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A New Notch for Lung Stem Cells

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Homeostasis and repair of different lung compartments require the selective activation of specific progenitor/stem cells and their regulated differentiation to yield cell types that fulfill specialized regional functions. In this issue of *Cell Stem Cell*, Pardo-Saganta et al. (2015) identify Notch signaling as a determinant of basal cell heterogeneity and fate decisions in pseudostratified airways.

The epithelial lining of the lung can simplistically be divided into three anatomical regions—tracheobronchial airways, bronchiolar airways, and the alveolar gas-exchange region. In each region, specific resident stem/progenitor cells have been identified that are capable of long-term self-renewal and local replacement of specialized cell types. Basal cells fulfill this role in the proximal pseudostratified epithelium, Club cells do so in the bronchiolar region, and type II cells do so in the alveolar epithelium. Each of these stem/progenitor cell types is characterized by a low cell turnover under normal physiological conditions yet they respond promptly to injury for restoration of epithelial integrity and function (Hogan et al., 2014). However, mechanisms that control stem/progenitor cell renewal and differentiation for appropriate replacement of specialized cell types are only now being uncovered. In this issue of *Cell Stem Cell*, Pardo-Saganta et al. (2015) establish that differential expression of c-myb and the active signaling moiety of Notch2, Notch2 intracellular domain (N2ICD), lead to pre-determined heterogeneity among basal cells of the mouse tracheal epithelium that directs basal cell fate toward ciliated and secretory luminal cell types, respectively (Figure 1B). As these differentiated cell types have been thought to arise from a multipotent, CK8+ luminal progenitor (Figure 1A; Rock et al., 2011), these findings shed new light on mechanisms contributing to maintenance and replacement of functionally diverse cell types within complex epithelial tissues.

Despite the reported multipotency of basal cells and capacity for long-term self-renewal, functionally distinct subsets of basal cells have been demonstrated that may reflect either stochastic differentiation or intrinsic heterogeneity of a seemingly homogeneous population (Hong et al., 2004; Teixeira et al., 2013). Pardo-Saganta and colleagues were able to address the question of basal cell heterogeneity through use of tyramide amplification to boost the sensitivity of immunofluorescence detection and reveal three subsets of quiescent basal cells in the mouse tracheal epithelium (Figure 1B). Basal cell subsets were distinguished by the non-overlapping presence of c-myb or N2ICD immunoreactivity or the absence of either antigen. C-myb-expressing basal cells were found to be pre-disposed toward differentiation into ciliated cell fates after airway injury; this finding is consistent with the recent demonstration of roles for c-myb in the regulation of ciliated cell differentiation. Furthermore, these data are also consistent with the proposed role for Notch in suppressing c-myb expression and hence the generation of ciliated cell progeny (Pan et al., 2014). In contrast, N2ICD immunoreactivity was shown to define a subpopulation of basal cells that preferentially generated luminal secretory cell fates in response to injury.

Roles for canonical Notch in the regulation of the fate of this subset of N2ICD-immunoreactive basal cells were evaluated through use of conditional loss of function (LOF) of either Rbpj, a key transducer of canonical signaling, or mind-bomb (Mib1), a key enzyme for functional processing of Notch ligands. In each case, loss of Notch signaling within basal cells and their progeny resulted in failure to generate secretory luminal cells in favor of the overrepresentation of ciliated cells. This experiment demonstrates a critical role for canonical Notch signaling in the generation of luminal secretory cells. Pardo-Saganta et al. found that basal cells generated CK8+ luminal cells despite inhibition of Notch signaling. This suggests that, despite the clear impact of Rbpj and Mib1 LOF on basal cells, these LOF alleles will be transmitted to the luminal cell progeny and impact their behavior in the pseudostratified epithelium. Despite this caveat, it is clear that molecular heterogeneity of basal cells is established prior to their differentiation into immature luminal cell progeny and that the process of basal to luminal differentiation is independent of Notch signaling.

Previous work by Rock et al. demonstrated that inhibition of Notch signaling using γ-secretase inhibitors in 3D culture assays led to the expansion of basal cells in the absence of differentiation (Rock et al., 2011). Mori et al. (2015) recently showed that Notch3 may specifically be responsible for regulating the pool of basal cells available for differentiation (Figure 1B). However, there is disagreement between Pardo-Saganta et al. and Mori et al. over the impact of Notch LOF on basal cell self-renewal. This discrepancy may result from differences in model systems used to evaluate Notch signaling within basal cells. Pardo-Saganta et al. use in vivo models to argue that inhibition of Notch signaling within basal cells simply boosts the abundance of ciliated cell progeny at the expense of secretory cell progeny, with no changes in the abundance of basal cells. On the other hand, Mori

