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Paradigms that define lung epithelial progenitor cell fate in development and regeneration

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Abstract

Purpose of Review: Throughout the lifespan, lung injury impedes the primary critical function essential for life-respiration. To repair quickly and efficiently is critical and is orchestrated by a diverse repertoire of progenitor cells and their niche. This review incorporates knowledge gained from early studies in lung epithelial morphogenesis and cell fate and explores its relevance to more recent findings of lung progenitor and stem cells in development and regeneration.

Recent Findings: Cell fate in the lung is organized into an early specification phase and progressive differentiation phase in lung development. The advent of single cell analysis combined with lineage analysis and projections is uncovering new functional cell types in the lung providing a topographical atlas for progenitor cell lineage commitment during development, homeostasis, and regeneration.

Summary: Lineage commitment of lung progenitor cells is spatiotemporally regulated during development. Single cell sequencing technologies have significantly advanced our understanding of the similarities and differences between developmental and regenerative cell fate trajectories. Subsequent unraveling of the molecular mechanisms underlying these cell fate decisions will be essential to manipulating progenitor cells for regeneration.

Keywords

Lung development; progenitor cells; cell fate; lung regeneration; differentiation

Introduction

The mammalian lung is an origami of epithelial and vascular tubes folded into a matrix of mesenchyme. This structure allows an organization of over 40 diverse cell types to

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Conflict of Interest

Aravind Sivakumar and David B. Frank declare that they have no conflict of interest

Human and Animal Rights and Informed Consent

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orchestrate the fundamental functions of the lung including respiration, inflammation sensing and barrier protection, and metabolism and homeostasis (Table 1) [1]. Building and rebuilding a complex structure requires precision. A critical challenge for this is the coordination of cellular differentiation and maintenance with the patterning/morphogenesis programs that are specific and unique to lung development and regeneration.

Evolution has shaped development in multiple organ systems including the lung. The lung employs a spatiotemporal system of morphogens to not only pattern the lung but also simultaneously specify and lineage restrict progenitor cells. By deploying expression gradients, morphogens can multitask via inductive, autocrine, short-range and long-range signaling to instruct cell identity and structural morphogenesis in a coordinated fashion. This paradigm ensures a stereotypical roadmap for lung development and a guide for regeneration after lung injury.

In this review, we will summarize the essential elements of lung development. We will focus on some of the molecular mechanisms governing tissue interactions and epithelial progenitor cell fate projection during development. Finally, we will highlight how similar fetal programs may underlie the innate plasticity observed in progenitor cells in the adult lung that differ from progenitors present during development and carry out normal lung homeostatic functions.

Specification of multipotent epithelial progenitor cells during lung development

The lung undergoes a series of developmental phases that include the embryonic, pseudoglandular, canalicular, saccular, and alveologenesis stages. In the mouse, lung identity is first established during the embryonic phase of lung development when morphogens in the surrounding mesoderm induce expression of the transcription factor, NKX2-1, in the ventral anterior foregut endoderm around embryonic day 9.0 (E9.0) in the mouse. NKX2-1 expression not only assigns lung identity to the endoderm, but it is also a pioneering transcription factor required for lung development [2]. Soon after lung identity is established, outpouching of the endoderm into the surrounding mesoderm to form the primitive trachea and subsequent growth of paired lung buds occurs (Figure 1A, B).

Commencing around E12.5 during the pseudoglandular stage of development, reciprocal mesodermal-endodermal interactions govern branching morphogenesis and specification of multipotent progenitor cells into a proximal or distal fate. This sets in motion the establishment of proximal or conducting airway lineages marked by expression of SOX2 and those of the distal alveolar epithelial region expressing the transcription factors ID2 or SOX9 (Figure 1C) [3, 4].

As branching morphogenesis progresses into the canalicular stage around E15.5 of lung development, the proximal SOX2+ progenitor cells are specified and presumably differentiate into many of the epithelial cells of the conducting airway and trachea including the pulmonary neuroendocrine cells, secretory or club cells, multiciliated cells, basal cells, goblet cells, tuft cells, and newly described ionocyte cells (Table 1) [5–12]. Whether all

SOX2+ progenitor cells at this stage are multipotent or exist as multiple subsets of oligopotent or even unipotent progenitor cells is unknown (Figure 1D).

