Lung epithelium development and airway regeneration

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The lung is composed of a highly branched airway structure, which humidifies and warms the inhaled air before entering the alveolar compartment. In the alveoli, a thin layer of epithelium is in close proximity with the capillary endothelium, allowing for an efficient exchange of oxygen and carbon dioxide. During development proliferation and differentiation of progenitor cells generates the lung architecture, and in the adult lung a proper function of progenitor cells is needed to regenerate after injury. Malfunctioning of progenitors during development results in various congenital lung disorders, such as Congenital Diaphragmatic Hernia (CDH) and Congenital Pulmonary Adenomatoid Malformation (CPAM). In addition, many premature neonates experience continuous insults on the lung caused by artificial ventilation and supplemental oxygen, which requires a highly controlled mechanism of airway repair. Malfunctioning of airway progenitors during regeneration can result in reduction of respiratory function or (chronic) airway diseases. Pathways that are active during development are frequently re-activated upon damage. Understanding the basic mechanisms of lung development and the behavior of progenitor cell in the ontogeny and regeneration of the lung may help to better understand the underlying cause of lung diseases, especially those occurring in prenatal development or in the immediate postnatal period of life. This review provides an overview of lung development and the cell types involved in repair of lung damage with a focus on the airway.

INTRODUCTION

After fertilization, tightly controlled processes and cell fate decisions guide the development of a full-grown organism from a single cell embryo. Axis formation and specification is followed by gastrulation, a highly complex process leading to the determination of the three germ layers, ectoderm, mesoderm and endoderm. The epithelial cells of the trachea, airway, and alveoli are derived from the endodermal lineage, whereas the lung mesoderm develops and generates various cell lineages like, vascular cells, smooth muscle cells, pericytes and cartilage precursors. The lung
FIGURE 1
Lung specification, primary lung bud formation and growth. (A) During lung specification, Nkx2-1 expression is restricted to the ventral side and Sox2 to the dorsal side of the foregut endoderm. Retinoic acid (RA)-signaling activates RA receptors in the surrounding mesoderm driving cells to secrete Sonic Hedgehog (SHH) in the ventral foregut mesoderm. SHH-responsive cells subsequently trigger activation of GLI2 and GLI3 transcription factors in the ventral mesoderm, which stimulate expression of WNT2/2b and BMP4 (Rankin et al., 2016). Odd-skipped related zinc finger transcriptional repressor, OSR, and SHH signaling target TBX5 are important modulators of WNT2/2b and BMP4 signaling (Han et al., 2017; Steimle et al., 2018). The transcription factor, BARX1, is expressed in the dorsal mesenchyme thereby repressing WNT signaling (Woo et al., 2011).

(B) FGF10 from the ventral mesoderm is essential in lung bud formation, and is regulated by RA and TGF-β signaling. TBX transcription factors present in the foregut mesoderm has shown to be essential in regulating FGF10 expression as well (Sakiyama et al., 2003; Arora et al., 2012).

E = embryonic day, pcw = post-conceptional week (C-1) Several reciprocal interactions between mesoderm and endoderm regulate the expansion of the distal tip through proliferation and suppression of Sox2 expression. SHH is expressed in a gradient with the highest expression in the distal bud. SHH inhibits mesenchymal FGF10 expression just proximal of the distal bud. At high concentrations, SHH induces expression of, HH inhibitory protein (HHIP) in the distal mesenchyme to allow for FGF10 expression via regulation of GLI3 and FOXF1 (Morrisey and Hogan, 2010). Proliferation of progenitor cells is positively regulated via BMP4 induction or inhibited via its antagonist SPRY2 (Weaver et al., 2000; Mailleux et al., 2001; Hyatt et al., 2004; Eblaghie et al., 2006). Sox2 expression is inhibited via Wnt-β-Catenin and BMP4 signaling (Volckaert et al., 2015; Wang et al., 2013). Ω = proliferation. (C-2) Knock-out mouse models of WNT ligands demonstrated defects in lung development; WNT2/2b (canonical) (Hrycaj et al., 2015) (Continued)
mesoderm and endoderm reciprocally interact, thereby affecting the development and differentiation of each other during all stages of development (Cardoso and Lu, 2006; Swarr and Morrissey, 2015). The pulmonary vasculature is already present early during lung development and expands as the lung grows (Canis Parera et al., 2005). Here, we will focus on the development of lung epithelium from endodermal progenitor cells.

