mucins and are believed to confer biophysical properties (aggregation, gel formation) of mucins and mucus. However, there is little direct evidence that defines the functional properties of specific carbohydrate structures or specific motifs in mucin protein backbones. In addition to unique structural characteristics of mucins, expression of specific mucins is restricted to different secretory cell types. For example, MUC5AC is localized to goblet cells and MUC5B is localized to mucous cells of submucosal glands in the healthy airway. The impact of mucin segregation in different secretory cells on secreted mucus function in healthy airways is not known (49).

The secretory cells are implicated in lung diseases as mucus hypersecretion/overproduction, goblet cell hypertrophy and hyperplasia, and submucosal gland hypertrophy, which are common pathologic features of asthma, COPD, and CF. In a mouse model of asthma, it has been demonstrated that blocking mucus hypersecretion relieves airway obstruction (50), providing an important rationale for investigating secretory cell remodeling in chronic inflammatory airway diseases. However, there is not a common paradigm underlying secretory cell hyperplasia because these mechanisms likely vary for each chronic inflammatory disease process (49), and also vary by the severity of the insult. For example, airway epithelial cell proliferation varies directly with disease severity in asthma (51), yet goblet cell hyperplasia is present in patients with mild and severe asthma (52). Mouse models of asthma do not demonstrate increased airway epithelial proliferation and data support the hypothesis of "transdifferentiation" from either ciliated (53) or Clara cells (54) to goblet cells to explain secretory cell remodeling. In contrast, after naphthalene-induced epithelial injury and loss, lineage-tagged basal cells proliferate and differentiate to repopulate ciliated, goblet, Clara, and basal cell populations (24), and transdifferentiation of ciliated to goblet cells does not occur (32).

Distinct signaling pathways also regulate goblet cell hyperplasia and differ according to the disease and the environmental insult. In asthma, IL-13 and epidermal growth factor receptor (EGFR) activate independent pathways to regulate goblet cell hyperplasia (53). In COPD, tobacco smoke or reactive oxygen species regulate goblet cell hyperplasia by either EGFR-dependent or EGFR-independent pathways (55, 56). In mice, overexpression of the epithelial sodium channel creates CF-like primary defects of increased sodium absorption and diminished airway surface liquid volume, resulting in goblet cell metaplasia and mucus obstruction of airways (57). Finally, a profile of secretory cell positive and negative regulatory transcription factors is emerging with FoxA2 (58), a negative regulatory factor for goblet cell metaplasia and catenin and SAM pointed domain containing ETS transcription factor [SPDEF], positive regulatory factors for goblet cell metaplasia (58). Although progress has been made in understanding mechanisms of secretory cell metaplasia in the mouse, in humans there are many outstanding questions concerning the fundamental properties of airway mucins and mechanisms of secretory cell remodeling in chronic inflammatory airway diseases.

Among the important questions to be answered regarding the interactions of mucous and ciliated cells are the following:

1. What are the MUC species and their relative amounts in normal mucus? How does this profile change in disease states and what is the role of post-transcriptional and epigenetic regulation?
2. What are the functions of secreted mucins? What is the role of the carbohydrate diversity and the role of specific motifs in mucin protein backbones?
3. How does mucus interact with cilia?
4. What is the machinery required for constitutive versus stimulated exocytosis in secretory cells?
5. Are there local environmental or structural factors that regulate normal development of goblet cells or submucosal gland cells?
6. How are goblet cell hyperplasia and submucosal gland remodeling regulated and what is the relative contribution of gland secretions to abnormal mucus?
7. What is the role of proliferation and differentiation versus transdifferentiation in goblet cell hyperplasia and which cells are the precursor cells?
8. Can secretory cell remodeling be reversed?

RARE CELL TYPES

Mary Sunday (Duke University) discussed pulmonary neuroendocrine cells (PNECs), a rare, but likely very important cell of the airway epithelium. Although there are a number of other important rare cell types, such as neural cells, PNECs appear to play a number of roles that are unique in lung physiology in health and disease. Their normal function and their role in lung disease are only beginning to be understood. The cells have dense-core neurosecretory granules containing serotonin and numerous bioactive peptides, including calcitonin and gastrin-releasing peptide (GRP). The relative numbers of PNECs and tissue levels of GRP and GRP mRNA are highest at midgestation in human fetal lung, which corresponds to the canalicular phase of development. Recent studies have shown that GRP promotes proliferation and differentiation of type II cells and PNECs in lung explants from mice, humans, and baboons and mice *in vivo*. Focusing on the PNEC hyperplasia associated with bronchopulmonary dysplasia (BPD), studies in premature baboon models showed that increased GRP production and elevated numbers of PNECs occur within days of birth, and correlate with elevated urine GRP levels. In contrast, clinical and pathologic evidence of BPD takes weeks to develop. In premature infants who went on to develop BPD months later, mean urine GRP levels doubled in the first 5 days of life. To

address the mechanisms underlying this association, premature baboons were treated with a blocking anti-GRP antibody, which resulted in abrogation of both clinical and pathologic parameters of BPD. These studies help define GRP as a mediator of lung injury in BPD and suggest that PNECs function as part of the innate immune system.

