New Rules for Club Development: New Insights into Human Small Airway Epithelial Club Cell Ontogeny and Function

The human airways are lined with a pseudostratified epithelium comprised of a number of distinct populations of cells with specialized effector functions (1, 2). These include ciliated cells, secretory cells (goblet and club), basal cells, and neuroendocrine cells, although the abundance and prevalence of each distinct population vary considerably throughout the proximal–distal axis of the airways and also exhibit significant interspecies differences. Pathological airway remodeling is a prominent feature of many chronic lung diseases, including chronic obstructive pulmonary disease, asthma, and cystic fibrosis, and encompasses substantial pathological alterations in the airway epithelium. However, the etiology of these pathological changes is poorly understood, owing in part to a lack of basic knowledge regarding the mechanisms that regulate differentiation and repair of these epithelial populations. Accordingly, elucidating the specific roles of resident stem cells or progenitors that are responsible for differential postnatal growth, maintenance of homeostasis, and regeneration of the airway epithelium is of fundamental importance (3). It is clear that the lungs are capable of intrinsic repair, and, given the right circumstances, this opens up the possibility of designing and implementing regenerative medicine strategies to repair lung damage across a broad range of diseases. constitute

Club cells represent the major secretory cells of the small-airway epithelium in humans, constituting approximately 20% of such cells, and are clearly distinguishable morphologically by their smooth, dome-shaped apical surface that extends into the lumen, and their expression of SCG1A1 protein (also known as club cell 10) (4). Much of our knowledge concerning the ontogeny and function of these specialized epithelial cells originates from studies conducted in mice; however, there are substantial anatomical differences between human and murine airways that complicate translatability (5). Importantly, basal cells, a recognized stem cell population in both mouse and human lungs, are only present in the trachea of mouse lungs, whereas in humans, the pseudostratified epithelium containing basal cells extends much farther down the respiratory tree, as far as the respiratory bronchioles; thus, the mouse trachea more closely resembles the smaller airways seen in humans. Furthermore, club cells line all conducting airways of the murine lung, but are restricted to just the small airways in humans. In the pseudostratified epithelium of the mouse trachea, basal cells act as the primary progenitor cells, capable of self-renewal and differentiation into club and ciliated cells (6–8). The club cells of the mouse trachea exist as a transiently amplifying population, but their capacity for self-renewal and multilineage differentiation is enhanced after injury (6). Conversely, a self-renewing population of club cells maintain the basal cell–deficient epithelium of the distal bronchial and bronchiolar airways in mice, functioning as progenitors to ciliated cells and mucin-secreting goblet cells (6, 9). Furthermore, studies in mice have highlighted that club cells play important protective roles by participating in immune modulation, oxidative stress reduction, and xenobiotic metabolism (10–13). However, given the marked differences in the composition of cells lining the mouse and human airways, it is vital that we clarify the origin and roles of human airway club cells in homeostasis and repair before we move further along the translational pathway.

Although basal cells are progenitors of ciliated and mucus-producing cells in human airways (8, 14), the ontogeny and function of human small airway club cells are unknown, and this is the subject of the elegant study reported by Zuo and colleagues (pp. 1375–1388) in this issue of the *Journal* (15). The authors used principal component gradient analysis to demonstrate an ontological link between small airway basal cells and club cells in healthy nonsmokers, and subsequently showed that isolated basal cells differentiated into club cells in air–liquid interface (ALI) cultures. Thus, in keeping with findings from the mouse trachea, basal cells were convincingly shown to be a progenitor to club cells under steady-state conditions in human small airways. Although this conclusion is compelling, it should be acknowledged that the *in vitro* culture and differentiation of basal cells into a pseudostratified epithelium at the ALI may never truly reflect the complex microenvironmental cues and interactions that regulate airway epithelial homeostasis in the *in vivo* context of the human lung, as epitomized by the overrepresentation of basal cells seen in the ALI cultures. Nor does it preclude the potential for club cell self-renewal, as seen in mice and supported by previous observations of club cell proliferation in human small airways (4). Furthermore, given studies in mice, it would be prudent to question whether the relative significance of basal cells as club cell progenitors during postnatal lung growth is different from that observed after lung injury. Are club cells that arise from basal cells functionally the same as those that arise from existing club cells, and is differentiation aberrant or subverted in chronic lung disease? As discussed above, studies in mice demonstrated that club cells not only self-renew but also have the capacity to differentiate into multiple mature epithelial cell types, including ciliated and mucus-producing cells (6, 9). Accordingly, it is intriguing that transcriptome and ALI culture analyses defined subsets of

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SCGB1A1$^+$ cells expressing the ciliated cell marker β-tubulin IV and goblet cell marker MUC5AC. Could these represent intermediary, transitional stages in the development of pure ciliated or goblet cell populations, supporting the idea that human small airway club cells are also multipotent? Alternatively, are these distinct, novel populations of cells within human small airways? Future studies should seek to further define the ontogeny, fate potential, and functional significance of these cells, and examine whether these populations are overrepresented after injury/stress or in the diseased state, along with conceivable pathological implications.