Origin and specification of the trachea and primary lung bud formation

Specification of lung and esophagus starts from the anterior foregut endoderm. Sry-related HMG box 2 positive (SOX2+) dorsal esophagus progenitors are separated from ventral, NK2 Homeobox 1 positive (NKX2-1+) lung progenitors (Minoo et al., 1999; Que et al., 2007) (for details see Figure 1A). Reciprocal signaling cues between mesoderm and endoderm contribute to a proper localization of Nkx2-1 expression (Figure 1A) (Swarr and Morrissey, 2015; Billmyre et al., 2015; Morrissey and Rustgi, 2018; Kishimoto et al., 2018; Kiyokawa and Morimoto, 2021). Nkx2-1 expression is induced by canonical Wingless and Int1 (WNT2) and WNT2b ligands from the ventral mesoderm and by Fibroblast Growth Factor 2 (FGF2) secretion from adjacent developing cardiac mesoderm (Serls et al., 2005; Goss et al., 2009; Harris-Johnson et al., 2009). Sox2 expression is repressed in the ventral foregut endoderm due to the secretion of Bone Morphogenetic Protein 4 (BMP4) from the ventral mesoderm (Domyan et al., 2011). The BMP antagonist NOGGIN is secreted by cells of the notochord, suppressing BMP signaling in the dorsal mesoderm and allowing Sox2 expression (Que et al., 2006; Li et al., 2007). Sox2 represses Nkx2-1 expression, thereby restricting its expression to the ventral foregut endoderm (Figure 1A) (Domyan et al., 2011). In addition, canonical WNT signaling induces Wnt7b expression in the endoderm, which in turn activates Tbx4 in the surrounding mesoderm (Kishimoto et al., 2020). T Box transcription factor 4 (TBX4) activates the branch inducing growth factor, FGF10, and is involved in fibrolast maturation (Sakiyama et al., 2003; Masafumi et al., 2018). Inactivation of mesodermal WNT signaling leads to cartilage agenesis as well as malformation of the circumferential smooth muscle cell layer (Kishimoto et al., 2020). SOX2 and NKK2-1 demarcate the Dorsal-Ventral (D-V) boundary of the foregut endoderm and are important in separating the trachea from the esophagus. Mouse models with reduced expression Sox2 or absence of Nkx2-1 resulted in separation defects, resembling the human congenital condition called tracheoesophageal fistula (TEF), where the airway is connected with the stomach and/or esophageal atresia (EA), a short and blunted esophagus (Minoo et al., 1999; Que et al., 2006; Que et al., 2007). Multiple factors contributing to trachea and esophagus D-V patterning have been identified using genetic mouse models, such as Nkx2-1-/- (Canis Parera et al., 2005), Sox2-/-/Fgfr2b+/+ (Que et al., 2007), Bmp4-/- (Li et al., 2008), Barx1-/- (Woo et al., 2011), Noggin-/- (Que et al., 2006; Li et al., 2007), Gli2/3 (Motoyama et al., 1998) and Shh (Littington et al., 1998; Pepicelli et al., 1998), or through genetic screens of human infants born with EA/TEF, such as NOGGIN (Murphy et al., 2012) and SOX2 (Williamson et al., 2006) (Figure 1A) (Que et al., 2006; Billmyre et al., 2015). Although genetic analyses of human EA/TEF patients and animal models revealed genes associated with EA/TEF, the cellular mechanisms causing the separation defect are poorly understood (Brosens et al., 2020; Brosens et al., 2021).

After specification of lung progenitors, the single common foregut tube begins to compartmentalize (Cardoso and Lu, 2006; Schittay, 2017; Whitsett et al., 2019; Zepp and Morrissey, 2019). A timed and localized expression of retinoic acid (RA) induces mesenchymal expression of FGF10, which activates NKK2-1+ lung progenitor cells by binding to its receptor FGFR2B and subsequently induces lung bud formation (Malpel et al., 2000;
Branching morphogenesis

A complex tree-like structure of airways is formed at the pseudoglandular stage, with a repetitive pattern of formation of new buds, bifurcation and outgrowth of buds (Metzger et al., 2008). During branching of the airways, SOX9+ Inhibitors of DNA binding 2+ (ID2+) progenitor cells reside at the branching distal tips. These tip progenitors, are multipotent and give rise to the SOX2+ progenitor cells which will form the airway epithelium (Gontan et al., 2008; Rawlins et al., 2009a; Que et al., 2009). In contrast to the mouse branching airways, in human lung the tip progenitors express both SOX9 and SOX2 (Figure 1D) (Nikolic et al., 2017; Danopoulos et al., 2018; Eenjes et al., 2021).

Maintaining a proximal-distal patterning during lung development is crucial for a proper branching of the airways. We previously illustrated formation of cystic airway structures in a mouse model where Sox2 expression was induced in the distal tip progenitor cells (Gontan et al., 2008). During the last decades, the use of transgenic mouse models contributed highly to the identification of multiple epithelial-mesenchymal signaling pathways important for maintaining a proximal-distal patterning and coordinating initiation and outgrowth of lung buds [see (Morrissey and Hogan, 2010; Whitsett et al., 2019; Zepp and Morrissey, 2019) and Figure 1C]. FGF10 is important for primary bud formation, and continues to be present in the mesenchyme surrounding the outgrowing buds during branching morphogenesis (Belluscio et al., 1997; Yuan et al., 2018). The localized source of FGF10 within the “tip-microenvironment” regulates multiple factors to control expansion of the bud by inducing proliferation and suppressing Sox2 expression to prevent differentiation (Figure 1C) (Hyatt et al., 2004; Shu et al., 2005; Volckaert et al., 2013; Wang et al., 2013; Chao et al., 2019). As the lung bud grows, cells become displaced from the FGF10 source and differentiate to SOX2+ airway progenitor cells. FGF10 plays a central role in branching morphogenesis of mouse lungs, however, FGF10 is not essential for branching of human fetal lungs in vitro (Danopoulos et al., 2019).

Development of proximal airway and distal alveolar lineages

During branching morphogenesis, SOX2+ progenitor cells proliferate but also start to differentiate into proximal airway cell lineages (Figure 1D). SOX2 positive cells demarcates the airway epithelium after progenitor cells differentiate, and deletion of SOX2 during development shows a severe reduction in basal, ciliated and secretory cells (Que et al., 2009).

Differentiation commences with the appearance of a few basal cells (Transformation-related protein 63) at E9.5 in the trachea and in proximal regions of the lung bud in mice. Lineage tracing studies using Trp63-CreERT shows that presumptive basal cells genetically labeled before E9.5 give rise to both airway and alveolar epithelial cells (Figure 1D). Lineage-labeling from E10.5 onward showed that the basal cells only serve as progenitors for the cells in the pseudostratified epithelium of the extrapulmonary airways (trachea and main bronchi) (Yang et al., 2018). Vice versa, lineage tracing of tip progenitor cells using Sox9-Cre or Id2-Cre induced before E9.5, shows that tip progenitor cells give rise to airway epithelial cells both in the extra- and intra-pulmonary airways, whereas induction at E11.5 shows that tip progenitor cells only give rise to the intrapulmonary airways (Rawlins et al., 2009a; Yang et al., 2018). So, during lung specification and lung bud formation (E8.5–E9.5), two complementary lineages are defined early in trachea/lung development, both contributing to the epithelial cells of the respiratory tract.

