Airway Basal Cells in Chronic Obstructive Pulmonary Disease: A Continuum or a Dead End?

Progressive airflow limitation in chronic obstructive pulmonary disease (COPD) develops because of stable changes in airway structure known as airway remodeling. Common features of airway remodeling in COPD include airway wall thickening, narrowing of the airway lumen and its occlusion by excessively produced mucus, and loss of small bronchioles (1). Some of these changes emerge relatively early during COPD development before emphysema and before airway obstruction is clinically detectable (2). Cigarette smoking, the major risk factor for COPD, causes many components of airway remodeling relevant to COPD. However, specific mechanisms driving the transition of otherwise reversible smoking-induced lesions into stable airway remodeling that underlies persistent airway obstruction in COPD remain unknown.

Among multiple cell types that may contribute to pathogenesis of airway remodeling in COPD, basal cells (BCs) have recently received particular attention. As resident stem cells, BCs are responsible for maintenance and regeneration of airway epithelium (3), the primary target of smoking-induced injury. Because of their ability to self-renew, BCs maintain the homeostatic stem cell pool in the airway epithelium. Some BC-derived progenitors differentiate to produce ciliated and secretory cells (Figure 1A). Numbers of all these cell types, including BCs themselves, are altered in smoking-induced lesions, such as BC hyperplasia and mucous hyperplasia, implying a possible role of stable changes in BC function in the evolution of these lesions into persistent airway remodeling (4). Located in the basal epithelial layer, right above the basement membrane that separates airway epithelium from the underlying mesenchyme (Figure 1B), BCs can interact with stromal and immune cells present in the subepithelial compartment, potentially relevant to airway fibrosis and inflammation (5, 6). Furthermore, as self-renewing stem cells, BCs are at risk of accumulating genetic or epigenetic alterations that may determine long-term changes in their function, enabling these progenitors to continuously produce remodeling patterns (4). However, little is known about specific changes that occur in airway BCs during COPD development.

To tackle this problem, in this issue of the *Journal*, Wijk and colleagues (pp. 103–113) conducted a study in which gene expression and clonogenic function of BCs—indicative of their self-renewal ability—were assessed immediately after their isolation from airway biopsy samples of subjects with or without COPD (7). To isolate BCs, they employed a FACS sorting strategy, which selects cells expressing NGFR (nerve growth factor receptor), a cell-surface marker of airway BCs (3), and also exhibits high forward scatter profile. Targeted gene expression analysis of individual isolated cells confirmed that all of them expressed the classical BC markers, keratin 5 and tumor protein TP63. About a third of BCs isolated from healthy airways expressed markers of secretory (*SCGB1A1*) or ciliated (*FOXJ1*) lineages, which likely represent committed progenitors (Figure 1A). This observation is in line with recent single-cell RNA-sequencing (scRNA-seq) studies that identified airway BC subsets with transitional gene expression patterns indicative of early differentiation (8, 9).

To evaluate the impact of cell culture, commonly used to expand BCs in *in vitro* studies, Wijk and colleagues (7) evaluated changes occurring in airway BCs after passaging them in culture. After the initial passage, the clonogenic capacity of BCs increased multifold, paralleled by downregulation of NGFR and early differentiation markers. This was accompanied by upregulation of *MKI67*, a cell proliferation marker, and *KRT14*, a keratin expressed by activated BCs in the injured airway epithelium or in association with squamous metaplasia (10). Thus, whereas freshly isolated cells contain the spectrum of homeostatic biological states of BCs ("state 1" in Figure 1C), BC culture reduces their natural heterogeneity, inducing or selecting for an activated BC state similar to that found in the injured or repairing airway epithelium ("state 2" in Figure 1C). How does airway BC heterogeneity change in COPD? To address this question, Wijk and colleagues (7) compared the transcriptomes of airway BCs freshly isolated from patients with COPD to those without disease using scRNA-seq analysis. This analysis revealed remarkable heterogeneity of airway BCs, which, based on their global transcriptional profiles, formed four clusters. Two of these clusters were dominated by BCs isolated from "healthy airways" and included minor subsets of BCs from patients with COPD, whereas the remaining two clusters were almost exclusively composed of BCs isolated from airways of patients with COPD. Together, these clusters formed a continuum of biological states of BCs, from healthy to COPD, which, as the authors hypothesize, may represent a trajectory of molecular phenotypes of BCs that gradually evolve as COPD develops.

The first question that comes to mind is, what genes change their expression in airway BCs following the "logic" of disease development and contribute to the "COPD BC trajectory" identified by Wijk and colleagues (7)? Strikingly, none of the genes that marked physiological or culture- induced airway BC states were found by the authors to be differentially expressed in COPD BCs, except for *NGFR*, which was upregulated. Instead, the identified COPD BC trajectory was found to be driven by progressively increased expression of a set of cellular stress response genes commonly upregulated in growth arrest conditions and in response to DNA damage and those associated with oxidative stress, hypoxia, and apoptosis. This finding suggests the possibility that in COPD, airway BCs, because of continuous oxidative damage and tissue injury, become progressively