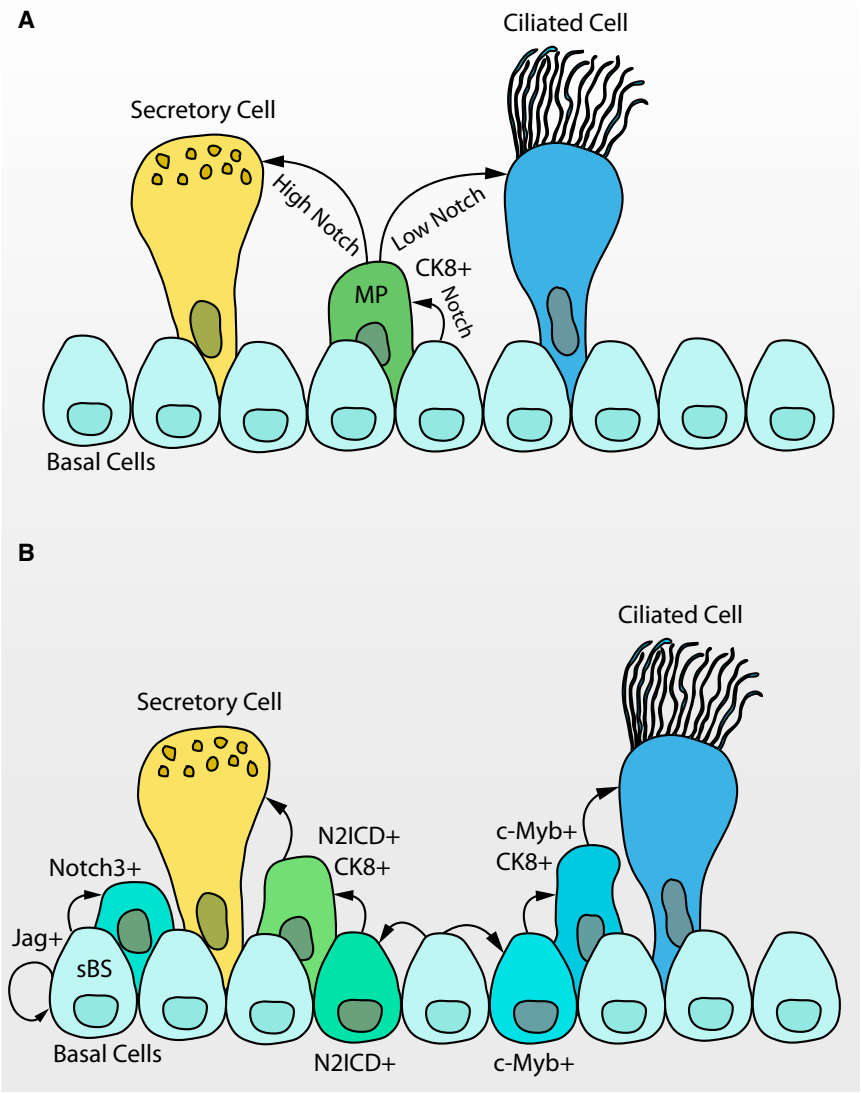

Figure 1. Schematic of Stem/Progenitor Cells in the Tracheobronchial Airways
(A) The current model for the differentiation of luminal cells contemplates the existence of multipotent CK8+ progenitor (MP) cells. MP cells can differentiate into secretory and ciliated cells depending on the level of Notch signaling.
(B) The new model describes three different subsets of basal cells: self-renewing basal cells (sBS), which could rely on Notch3 signaling from luminal cells for their maintenance; N2ICD+ basal cells that give rise to secretory cells; and c-myb+ basal cells that constitute the precursors of ciliated cells.

et al. show that in the absence of Notch3 there is an expansion of basal cell progenitors. The findings of Mori and colleagues, using 2D air-liquid interface cultures, show that Notch LOF expands the population of basal cells while also leading to selective differentiation of progeny toward ciliated rather than secretory cell fates. A consensus model emerging from these studies is that basal cell fate is pre-determined and reflects intrinsic heterogeneity within this population of epithelial basal stem/progenitor cells. Furthermore, whereas basal cells include a subset showing N2ICD immunoreactivity, Notch is not required for the generation of early luminal cell progeny but is a determinant of secretory cell fate.

These new findings highlight the complexity of cell-cell signaling involved in regulation of fate decisions within pseudostratified epithelium of proximal airways. It is clear from this and related work that Notch signaling plays a central role in regulating the balance between proliferation and differentiation of basal cells, and its differential activation among basal cells contributes to their functional heterogeneity. Given the availability of specific drugs that can target Notch signaling, a better understanding of this pathway in lung homeostasis and disease may yield novel approaches to promote tissue regeneration over aberrant remodeling. Recently, Vaughan et al. showed that Notch signaling is required to promote expansion of a novel lineage-negative distal lung epithelial progenitor cell (LNEP), yet directs the fate of this novel progenitor cell type toward proximal rather than distal cell phenotypes. They found that pharmacologic inhibition of Notch signaling during repair after influenza virus infection restored regionally appropriate epithelial cell types (Vaughan et al., 2014). From this work it is clear that there are downsides to Notch signaling; aberrant pathway activation has the potential to direct ectopic differentiation and tissue remodeling.