At E13.5, as the bronchial tree is expanding, SOX2+ progenitor cells give rise to neuroendocrine (NE) cells and non-NE cells (Figure 1E). Precursors of NE cells, are first scattered throughout the proximal airway epithelium and subsequently migrate to form NE clusters, which are mostly located at the bifurcations of airways (Kuo and Krasnow, 2015; Noguchi et al., 2015; Noguchi et al., 2020). Notch activity controls the choice between NE and non-NE cell fate (Ito et al., 2000; Jia et al., 2015; Shue et al., 2022). Inhibition of Notch signaling results in an increase in NE cells, but also in an increase in ciliated cells at the expense of secretory cells. This showed that at later stages in development (after E13.5), Notch signaling balances the differentiation between secretory and ciliated cells (Figure 1E) (Rawlins et al., 2007; Tsao et al., 2009; Morimoto et al., 2012). NE cell hyperplasia is associated with CDH, but whether this contributes to the onset or specific pathology related to CDH is not yet investigated (Jisselstijn et al., 1997). Previously, it was shown that overexpression of Sox2 during lung development resulted in increased basal cell numbers, but also to an increase in NE cells. However, the underlying molecular mechanisms that guide the SOX2+ airway progenitor to differentiate to basal or NE cells is not yet understood (Gontan et al., 2008).

Mature alveoli exist of cuboidal surfactant producing alveolar type 2 cells (ATII) and flattened alveolar type I (ATI) cells. The first specification of SOX9+ tip progenitors to either ATI or ATII cells is observed at E13.5 (Figures 1D,E) (Frank et al., 2019). From E15.5 onward, SOX9+ progenitors are still involved in
branching of distal tips, but cells in this recently branched epithelium do not express SOX2, as they do early in development, but rather express the ATI marker, Homeodomain-Only Protein homeobox (HOPX) (Figure 1D) (Alanis et al., 2014; Frank et al., 2019). In addition, bipotent progenitor cells expressing both ATI and ATI markers, can be found in the distal bud but they show only minor contribution to the alveolar compartment during development (Figure 2) (Desai et al., 2014; Treutlein et al., 2014; Frank et al., 2019; Zepp et al., 2021). In human lung development, distal tip progenitors loose SOX2 expression and remain only SOX9+ in the canalicular and saccular stage (Figure 1D). However, tip progenitor cells already start to express both markers of ATI and ATII cells 5 weeks prior to the canalicular stage and in co-expression with SOX2 (Nikolic et al., 2017). The functional significance of SOX2 expression in human tip progenitor cells during the pseudoglandular stage is currently unknown.

**Epithelial lineage diversification and cell plasticity upon airway regeneration**

As a result of lung development, the airway epithelium is aligned with a wide range of cell types (Figure 2). During steady state, the airway epithelium is a low turnover tissue, but upon severe damage, quiescent progenitor cells can regenerate the airway epithelium. Lineage tracing studies in mice demonstrated that within the airway epithelium, most adult epithelial cells retain plasticity to dedifferentiate or transdifferentiate under stress or damage conditions. Ciliated cells seems to be an exception, which have no apparent potential to proliferate or differentiate after injury (Rawlins et al., 2007) The interaction with the underlying mesenchyme and vasculature is important in the differentiation and regeneration of the epithelium [reviewed in (Mammoto and Mammoto, 2019; Tsuchiya et al., 2020)]. For instance, Dil4 deficient mice resulted in microvascular defects and subsequent impaired alveolarization (Xia et al., 2021). In mouse model of regenerative alveolarization, it was shown that capillary endothelial cells were stimulated to secrete growth factors that would induce epithelial proliferation (Ding et al., 2011). Recent work has described distinct processes and specialized AT2 cells that contribute to alveolar regeneration after induced damage in mice (Paisley et al., 2014; Choi et al., 2020; Kobayashi et al., 2020; Hurskainen et al., 2021). Like for the alveolar compartment, the epithelial cells of the airways are also subjected to signaling from the underlying mesenchymal cells. Upon injury, epithelial cells secreted Wnt7b, which subsequently induced the mesenchymal smooth muscle cells to express Fgf10 and thereby activating the basal cells (Volkcaert et al., 2017). Here, we focus on the main adult airway cell types that are known to contribute to repair after injury. A more extensive description of lung regeneration and *in vitro* models to study adult airway epithelium was reviewed previously (Schilders et al., 2016; McQualter, 2019).

**Basal cells**

The basal cell is one of the most studied cell types of the lung regarding regeneration. In mouse lung, basal cells are mainly located in the extrapulmonary airway epithelium, while the distribution in the human lung ranges from the trachea down to the smallest airways (Figure 2) (Rock et al., 2010). *In vitro* cultures using isolated mouse and human basal cells has shown that these cells could self-renew and are multipotent, meaning that they could differentiate to secretory and ciliated cells (Rock et al., 2009; Eenjes et al., 2018).

Human and mouse basal cells are characterized by the expression of *Trp63*, and *Trp63* knock-out mice completely lack basal cells (Mills et al., 1999; Yang et al., 1999; Daniely et al., 2004). Besides *Trp63* expression, all basal cells also express *Cytokeratin 5* (*Krt5*), and a subpopulation of basal cells express *Cytokeratin 14* (*Krt14*), which greatly expands upon injury (Hong et al., 2004a; Hong et al., 2004b). In human airway epithelium, *KRT14* also shows a more restricted expression pattern than *KRT5*, but increases in regions of squamous metaplasia in COPD patients (Rock et al., 2010). However, a functional difference between *KRT14*+ and *KRT14*-basal cells is not yet explored. Furthermore, basal cells are thought to be the source of lung squamous cell carcinoma through increased expression of both SOX2 and *TRP63* (Bass et al., 2009; Ferone et al., 2016). The regulation of basal cell maintenance, proliferation and differentiation in relation of SOX2 is poorly understood, although ectopic expression of SOX2 induced the emergence of basal cells (Gontan et al., 2008; Kapere Ochieng et al., 2014; Ochieng et al., 2014). Recent single cell RNA sequencing data revealed that potentially several basal cells, or basal-like cells exist in the lung, that could form a continuum of differentiation (Montoro et al., 2018; Plasschaert et al., 2018; Travaglini et al., 2020; Basil et al., 2022; Kadur Lakshminarasimha Murthy et al., 2022).

