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The Lung and Esophagus: Developmental and Regenerative Overlap

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Abstract

Lung and esophageal development and organogenesis involve a complex interplay of signaling pathways and transcriptional factors. Once the lung and esophagus do separate, their epithelial proliferation and differentiation programs share certain common properties that may fuel adaptive responses to injury and subsequent regeneration. Lung and esophageal tissue organogenesis and regeneration provide perspectives on squamous cell cancers and adenocarcinomas in each tissue.

Perspectives on Lung and Esophageal Development

The lung and esophagus share common and divergent properties during development and adult epithelial differentiation [1–3]. The purpose of this review is to: provide perspectives on lung and esophageal development, especially the factors that are permissive for separation of the two organs; describe how epithelial cells differentiate and the roles of stem cells/progenitor cells therein; delineate how both stem/progenitor cells and differentiated cells contribute to repair after injury; and indicate how proliferative and differentiated cells are susceptible to oncogenesis. This is important given that benign and malignant esophageal and lung diseases are prevalent. Furthermore, insights into fundamental mechanisms that underlie lung and esophageal organogenesis and regeneration may reveal new translational therapeutic approaches.

Lung Development

While there are important morphological and developmental distinctions between the lung and esophagus, both tissues arise from the anterior foregut endoderm (AFE) [2,3]. These distinctions begin around mouse embryonic day (E)9.5 when a ventral-dorsal gradient of signaling by Wnt and Bmp, which are critical in development, elicits expression of the pulmonary-restricted transcription factor Nkx2.1 on the ventral side of the foregut endoderm

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[4,5] (Figure 1). After initiation of Nkx2.1 expression, transcription factor expression is downregulated in the ventral portion of the AFE allowing pulmonary epithelial fate progression. Subsequent to evagination of the lung and tracheal **anlagen** (see Glossary), Sox2 expression re-emerges in the large proximal airways of the lungs where it is an essential transcriptional regulator of airway epithelial differentiation [6,7]. By contrast, Sox2 remains expressed at a high level in the dorsal foregut or esophageal anlagen [6,8].

From an evolutionary standpoint, the pulmonary system is a relatively late newcomer to the organ party. The general layout of the foregut was already in place in both invertebrates and vertebrates before the evolution of the pulmonary system. Many of the critical signaling and transcriptional pathways important for pulmonary specification and early development are likely to have been coopted in unique ways to define and promote the establishment of the lungs and trachea. For example, Wnt2/2b is essential for pulmonary endoderm specification in mice, as demonstrated by loss of Nkx2.1 expression in the ventral AFE and lung agenesis [5]. By contrast, in zebrafish that do not have lungs, Wnt2/2b is critical for liver and anterior foregut development [9]. In addition to Wnt signaling, one group has shown that Bmp signaling is essential for tracheal development in part through its ability to repress Sox2 expression in the ventral region of the foregut endoderm [4]. Moreover, these authors showed that loss of Bmp signaling in early foregut development leads to ectopic formation of lung buds, suggesting a role for Bmp signaling in both tracheal and lung bud spatial patterning.

One theory about the evolutionary ancestor of the lungs is that they developed from the swim bladder of fish, which itself develops from the foregut endoderm [10]. The swim bladder is located in an anterior-posterior position along the gut tube axis similar to that of the esophagus and lungs, supporting similarities in these two organ systems outside mammals. Molecular similarities between the swim bladder and the lungs are revealed through the similar coexpression of many important regulators of lung development, including sonic hedgehog and surfactant proteins [10,11]. However, the swim bladder buds from the dorsal not the ventral side of the foregut, suggesting that additional rewiring of AFE patterning was needed to evolve the lungs in terrestrial animals.