Several earlier studies indicate that distal lung specification of the SOX9/ID2+ progenitor cells is a progressive process that eventually leads to differentiation into the type 1 (AT1) and type 2 (AT2) alveolar epithelial cells (Table 1) [13, 14]. In addition, recent evidence suggests that specific alveolar cell fate specification programs are initiated concurrent with branching morphogenesis in the pseudoglandular and canalicular stages [15]. Alternatively, it has been proposed that the distal tip endodermal progenitor cells evolve into a bipotent progenitor cell capable of becoming AT1s or AT2s during the saccular stage (Figure 1E) [16, 17]. However, the relative contribution of bipotent cells to mature alveolar lineages appears to be minimal, and it may represent residual, progressively differentiating cells [15]. Nevertheless, alveolar epithelial cell fate is likely represented by early specification and acquisition of cell identity via predominantly unilineage progenitors with additional contribution through bilineage progenitor cells.

As our understanding grows of how progenitor cell fate identity is temporally and spatially established, a recurring theme arises in that a common set of molecular mechanisms and morphogens regulate both morphogenesis and cell specification. Evolution has equipped the lung with efficiency. While there is contribution from an abundant number of morphogens and their downstream signaling pathways, we outline below a few major players.

Role of WNT signaling pathway in coordinating epithelial differentiation

The signaling network comprising WNT family of ligands and receptors and their downstream effectors is one of the fundamental pathways that directs cell proliferation, cell polarity, and determination of cell fate throughout embryonic development and postnatal homeostasis and regeneration [18]. WNT proteins function as morphogens that are capable of both short- and long-range signaling. They are regulated by lipidation, membrane microdomain proteins, and distribution by heparan sulfate proteoglycans to regulate the ligand concentration, diffusion, and distribution through the aqueous extracellular space.

While both canonical and non-canonical pathways of WNT signaling regulate lung morphogenesis [19], canonical WNT/ β -catenin-dependent signaling plays a dominant role in airway specification during lung morphogenesis. The role of WNT in progenitor specification during the fetal and postnatal stages can be understood in the context of two temporally segregated waves of WNT signaling. The first wave of WNT signaling is concomitant with the specification of the foregut into the respiratory lineage and lasts up till mid pseudoglandular phase of lung development. WNT ligands emanating from the surrounding mesoderm signal to the anterior foregut endoderm to activate WNT signaling and specify initial lung identity [20–22]. Intriguingly, while β -catenin maintains NKX2–1 expression to repress SOX2 and gastrointestinal (GI) identity genes early after specification, its function is restricted to promotion of SOX9 expression and repression of GI genes only later in early development [21]. Later in lung development, loss of β -catenin or ectopic expression of the WNT inhibitor, DKK1, in the lung epithelium also affects airway endoderm identity with loss of distal epithelial lineages and proximalization of airways, a

process acting upstream of BMP4, FGF signaling and N MYC [23, 24]. Interestingly, ectopic activation of WNT signaling in a more distal and later context is dependent on the presence or absence of endogenous TCF-LEF factors [25, 26]. Towards the end of branching morphogenesis, WNT-responsiveness in the lung diminishes with a few responsive cells confined to the SOX9+/SOX2- distal lung progenitors [27]. However, following late sacculation occurring in the early postnatal period in mice, there is a re-emergence of WNT signaling and WNT-responsive AT2 cells that promotes postnatal alveolar progenitor expansion and balances the epithelial composition of the alveolus through control of AT2-AT1 progenitor differentiation [27].

FGF signaling in the mesenchyme coordinates epithelial progenitor specification and differentiation

Fibroblast growth factor (FGF) signaling components include eighteen ligands and four receptor tyrosine kinases that propagate signaling via a vast number of downstream effectors [28]. FGF signaling is critical through all stages of life from coordinating organogenesis to tissue regeneration by regulating proliferation, specification, survival and metabolism.