RECOMMENDATIONS RELEVANT TO THE AIRWAY EPITHELIUM

1. Using gene expression at the mRNA, protein, metabolic, and immunohistochemical levels, identify markers for each of the categories of airway epithelial differentiated cells. It is likely that there are subpopulations of airway epithelial cells with similar morphology, but with distinct differences in gene expression. Generate monoclonal antibodies specific for each cell type that can be used to detect and isolate specific airway epithelial cell types in mice and humans.
2. Develop a consensus as to the definitions of lung stem/progenitor cells and the assay criteria needed to define these populations. Isolate, characterize, and develop markers specific for airway epithelial stem/progenitor cells. Study the biology of airway epithelial cell "stemness," and define the complex signaling pathways that direct airway epithelial stem/progenitor cells to differentiate to distinct epithelial cell types.
3. Develop methods to isolate specific cell populations of the human airway epithelium, and use 'omics technologies to characterize the individual cell types in health, under environmental stress and in disease states.
4. Using morphologic and gene expression strategies, characterize the changes in the cell composition of the developing perinatal and childhood airway epithelium lung. Analogous to the efforts of the NHLBI-funded Lung Tissue Research Consortium for COPD and IPF, a fundamental step forward would be to identify and/or create a tissue bank of well-characterized human airway samples encompassing this critical developmental period.
5. Develop murine models to enable precise lineage mapping of airway epithelial stem/progenitor cells, and the differentiation of these cells in the normal lung and after injury.
6. Invest in new methodologies to lineage-tag specific human airway epithelial cells. This advance would provide a tool to definitively study specific cellular function and cellular adaptation under conditions of stress or disease in the context of the whole organ. It could be used to evaluate the impact of epigenetic regulation of genotypes in health and disease and to evaluate cell interactions between different tissues in the lung.