Once specified, the early lungs proceed through a process called branching morphogenesis to develop the arborized airway tree critical for generation of the large gas-exchange surface in the adult organ [2]. The respiratory system can be divided into two basic compartments: the proximal airways and the distal alveolus, the latter of which is the site of gas exchange. In the mouse, the trachea and main-stem bronchi contain basal cells with a subset of Trp63⁺/Krt5⁺ cells that act as the resident stem/progenitor cell population (Figure 2A) [12,13]. In the human lung, these TP63⁺/KRT5⁺ basal cells are thought to be found in more distal airways compared with the mouse airways and may act as stem/progenitor cells for a much larger region of the human airway tree [2].

The proximal airways develop in a unique manner and are derived from an early Sox2⁺ progenitor cell in the mouse [7,8]. Multiple pathways have been shown to play an important role in regulating these early Sox2 proximal progenitors, including epigenetic factors. Histone deacetylases 1/2 (HDAC1/2) are required for the early development of Sox2⁺

proximal progenitors in the mouse lung and lung endoderm-specific loss of these two factors results in an organ comprising solely Sox9⁺ distal lung endoderm progenitors [14]. This phenotype correlates with derepression of Bmp4, which is known to promote the distal endoderm progenitor fate during lung development. Interestingly, significant downregulation of Sox2 in the nascent esophagus also leads to abnormal development of the epithelium whereby the organized stratified squamous characteristics are lost and significant amounts of mucins are produced [6]. Whether HDAC1/2 also regulates esophageal development is unknown; however, it will be interesting to address this given the close regulation between HDAC1/2 and Sox2 in foregut development.

Esophageal Development

As mentioned, the separation between the lung and esophagus occurs at E9.5-E11.5 in mice and at around 4–6 weeks in the human embryo. Insights may be gleaned from a mouse foregut culture system to visualize the separation. A *SOX2-GFP* knock-in allele, as a means to monitor the fate of SOX2⁺ cells during development, was used to monitor the movement and an epithelial saddle was observed that is initiated at the lung-esophageal boundary in the E9.5 foregut. The saddle then moves in a caudal-cranial direction to split the trachea and esophagus, while both nascent organs extend caudally. Post-separation of the lung and esophagus, the esophageal epithelium is a single layer of columnar cells. On differentiation these cells stratify to form a multilayered squamous epithelium (Figure 2B).

Besides the importance of SOX2, the Trp63 transcription factor is also critical for the generation of a stratified squamous epithelium. Loss of Trp63 leads to failed conversion of simple columnar cells into stratified squamous epithelium [15]. The epithelium remains Krt8/18⁺ and does not express Krt5/14 (as would be the case for basal cells). While not much is known about the Trp63 isoforms in esophageal development, conditional deletion of the NTrp63 isoform results in defects in differentiation and stratification in the esophageal epithelium [16].

In addition to epithelial intrinsic cues, the mesenchyme may play a role in esophageal development. For example, FOXF1 heterozygous deletion contributes to EA/TEF formation [17], underscoring the importance of the mesenchymal compartment, and is reminiscent of defective sonic hedgehog signaling. Interestingly, Foxf1 plays an important role in lung mesenchymal development where it regulates lung vascular endothelial development and is a causative gene in the human syndrome alveolar capillary dysplasia [17–19]. The BARX1 transcription factor is present in the dorsal foregut mesenchyme and mesenchyme between the nascent esophagus and trachea. Deletion of the Barx1 gene results in expansion of canonical Wnt signaling and mutants demonstrate EA/TEF [20].

The Bmp and Wnt signaling pathways repress the esophageal fate in early foregut development. Later in esophageal development, Bmp signaling is evident and limited to suprabasal cells at E14.5 when expression of the secreted Bmp inhibitor **Noggin** is lost, but at E15.5 activity expands [21]. This is reinforced through treatment with Noggin, which fosters self-renewal of basal cells in **3D organoids** [22]; conversely, Bmp4 promotes squamous differentiation of basal cells. By contrast, Sonic hedgehog (SHH)-Gli2/3 and

retinoic acid (RA) are both critical for the growth of the esophagus and lung. RA signaling can modulate the expression of Shh in the AFE and RA inhibits TGF β , which may increase Bmp signaling activity, before lung bud specification [23].