Initial specification of lung endoderm relies on signals from the splanchnic mesoderm that includes the secondary heart field. Included in this group is FGF2 that is secreted from the cardiac mesoderm and has the potential to induce lung identity from foregut endoderm [29]. FGF10 is a morphogen secreted by the lung mesenchyme that plays a paramount role in the regulation of epithelial proliferation and lineage commitment during embryonic and postnatal development and regeneration post-injury [30, 31]. Lineage tracing FGF10+ cells in mice has revealed temporally segregated waves of FGF expression in the lung primordium [32]. The first wave initiates post lung specification during the early pseudoglandular stage with the FGF10+ cells localizing to distal (sub-mesothelial) mesenchyme surrounding the airways. While FGF10 plays an essential role in directing the epithelial branching, its role is more permissive than instructive [33]. Instead, localized FGF10/FGFR2 signaling in the distal lung is required to regulate the proximal-distal progenitor specification of the developing airway epithelia [34, 33]. The FGF10/FGFR2 signaling activates β -catenin signaling in the proliferating distal tip cells to suppress the expression of SOX2 and induce the expression of SOX9 and ID2. As this SOX9/ID2+ progenitor population expands, their daughter cells get displaced proximally and further away from the FGF10 signaling center concomitant with derepression of SOX2 and subsequent differentiation into bronchial epithelium. Towards the end of the pseudoglandular stage (E15.5), the second wave of FGF10+ cells is dispersed throughout the mesenchyme rather than localized to the distal tip [32]. This dispersion coincides with distal tip progenitors downregulating SOX9 and restricting to the alveolar fate [35]. Overexpression of FGF10, a hyperactive KRAS allele, or SOX9 overexpression from E15.5 blocks the alveolar epithelial differentiation program, maintains the branching program, and induces ectopic expression of proximal lineages [3, 35, 33]. Fascinating recent work has revealed a role for FGF signaling in alveolar epithelial differentiation regulated by fetal breathing movements. Distal progenitors are programmed by the fetal breathing movements to differentiate into the AT1 lineage, but many tip cells

have sustained FGF signaling to prevent flattening and retain an AT2-like rather than AT1 cell fate [36].

HIPPO-YAP regulates epithelial progenitor specification and epithelial morphogenesis

The core HIPPO pathway is an evolutionarily conserved serine/threonine kinase signaling cascade composed of MST1/2 kinases, which together with SAV1 and MOB1A/B activate the LATS1/2 kinases to promote phosphorylation of transcriptional coactivators known as YAP and TAZ. In the absence of phosphorylation, YAP and TAZ translocate into the nucleus and regulate expression of genes involved in mechanotransduction, cell proliferation, cell fate specification, and cell death [37]. Deletion of YAP using a variety of pan- and distal epithelial Cre drivers results in a variety of branching morphogenesis and structural defects [38, 39]. Deficiency of TAZ, on the other hand, produces a milder phenotype of abnormal alveolarization mimicking emphysema, suggesting partial overlapping and independent roles of YAP and TAZ [40].

HIPPO pathway's role in specification of progenitors during lung development came to light when epithelial specific conditional deletion of MST1 (STK4) and MST2 (STK3) resulted in permanent nuclear retention of YAP and TAZ and impaired alveolar epithelial differentiation and proximal multiciliated and secretory cells, while the branching morphogenesis was largely unaffected [41, 42]. Intriguingly, conditional deletion of LATS1/2 kinases, previously thought to be downstream of the MST1/2 pathway, resulted in a different phenotype, with precocious ectopic induction of AT1 cells accompanied by defective branching morphogenesis [43]. Similarly, deletion of YAP and TAZ in the lung epithelium led to a decrease in AT1s, indicating HIPPO signaling as an essential component for AT1 cell fate. Further evidence showing a lack of correlation between the phosphorylation states of LATS kinases and loss of MST1/2 in the lung suggests that MST1/2 might be exploiting a kinase other than LATS1/2 to modify YAP activity during lung development [43]. CLDN18 has also been shown to be an unexpected regulator of nuclear YAP activity that regulates epithelial progenitor proliferation and progenitor capacity in the lung [44]. The compartment-specific roles of the nuclear localization of YAP in later cytodifferentiation of the airways was identified when nucleocytoplasmic shuttling of YAP was shown to be dispensable mainly for both distal branching morphogenesis and distal differentiation, but crucial for proximal airway differentiation [45]. Importantly, loss of YAP in the proximal airways resulted in the increase of multiciliated cells and a decrease of secretory club cells, while constitutive activation of YAP abrogated airway differentiation [41, 45].