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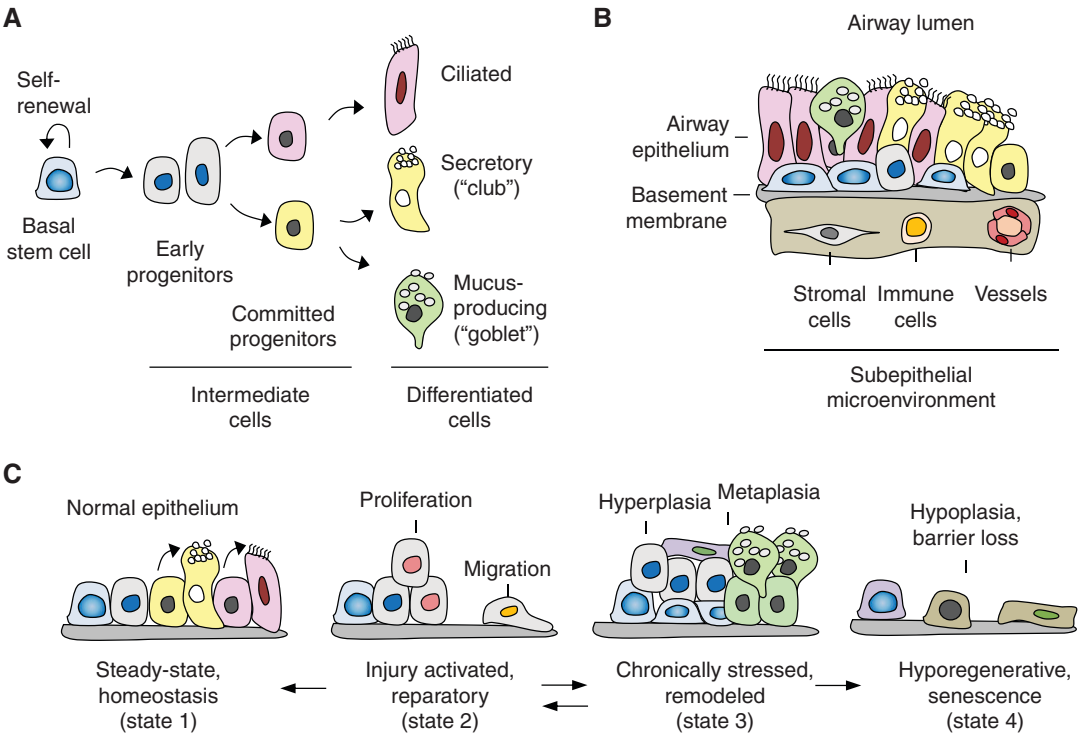

Figure 1. Airway basal cell (BC) heterogeneity and its contribution to airway remodeling in disease. (*A*) Stem/progenitor cell compartment of airway epithelium includes basal stem cells, which self-renew to maintain stem cell compartment, and "early" and "committed" progenitors also known as para-BCs, differentiating BCs, or intermediate cells, which differentiate to generate ciliated cells and secretory (mucus-producing and nonmucus club) cells. (*B*) Airway epithelium composed of BCs, intermediate cells, and differentiated cells. BCs are located in the basal epithelial layer, above the basement membrane, which separates airway epithelium from the subepithelial compartment, which contains stromal cells, immune cells, and vessels. These cells constitute the subepithelial microenvironment for airway BCs. (*C*) Heterogeneous biological states of airway BCs and BC-derived epithelial patterns: homeostatic (state 1; includes BCs, BC-derived differentiating progenitors, and differentiated cells), injury-activated reparatory (state 2; characterized by emergence of activated reparatory BC states that respond to injury by increased proliferation and migration), chronically stressed or remodeled (state 3; includes hyperplastic patterns of BC hyperplasia and mucus-producing cell hyperplasia and metaplastic patterns of mucous metaplasia and squamous metaplasia; these patterns constitute airway remodeling phenotypes), and hyporegenerative or senescent (state 4; characterized by loss of BC regenerative function, epithelial hypoplasia, and loss of epithelial barrier integrity).

"stressed out" and, at some point, lose their normal stem cell function (transition from "state 3" to "state 4" in Figure 1C). In support of this possibility, in previous studies, airway BCs of subjects with COPD have been found to be exhausted, with decreased capacity to self-renew and regenerate mechanically stable, normally differentiated airway epithelium (11, 12).

Although data generated by Wijk and colleagues (7) suggest a number of interesting possibilities about the biology of airway BCs in COPD, this study has several limitations. The first limitation relates to the small sample size of this study; BCs from only three patients with COPD were isolated for the scRNA-seq analysis that was used to build the pseudo–time trajectory. COPD is a heterogeneous disease with multiple endotypes, and not all of them may involve similar changes in airway BCs (4). Second, BCs were isolated from the fourth- to sixth-order bronchi, which is about 10 branching generations away from the small airways where COPD pathology develops (1). Changes observed in large airway BCs of patients with COPD may potentially reflect alterations occurring in these cells in association with chronic bronchitis, believed to be a pathophysiological precursor of COPD (13). Also, the analysis did not include samples from patients with mild or moderate COPD, which makes it impossible to confirm whether the identified trajectory indeed reflects disease evolution. Finally, the BC sorting strategy in this study was aimed at selecting cells that highly express NGFR and have a large size (high forward scatter), thus precluding the capture of BCs that do not express NGFR or are smaller, which may represent a considerable subset of airway BCs (14).

In sum, the study of Wijk and colleagues (7) stimulates innovative thinking about airway BC heterogeneity and its changes as a biomarker and mechanism of airway remodeling that mediates COPD progression. Alterations in airway BCs may be secondary to changes in stromal, immune, vascular, and other cell types that constitute their tissue-specific microenvironment, as recently shown at the single-cell level in patients with asthma (15). Studies that survey the entire diversity of cells and their interactions in airway regions that undergo disease-relevant remodeling in a large cohort of patients representing the broad spectrum of COPD heterogeneity, from pre- and early COPD to end-stage disease, should be the next step in the continuum of our search for new answers about mechanisms of disease. ■

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