

The Glandular Stem/Progenitor Cell Niche in Airway Development and Repair

Xiaoming Liu¹ and John F. Engelhardt^{1,2,3}

¹Department of Anatomy and Cell Biology, ²Department of Internal Medicine, and ³Center for Gene Therapy of Cystic Fibrosis and other Genetic Diseases, College of Medicine, University of Iowa, Iowa City, Iowa

Airway submucosal glands (SMGs) are major secretory structures that lie beneath the epithelium of the cartilaginous airway. These glands are believed to play important roles in normal lung function and airway innate immunity by secreting antibacterial factors, mucus, and fluid into the airway lumen. Recent studies have suggested that SMGs may additionally serve as a protective niche for adult epithelial stem/progenitor cells of the proximal airways. As in the case of other adult stem cell niches, SMGs are believed to provide the localized environmental signals required to both maintain and mobilize stem/progenitor cells, in the setting of normal cellular turnover or injury. Aberrant proliferation and differentiation of glandular stem/progenitor cells may be associated with several hypersecretory lung diseases, including chronic bronchitis, asthma, and cystic fibrosis. To better understand the molecular mechanisms that regulate the specification and proliferation of glandular stem/progenitor cells in lung diseases associated with SMG hypertrophy and hyperplasia, researchers have begun to search for the molecular signals and cell types responsible for establishing the glandular stem/progenitor cell niche, and to dissect how these determinants of the niche change in the setting of proximal airway injury and repair. Such studies have revealed certain similarities between stem/progenitor cell niches of the distal conducting airways and the SMGs of the proximal airways.

Keywords: stem cell niche; submucosal gland; airway; lung; development; repair

The adult human lung is lined by specialized types of airway epithelia that comprise the various trophic units of the lung: tracheobronchial, bronchiolar, and alveolar. Each of these trophic units is composed of distinct types of epithelial cells that are important for maintaining normal lung function. Given that the lung is directly exposed to the environment, it must react to inhaled pathogens and pollutants, and this must be done in a coordinated fashion for effective epithelial repair in the setting of injury. In certain genetic diseases, such as cystic fibrosis (CF), an enhanced predisposition of the lung to bacterial infection from the environment can lead to enhanced cellular turnover and aberrant proliferation of certain cell types of the conducting airways and submucosal glands (SMGs) of the lung. The ability of the lung to repair itself in the setting of injury is determined by the molecular events that control stem cell mobilization from niches within each of the trophic units. Unfortunately, the field is only beginning to understand these molecular signals and the phenotypes of stem cells in the various trophic units of the adult

lung. A fundamental understanding of stem cell niches in each of the trophic units is central to developing regenerative therapies and controlling abnormal injury responses by the lung. SMGs are one proximal tracheobronchial airway component that has been proposed to serve as an important airway stem cell niche. This article focuses on advances in our understanding of the biology of this glandular stem cell niche in the context of airway development and repair.

SMGs IN THE AIRWAY

SMGs are composed of a series of interconnecting tubules and ducts that are localized in the interstitium beneath the surface epithelium (1) and connect with the surface airway epithelium. Distal regions of this tubular network comprise serous acini and tubules. Secretory products move vectorially from the distal serous tubules, through mucous tubules, and then accumulate in collecting ducts, which, together with ciliated ducts, connect each glandular network to the airway lumen (2). Each of these spatially distinct SMG regions has specific cell types that control both the content/viscosity of secretory products and the timing of the expulsion of secretions in response to airway irritation and infection (2–5).

The development and distribution of SMGs in the airway has been examined in many mammals, including humans. The abundance, distribution, and anatomic structure of SMGs throughout the conducting airways varies greatly between species; in rodents (mouse and rat), SMGs are much less numerous than in other mammalian species (6–8). In mice, for example, SMGs are observed only in the larynx and proximal trachea, and their abundance in the trachea can vary between inbred strains (6, 7). This unique feature of murine SMGs has led to questions about the importance of these structures to lung biology in this species. An additional limitation of mouse models for the study of proximal airway cell biology is that goblet cells—the predominant secretory cell type in the proximal airways of humans—are replaced by the Clara cell (9, 10). Thus, whereas in humans the Clara cell is limited to the bronchioles, in mice it is distributed throughout all levels of the airway.