Lung and Esophageal Basal Cells

As reviewed above, the proximal airways of the lungs exhibit some similarity to the esophagus in both cellular composition and morphological patterning. In the pulmonary airways, Trp63⁺ basal cells underlie a **pseudostratified epithelium** that contains Krt8⁺ luminal cells (Figure 2A). The esophagus contains a classic **stratified epithelium** with Trp63⁺ basal cells on the basal side and suprabasal keratins overlying these basal cells (Figure 2B). There are many similarities between the Trp63⁺ basal cells in the esophagus and those in the lung airways (Figure 3). In both tissues, subpopulations of basal cells can self-renew and regenerate all other epithelial lineages in their respective locales. However, the pattern of basal cell distribution is strikingly different in rodent versus human airways. In humans, TP63⁺ basal cells are found throughout the majority of the branching airway tree [1]. In the mouse and rat lung, Trp63⁺ basal cells are only found in the trachea and main-stem bronchi and a little further distally into the branched airway network [1]. This difference in patterning could be due to several possibilities including the larger size of human airways requiring a more robust and stable stem/progenitor cell lineage or unique, as-yet-undiscovered functions for more distal airways in the human lung that require a stable stem cell population. Thus, basal cells can be considered regional specific stem/progenitor cells in both tissues but are spatially dispersed differently in the rodent and human lungs.

One distinguishing characteristic of the esophagus versus the proximal airways of the lungs is that the esophagus is lined with a true stratified epithelium whereas the proximal airways are lined with a pseudostratified epithelium. Perhaps a true stratified epithelium may be more resistant to the deleterious effects of injury. During esophageal development, the esophageal epithelium converts from a simple columnar epithelium (Krt7⁺, Krt8⁺) to a stratified squamous epithelium. Basal cells are Trp63⁺, Sox2⁺, Krt5⁺, Krt14⁺, suprabasal cells are Krt4⁺/Krt13⁺ (also Krt1⁺, Krt10⁺), and superficial squamous cells are **loricrin**⁺, **involucrin**⁺ [24]. This cell patterning and expression of keratins and other epithelial markers closely mimics the stratified epithelium in other organs, including the skin. However, protein marker expression is different in the lung airways and trachea, where basal cells are Krt5⁺/Trp63⁺ with a subset of basal cells also expressing Krt14. Luminal cells of the proximal airways express Krt8 but expression of markers of more differentiated squamous cells such as loricrin and involucrin have not been reported. This difference could be due to the constant physical and chemical bombardment that the esophagus is exposed to from eating, requiring a more robust and hierarchical cell composition that exists in a stratified epithelial lining. A recently defined long-lived progenitor cell subpopulation in the mouse esophageal basal cell compartment that has properties consistent with stem cells contributes to tissue regeneration following irradiation [25]. This premise is reinforced through genetic ablation of the subpopulation of these cells and impaired ability for tissue regeneration. Whether differentiated suprabasal cells contribute to tissue regeneration is unclear, although not inconceivable, and is not mutually exclusive with the finding that esophageal basal long-

lived progenitor cells may harbor such properties. Alternatively, it has been proposed that a single population of esophageal epithelial cells is unipotent and can respond to injury [26].

In addition, a transitional basal progenitor cell population (Krt5⁺, Krt7⁺, p63⁺) was identified recently at the gastroesophageal junction (GEJ). This progenitor population is responsible for maintaining the epithelium lining the transitional zone [24]. This unique basal progenitor population can give rise to intestinal-like cells with bile acid injury induced by jejunoesophageal anastomosis. By contrast, squamous basal progenitor cells (Krt5⁺, Krt7⁻, p63⁺) maintain stratified squamous cells throughout the esophagus [24].