Zuo and colleagues also used single-cell analysis to identify potentially novel functional roles for human small airway club cells in diverse biological activities. In keeping with previous studies in mice (10–13), the authors reveal an expanded role for human small airway club cells in host defense and xenobiotic metabolism, but they also highlight an exciting potential complicity in antiprotease defense, hereditary lung disorders, and pathogen recognition. The implications of these studies are potentially considerable and undoubtedly expand our appreciation of the role of small airway club cells in defining the balance between health and disease. However, the relative functional significance of these findings clearly needs to be interrogated and more fully defined, as does the relative significance of club cells versus other epithelial lineages in regulating these biological pathways throughout the proximal–distal axis of human airways. Furthermore, it would be intriguing to perform transcriptomic and relevant functional analyses of club cells from patients with chronic lung diseases to verify whether these pathways are aberrant. Nonetheless, these findings will serve as an excellent repository and resource to guide future interrogation, and the work by Zuo and colleagues can viewed as a seminal study in defining the ontogeny and biology of human club cells. ■

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References

1. 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.
2. Crystal RG, Randell SH, Engelhardt JF, Voynow J, Sunday ME. Airway epithelial cells: current concepts and challenges. *Proc Am Thorac Soc* 2008;5:772–777.
3. Rawlins EL, Hogan BL. Epithelial stem cells of the lung: privileged few or opportunities for many? *Development* 2006;133:2455–2465.
4. Boers JE, Ambergen AW, Thunnissen FB. Number and proliferation of Clara cells in normal human airway epithelium. *Am J Respir Crit Care Med* 1999;159:1585–1591.
5. Rock JR, Randell SH, Hogan BL. Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. *Dis Model Mech* 2010;3:545–556.
6. Rawlins EL, Okubo T, Xue Y, Brass DM, Auten RL, Hasegawa H, *et al*. The role of Scgb1a1+ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. *Cell Stem Cell* 2009;4:525–534.
7. Hong KU, Reynolds SD, Watkins S, Fuchs E, Stripp BR. In vivo differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L643–L649.
8. Rock JR, Onaitis MW, Rawlins EL, Lu Y, Clark CP, Xue Y, *et al*. Basal cells as stem cells of the mouse trachea and human airway epithelium. *Proc Natl Acad Sci USA* 2009;106:12771–12775.
9. Straume M, Johnson ML. Resolvability of free energy changes for oxygen binding and subunit association by human hemoglobin. *Biophys J* 1989;56:15–25.
10. Fanucchi MV, Murphy ME, Buckpitt AR, Philpot RM, Plopper CG. Pulmonary cytochrome P450 monooxygenase and Clara cell differentiation in mice. *Am J Respir Cell Mol Biol* 1997;17:302–314.
11. Mango GW, Johnston CJ, Reynolds SD, Finkelstein JN, Plopper CG, Stripp BR. Clara cell secretory protein deficiency increases oxidant stress response in conducting airways. *Am J Physiol* 1998;275:L348–L356.
12. Jones KG, Holland JF, Foureman GL, Bend JR, Fouts JR. Xenobiotic metabolism in Clara cells and alveolar type II cells isolated from lungs of rats treated with beta-naphthoflavone. *J Pharmacol Exp Ther* 1983;225:316–319.
13. Wang SZ, Rosenberger CL, Bao YX, Stark JM, Harrod KS. Clara cell secretory protein modulates lung inflammatory and immune responses to respiratory syncytial virus infection. *J Immunol* 2003; 171:1051–1060.
14. Gomi K, Arbelaez V, Crystal RG, Walters MS. Activation of NOTCH1 or NOTCH3 signaling skews human airway basal cell differentiation toward a secretory pathway. *PLoS One* 2015;10:e0116507.
15. Zuo W-L, Shenoy SA, Li S, O'Beirne SL, Strulovici-Barel Y, Leopold PL, *et al*. Ontogeny and biology of human small airway epithelial club cells. *Am J Respir Crit Care Med* 2018;198:1375–1388.

Palliative Care for Chronic Obstructive Pulmonary Disease

Signs of Progress, but Still a Long Way to Go

In 2015, in the United States, chronic obstructive pulmonary disease (COPD) was the third most common cause of death, with 155,041 people dying of the disease (1), whereas in Canada, it was the fourth most common cause of death, accounting for 12,573 deaths (2).

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Before death, patients with COPD have a progressive decline in health status, increasing symptoms, and increased reliance on family and carers to perform simple daily activities such as washing and dressing. Many patients would benefit from palliative care, an approach that centers on the management of symptoms, maintaining quality of life, and good communication. Palliative care has much to offer for people living with advanced COPD, but it includes more than just terminal care or symptom control and is not