Because of these differences in mouse proximal airway biology, efforts in other species such as the ferret have begun to contribute significantly to our understanding of SMG biology. Unlike mice, ferrets and humans share a remarkably similar proximal airway cytoarchitecture (11–13). As in humans, SMGs in the ferret are distributed throughout the cartilaginous airways (12, 14). Despite these similarities, however, ferret and human SMG biology differs in some respects—for example, in the timing of SMG development. In humans, airway SMG development initiates when clusters of surface epithelial cells invade the lamina propria of the proximal trachea during gestation (14). In ferrets, by contrast, SMG development initiates within the trachea during the first few postnatal weeks of life, a point at which this structure closely resembles the *in utero* human airway at gestation stages (12, 14). Although the ferret is the only known placental mammal in which substantial development of both the airway epithelium and SMGs

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Correspondence and requests for reprints should be addressed to John F. Engelhardt, Ph.D., Department of Anatomy and Cell Biology, University of Iowa School of Medicine, 51 Newton Road, Room 1-111 BSB, Iowa City, IA 52242. E-mail: john-engelhardt@uiowa.edu

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occur postnatally, these morphologic and developmental features of the ferret airway make it uniquely suited to serve as a model for studies pertaining to the development of tracheal SMGs.

From a functional standpoint, the interconnecting network of serous and mucous tubules of the SMGs secretes antibacterial factors, mucus, and fluid into the airway lumen. Many of these glandular secretory products, such as lysozyme and lactoperoxidase, are critical to maintaining sterility of the proximal airway (15, 16). Furthermore, *ex vivo* models of airways with and without SMGs suggest that the presence of SMGs significantly influences bioelectric and fluid transport properties of the airway (13, 17). SMGs are also believed to play an important role in the pathogenesis of a number of progressive lung diseases—such as CF, chronic bronchitis, and asthma—which are characterized by severe hypertrophy and hyperplasia of the SMGs (18–23). A common feature of these diseases is an expansion of SMGs (as an increase in glandular mass of each gland and potentially also an increase in the number of glands), which leads to abnormally high levels of mucus production in the airways. SMG hyperplasia (increase in the number of glands) has also been reported in mouse models of CF (24). However, whether disease-associated alterations in the SMG cellular architecture involve abnormal proliferative responses by glandular stem/progenitor cell compartments remains to be investigated.

STEM CELL NICHES IN THE ADULT LUNG

Stem cell fate and the maintenance of stem cell populations are regulated by local anatomically and chemically defined micro-environments called niches. These discrete regions of specialized cell types, cell matrix, and diffusible factors (e.g., cytokines and growth factors) are critical for maintaining stem cells, as well as for promoting appropriate cell fate and migration decisions (25). To fully appreciate the role SMGs play in lung stem cell biology, it is useful to compare glandular niches to other stem cell niches of the airway. Most of our current understanding of the progenitor/progeny relationships and stem cell phenotypes in the adult lung originates from research using lung injury models in the mouse and in epithelial xenograft reconstitution models (involving multiple species). These studies have led to the identification of candidate progenitors that have a limited capacity to differentiate, and candidate stem cells with the capacity to differentiate into all cells within a trophic unit of the lung.

Because stem cells are believed to divide very infrequently, it has been necessary to injure the lung to study lung stem cell phenotypes and the stem cell niches of the airway. The slow-cycling feature of stem cells has, however, been advantageous in that it allows for the use of DNA labeling with detectable nucleotide analogs to track candidate stem cells *in situ*. Bromodeoxyuridine (BrdU) is one such common nucleotide analog, and is classically used to track so-called labeling-retained cells (LRCs) after a prolonged “washout” period. After BrdU pulse labeling, both cycling stem cells and transient amplifying cells incorporate the nucleotide into their DNA. During the washout period, the label is diluted more rapidly from the transient amplifying cell pool, leaving less frequently dividing stem cells to retain more label. First developed for studying stem cell niches in the skin (26), this technique has more recently been applied to the mouse lung. Although LRC localization is useful in determining the location of candidate stem cells, this method should also be used with caution when defining stem cell phenotypes. As discussed later in this article, certain cells, such as pulmonary neuroendocrine cells, can also proliferate after

lung injury and retain nucleotide label, despite the fact they cannot differentiate into all epithelial cell types in the airway.