Differentiated Cells: Capacity for Repair and Regeneration

In rodent pulmonary airways, both basal cells and certain populations of luminal secretory epithelial cells can regenerate airway epithelium after injury [12,13,27–30]. This ability of differentiated secretory cells of the airway epithelium to reconstitute lost cells after injury may be an adaptation in the rodent lung to the fact that the more distal airways do not contain Trp63⁺ basal cells. Surprisingly, recent evidence has revealed that these secretory cells can also dedifferentiate into basal cells in the large airways of the mouse lung after genetic depletion of the Trp63⁺ basal cells [31]. This finding raises the concept that luminal epithelial cells, such as the secretory cells, and basal cells may not be as dissimilar as previously believed. While this secretory cell-basal cell plasticity has been demonstrated in genetic cell depletion studies, it will be important to determine whether this type of plasticity or something similar is exhibited in human disease or oncogenic states. There is emerging evidence that basal stem cells in the lung airways generate niche or microenvironmental signals required for secretory cell maintenance [32]. Signals such as Notch appear to provide a cell-cell context-dependent inhibitory signal to restrict or promote certain cell-fate decisions in a basal stem cell niche. If both basal cells and secretory cells can act to regenerate lost epithelium in the airways of the lung, and stem cells can generate niche-supporting signals for differentiated daughter cells, the definition of both ‘niche’ and ‘stem cell’ in the lung or other tissues with pseudostratified or true stratified epithelium is likely to require some rethinking. Basal cells are found not only in the esophagus, skin, and lung but also in other organs, including the prostate [33], thereby suggesting that such plasticity may be present broadly in many tissues. Moreover, the molecular pathways important for such plasticity will be important to define as they may contribute towards both an effective regenerative response and tumorigenesis after epithelial stress. Disruption or activation of these pathways could provide possible therapeutic approaches for cancer and tissue regeneration, respectively.

This paradigm of extensive epithelial plasticity could be extended to other stem cell niches in the lung, including the alveolus, which contains two major epithelial lineages: alveolar type 1 (AT1) and 2 (AT2) cells. Previous work has indicated that there is plasticity between these two lineages; however, what functional role this plasticity plays in regeneration of the gas-exchange interface in the lung is unknown [34,35]. The recent identification of an evolutionarily conserved alveolar epithelial progenitor in the adult mouse and human lung may help to clarify whether such plasticity contributes to a significant amount of alveolar

regeneration or whether it constitutes a minor regenerative process and whether such processes are injury and disease specific [36].

Whether differentiated esophageal suprabasal cells contribute to tissue regeneration is unclear, although it is not inconceivable and is not mutually exclusive with the finding that esophageal long-lived progenitor cells contribute to regeneration [25,37]. Moreover, whether differentiated suprabasal cells can replenish depleted esophageal long-lived progenitor cells during injury is unclear. Studies similar to those performed in the lung airways including basal cell depletion and careful lineage tracing should shed light on these questions. Together these studies point to an emerging concept that many stem/progenitor cell niches require reconsideration regarding the relationship between these stem cells and their differentiated progeny. The ability of differentiated cells to dedifferentiate into more defined stem/progenitor lineages could provide an important backup system for many tissues including the lung and esophagus. However, this plasticity could also engender increased oncogenic risk, which is consistent with the high frequency of lung and esophageal cancers.