A very small population of *Trp63* expressing cells reside in the mouse intrapulmonary airways. The number of these distal basal cells substantially increases upon severe lung injury. Lineage tracing showed that these cells contributed to both alveolar and airway lineages, showing the high potential of distal *TRP63*+ cell population (Vaughan et al., 2015; Zuo et al., 2015; Yang et al., 2018). Although, a similar population of basal cells was identified in human terminal bronchioles, its expansion or differentiation potential and contribution to airway regeneration is still uncertain (Vaughan et al., 2015).

**Submucosal glands**

Submucosal glands (SMGs) are specialized secretory glands with a grape like structure embedded within the connective
tissue, just underneath the proximal tracheal epithelium of the mouse and the cartilaginous airways of the human (Figure 2) (Tata and Rajagopal, 2017). The submucosal glands can be subdivided in the ducts and acini. The ducts contain a similar cellular composition as the surface epithelium of the airways. The acini contain basally located myoepithelial cells expressing Krt14, Krt5, and smooth muscle actin 2 (Acta2), and luminal cells secreting mucous and fluids rich in antimicrobial enzymes (Hegab et al., 2011; Lynch and Engelhardt, 2014). Upon injury, basal myoepithelial cells migrate to the surface epithelium of the trachea and aid in repopulating the airway due to proliferation and differentiation to basal, ciliated and secretory cells (Lynch et al., 2018; Tata et al., 2018). In pigs, similar to human, SMGs are present throughout the cartilaginous airways and exposure to chlorine gas showed that SMG derived cells contributed to the repair of the airway (Tata et al., 2018).

Secretory cells

Secretory (Club) cells produce mucins and microbial peptides to capture inhaled substances, which are propelled out of the lung through cilia movement. Different subsets of secretory cells in mouse and human airways are identified by the secretion of different members of secretoglobins; SCGB1A1, SCGB3A1 or SCGB3A2 (Reynolds et al., 2002) (Figure 2). Lineage tracing studies, using secretory cell marker SCGB1A1, showed that besides the protective function, secretory cells have the potency to self-renew, differentiate to ciliated cells, and de-differentiate to basal cells (Rawlins et al., 2009b; Tata et al., 2013).

Naphthalene-induced injury is a frequently used mouse model to study airway regeneration (Van Winkle et al., 1995). Secretory cells are most vulnerable to naphthalene exposure due to their expression of cytochrome P450 enzyme (Cyp2f2), which converts naphthalene to a cytotoxic product (Plopper et al., 1992). A subset of secretory cells, the variant club cells, was identified because they lack Cyp2f2 expression, and survive naphthalene exposure (Reynolds et al., 2000; Hong et al., 2001). The variant club cell is closely located to neuroendocrine cell clusters, and expresses besides Sgb1a1, also Uropakin3a (UPK3a) (Figure 2) (Guha et al., 2017). A similar localization of UPK3a+ secretory cells near neuroendocrine cells was observed in human lung sections, suggesting a similar progenitor cell population might be present (Guha et al., 2017).
**Neuroendocrine cells**

Neuroendocrine (NE) cells are a rare population of cells in the airway epithelium and act as chemosensory cells, communicating with the nervous system and influencing smooth muscle tone as well as regulating immune response (Branchfield et al., 2016; Sui et al., 2018; Garg et al., 2019; Noguchi et al., 2020). NE cells also have the ability to contribute to airway epithelial repair after naphthalene induced injury (Song et al., 2012; Ouadah et al., 2019). As mentioned, hyperplasia of NE cells has been implicated in a number of lung diseases, which some of them are pediatric lung diseases, like BPD and CDH (Ijsselstijn et al., 1997; Cutz et al., 2007). Furthermore, NE cell markers are found in small cell lung cancer (SCLC) (van Meerbeeck et al., 2011), and in vivo studies in mouse showed the NE cells are the origin for SCLC development (Song et al., 2012; Ouadah et al., 2019). How and why NE cells associate with such a wide range of lung diseases is unknown and therefore an interesting airway population to study.

**Bronchioalveolar stem cells**

In the zone where bronchiole transition to the alveoli, epithelial cells reside carrying both the secretory cell marker SCGB1A1 and ATII marker SFTPC (Kim et al., 2005) (Figure 2). These, so called Broncho-Alveolar Stem Cells (BASCs), showed self-renewal potential and were able to differentiate to bronchiolar and alveolar cell types in vitro (Kim et al., 2005; Lee et al., 2014; Lee et al., 2017). A recent dual-lineage tracing approach, showed that SFTPC+SCGB1A1+ cells contribute to bronchiolar and alveolar epithelium after naphthalene-induced airway injury or bleomycin-induced alveolar injury, respectively (Liu et al., 2019; Salwig et al., 2019). However, BASCs are relatively stable in normal lung homeostasis, showing that BASCs are only activated upon injury (Liu et al., 2019; Salwig et al., 2019). In addition, lineage tracing studies using Scgb1a1-Cre showed that; SCGB1A1+ cells did not contribute to alveolar repair after hyperoxic aveolar injury (Rawlins et al., 2009b), suggesting that contribution of SCGB1A1+ cells to alveolar repair depends on the type and possibly severity of injury. Interestingly, recently a progenitor cell was described residing in the human terminal and respiratory bronchioles that shared an expression profile with SCGB1A1+ secretory cells and AT2 cells (Basil et al., 2022; Kadur Lakshminarasimha Murthy et al., 2022).