On the basis of their expected nucleotide label retaining characteristics, several candidate adult stem/progenitor cells and niches have been identified in reconstituted airway epithelial models and *in vivo* mouse models of lung injury. In the most proximal portions of the mouse trachea where SMGs reside, LRCs localize to the gland ducts after SO₂- (27) or naphthalene-induced airway injury (Figure 1). This finding suggests that SMGs may serve as a protective niche for stem/progenitor cells in the proximal airways (28) (niche 1 in Figure 2). In the surface airway epithelium of the lower mouse trachea (which has no SMGs), a subset of BrdU-label-retaining basal and columnar cells resides in the intercartilaginous zone of mouse trachea 95 days after SO₂- or polidocanol-induced injury (27). Phenotyping studies in mice further revealed that basal cells expressing a transgenic K5 promoter-driven enhanced green fluorescent protein (EGFP) construct have a higher colony-forming efficiency, as well as a greater capacity to generate large colonies, than their EGFP-negative counterparts (29). Overall, these findings suggest that a subset of K5-positive basal cells are potential stem/progenitor cells in the lower trachea, and that they reside within an intercartilaginous niche (27, 29) (niche 2 in Figure 2). Another important observation from this study was that calcitonin gene-related peptide (CGRP)-positive pulmonary neuroendocrine cells (PNECs) also frequently resided at the intercartilaginous zone, the region in which the LRCs of the distal mouse trachea were found. Although the LRCs did not express CGRP and thus are not the PNECs themselves, the association of PNECs with LRCs in the distal trachea was similar to findings in the distal airways (discussed *below*).

A number of studies have suggested that, in contrast to the situation in the proximal airways, a subset of Clara cells is the population that serves as local stem/progenitor cells in the distal airways (30, 31). Studies using naphthalene to deplete the lung of Clara cells have revealed that BrdU- and/or [³H]-labeled LRCs are a subset of Clara cells that are positive for the Clara cell secretory protein (CCSP) but resistant to naphthalene. This subpopulation has been termed a variant Clara cell (Clara^v). Clara^v cells also retained characteristics of stem cells, including the ability to efflux Hoechst dye and to express stem cell antigen (Sca)-1 (31). Although PNECs associated with neuroendocrine bodies (NEBs) of the bronchiolar airways also retained nucleotide label, and therefore are technically LRCs, genetic studies in which Clara cells were conditionally ablated demonstrated that PNECs do not have the capacity to regenerate a ciliated airway epithelium; thus, PNECs are not the stem cell of the bronchiolar airways (30, 32). These studies strongly suggested that Clara^v cells are the local stem/progenitor cell population of the bronchiolar airways, and that NEBs serve as stem cell niches in this region (niche 3 in Figure 2).

Recent studies using a mouse naphthalene model have suggested that a subset of naphthalene-resistant, CCSP and surfactant protein C double-positive, CGRP-negative Clara^v cells may be a bronchioalveolar stem cell (BASC) population (33). BASCs are not associated with NEBs but instead reside at the bronchioalveolar duct junction (BADJ) of the adult mouse lung, a region that is believed to play a role in both the maintenance and repair of bronchiolar and alveolar cells (33, 34). Therefore, BASCs are potentially a local stem cell population of the terminal bronchioles, and the BADJ appears to be an additional region-specific stem cell niche of the lung (niche 4 in Figure 2). In addition to BASCs, a subpopulation of type II alveolar epithelial cells has also been considered as a stem/progenitor cell of the alveolar epithelium, based on their