Transcription Regulatory Networks and Signaling Pathways That Impact on Cell Lineages

What transcriptional regulators and signaling pathways distinguish the respiratory endoderm from the esophageal endoderm? As noted above, Wnt2/2b signaling is essential for specification of the Nkx2.1⁺ respiratory endoderm. Activation of Wnt/beta-catenin signaling dramatically expands the Nkx2.1⁺ endoderm domain to include the esophagus and proximal stomach [4,5]. More recent evidence has revealed that RA and SHH signaling cooperate with each other to regulate early specification of the Nkx2.1⁺ lung endoderm [23]. RA patterns the **lateral plate mesoderm** surrounding the anterior foregut, which leads to induction of SHH expression in the adjacent endoderm. This in turn promotes Wnt2/2b and Bmp4 expression in the mesoderm, which cycles back to induce Nkx2.1 expression and the specification of early lung endoderm progenitors. Bmp4 expression in turn is modulated by the transcriptional repressors Osr1 and Osr2, which are required for induction of Wnt2/2b expression in the ventral mesoderm that flanks the foregut endoderm [38]. This complex interplay between paracrine signaling and downstream transcriptional effectors helps to pattern the anterior foregut in a specific ventral dorsal manner.

The role for Wnt signaling in esophageal and lung regeneration remains less clear. Several studies have reported conflicting data indicating that Wnt/beta-catenin signaling is dispensable for airway secretory cell regeneration or that it is critical in context-dependent ways [39–45]. Nonetheless, suppression of Wnt/beta-catenin signaling is required to generate proximal airway epithelial cell lineages from pluripotent stem cells [46,47]. Thus, Wnt/beta-catenin signaling may antagonize the generation and regeneration of proximal airway epithelial cells. However, the role of Wnt signaling specifically in airway basal cells versus esophageal basal cells has not been tested. If the developmental paradigms are conserved between these two tissues, Wnt may play a key role in airway basal cell self-renewal, maintenance, or differentiation. Such a paradigm would also predict that Wnt may not be particularly important for the homeostasis of esophageal basal cells, although

components of Wnt signaling are altered in esophageal squamous cell cancers [48]. While Wnt signaling is often disrupted in cancers of the gastrointestinal system, it has only rarely been reported as a key mutational driver pathway in lung cancers [49]. Whether lung epithelial cells are more resistant and thus do not transform as readily as gastrointestinal lineages on activated Wnt signaling is unclear. This correlative finding may suggest that Wnt signaling is more important during early lung epithelial development but after this stage the epithelium becomes less responsive to this pathway.

In addition to Wnt signaling, Notch signaling plays a key role in lung airway development and basal stem cell activation and differentiation. Increased Notch activity promotes secretory cell differentiation whereas reduced Notch activity promotes multiciliated cell differentiation during lung airway development [50–52]. In adult airway basal cells, Notch signaling is important for basal cell self-renewal and differentiation to the secretory cell lineage [53]. Thus, Notch signaling helps to promote a tight stem cell niche in the airway epithelium by balancing basal cell self-renewal and secretory cell differentiation. How Notch signaling activity is quantitatively controlled in airway and tracheal basal cells is not understood but the Notch pathway could be a target for diseases that exhibit significant airway remodeling due to mucinous goblet cell hyperplasia, such as asthma [54].

Notch 1 is important for cell-fate decisions emerging from esophageal basal cells. The intracellular domain of Notch1 (ICN1) drives post-mitotic terminal differentiation in stratified squamous epithelium of the esophagus in concert with Notch3, one of the Notch receptor **paralogs** and a direct transcriptional target for ICN1-mediated transcription [55]. Loss of Notch signaling in *K14Cre;DNMAML1* mice results in perturbation of esophageal squamous differentiation with loss of Notch3 expression and basal cell hyperplasia. Loss of Notch3 prevents esophageal basal cells from undergoing terminal differentiation, with concurrent suppression of the transcription factor KLF4, and loss of Notch 3 facilitates epithelial-mesenchymal transition, a feature of tumorigenesis, via transcription factors ZEB1 and ZEB2 [56]. Additionally, Notch1 activation mediates replicative senescence in esophageal basal cells whereas pharmacological Notch inhibition extends replicative lifespan in the presence of intact cell cycle checkpoint functions [57]. Interestingly, Notch inhibition plays a role in the development of intestinal metaplasia in the esophageal squamous epithelium, one feature of which is goblet cell hyperplasia [25].