In conclusion, airway epithelial cells have a great ability to regenerate the airway epithelium and the contribution of different cell types can be assessed by the use of lineage tracing tools, and different injury models. However, the identification of progenitor lineages is much faster than the understanding of underlying mechanisms in the contribution of each cell type to regeneration. Increasingly sensitive methods, such as single cell RNA sequencing, spatial transcriptomics, ATAC-sequencing, and other multi-omics approaches, are being employed to analyze improved and newer models to study the role of the different cell types in development and regeneration (Krassowski et al., 2020; Subramanian et al., 2020). Furthermore, most of airway epithelial cell plasticity is observed in mouse models, translating these findings to either; the quiescent human airway epithelium; or the mis-regulation of cellular plasticity upon disease will be a great challenge. Importantly, the fast growth in the development of in vitro lung models, such as lung organoids, air-liquid interphase cultures and lung-on-a-chip model, may contribute to increase our understanding of human airway plasticity in development, homeostasis and disease (Schilders et al., 2016; McQualter, 2019).

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**Conflict of interest**

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References

Alanis, D. M., Chang, D. R., Akiyama, H., Krasnow, M. A., and Chen, J. (2014). Two nested developmental waves demarcate a compartment boundary in the mouse lung. Nat. Commun. 5, 3923. doi:10.1038/ncomms4923

Arora, R., Mettger, R. J., and Papasavvou, V. E. (2012). Multiple roles and interactions of Tbx4 and Tbx5 in development of the respiratory system. PLoS Genet. 8, e1002866. doi:10.1371/journal.pgen.1002866

Basil, M. C., Cardenas-Diaz, F. L., Kathiriya, J. J., Morley, M. P., Carl, J., Brummell, A. N., et al. (2022). Human distal airways contain a multipotent secretory cell that can regenerate alveoli. Nature 604, 120–126. doi:10.1038/s41586-022-04552-0

Bass, A. J., Watanabe, H., Mermel, C. H., Yu, S., Perner, S., Verhaak, R. G. et al. (2009). SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. Nat. Genet. 41, 1238–1242. doi:10.1038/ng.465

Belluscio, S., Grindley, J., Emoto, H., Itoh, N., and Hogan, B. L. (1997). Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung. Development 124, 4867–4878. doi:10.1242/dev.124.23.4867

Bilmiry, K. K., Hutsom, J., and Klingensmith, J. (2015). One shall become two: Separation of the esophagus and trachea from the common foregut tube. Science 351, 707–710. doi:10.1126/science.aad9796

Brosen, E., Brouwer, R. W. W., Douben, H., van Bever, Y., Brooks, A. S., Wijnen, R. M. H., et al. (2021). Heritability and de novo mutations in oesophageal atresia and tracheoesophageal stenosis. Am. J. Hum. Genet. 1016/j.ajhg.2020.06.020

Danopoulos, S., Shiosaki, J., and Al Alam, D. (2019). FGF signaling in lung development and disease. Human versus mouse. Front. Genet. 10, 170. doi:10.3389/fgene.2019.00170

Desai, T. J., Brownfield, D. G., and Krasnow, M. A. (2014). Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature 507, 190–194. doi:10.1038/nature12980

Desai, T. J., Malpel, S., Flentzke, G. R., Smith, S. M., and Cardoso, W. V. (2004). Retinoic acid selectively regulates Fltg10 expression and maintains cell identity in the prospective lung field of the developing foregut. Dev. Biol. 273, 402–415. doi:10.1016/j.ydbio.2004.03.039

Ding, B.-S., Nolan, D., Gao, P., Babazadeh, Alexander O., Cao, Z., Rosenwaks, Z., et al. (2011). Endothelial-derived angiogenic signals induce and sustain regenerative lung alveolarization. Cell 147, 539–555. doi:10.1016/j.cell.2011.10.003

Domyan, E. T., Ferretti, E., Throckmorton, K., Mishina, Y., Nicolas, S. K., and Sun, X. (2011). Signaling through BMP receptors promotes respiratory identity in the foregut via repression of Sox2. Development 138, 971–981. doi:10.1242/dev.053694

Eblaghie, M. C., Reedy, M., Oliver, T., Mishina, Y., and Hogan, B. L. (2006). Evidence that autocrine signaling through Bmp1a regulates the proliferation, survival and morphogenetic behavior of distal lung epithelial cells. Dev. Biol. 291, 67–82. doi:10.1016/j.ydbio.2005.12.006

Eenjes, E., Buscop-van Kempen, M., Boereema-de Munck, A., Eddel, G. G., Benthem, F., de Kreij-de Brun, L., et al. (2021). SOX21 modulates SOX2-initiated differentiation of epithelial cells in the extrapulmonary airways. Elife 10, e57325. doi:10.7554/eLife.57325

Eenjes, E., Mertens, T. C. J., Buscop-van Kempen, M. J., van Wijck, Y., Taube, C., Rottier, R. J. F., et al. (2018). A novel method for expansion and differentiation of mouse tracheal epithelial cells in culture. Sci. Rep. 8, 7349. doi:10.1038/s41598-018-25799-6

Ferone, G., Song, I. Y., Sutherland, K. D., Bhaskaran, R., Monkhorst, K., Lambooij, E. J. P., et al. (2016). SOX2 regulates epithelial progenitor function and self-renewal in mouse squamous cell carcinoma from different cells of origin. Cancer Cell 30, 519–532. doi:10.1016/j.ccell.2016.09.001

Frank, D. B., Penkala, I. J., Zepp, J. A., Sivakumar, A., Linares-Saldana, R., Zacharias, W. J., et al. (2019). Early lineage specification defines alveolar epithelial ontogeny in the murine lung. Proc. Natl. Acad. Sci. U. S. A. 116, 4362–4371. doi:10.1073/pnas.1813952116