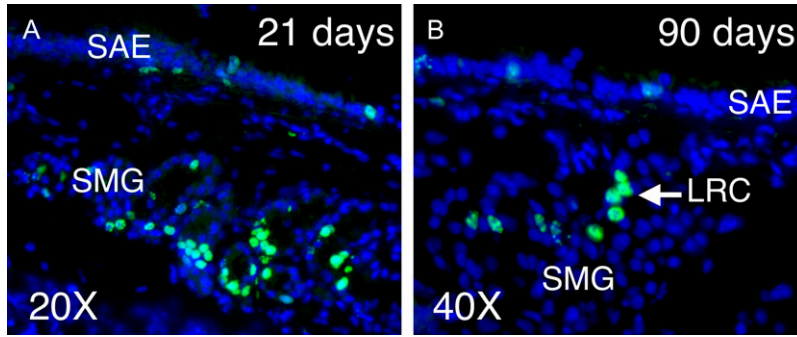


Figure 1. Label-retaining cells localize to submucosal glands in mouse trachea. Naphthalene-induced injury to mouse tracheal epithelia was followed by systemic bromodeoxyuridine (BrdU) labeling for 3 days. BrdU-labeled cells (termed labeling-retained cells [LRCs]) were localized in tracheal tissue sections using fluorescein isothiocyanate-labeled anti-BrdU antibodies. (A) Submucosal glands (SMGs), with relatively frequent cell labeling with BrdU at 21 days post-naphthalene injury and pulse labeling with BrdU. These labeled cells likely include both labeled transient amplifying progenitor cells and stem cells. (B) By 90 days post-naphthalene injury and BrdU labeling, BrdU-positive cells are seen less frequently in SMGs. These LRCs are considered as candidate stem cells of the glands. Nuclei are counterstained with DAPI. SAE = surface airway epithelium. Original magnification of microscope objective is given for each panel.

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ability to replicate as well as to differentiate into squamous type I alveolar epithelial cells after injury (35). Therefore, alveoli may serve as a niche for local stem/progenitor cells in the most distal regions of the lung (niche 5 in Figure 2).

Molecular Mechanisms That Specify Formation of the SMG Stem Cell Niche

As briefly discussed above, Borthwick and colleagues localized LRCs after SO₂ injury of the mouse lung to the ducts of SMGs in the proximal trachea (27). The authors further investigated the regenerative capacity of stem/progenitor cells in the gland

ducts using a xenograft reconstitution model (27). In these studies, mouse tracheal airways were denuded of surface airway epithelial cells, transplanted into immunocompromised mice as subcutaneous implants, and allowed to regenerate for various lengths of time. Findings from these studies demonstrated that the regenerating surface airway epithelium emerged from cells migrating from the gland ducts. An alternative approach using human bronchial xenografts, in which surface airway epithelial cells are harvested from human bronchus and transplanted onto denuded rat tracheal subcutaneous implants, has also suggested that an infrequent epithelial cell type exists in the proximal airway with multipotent capacity for differentiation (36). This

