

Stem Cell Niches in the Mouse Airway

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Stem cell research has progressed to new levels of understanding over the last decade. Many adult tissues undergo continuous self-renewal and hence require resident stem cells with the capacity for multipotent differentiation in that organ (1). Such adult stem cells are permanently established in an organ following embryogenesis. Stem cells are most often slow-cycling and give rise to transient amplifying cells (TAC), which impart the majority of tissue renewal in the setting of injury. Unlike stem cells, which by definition have unlimited proliferative capacity, TAC are restricted in their capacity to divide and cannot proliferate indefinitely. Although stem cells and TAC are both self-renewing populations, TAC are primarily thought to be the major progenitor population which gives rise to terminally differentiated cell types of an adult organ. While the concept of lineage restricted stem cells in adult organs has a long history, recent research isolating stem cells from different organs with multipotent capacity for “trans-differentiation” into various tissue lineages has begun to redefine “stem cell” characteristics (2). For example, neural stem cells have been found to have the capacity to cross tissue-specific lineages in the production of blood derived hematopoietic cell types (3). Similarly, bone marrow-derived myogenic progenitors have also been demonstrated to have the capacity to regenerate muscle (4, 5). These findings have reorganized thinking in the field beyond the traditional viewpoint that stem cells of adult tissues are limited to cell lineages present only in the organ from which they are derived. The concept of whether all organs retain stem cells with multipotent capacity for trans-differentiation across tissue lineages remains to be determined. However, these findings suggest that stem cells can respond to the unique biochemical cues of a niche in which they reside.

Stem Cell Niches and Microenvironmental Influences

Major underlying questions regarding adult stem cell research include the extent to which intrinsic characteristics and the microenvironment influence stem cell commit-

ment to specific lineages at their place of residence. Identifying the intrinsic and extrinsic molecular cues responsible for defining stem cell characteristics are of paramount importance to fully understanding stem cells in a given organ. Several intrinsic factors that influence stem cell fates in epithelial organs, such as the intestine and skin, have been identified. These studies have demonstrated that *Lef/Tcf/β-catenin* complexes are important factors in controlling stem cell maintenance and/or cell commitment to differentiation (6, 7). Epithelial stem cells in many organs, including the dermis, intestine, and hair follicle, are often confined to discretely localized niches that are protected from environmental insults. These niches likely also provide important extrinsic cues, which control stem cell proliferation and commitment into more differentiated TAC. Such cues may arise from other cell types in the niche (mesenchymal or epithelial in nature) or from the extracellular matrix. In the context of the lung, it has been unclear whether such confined niches serve as a reservoir for proliferating stem cells. This *Perspective* will review new findings presented in two manuscripts in this issue (Borthwick and colleagues [14] and Hong and coworkers [15]), which have elucidated important aspects regarding stem cell niches in the proximal and distal airways of mice.

Multipotent Tissue-specific Epithelial Stem Cells in the Airway

Stem cell research in the lung has progressed rather slowly due to the anatomical and functional complexities associated with numerous distinct cell types. Furthermore, differences in the cell biology of rodents and primates have also hindered consensus research defining cross-species phenotypes of stem cells in the lung. This organ must be divided into various anatomical regions when considering multipotent progenitor or stem cells. Evidence clearly suggests that multipotent progenitors of the conducting airway epithelium and gas-exchange alveolar regions are derived from different populations of stem cells that are anatomically separated in the lung. Furthermore, evidence by Borthwick and colleagues and Hong and coworkers suggests that stem cell niches in the conducting airways must also be uniquely divided between the proximal and distal regions.

Given the field's relatively infantile understanding of potential stem cell characteristics in the adult lung and progenitor-progeny relationships, more research is needed to define molecular markers for stem cells and the regulatory processes controlling stem cell differentiation. Because of the low abundance of stem cells, characterization of their phenotype and niches has been challenging. In this issue,

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Abbreviations: Clara cell secretory protein, CCSP; calcitonin gene related peptide, CGRP; label retaining cells, LRC; neuroepithelial bodies, NEBs; pulmonary neuroendocrine cell, PNEC; transient amplifying cells, TAC.

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Borthwick and colleagues describe studies beginning to elucidate the characteristics of potential stem cell niches in the tracheal airway of mice. In this study, the authors used several defining characteristics to support the identification of stem cells in the airway following injury. First, it is expected that stem cells undergo minimal expansion during regeneration by giving rise to TAC populations. This slow-cycling phenotype can be used to mark potential stem cell compartments *in vivo* following the incorporation nucleotide analogs, such as BrdU, which are maintained within genomic DNA for long periods of time. This marking gives rise to label retaining cells (LRC), which presumably include the stem cell population. Second, airway stem cell populations should be capable of regenerating all cellular phenotypes present in the proximal airway. Because of the low basal rate of stem cell division and turnover of differentiated cells in the normal airway, injury models were required to study stem cell proliferation. Using a model of SO₂ injury-induced regeneration in the mouse trachea, Borthwick and colleagues have identified specific niches of stem cell expansion that are marked by distinct zonal boundaries. In the proximal glandular con-

taining trachea, LRC expanding zones were confined to the ducts of submucosal glands, while in more distal trachea and bronchi, which do not contain glands, these LRC expanding stem cells were located in systematically arrayed foci along the surface airway epithelium. These foci in the distal trachea also appeared to be localized at cartilage–intercartilage junctions. To test the hypothesis that gland ducts may be capable of regenerating the surface airway epithelium and hence may be a compartment for airway stem cells, the investigators analyzed tracheal xenografts in which the surface airway epithelium was removed by protease digestion, followed by subcutaneous transplantation in T-cell deficient mice. These studies clearly demonstrated that regenerating surface airway epithelium could emerge from within gland ducts and supports the notion that this region may be stem cell niche.