Garg, A., Sai, P., Verheyden, J. M., Young, L. R., and Sun, X. (2019). Consider the lung as a sensory organ: A tip from pulmonary neuroendocrine cells. Curr. Top. Dev. Biol. 132, 67–80. doi:10.1016/bs.ctdb.2018.12.002

Guha, A., Deshpande, A., Jain, A., Sebastiani, P., and Cardoso, W. V. (2017). Uropylaksin (34-) cells are a distinctive population of epithelial progenitors that contribute to airway maintenance and post-injury repair. Cell Rep. 19, 245–254. doi:10.1016/j.celrep.2017.03.051

Hegab, A. E., Ha, V. L., Gilbert, J. L., Zhang, K. X., Malkoski, S. P., Chon, A. T., et al. (2011). Novel stem/progenitor cell population from murine tracheal submucosal gland ducts with multipotent regenerative potential. Stem Cells 29, 1283–1293. doi:10.1002/stem.680

Hodgkinson, K. S., Domany, E. T., Verina, C. M., and Sun, X. (2009). Beta-Catenin promotes respiratory progenitor identity in mouse foregut. Proc. Natl. Acad. Sci. U. S. A. 106, 16287–16292. doi:10.1073/pnas.0902274106

Horn, K. U., Reynolds, S. D., Giangreco, A., Hurley, C. M., and Stripp, B. R. (2001). Clara cell secretory protein-expressing cells of the airway neuroepithelial body microenvironment include a label-retaining subset and are critical for epithelial renewal after progenitor cell depletion. Am. J. Respir. Cell Mol. Biol. 24, 671–681. doi:10.1165/rcmb.24.4.4498

Horn, K. U., Reynolds, S. D., Watkins, S., Fuchs, E., and Stripp, B. R. (2004). Basal cells are a multipotent progenitor capable of renewing the bronchial epithelium. Am. J. Pathol. 164, 577–588. doi:10.1016/S0002-8402(10)63147-1

Horn, K. U., Reynolds, S. D., Watkins, S., Fuchs, E., and Stripp, B. R. (2004). In vivo differentiation potential of tracheal basal cells: Evidence for multipotent and unipotent subpopulations. Am. J. Physiol. Lung Cell. Mol. Physiol. 286, L643–L649. doi:10.1152/ajlpc.00155.2003

Hrycza, S. M., Dye, B. R., Baker, N. C., Larsem, B. M., Burke, A. C., Spence, J. R., et al. (2015). Hoxg5 genes regulate the Wnt7b/2b-hnmp4-signaling Axis during lung development. Cell Rep. 12, 903–912. doi:10.1016/j.celrep.2015.07.020

Inman, E. T., Ferranti, E., Throckmorton, K., Mishina, Y., Nicolas, S. K., and Sun, X. (2011). Signaling through BMP receptors promotes respiratory identity in the foregut via repression of Sox2. Development 138, 971–981. doi:10.1242/dev.053694
Lung stem cell differentiation in mice directed by endothelial cells via a_shh and fgf10 signaling during lung development. 2017.07.028

Lesage, F., et al. (2021). Single cell transcriptomic analysis of murine lung development on hyperoxia-induced damage. Nat. Commun. 12, 11565. doi:10.1038/s41467-020-21865-2

Li, J., Luo, Z., Jiang, L., Li, Y., and Zhang, Y. (2020). Persistence of a regeneration-associated, transiton alveolar epithelial differentiation of fetal mouse pulmonary epithelium. Dev. Cell 22, 779. doi:10.1016/j.devcel.2018.04.007

Lynch, T. J. and Engelhardt, F. J. (2014). Progenitor cells in proximal airway epithelial development and regeneration. J. Cell Biol. 115, 1637–1645. doi:10.1002/jcb.24834

Maclellan, A. A., Tefti, D., Ndaye, D., Itoh, N., Thiey, J. P., Warburton, D., et al. (2001). Evidence that SPROUTY2 functions as an inhibitor of mouse embryonic lung growth and morphogenesis. Mech. Dev. 102, 81–94. doi:10.1016/s0925-4773(01)00286-6

Malped, S., Mendelsohn, C., and Cardoso, W. V. (2000). Regulation of retinoic acid signaling during lung morphogenesis. Development 127, 3057–3067. doi:10.1242/dev.127.18.3057

Mammoto, A., and Mammoto, T. (2019). Vascular niche in lung alveolar development, homeostasis, and regeneration. Front. Biosci. 7, 318. doi:10.3389/fbioe.2019.00318

Matsafumi, H., Naoya, M., Yu, M., Satoshi, N., Yasuhiro, Y., Maho, S., et al. (2018). TBX4 is involved in the super-enhancer-driven transcriptional programs underlying features specific to lung fibroblasts. Am. J. Physiol. Lung Cell. Mol. Physiol. 314, L177–L191. doi:10.1152/ajplung.00193.2017

McQualter, J. J. (2019). Endogenous lung stem cells for lung regeneration. Expert. Opin. Biol. Ther. 19, 539–546. doi:10.1080/17470383.2019.1652636

Metzger, R. J., Klein, O. D., Martin, G. R., and Krasnow, M. A. (2008). The branching programme of mouse lung development. Nature 453, 745–750. doi:10.1038/nature07005

Mills, A. A., Zheng, B., Wang, X. J., Vogel, H., Roop, D. R., and Bradley, A. (1999). p63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 398, 708–713. doi:10.1038/19531

Minino, P., Su, G., Drum, H., Bringas, P., and Kimura, S. (1999). Defects in tracheoepithelial and lung morphogenesis in Nkx2.1−/− mouse embryos. Dev. Biol. 209, 60–71. doi:10.1006/dbio.1999.9234