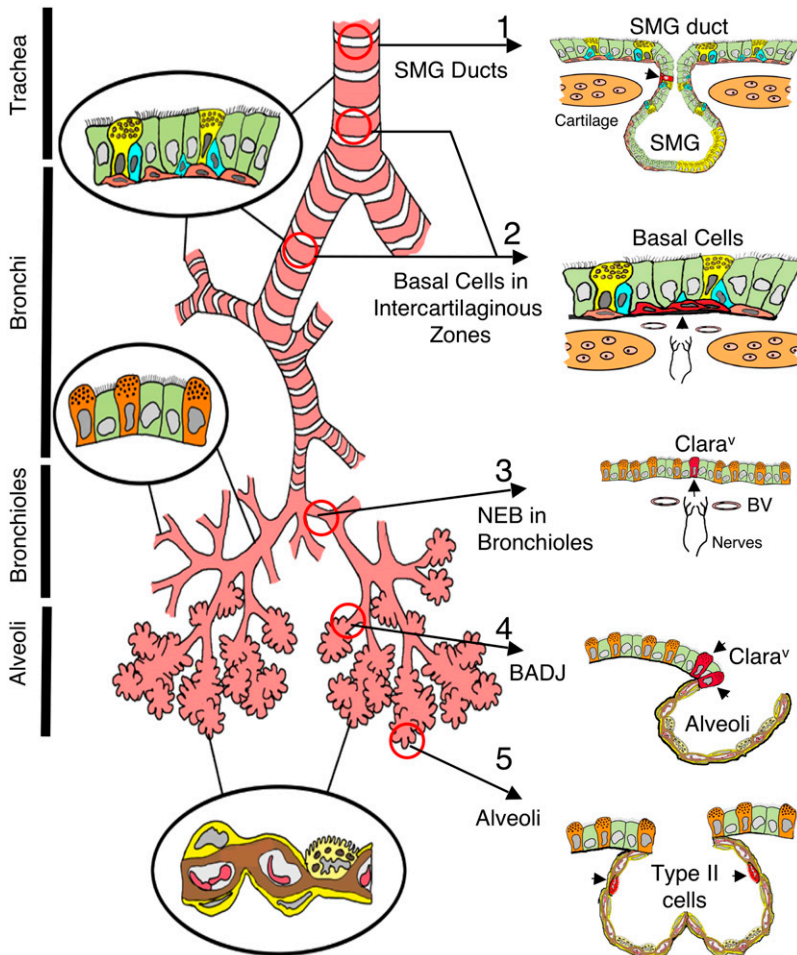


Figure 2. Illustration of putative stem cell niches in the adult mouse lung. Epithelia of the adult mouse lung can be divided into four major, biologically distinct trophic units (trachea, bronchi, bronchioles, and alveoli), each of which encompasses unique types of airway epithelial cells (epithelia relevant to each unit are shown inside circles). Five potential stem cell niches for these various trophic units are shown on the right, with locations of candidate stem cells marked by arrowheads (cells are in red). Stem cells and niches include the following: (1) an unknown cell type in the submucosal gland (SMG) ducts of the proximal trachea, (2) basal cells in the intercartilaginous zones of the lower trachea and bronchi (these structures may also be associated with innervated neuroendocrine bodies [NEBs]), (3) variant Clara cells (Clara^v) associated with NEBs in bronchioles, (4) Clara^v cells associated with bronchiolar alveolar duct junctions (BADJ), and (5) alveolar type II cells of the alveoli.

approach used retrovirally mediated expression of marker transgenes to perform clonal analysis of expanding progenitors and stem cells within a regenerating xenograft airway epithelium. This approach demonstrated an incredible diversity in the repertoire of progenitor cells with differing capacities for proliferation and differentiation into basal, ciliated, goblet, and intermediate cells (36). Another important finding from these clonal analysis studies in xenografts was that transgene-marked SMGs developed, albeit infrequently. Transgene-expressing glands were always associated with multipotent transgene-expressing clones in the surface airway epithelium (i.e., composed of basal, ciliated, goblet, and intermediate cells); thus, a subset of stem/progenitor cells with a capacity to differentiate into all surface airway epithelial cell types and SMGs appears to exist in the proximal human airway epithelium (36).

Little is currently known about the phenotypes of SMG duct stem/progenitor cells. However, we have observed that glandular LRCs express high levels of CCSP and appear to be resistant to naphthelene injury (unpublished results). Furthermore, glandular LRCs also appear to often be associated with CGRP-expressing cells in the glands (unpublished results). These findings suggest that the glandular stem/progenitor cells of the proximal airways and the Clara^v cells of the distal airways may have certain similarities. Despite these interesting observations, however, relatively little is known about the phenotypes of glandular stem/progenitor cells and their niche.

Studies elucidating the molecular events that control the formation of SMG stem/progenitor cell niches may help to shed light on the molecular signals that are important for the maintenance of stem/progenitor cells and the mobilization of their differentiating progeny from the glandular niche. Thus, we have focused on elucidating early events involved in SMG morphogenesis. The morphogenesis of glandular organs includes distinct steps of epithelial invagination, ductal elongation, and branching. Each step is a tightly regulated process that requires complex reciprocal interactions between the glandular epithelium and the underlying mesenchyme, and the stimulation of signal transduction cascades that regulate transcription of the genes required to achieve epithelial cell movement, proliferation, and differentiation.

Although relatively little is known about the epithelial-mesenchymal interactions that regulate SMG formation in the airway itself, studies of such interactions in other bud-forming organs has revealed some recurrent themes. To date, a number of signaling pathways (including those involving wingless + int-1 [Wnt], bone morphogenetic protein [BMP], fibroblast growth factor [FGF] Notch, and Hedgehog) and their downstream effectors or modulators have been identified as important mediators of cell-fate specification during the morphogenesis of glandular organs. For example, the HMG-box transcriptional factor lymphoid enhancer binding factor (Lef-1) of the Wnt pathway is involved in the initiation of the development of SMGs and a wide range of other epithelial, bud-forming organs, including mammary glands, teeth, and hair follicles (37–41). Like Lef-1, other transcription factors, such as Pax6 and Msx1/2, have been shown to be involved in bud formation of glandular organs (42, 43). For example, in the absence of Msx1 and Msx2, mammary gland and tooth development halts at the bud stage, shortly after initiation (43). Similarly, Indian Hedgehog (Ihh) and sonic Hedgehog (Shh) are required for the polarization of mammary glandular ducts, and mammary glands deficient for the Hedgehog signaling mediator Gli2 display glandular duct abnormalities (44). Expression of mRNAs for Shh, the Hedgehog intracellular regulator Gli1, and the Hedgehog receptor Ptch has also been detected in the mouse nasal gland and regenerating airway epithelium, suggesting that Hedgehog

signaling may be important for glandular function as well for airway regeneration (45–47). Of the molecular markers present in SMGs, Lef-1, Shh, and Ptch appear to be the most specifically linked to bud formation of these organs.

