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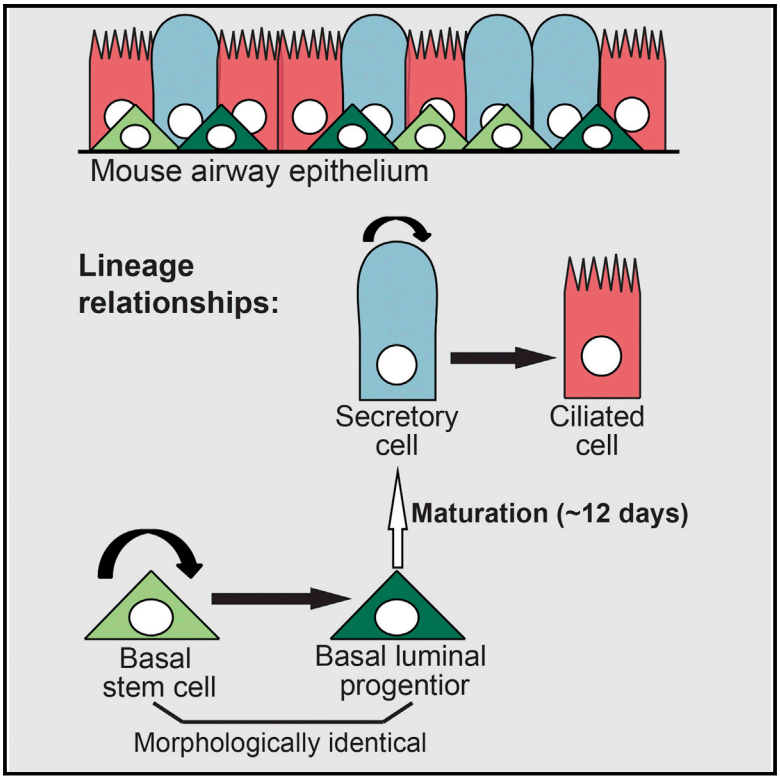
Clonal Dynamics Reveal Two Distinct Populations of Basal Cells in Slow-Turnover Airway Epithelium

Graphical Abstract



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In Brief

Using clonal analysis, mathematical modeling, and single-cell qRT-PCR, Watson et al. define the homeostatic tracheal epithelial lineage. The epithelium contains two major, equally distributed subpopulations of basal cells: stem cells and long-lived precursors that are already committed to differentiation.

Highlights

- Clonal analysis for determining homeostatic tracheal epithelial cell hierarchy
- Basal cells comprise two subpopulations: stem cells and luminal precursors
- Luminal secretory cells are short-lived and self-renewing
- Secretory cells are the major steady-state source of new ciliated cells

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Clonal Dynamics Reveal Two Distinct Populations of Basal Cells in Slow-Turnover Airway Epithelium

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SUMMARY

Epithelial lineages have been studied at cellular resolution in multiple organs that turn over rapidly. However, many epithelia, including those of the lung, liver, pancreas, and prostate, turn over slowly and may be regulated differently. We investigated the mouse tracheal epithelial lineage at homeostasis by using long-term clonal analysis and mathematical modeling. This pseudostratified epithelium contains basal cells and secretory and multiciliated luminal cells. Our analysis revealed that basal cells are heterogeneous, comprising approximately equal numbers of multipotent stem cells and committed precursors, which persist in the basal layer for 11 days before differentiating to luminal fate. We confirmed the molecular and functional differences within the basal population by using single-cell qRT-PCR and further lineage labeling. Additionally, we show that self-renewal of short-lived secretory cells is a feature of homeostasis. We have thus revealed early luminal commitment of cells that are morphologically indistinguishable from stem cells.

INTRODUCTION

The mouse trachea contains three major cell types: TRP63$^+$, KRT5$^+$ basal cells (BCs); luminal secretory cells (SecCs, mostly Scgb1a1$^+$ Club/Clara-like cells); and luminal ciliated cells (CCs) (Rock et al., 2010). Previous population-level lineage tracing using transgenic *Tg(KRT5-CreER)* mice demonstrated that BCs include self-renewing stem cells involved in tracheal growth, homeostasis (at least for up to 16 weeks), and repair (Rock et al., 2009). However, it is not known if BCs are a functionally heterogeneous population. A subset of tracheal BCs (<20%) expressing *Krt14* (*Keratin 14*) was suggested to be a unipotent self-renewing subpopulation at homeostasis (Ghosh et al., 2011). Similar unipotent BCs have been postulated following injury and in xenografts (Engelhardt et al., 1995; Ghosh et al., 2011; Hong et al., 2004). Other repair studies described an early progenitor (EP) cell as a proliferative KRT8$^+$ (luminal type cytokeratin), TRP63$^-$ cell derived from BCs and controlled by Notch signaling (Paul et al., 2014; Rock et al., 2011). In development, KRT5$^+$ TRP63$^-$ cells with basal morphology have recently been described in germline *Notch3* mutants and in embryonic lungs deleted for *Ezh2* (Mori et al., 2015; Snitow et al., 2015), leading to the speculation that these are precursors of luminal cells. Subsequently, an independent study showed that a population of adult BCs (~12% of steady-state total), which express low levels of transcription factors usually found in more differentiated cells, are able to contribute disproportionally to regeneration following injury (Pardo-Saganta et al., 2015). However, none of these studies investigated the adult airway lineage at steady state, leaving key questions unanswered. In particular, is there is an engrained proliferative heterogeneity in the steady-state basal layer? If so, what is the lineage relationship of cells within the basal layer, and how do they connect to the luminal compartments? How do distinct subpopulations of BCs function to maintain normal homeostasis?

Within luminal cells, population lineage-labeling studies had shown that SecCs can self-renew and generate CCs, but their relative contribution to homeostasis was unclear (Rawlins et al., 2009). CCs are post-mitotic, with an average loss-rate of ~6 months in the trachea (Rawlins and Hogan, 2008; Rawlins et al., 2007). Molecular signals controlling the tracheal epithelium are being determined (Brechbuhl et al., 2011; Giangreco et al., 2012; Lu et al., 2013; Paul et al., 2014; Rock et al., 2011; Zhao et al., 2014). However, the lack of a clearly defined epithelial lineage impedes analysis of molecular function at cellular resolution. Human airways have a very similar cell lineage to mouse trachea (Engelhardt et al., 1995; Hackett et al., 2011; Hajj et al., 2007; Teixeira et al., 2013), but the limited resolution for lineage studies in human means that complementary mouse analysis is required to determine the detailed cellular hierarchy. Here, we use clonal lineage labeling, coupled with biophysical