One remaining important aspect of LRCs in the proximal airway will be to determine their phenotype with regard to molecular markers. Interestingly, in this study by Borthwick and colleagues, LRCs did not express the pulmonary neuroendocrine cell (PNEC) marker calcitonin gene related peptide (CGRP); however, it was noted that

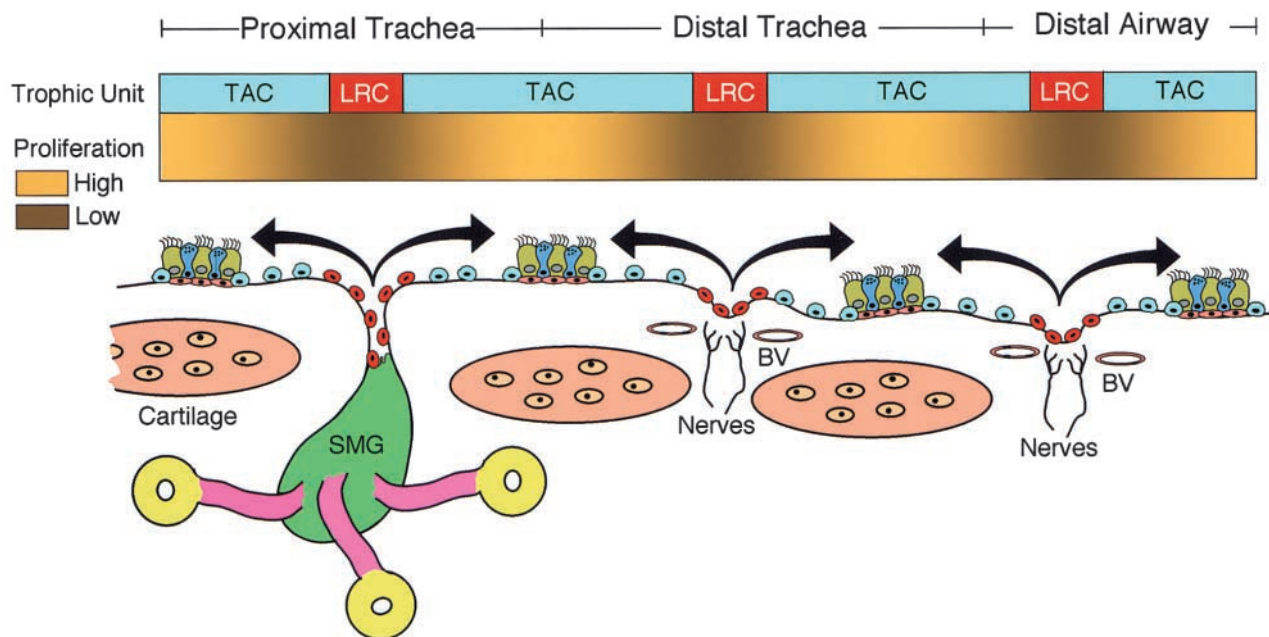


Figure 1. Schematic representation of stem cell niches in the proximal and distal airways of mice. For simplicity, two trophic units in the airway have been classified as transient amplifying cells (TAC) and label retaining cells (LRC). LRC represent the candidate slow-cycling stem cells and are denoted in red, while undifferentiated TAC (denoted as light blue cells) have a much higher proliferative rate and capacity for regeneration. Following SO₂ injury, expanding LRC populations in the proximal glandular trachea were found in the ducts of submucosal glands (SMG) and the immediate surrounding surface airway epithelium. This uniquely protected niche represents an area for expansion of LRC into undifferentiated TAC as they migrate away from gland ducts, proliferate, and differentiate into a mature ciliated epithelium. In the distal nonglandular trachea and distal bronchiolar airways, similar niches for LRC were seen following TAC ablation. However, in contrast to the proximal glandular trachea, these expanding LRC niches were associated with innervated NEBs containing CGRP-expressing PNECs (PNEC cells are not shown). In the distal nonglandular trachea, LRC expansion was confined predominantly to inter-cartilaginous junctions associated with vascularized (BV) and innervated regions of the airway (sites often associated with NEBs). In the distal noncartilaginous bronchioles, LRC were also associated with NEBs. These NEBs may serve as a niche in the distal trachea and bronchiolar airways for maintaining stem cells, which are likely a subpopulation of CCSP-expressing cells. Although CGRP-expressing PNECs were associated with LRC in this region, the evidence indicates that PNECs are not the airway stem cell and nor are they capable of multipotent differentiation into all surface airway epithelial cell phenotypes.