The Wnt/ β -catenin/TCF (T-cell factor) signaling pathway is one of the most extensively studied transcriptional cascades involved in various types of organogenesis. In the context of canonical Wnt pathways, extracellular Wnt binds to receptors and coreceptors and this leads to the accumulation of β -catenin in the nucleus. There it interacts with TCF/Lef-1 to modulate the transcriptional activities of Wnt target genes. The roles of Wnt signaling in mammary gland development, tumorigenesis, and stem/progenitor cell specification have been widely studied (48–55). Indeed, the vertebrate Wnt homolog was first discovered during studies of the integration of mouse mammary tumor virus (MMTV), when one particular integration found to cause pregnancy-dependent mammary tumors in mice (designated as Int-1) led to the identification of a gene with a sequence of high identity to that of the *Drosophila* Wingless (Wg) gene (56). The designation Wnt arose from the combination of Wingless and Int-1. Expression of several Wnt ligands during the morphogenesis and tumorigenesis of mammary glands has been reported (57, 58), and canonical Wnt signaling is essential for mammary gland development (59). Mammary glands in genetically manipulated murine models that either lack or overexpress Wnt signaling components (e.g., Lef-1 [41, 59, 60], β -catenin [52], lipoprotein receptor-related protein [LRP]5/6 [50], Axin [51], Dickkopf protein [DKK]1 [61, 62]) are abnormal during either morphogenesis or pregnancy. The latter defects are due to the fact that Wnt signaling promotes mammary ductal branching in early pregnancy and is also required for proliferation and survival of lobuloalveolar progenitor cell in later pregnancy (48, 51, 52). Numerous studies have also provided solid evidence for a link between Wnt signaling, mammary stem/progenitor cells, and Wnt-induced carcinogenesis (8, 50, 54, 63, 64). For example, the population of mammary progenitor cells is significantly increased in preneoplastic hyperplasias of transgenic mice that overexpress Wnt-1 and β -catenin (8, 54, 63), and mammary ductal cells isolated from MMTV-Wnt-1 transgenic mice show increases in the fraction of cells with stem cell activity (54). Furthermore, mice lacking the Wnt coreceptor LRP5 show resistance to Wnt-1-induced tumorigenesis (50). These studies strongly suggest that Wnt signaling plays key roles in mammary gland formation and tumorigenesis, and that it acts by influencing the specification of mammary stem/progenitor cells. Although much more is known about the involvement of Wnt signaling in the morphogenesis and tumorigenesis of the mammary gland than about the same processes in the SMG, it is clear that some similarities exist (Table 1).

Lef-1 is thus far the earliest-acting transcription factor demonstrated to be involved in SMG bud formation (38). Mice lacking the Lef-1 gene are impaired in bud formation of the mammary gland, tooth, vibrissa, hair, and airway/nasal SMGs, suggesting that Lef-1 expression is required for formation of these organs (38–41). In the context of airway SMG development, expression of Lef-1 at both the mRNA and protein levels is highly induced during the early bud stage, in which three to five cells aggregate within the surface epithelium before invagination (37–40). During later stages of gland development, Lef-1 expression is restricted to the cells in the most distal invading tips of the tubules. In the ferret xenograft model of the tracheal airway, inhibition of Lef-1 expression with antisense oligonucleotides decreases the abundance and branching morphogenesis of SMGs (37, 38). These characteristics, together with the fact that SMG development can be rescued in Lef-1-deficient mice by airway-specific expression of a Lef-1 cDNA

TABLE 1. Wnt SIGNALING INVOLVED IN ORGANOGENESIS AND/OR TUMORIGENESIS OF MAMMARY GLANDS AND AIRWAY SUBMUCOSAL GLANDS

Signaling Molecule	Function in Wnt Pathway	Functional Evidences	References
Wnt1, Wnt2, Wnt3a, Wnt4, Wnt5a, Wnt5b, Wnt6, Wnt7b, Wnt10b	Ligands	Expression detected during gland morphogenesis. Overexpression of Wnt induces abnormal glandular development and tumorigenesis. Mice deficient for certain Wnt molecules have reduced ductal branching in mammary glands and reduced SMG formation.	(40, 48, 49, 53–55, 57, 58, 63)
Lef-1	Activator	Required for glandular morphogenesis and the mesenchymal–epithelial transition of Wnt signal.	(38–41, 60)
TCF4	Activator	Expression in early SMG buds, in the mammary epithelium and in tumors.	(40, 68)
DKK1	Inhibitor	Overexpression leads to a failure to form mammary placodes.	(61, 62)
LRP5	Coreceptor	Deficiency leads to smaller mammary placodes and a reduction of the primitive ductal tree.	(50)
Axin	Inhibitor	Inducible expression impairs mammary gland development.	(51)
β -Catenin	Modulator	Expression of dominant negative or active β -catenin leads to impaired development or hyperplasia of mammary glands, respectively.	(52, 54)