TGF- β superfamily member, BMP, patterns the developing airway

The TGF- β superfamily is composed of 33 polypeptides including multiple TGF- β isoforms, activins, nodal, bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs). These ligands form homo- and heterodimers and signal through a variety of type II and type I receptor complexes to generate diverse cellular effects via SMAD effector proteins [46]. Appropriate TGF- β signaling is essential for normal lung development with distinct roles in epithelial and mesenchymal compartments. Deletion or overexpression of

ligands, ligand-binding partners, receptors and downstream signaling SMADs result in a diverse array of lung structural phenotypes [47].

BMP signaling's role has been more refined. It is one of the earliest pathways discovered to regulate dorsal-ventral patterning for esophageal and tracheal identity and respiratory specification and morphogenesis [48–50]. Subsequently, BMP was also found to regulate proximal-distal differentiation of epithelial progenitors within the lung. Temporal-dependent inhibition of BMP-BMPR-SMAD signaling in lung development results in various phenotypes including abrupted airway branching morphogenesis and severe reduction in distal epithelial cell types concurrent with an increase in proximal cell types [51–53].

NOTCH signaling is predominantly active in proximal airway progenitor specification

NOTCH signaling is a tightly controlled pathway that regulates precise cell-cell communication during development to regulate survival, proliferation, differentiation and patterning of multiple cell types via cell autonomous as well as cell non-autonomous mechanisms. NOTCH signaling pathway genes are expressed in the developing lung as early as bud formation. Expression of NOTCH1, JAG1 AND JAG2 are restricted to the distal end of the growing bud, while DLL1 expression is found in the proximal region [54].

NOTCH signaling is a critical regulator of progenitor cell specification in early lung development, working predominantly to establish proximal cell lineages. Early explant experiments using NOTCH receptor inhibitors resulted in expansion of distal progenitors, suggesting an importance in proximal-distal patterning [55]. However, subsequent experiments with genetic pan-epithelial deletion of NOTCH components such as POFUT, RBPJK, and JAG1 illustrated a preponderance in directing proximal cell fate [56, 7, 57]. Particularly, there was a drastic loss of secretory cells and a significant increase in multiciliated and neuroendocrine cells, establishing an important role in proximal cell fate. In addition, the NOTCH target gene, *Hes1*, was downregulated, corroborating other genetic studies detailing a HES1-ASCL1-IMSL1 transcriptional regulatory network regulating early non-neuroendocrine versus neuroendocrine fate [58, 12, 59]. NOTCH signaling also demonstrates a ligand-dependent role in progenitor cell fate. While NOTCH2 predominantly drives secretory versus multiciliated cell fate, NOTCH1/2/3 play a combinatorial role in specification of neuroendocrine cells [60]. Conversely, misexpression of the NOTCH1 intracellular domain in early lung epithelium expanded secretory cells distally in the lung and decreased multiciliated and increased goblet cells in the trachea [61]. NOTCH3 has also been implicated the balance between TRP63+ basal progenitor and luminal progenitors in a JAG1/JAG2-dependent manner, and it also potentially serves to activate NOTCH1 and NOTCH2 later for secretory and multiciliated cell fate selection in pseudostratified epithelium [62].

Resident epithelial progenitors in the adult lung

The homeostasis and regeneration of the adult lung epithelium is maintained by a variety of cells (Table 2). Homeostasis is an active process with cells required to carry out basic

functions of respiration, barrier function, metabolism, and immunity. The cells of the lung have evolved to not only carry out these daily cellular tasks but also simultaneously possess the potential to regenerate tissue following severe injury. Frequently fetal programs are rederived to allow a resident cell to proliferate and differentiate to replace cells at homeostasis and after injury. The terms facultative progenitor, progenitor, and stem cell have all been used to describe resident cells of the adult lung that maintain the normal cellular turnover and regenerate the lung following injury. There is ongoing debate on how to accurately classify these cells, and we refer the reader to a recent review on their description in the lung [63]. For simplicity, we will use the term progenitor cell to describe this group of cells.