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References

1. Weibel ER. Morphometry of the human lung. New York: Academic Press; 1963.
2. Mercer RR, Russell ML, Roggli VL, Crapo JD. Cell number and distribution in human and rat airways. *Am J Respir Cell Mol Biol* 1994;10:613–624.
3. Weibel ER. Design and morphometry of the pulmonary gas exchanger. In: Crystal RG, West JB, Weibel ER, Barnes PJ, editors. *The lung: Scientific foundations*, 2nd ed. Philadelphia: Lippincott-Raven; 1997. pp. 1147–1157.
4. Knight DA, Holgate ST. The airway epithelium: structural and functional properties in health and disease. *Respirology* 2003;8:432–446.
5. Thompson AB, Robbins RA, Romberger DJ, Sisson JH, Spurzem JR, Teschler H, Rennard SI. Immunological functions of the pulmonary epithelium. *Eur Respir J* 1995;8:127–149.
6. Puchelle E, Zahm JM, Tournier JM, Coraux C. Airway epithelial repair, regeneration, and remodeling after injury in chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2006;3:726–733.
7. Holgate ST. Epithelial damage and response. *Clin Exp Allergy* 2000;30:37–41.
8. Bowden DH. Cell turnover in the lung. *Am Rev Respir Dis* 1983;128:S46–S48.
9. Erjefalt JS, Erjefalt I, Sundler F, Persson CG. In vivo restitution of airway epithelium. *Cell Tissue Res* 1995;281:305–316.
10. Erjefalt JS, Persson CG. Airway epithelial repair: breathtakingly quick and multipotentially pathogenic. *Thorax* 1997;52:1010–1012.
11. Persson CG. Epithelial cells: barrier functions and shedding-restitution mechanisms. *Am J Respir Crit Care Med* 1996;153:S9–S10.
12. Heguy A, Harvey BG, Leopold PL, Dolgalev I, Raman T, Crystal RG. Responses of the human airway epithelium transcriptome to in vivo injury. *Physiol Genomics* 2007;29:139–148.
13. Kim CF, Jackson EL, Woolfenden AE, Lawrence S, Babar I, Vogel S, Crowley D, Bronson RT, Jacks T. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005;121:823–835.
14. Rawlins EL, Hogan BL. Epithelial stem cells of the lung: privileged few or opportunities for many? *Development* 2006;133:2455–2465.
15. Clayton E, Doupe DP, Klein AM, Winton DJ, Simons BD, Jones PH. A single type of progenitor cell maintains normal epidermis. *Nature* 2007;446:185–189.
16. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, *et al*. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 2007;449:1003–1007.
17. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 2004;429:41–46.
18. Kotton DN, Fine A. Lung stem cells. *Cell Tissue Res* 2008;331:145–156.
19. Donnelly GM, Haack DG, Heird CS. Tracheal epithelium: cell kinetics and differentiation in normal rat tissue. *Cell Tissue Kinet* 1982;15:119–130.
20. Breuer R, Zajicek G, Christensen TG, Lucey EC, Snider GL. Cell kinetics of normal adult hamster bronchial epithelium in the steady state. *Am J Respir Cell Mol Biol* 1990;2:51–58.
21. Liu JY, Nettesheim P, Randell SH. Growth and differentiation of tracheal epithelial progenitor cells. *Am J Physiol* 1994;266:L296–L307.
22. Borthwick DW, Shahbazian M, Krantz QT, Dorin JR, Randell SH. Evidence for stem-cell niches in the tracheal epithelium. *Am J Respir Cell Mol Biol* 2001;24:662–670.
23. Engelhardt JF, Schlossberg H, Yankaskas JR, Dudus L. Progenitor cells of the adult human airway involved in submucosal gland development. *Development* 1995;121:2031–2046.
24. Hong KU, Reynolds SD, Watkins S, Fuchs E, Stripp BR. Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. *Am J Pathol* 2004;164:577–588.
25. Schoch KG, Lori A, Burns KA, Eldred T, Olsen JC, Randell SH. A subset of mouse tracheal epithelial basal cells generates large colonies in vitro. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L631–L642.
26. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet* 2004;364:709–721.
27. Nicod LP. Mechanisms of airway obliteration after lung transplantation. *Proc Am Thorac Soc* 2006;3:444–449.
28. Soubani AO, Uberti JP. Bronchiolitis obliterans following haematopoietic stem cell transplantation. *Eur Respir J* 2007;29:1007–1019.
29. Van Winkle LS, Buckpitt AR, Nishio SJ, Isaac JM, Plopper CG. Cellular response in naphthalene-induced Clara cell injury and bronchiolar epithelial repair in mice. *Am J Physiol* 1995;269:L800–L818.
30. Hong KU, Reynolds SD, Giangreco A, Hurley CM, Stripp BR. Clara cell secretory protein–expressing cells of the airway neuroepithelial body microenvironment include a label-retaining subset and are