Montoro, D. T., Haber, A. L., Biton, M., Vinarsky, V., Lin, B., Birckett, S. E., et al. (2018). A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. Nature 560, 319–324. doi:10.1038/s41586-018-0339-3

Mormito, M., Nishikamuraka, R., Saga, Y., and Koyan, R. (2012). Different assemblies of Notch receptors coordinate the distribution of the major bronchial Clara, climated and neuroendocrine cells. Development 139, 4365–4373. doi:10.1242/dev.089340

Morrissey, E. E., and Hogan, B. L. (2010). Preparing for the first breath: Genetic and cellular mechanisms in lung development. Dev. Cell 18, 8–23. doi:10.1016/j.devcel.2009.12.010

Morrissey, E. E., and Rustgi, A. K. (2018). The lung and esophagus: Developmental and regenerative overlap. Trends Cell Biol. 28, 738–748. doi:10.1016/j.tcb.2018.04.007

Motomaya, Y., Liu, J., Mo, R., Ding, Q., Post, M., and Hui, C. C. (1999). Essential function of Gli2 and Gli3 in the formation of lung, trachea and oesophagus. Nat. Genet. 20, 54–57. doi:10.1038/1711

Murphy, A. J., Li, Y., Pietsch, J. B., Chiang, C., and Lovvorn, H. N., 3rd (2012). Mutational analysis of NOG in esophageal atresia and tracheoepithelial fistula patients. Pediatr. Surg. Int. 28, 335–340. doi:10.1007/s00383-011-3222-1

Nasr, T., Mancini, P., Rankin, S. A., Edwards, N. A., Agricola, Z. N., Kenny, A. P., et al. (2019). Endosome-Mediated epithelial remodeling downstream of hedgehog signaling. Dev. Cell 51, 665–674. doi:10.1016/j.devcel.2019.11.003

Nakae, M. Z., Carig, O., Jeng, Q., Johnson, J. A., Sun, D., Howell, K. J., et al. (2017). Human embryonic lung epithelial tips are multipotent progenitors that can be expanded in vitro as long-term self-renewing organoids. Elife 6, e26575. doi:10.7554/eLife.26575

Noguchi, M., Furukawa, K. T., and Morimoto, M. (2020). Pulmonary neuroendocrine cells: Physiology, tissue homeostasis and disease. Dis. Model. Mech. 13, dmm046920. doi:10.1242/dmm.046920

Noguchi, M., Suniyama, K., and Morimoto, M. (2015). Directed migration of pulmonary neuroendocrine cells toward airway branches organizes the stereotopic location of neuroendophilic bodies. Cell Rep. 13, 2679–2686. doi:10.1016/j.celrep.2015.11.058

Noguchi, M., Sumiyama, K., and Morimoto, M. (2015). Directed migration of pulmonary neuroendocrine cells toward airway branches organizes the stereotopic location of neuroendophilic bodies. Cell Rep. 13, 2679–2686. doi:10.1016/j.celrep.2015.11.058
noggin and bmps.

Morphogenesis of the trachea and esophagus: Current players and new roles for differentiation of anterior foregut endoderm.

et al. (2007). Multiple dose-dependent roles for Sox2 in the patterning and the developing and adult mouse trachea.

Development 136, 1899–1907. doi:10.1242/dev.034829

Peeper, C. V., Lewis, P. M., and McMahon, A. P. (1998). Sonic hedgegog regulates branching morphogenesis in the mammalian lung. Curr. Biol. 8, 1083–1086. doi:10.1016/S0960-9822(98)70446-4

Plasschaert, L. W., Zálois, R., Choo-Wong, R., Savova, V., Knehr, J., Roma, G., et al. (2018). A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. Nature 560, 377–381. doi:10.1038/s41586-018-0394-6

Plopper, C. G., Suverkropp, C., Morin, D., Nishiou, S., and Buckpitt, A. (1992). Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. I. Histopathology 136, 3741–3745. doi:10.1242/dev.037317

Steimle, J. D., Rankin, S. A., Slagle, C. E., Beken, J., Rydeen, A. B., Chan, S. S., et al. (2018). Evolutionarily conserved Tbx5-Wnt2b pathway orchestrates cardiopulmonary development. Proc. Natl. Acad. Sci. U. S. A. 115, E10615–E10624. doi:10.1073/pnas.1801124115

Subramanian, I., Verma, S., Kumar, S., Jere, A., and Anamika, K. (2020). Multi-omics data integration, interpretation, and its application. Bioinform. Biol. Insights 14, 1797322819999501. doi:10.1080/17557990.2020.1772254

Sui, P., Wiesner, D. L., Xu, J., Zhang, Y., Lee, J., Van Dyken, S., et al. (2018). Pulmonary neuroendocrine cells amplify allergic asthma responses. Science 360, eaan8546. doi:10.1126/science.aan8546

Swarr, D. T., and Moriarty, E. E. (2015). Lung endoderm morphogenesis: Gasping for form and function. Annu. Rev. Cell Dev. Biol. 31, 553–573. doi:10.1146/annurev-cellbio-100814-125249

Tata, A., Kobayashi, Y., Chow, D. R., Tran, J., Desai, A., Masri, A. J., et al. (2018). Mysophosphatidic cells of submucosal glands can function as reserve stem cells to regenerate airways after injury. Cell Stem Cell 22, 668–683. doi:10.1016/j.stem.2018.03.018

Tata, P. R., Mou, H., Fardo-Sagatia, A., Zhao, R., Prabhhu, M., Law, B. M., et al. (2013). Dedifferentiation of committed epithelial cells into stem cells in vivo. Nature 503, 218–223. doi:10.1038/nature12777

Tata, P. R., and Rajagopal, J. (2017). Plasticity in the lung. Making and breaking cell identity. Development 144, 755–766. doi:10.1242/dev.143784