Definition of abbreviations: Lef-1 = lymphoid enhancer binding factor 1; SMG = submucosal gland.

under the direction of the CC10 promoter (38), strongly suggest that a subset of Lef-1–expressing cells may be a stem/progenitor cell population important for airway gland development. However, ectopic Lef-1 expression in the proximal airways of these CC10–Lef-1 transgenic mice was insufficient to increase SMG abundance, suggesting that signals in addition to Lef-1 are required for glandular development in the airway.

A better understanding of the molecular regulation of the Lef-1 gene could provide insights both into the transcriptional processes that regulate stem cell commitment to SMG formation and into the biology of the glandular niche. Progress on this front has begun, and initial findings on the signals that regulate the Lef-1 gene during airway SMG formation have been reported. *In vitro* studies dissecting the human Lef-1 promoter have demonstrated that a 110-bp segment in the promoter region (termed the Wnt responsive element [WRE]) is required for the transcriptional responsiveness of the Lef-1 promoter to Wnt3A/ β -catenin (65). More specifically, a Lef-1 promoter lacking a WRE exhibited increased basal levels of transcription and a loss of Wnt responsiveness, suggesting that the WRE acts as a repressor in the absence of Wnt signals. Furthermore, chromatin immunoprecipitation (ChIP) assays have suggested that TCF4 can bind the WRE in airway epithelial cells (40) and other cell types (66). The analysis of mice expressing β -galactosidase or EGFP reporter transgenes under the control of the 2.5-kb Lef-1 promoter fragment has indicated that this promoter segment alone is sufficient to regulate expression in tracheal and nasal SMG buds (39, 40). Furthermore, in this model system, the deletion of the 110-bp WRE from the Lef-1 promoter led to a lack of expression in gland buds (39). Interestingly, the expression of endogenous Lef-1 and a Lef-1 promoter–driven reporter gene was significantly reduced or absent in SMG buds of Wnt3A-deficient mice (40). Additional evidence for the activation of the β -catenin/TCF complex during early stages of SMG bud formation includes strong TCF4 and Wnt-responsive TOPGal reporter (three consensus Lef-1/TCF-binding motifs upstream of a minimal *c-fos* promoter control β -galactosidase reporter gene expression) expression in early-stage SMG buds. These findings suggest that the Wnt3a/ β -catenin pathways may play an important role in regulating Lef-1 gene expression during the early stages of gland development and, therefore, they may also play an important role in glandular stem cell specification and in formation of the SMG stem cell niche.

The canonical Wnt/ β -catenin signaling pathway has been shown to play a critical role in the maintenance of stem/progenitor cell homeostasis and/or cell commitment to differentiation. For

example, Wnt3A has also been shown to stimulate the proliferation and self-renewal of hematopoietic stem cells (67). This latter finding is of particular interest in light of the importance of both the Wnt3A and Lef-1 genes in glandular development and in the establishment of the glandular stem cell niche. Whether Lef-1 or an upstream signal that controls the transcriptional activation of the Lef-1 gene is required for stem cell maintenance and/or activation after airway injury remains to be investigated. However, given the similarities between Wnt/TCF/ β -catenin involvement in stem cell maintenance and/or stem cell commitment in other glandular organs, this pathway may be a good candidate for a regulator of stem cell niches in the SMGs.

CONCLUSIONS

The lung is believed to have several anatomically distinct niches in which multipotent adult stem cells destined for the various trophic units of the lung reside. These niches orchestrate environmental cues that control stem/progenitor cell responses in the settings of both airway injury and normal cellular turnover. SMGs represent one such niche that appears to be important in the proximal airway. Defining the intrinsic and extrinsic cues required for the proliferation and differentiation of glandular stem/progenitor cells may improve our understanding of lung diseases with associated glandular abnormalities, and may ultimately provide new avenues for their treatment.

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