Many transcriptional regulators, including Sox2 and Foxp1, are expressed in both the epithelium of the proximal airways and the esophagus later in development and in the adult [58]. Sox2 is required for proper differentiation of luminal epithelial cells in both lung and esophageal development [6–8]. While most of the epithelium in the large airways of the lung express Sox2 [6,7], it remains unclear what role this transcription factor plays in airway regeneration. However, given the role of Sox transcription factors in cellular fate decisions, including phenotypic reprogramming [59], it is likely that Sox2 helps to integrate multiple cell-fate decisions, including those that are regulated in a cell-cell manner in the airway basal cell niche, to direct both self-renewal and daughter cell differentiation. In this manner, Sox2 is likely to play a similar role in esophageal basal cell regeneration. While Sox2 may play a critical role in injury-induced regeneration of basal cells in both the lung and the

esophagus, its role in homeostatic maintenance of the basal cell population may be even more important.

The Foxp1/2/4 family of forkhead transcription factors are all expressed in overlapping patterns in the lung and esophagus [58,60]. Mouse genetic knockout lines have revealed both distinct and cooperative functions for these forkhead genes in lung and esophageal development. In particular, Foxp1/2 act cooperatively to regulate lung alveologenesis and esophageal smooth muscle development [58]. Complete loss of Foxp1/2/4 in the foregut endoderm reveals the requirement of these factors in the promotion and maintenance of the lung endoderm [61]. Foxp1/2/4 triple mutants exhibit ectopic activation of an entire non-pulmonary gene expression program, including genes highly expressed in esophageal epithelium such as Pax9. Whether Foxp1/2/4 regulates basal cell differentiation in the adult lung or esophagus remains to be determined. However, given their broad expression in both tissues, Foxp1/2/4 likely plays multiple roles in both lung and esophageal endoderm and mesoderm development.

Oncogenic Susceptibility

Given the similarities in the developmental origin and morphological structure of the esophagus and the large airways of the lung, it is unsurprising that similar expression patterns and molecular mechanisms drive the development, repair, and oncogenic outcomes of these tissues [2,3]. These genes, besides Sox2, Pax9, and KLF4/KLF5, are expressed in a restricted fashion in the esophagus and the proximal large airways of the lung. Sox2 is particularly conserved as an oncogene in both esophageal and lung squamous cell carcinomas. Mice with Sox2 overexpression in the lung and esophagus exhibit hyperplasia and eventual formation of adenocarcinomas and squamous cell carcinomas, respectively [62,63].

An analysis of differential gene expression, DNA methylation, and noncoding RNA expression between esophageal adenocarcinoma (EAC) and lung adenocarcinoma (LAC) and esophageal and lung squamous cell carcinoma (ESCC, LSCC) revealed over 1700 genes, nearly 350 CpG sites, and approximately 40 miRNAs in common between organ sites, suggesting that specific adenocarcinoma-associated and squamous cell carcinoma-associated molecular patterns are conserved across organs [61]. As examples of this type of analysis, the nuclear receptors LXR/RXR (note the importance of RA in esophageal and lung development) are upregulated in EAC and LAC and p38/MAPK and paxillin signaling are downregulated. In ESCC/LSCC, the PCP, basal cell carcinoma, Wnt signaling, Notch signaling, and TGF β signaling pathways are upregulated; conversely, the LXR/RXR pathway is activated [64]. This type of analysis may pave the way for common therapeutic approaches for ESCC/LSCC versus EAC/LAC.