In conclusion, it will be important to further explore details of Notch pathway modulation of regional progenitor cells in lung epithelial homeostasis and remodeling. In basal cells of pseudostratified airways it would help to better understand interactions between Notch, which directs secretory cell fates, and programs that regulate ciliated cell fate. It remains to be determined precisely how distinct Notch receptors and ligands interact to differentially modulate fate decisions made by regional epithelial progenitor cell types of conducting airways. Finally, additional studies are needed to determine how Notch modulates progenitor cell types, including the recently described LNEPs, that contribute to homeostatic maintenance, repair, and remodeling of the epithelial lining of the alveolar gas-exchange region of the distal lung. Further basic studies aimed at revealing the molecular circuitry of cellular fate decisions within airways will provide exciting translational opportunities with potential to expand treatment options for patients with chronic lung disease.

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Intimacy of the Niche: Perivascular Remodeling Cuddles Incoming HSCs

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Stem cells reside in "niches," which provide signaling cues necessary for self-renewal. In a recent issue of *Cell*, Tamplin et al. (2015) perform live imaging of hematopoietic stem and progenitor cells (HSPCs) and find dynamic remodeling of endothelial cells is triggered upon arrival of HSPCs at the caudal hematopoietic tissue.

Tissue stem cells underlie the development and homeostasis of each major organ system. They reside in unique microenvironments known as "niches," which are necessary to maintain stem cell survival and self-renewal over the lifetime of the organism. Over the last decade, we have gained a better understanding of the cell types and signals necessary for stem cell retention in a tissue. Many features are conserved across diverse organs and organisms, and paradigms of how stem cell niches operate have emerged (reviewed in Morrison and Spradling, 2008). However, we currently lack a detailed understanding of the cellular interactions and steps required for hematopoietic stem and progenitor cell (HSPC) niche formation and function, in part due to the difficulty of live cell imaging in vivo. Recent advances in imaging approaches have opened new avenues of inquiry into the in vivo behavior of murine HSPCs (Lo Celso et al., 2009; reviewed in Joseph et al., 2013). Similarly, in a recent issue of *Cell*, Tamplin et al., (2015) have exploited the transparency of the zebrafish embryo to perform high-resolution imaging of HSPC interactions with cells in the caudal hematopoietic tissue (CHT), where nascent HSPCs first establish residency. They discover a stereotyped cellular behavior that is triggered in endothelial cells of the CHT upon HSPC arrival. Importantly, this behavior appears to be conserved over evolution, as Tamplin and colleagues observe similar interactions between nascent HSPCs and their vascular niche in the murine fetal liver ex vivo.

HSPCs give rise to all adult blood cell types, including the myeloid, lymphoid, and erythroid lineages. Vertebrates share a high degree of conservation in the molecular and cellular processes required for the genesis, maturation and maintenance of HSPCs (reviewed in Clements and Traver, 2013). After specification in the floor of the dorsal aorta, HSPCs migrate through transitory niche sites, which include the fetal liver and spleen in mammals and the CHT in teleosts. In these transient tissues, HSPCs expand and mature before colonizing their adult niche sites. Live imaging has significantly enhanced our understanding of biological processes, providing information that would be unattainable through still images. Here, Tamplin et al. (2015) use the translucent zebrafish embryo to obtain high-speed, high-resolution images and gain a better understanding of the complex cellular interactions that occur between the stem cell and its endothelial niche.

Tamplin and colleagues first generate a highly specific marker for HSPCs. They establish a transgenic zebrafish line with the well-characterized murine Runx1 +23 enhancer element (Bee et al., 2009), which drives the expression of a reporter EGFP or mcherry (*Runx:EGFP*, *Runx:mcherry*) fluorophore. Importantly, serial dilution transplantation assays demonstrate the ability of runx:EGFP+ cells to reconstitute the adult marrow of recipients. This demonstrates that Runx+ HSPCs possess the hallmarks of HSCs, namely self-renewal and multipotency. These functional studies represent an important advance in the study of HSCs in the zebrafish embryo, something that has previously been lacking.

To directly image the cellular interactions between Runx:EGFP+ HSPCs and the transient niche in the CHT, time-lapse imaging using spinning disk confocal microscopy was performed. HSPCs are observed to exit from circulation and lodge upon the outer wall of the endothelium. Within minutes, a small group