PNECs preferentially localized to niches containing LRC expansion. These studies suggest that PNECs may play a role in the microenvironment necessary for stem cell expansion, but are not likely themselves to be the airway stem cell. PNECs are predominantly organized into discrete innervated zonal locations within neuroepithelial bodies (NEBs) in the airway. NEBs have been thought to play important roles in regulating embryonic lung growth and maturation through the action of a number of neuropeptides (8). These aspects of NEB function are interesting when considering potential roles as a niche for airway stem cells and suggest that PNECs may play an important extrinsic role in modulating the stem cell phenotypes and commitment to TAC following injury.

Also in this issue, Hong and coworkers describe similar work evaluating the stem cell phenotype in the distal airway of mice. Previous findings by these authors demonstrated that naphthalene-induced ablation of Clara cells caused LRC expansion in regions localized to NEBs of the distal airway (9). Two populations of LRCs proliferated within NEBs following injury and hence could be candidate stem cells in the mouse airway. These included Clara cell secretory protein (CCSP)-expressing cells and CGRP-expression PNECs. Hence, the authors hypothesized that either PNECs or CCSP-expressing cells (i.e., Clara cells) might represent the stem cell pool with pluripotent capacity for airway epithelial renewal following injury. To test the hypothesis, the authors utilized a novel transgenic mouse model, in which the thymidine kinase gene is expressed under the direction of the CCSP promoter (CCtk). This model afforded the ability to temporally ablate all CCSP-expressing cells in the airways of mice. Using this strategy, it was possible to analyze the phenotype of regenerating epithelium in the absence of CCSP-expressing cells. Findings from these studies demonstrated that ablation of CCSP-expressing cells led to hyperplasia of PNECs. However, this CGRP-expressing population of cells was unable to effectively repopulate a differentiated ciliated epithelium. These studies support the notion that a subpopulation of CCSP-expressing cells, but not PNECs, may represent the stem cell in the mouse distal airway. The influence of the NEB microenvironment and the role to which PNECs might play in maintenance of the CCSP-expressing stem cell phenotype remain to be elucidated.

Similarities between the reports by Hong and coworkers and Borthwick and colleagues are notable since both studies demonstrated LCR expansion within NEB zonal boundaries (Figure 1). Despite the fact that proximal tracheal stem niches were confined to submucosal gland ducts, NEB associated expansion of stem cells in the distal trachea found by Borthwick was strikingly similar to that seen in the distal airways of Hong's study. However, PNEC hyperplasia was not seen in the Borthwick study and although PNECs were confined to regions of LRC in this study, they were only infrequent cell types. PNEC hyperplasia seen in the study by Hong may likely represent a compensatory mechanism to repopulate the airway in the setting of total stem cell ablation. Given that Clara cells are distributed throughout all levels of the mouse airway, it is conceivable that CCSP-expressing cells in both the trachea and distal airways represent a similar stem cell

phenotype. Although morphologic counterparts to Clara cells in the human airway are confined to only the distal airways, it is notable that CCSP-expressing cells are also seen throughout the bronchial and distal airways of the human lung (10). Hence, despite the divergent cell biology of mouse and human airways, the intrinsic phenotype that defines the airway stem cell compartments in mouse and human may be more similar than previously thought. Although speculative, this anecdotal finding highlights the fact that CCSP-expression does not in and of itself define the Clara cell morphologic phenotype.

Teleologically, the concept that airway stem cells exist in discrete stem cell niches does hold significant merit. One might expect that stem cell niches in the airway would be best positioned in anatomically protected regions of the lung. Gland ducts provide direct access to the airway surface and are nestled beneath the airway in a protected environment not directly exposed to inhaled toxins. Furthermore, evidence using retroviral lineage tracing also suggests that there are multipotent progenitors in the adult human proximal airway with the capacity for both glandular and surface airway epithelial differentiation (11). Interestingly, a Tcf family member, called Lef-1, was also shown to be highly expressed by this glandular stem cell at the time of commitment to form a gland (12), and was absolutely necessary for submucosal gland development in the mouse and ferret airways (13). These findings are reminiscent of Lef/Tcf/ β -catenin involvement in stem cell maintenance and/or cell commitment to TAC differentiation in the intestine and skin.

From a therapeutic standpoint, the identification of stem cells in the airway will undoubtedly increase the field's capacity for targeted genetic manipulation of this cell type for the treatment of genetic diseases such as cystic fibrosis. Long lasting treatments for such inherited diseases using gene therapy must efficiently target these stem cell compartments in the airway. The present studies demonstrating unique aspects of stem cell niches in the airway have begun to focus the field's understanding of the intrinsic properties of stem cells defining their unique characteristics. Such intrinsic characteristics may ultimately identify cell specific surface markers, which could conceivably be used to enhance gene targeting to these cell types. Such applications of stem cell research underscore the unique importance of basic cell biology to molecular medicine.

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