Concluding Remarks and Future Directions

Given the similarities in the cell composition and structure of the proximal airways of the lung and the esophagus, especially in the respective activities of the resident basal cells, future investigations to define common and differential molecular pathways may provide

unique insights into how regeneration occurs in a tissue-specific manner, which is an outstanding question. At this juncture one should consider also the roles of differentiated cells in lung and esophageal epithelial regeneration. These collective approaches may allow 3D models (e.g., 3D organoids) and drug testing (personalized medicine). Moreover, such investigations are likely to be important for the derivation of airway- and esophageal-specific basal cells and other lineages from iPSCs. Knowledge of the molecular pathways used *in vivo* to promote regeneration of the esophagus and the airways of the lungs will be critical (see Outstanding Questions); namely, for efficient derivation of tissue-specific basal cells, which would be pivotal for eventual cell replacement therapies.

Glossary

3D organoids

in vitro culture system using multiple cell types to mimic *in vivo* cell-cell differentiation and structure.

Anlagen

the earliest rudimentary portion of an organ as it develops.

Involucrin

protein that contributes to the superficial squamous layer.

Lateral plate mesoderm

early mesoderm found on the lateral sides of the developing vertebrate embryo that contributes to mesodermal derivatives in foregut-derived tissues including the lung and esophagus.

Loricrin

protein that contributes to the superficial squamous layer.

Noggin

a secreted inhibitor of Bmp signaling.

Paralogs

genes related by an evolutionary duplication event. They generally evolve new functions but may or may not retain similar functions.

Pseudostratified epithelium

an epithelial tissue where all cells, both basal and luminal, make contact with the basal lamina.

Stratified epithelium

an epithelial structure where distinct luminal cell types do not make contact with the basal lamina and instead overlie basal cells.

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Highlights

Nkx2.1 and Sox2 are critical in lung and esophageal development.

Wnt signaling and Bmp signaling play pivotal roles in the fate of lung and esophageal organogenesis.

The lung and esophageal basal cells share common properties.

Lung and esophageal basal cells participate in regeneration after injury. This has been also observed in differentiated lung cells.

Regulatory networks in lung and esophageal development, organogenesis, and regeneration re-emerge during oncogenesis.

Outstanding Questions

What are the regulatory networks underlying the separation of the lung and esophagus during development?

How do lung and esophageal basal stem and progenitor cells participate in regeneration?

Can the key molecular pathways involved in lung and esophageal organogenesis be coopted in oncogenesis?

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Figure 1. Patterning of the Anterior Foregut to Generate the Lung and Esophagus.

Multiple signal transduction pathways including Wnt, Bmp, and Shh are essential for proper patterning of the anterior foregut endoderm into esophageal and lung progenitors. A distinct dorsal-ventral polarity is generated through the combined action of Wnt and Bmp signaling and through other regulators [Barx1, retinoic acid (RA), Gli2/3, Osr1/2]. This results in Sox2 expression being highest in the dorsal endoderm with Nkx2.1 expression restricted to the ventral endoderm.

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Figure 2. Cellular Composition of the Trachea and Large Airways Compared with the Esophagus.

(A) The trachea and large main-stem bronchi of the mouse comprise a pseudostratified epithelium containing the cell lineages shown. Basal cells act as resident stem cells that can regenerate lost secretory and multiciliated epithelia after injury either directly or indirectly (broken arrows). This is regulated, in part, through active Notch signaling. (B) The esophagus contains a true stratified squamous epithelium with basal cells capable (purple) of generating differentiated lineages (suprabasal cells and superficial squamous epithelial cells)

(black arrows). Such cells are also referred to as keratinocytes. Notch signaling is important in the switch from proliferative basal cells to differentiated suprabasal cells.

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Figure 3. Hierarchical Comparison between Airway and Esophageal Basal Cells and Their Progeny.

While esophageal and airway basal cells express similar markers (Sox2, Trp63, Krt5), their differentiated progeny differ between the two organs. In the airway, basal cells generate secretory and multiciliated epithelium as well as more specialized epithelial cells such as goblet and neuroendocrine cells. In the esophagus, basal cells generate the stratified epithelium through a multistep process including a classic transient amplifying stage before conversion into fully differentiated luminal keratinocytes. Critical marker genes including transcription factors and keratins are noted for each lineage.