critical for epithelial renewal after progenitor cell depletion. *Am J Respir Cell Mol Biol* 2001;24:671–681.
31. Park KS, Wells JM, Zorn AM, Wert SE, Laubach VE, Fernandez LG, Whitsett JA. Transdifferentiation of ciliated cells during repair of the respiratory epithelium. *Am J Respir Cell Mol Biol* 2006;34:151–157.
32. Rawlins EL, Ostrowski LE, Randell SH, Hogan BL. Lung development and repair: contribution of the ciliated lineage. *Proc Natl Acad Sci USA* 2007;104:410–417.
33. Giangreco A, Reynolds SD, Stripp BR. Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. *Am J Pathol* 2002;161:173–182.
34. Fuchs E, Tumbar T, Guasch G. Socializing with the neighbors: stem cells and their niche. *Cell* 2004;116:769–778.
35. Scadden DT. The stem-cell niche as an entity of action. *Nature* 2006;441: 1075–1079.
36. Barker N, Clevers H. Tracking down the stem cells of the intestine: strategies to identify adult stem cells. *Gastroenterology* 2007;133: 1755–1760.
37. Fuchs E. Skin stem cells: rising to the surface. *J Cell Biol* 2008;180: 273–284.
38. Liu X, Driskell RR, Engelhardt JF. Stem cells in the lung. *Methods Enzymol* 2006;419:285–321.
39. Engelhardt JF. Stem cell niches in the mouse airway. *Am J Respir Cell Mol Biol* 2001;24:649–652.
40. Oppenheimer EH, Esterly JR. Pathology of cystic fibrosis review of the literature and comparison with 146 autopsied cases. *Perspect Pediatr Pathol* 1975;2:241–278.
41. Borthwick DW, West JD, Keighren MA, Flockhart JH, Innes BA, Dorin JR. Murine submucosal glands are clonally derived and show a cystic fibrosis gene–dependent distribution pattern. *Am J Respir Cell Mol Biol* 1999;20:1181–1189.
42. Hackett NR, Heguy A, Harvey BG, O'Connor TP, Luettich K, Flieder DB, Kaplan R, Crystal RG. Variability of antioxidant-related gene expression in the airway epithelium of cigarette smokers. *Am J Respir Cell Mol Biol* 2003;29:331–343.
43. Heguy A, Harvey BG, O'Connor TP, Hackett NR, Crystal RG. Sampling-dependent up-regulation of gene expression in sequential samples of human airway epithelial cells. *Mol Med* 2003;9:200–208.
44. Harvey BG, Heguy A, Leopold PL, Carolan BJ, Ferris B, Crystal RG. Modification of gene expression of the small airway epithelium in response to cigarette smoking. *J Mol Med* 2007;85:39–53.
45. Ammous Z, Hackett NR, Butler MW, Raman T, Dolgalev I, O'Connor TP, Harvey BG, Crystal RG. Variability in small airway epithelial gene expression among normal smokers. *Chest* 2008;133:1344–1353.
46. Tilley AT, O'Connor TP, Hackett NR, Zhou XK, Strulovici Y, Raman Y, Crystal RG. Variability of the small airway epithelium gene expression in response to cigarette smoke among the healthy population and individuals with COPD. *Am J Respir Crit Care Med* 2008;177:A900.
47. Harvey B-G, Heguy A, Dolgalev I, Raman T, Santilli A, Vanni H, Kazeros A, Crystal RG. Ancestral differences in the small airway epithelium gene expression response to the insult of cigarette smoking. *Am J Respir Crit Care Med* 2007;175:A821.
48. Finkbeiner WE. Physiology and pathology of tracheobronchial glands. *Respir Physiol* 1999;118:77–83.
49. Rose MC, Voynow JA. Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiol Rev* 2006;86:245–278.
50. Agrawal A, Rengarajan S, Adler KB, Ram A, Ghosh B, Fahim M, Dickey BF. Inhibition of mucin secretion with MARCKS-related peptide improves airway obstruction in a mouse model of asthma. *J Appl Physiol* 2007;102:399–405.
51. Cohen L, Xueping E, Tarsi J, Ramkumar T, Horiuchi TK, Cochran R, DeMartino S, Schechtman KB, Hussain I, Holtzman MJ, *et al.* Epithelial cell proliferation contributes to airway remodeling in severe asthma. *Am J Respir Crit Care Med* 2007;176:138–145.
52. Ordonez CL, Khashayar R, Wong HH, Ferrando R, Wu R, Hyde DM, Hotchkiss JA, Zhang Y, Novikov A, Dolganov G, *et al.* Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am J Respir Crit Care Med* 2001;163:517–523.
53. Tyner JW, Kim EY, Ide K, Pelletier MR, Roswit WT, Morton JD, Battaile JT, Patel AC, Patterson GA, Castro M, *et al.* Blocking airway mucous cell metaplasia by inhibiting EGFR antiapoptosis and IL-13 transdifferentiation signals. *J Clin Invest* 2006;116:309–321.
54. Evans CM, Williams OW, Tuvim MJ, Nigam R, Mixides GP, Blackburn MR, DeMayo FJ, Burns AR, Smith C, Reynolds SD, *et al.* Mucin is produced by Clara cells in the proximal airways of antigen-challenged mice. *Am J Respir Cell Mol Biol* 2004;31:382–394.
55. Casalino-Matsuda SM, Monzon ME, Forteza RM. Epidermal growth factor receptor activation by epidermal growth factor mediates oxidant-induced goblet cell metaplasia in human airway epithelium. *Am J Respir Cell Mol Biol* 2006;34:581–591.
56. Gensch E, Gallup M, Sucher A, Li D, Gebremichael A, Lemjabbar H, Mengistab A, Dasari V, Hotchkiss J, Harkema J, *et al.* Tobacco smoke control of mucin production in lung cells requires oxygen radicals AP-1 and JNK. *J Biol Chem* 2004;279:39085–39093.
57. Mall M, Grubb BR, Harkema JR, O'Neal WK, Boucher RC. Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. *Nat Med* 2004;10:487–493.
58. Maeda Y, Dave V, Whitsett JA. Transcriptional control of lung morphogenesis. *Physiol Rev* 2007;87:219–244.