Travaglini, K. J., Nahban, A. N., Penland, L., Sinha, R., Gilchrist, A., Sit, R. V., et al. (2020). A molecular cell atlas of the human lung from single-cell RNA sequencing. Nature 587, 639–652. doi:10.1038/s41586-020-2922-4

Treutlein, B., Brownfield, D. G., Wu, A. R., Neff, N. F., Mantalas, G. L., Espinosa, F. H., et al. (2014). Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-sequencing. Nature 509, 371–375. doi:10.1038/ nature13173

Tao, T. P., Van盛世通, M., Levkovsky, K. L., Quan, J., Li, J., and Cardoso, W. V. (2009). Notch signaling controls the balance of ciliated and secretory cell fates in developing airways. Development 136, 2297–2307. doi:10.1242/dev.034884

Tsuchiya, T., Todi, R., Obata, T., Hatachi, G., and Nagayasu, T. (2020). Lung microvascular niche, repair, and engineering. Front. Bioeng. Biotechnol. 8, 105. doi:10.3389/fbioe.2020.00105

van Meerbeeck, J. P., Fennell, D. A., and De Ruyscher, D. K. (2011). Small-cell lung cancer. Lancet 378, 1741–1755. doi:10.1016/S0140-6736(11)60165-7

Van Winkle, L. S., Buckpitt, A. R., Nishio, S. J., Isaac, J. M., and Plopper, C. G. (1995). Cellular response in naphthalene-induced Clara cell injury and bronchiolar epithelial repair in mice. Am. J. Physiol. Lung Cell. Mol. Physiol. 278: L1256. doi:10.1152/ajplung.1995.278.6.L1256

Volckaert, T., Campbell, A., Dill, E. L., Li, C., Mimoos, P., and De Lange, S. (2013). Localized Fgfl0 expression is not required for lung branching morphogenesis but prevents differentiation of epithelial progenitors. Development 140, 3731–3742. doi:10.1242/dev.096560
Volckaert, T., and De Langhe, S. P. (2015). Wnt and FGF mediated epithelial-mesenchymal crosstalk during lung development. *Dev. Dyn.* 244, 342–366. doi:10.1002/dvdy.24234

Volckaert, T., Yuan, T., Chao, C. M., Bell, H., Sitaula, A., Srimmtenings, L., et al. (2017). Fgf10-Hippo epithelial-mesenchymal crosstalk maintains and recruits lung basal stem cells. *Dev. Cell* 43, 48–59. doi:10.1016/j.devcel.2017.09.003

Wang, Y., Tian, Y., Morley, M. P., Lu, M. M., Demayo, F. J., Olson, E. N., et al. (2013). Development and regeneration of Sox2+ endoderm progenitors are regulated by a Hdac1/2-Bmp4/Rb1 regulatory pathway. *Dev. Cell* 24, 345–358. doi:10.1016/j.devcel.2013.01.012

Weaver, M., Dunn, N. R., and Hogan, B. L. (2000). Bmp4 and Fgf10 play opposing roles during lung bud morphogenesis. *Development* 127, 2695–2704. doi:10.1242/dev.127.12.2695

Whitsett, J. A., Kalin, T. V., Xu, Y., and Kalinichenko, V. V. (2019). Building and regenerating the lung by cell. *Physiol. Rev.* 99, 513–554. doi:10.1152/physrev.00001.2018

Williamson, K. A., Hever, A. M., Rainger, J., Rogers, R. C., Magee, A., Fiedler, Z., et al. (2006). Mutations in SOX2 cause anophthalmia-esophageal-genital (AEG) syndrome. *Hum. Mol. Genet.* 15, 1413–1422. doi:10.1093/hmg/ddl064

Woo, J., Miletich, I., Kim, B. M., Sharpe, P. T., and Shvidanasri, R. A. (2011). Barx1-mediated inhibition of Wnt signaling in the mouse thoracic foregut controls tracheo-esophageal septation and epithelial differentiation. *PLoS One* 6, e22493. doi:10.1371/journal.pone.0022493

Xia, S., Menden, H. L., Townley, N., Mahry, S. M., Johnston, J., Nyp, M. F., et al. (2021). Delta-like 4 is required for pulmonary vascular arborization and alveolarization in the developing lung. *JCI Insight* 6, 134170. doi:10.1172/jci.insight.134170

Yang, A., Schweitzer, R., Sun, D., Kaghad, M., Walker, N., Bronson, R. T., et al. (1999). p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. *Nature* 398, 714–718. doi:10.1038/19539

Yang, Y., Riccio, P., Schotsaert, M., Mori, M., Lu, J., Lee, D. K., et al. (2018). Spatial-temporal lineage restrictions of embryonic p63(+) progenitors establish distinct stem cell pools in adult airways. *Dev. Cell* 44, 752–761. doi:10.1016/j.devcel.2018.03.001

Yuan, T., Volckaert, T., Chanda, D., Thannickal, V. J., and De Langhe, S. P. (2018). Fgf10 signaling in lung development, homeostasis, disease, and repair after injury. *Front. Genet.* 9, 418. doi:10.3389/fgene.2018.00418

Zepp, J. A., Morley, M. P., Loebel, K., Kremp, M. M., Chaudhry, F. N., Basil, M. C., et al. (2021). Genomic, epigenomic, and biophysical cues controlling the emergence of the lung alveolus. *Science* 371, eabc3172. doi:10.1126/science.abc3172

Zepp, J. A., and Morrisey, E. E. (2019). Cellular crosstalk in the development and regeneration of the respiratory system. *Nat. Rev. Mol. Cell Biol.* 20, 551–566. doi:10.1038/s41580-019-0141-3

Zuo, W., Zhang, T., Wu, D. Z., Guan, S. P., Liew, A. A., Yamamoto, Y., et al. (2015). p63(+)Krt5(+) distal airway stem cells are essential for lung regeneration. *Nature* 517, 616–620. doi:10.1038/nature13903