Proximal airway progenitor cells

Submucosal glands—Recent evidence has confirmed the existence of progenitor cells within the submucosal glands of the trachea and lung. The submucosal glands, consisting of ducts and acini, lie within the stromal tissue of the cartilaginous airways throughout the conducting airway in humans, but only in the very proximal portion of the trachea in mice [64]. The glands are lined with epithelial cells including basal, secretory, and multiciliated cells, in addition to a unique myoepithelial cell. Recent studies have implicated this novel myoepithelial progenitor cell in tissue regeneration. Single cell RNA sequencing and cell lineage analysis of ACTA2+ myoepithelial cells within the submucosal glands revealed that these cells obtain a basal cell-like transition with the ability to differentiate into secretory and multiciliated cells [65, 66]. However, this state was only seen in the presence of severe injury, with little to no role in normal homeostasis or mild injury.

Basal cells—BCs are marked by expression of TRP63 and KRT5 and are juxtaposed near the basement membrane of the epithelial layer overlying the cartilaginous airways of the trachea and mainstem bronchi during homeostasis in mice but can extend to the smaller bronchioles in humans [67, 68]. Developmentally, the cells are lineage restricted to their native adult progenitor state and location during branching morphogenesis and can give rise to both secretory and multiciliated cells [69]. At homeostasis *in vivo*, BCs contribute to the normal cellular turnover of the basal, secretory, and multiciliated cell lineages [68, 70]. One mechanism of maintenance is dependent on mesenchymal-epithelial cross-talk mediated by mesenchymally-derived FGF10 and WNT7B and YAP in the epithelium [71, 33, 72, 73].

Recent studies have expanded BC contribution to additional and novel cell lineages. Single cell RNA sequencing on airway epithelium identified a rare cell type called an ionocyte that is marked by high expression of cystic fibrosis transmembrane conductance regulator (*Cftr*) gene, indicating a novel cell type for regulation of fluid and ions within the airways [6, 5]. Lineage tracing KRT5+ cells confirmed its presence as a descendent of the basal cell lineage. Similarly, they identified tuft cells and solitary neuroendocrine cells as progeny of basal cells.

Our understanding of basal cell progenitor cell specification derives largely in part from studies of lung regeneration where they aid in re-establishing the airway architecture. In acute injury, BCs proliferate and self renew in addition to differentiating into secretory and

repertoire of both AT2s and secretory cells. Interestingly, SCA1, a putative marker for BASCs in the lung, was expressed rarely on BASCs by flow cytometry, indicating that it may not be a sufficient marker for all BASCs. Interestingly, there was low expression of the naphthalene metabolizing enzyme, *Cyp2f2*, which may explain their resistance to naphthalene injury.

Distal airway progenitor cells

Alveolar epithelial cells—The adult alveolus is comprised of two types of epithelium. AT1s are elongated, flat cells that cover the alveolar surface and exchange oxygen with the capillary network. The AT2s are a cuboidal, highly metabolic cell with multiple functions including the production of surfactant. Historically, descriptive studies suggested that AT2s could differentiate into AT1s in the presence of injury [91]. More recently, confirmatory experiments using fate mapping of AT2 revealed that AT2s underwent self-renewal with rare AT1 differentiation during homeostasis [92]. AT1 lineage commitment from AT2s was significantly increased following bleomycin-induced alveolar injury or AT2 depletion [92, 93]. Conversely, the AT1 plasticity has also been illustrated in a pneumonectomy model of alveolar growth in that lineage labeled AT1s exhibit the ability to differentiate into AT2s [94]. While mechanisms of plasticity in AT1s is largely unclear, AT2 progenitor cell proliferation and differentiation following injury is in part regulated by a number of signaling pathways including the BMP, NOTCH, and HIPPO signaling [95–97].

Data also indicate the presence of several novel alveolar epithelial lineages. A population of SFTPC-, $\alpha6\beta4+$ epithelial cells reside in the alveolus and bronchoalveolar ductal junction [98]. Following injury or in organoid modeling systems, the cells appear to proliferate and differentiate into AT2s. However, these data were generated using novel *ex vivo* systems rather than classical fate mapping of this specific lineage. As such, confirmatory studies using recent dual lineage labeling strategies would make it possible to track this potential progenitor cell *in vivo*.

More recently, subsets of alveolar epithelial progenitor cells were identified based on single cell and population-based RNA sequencing and their ability to respond to a WNT signal [99, 100]. This WNT-responsive alveolar epithelial lineage arises as a subset of AT2s (AT2^{Axin2}) during alveologenesis and orchestrates the AT2 pool through enhanced proliferation and inhibition of AT1 differentiation [27]. During alveologenesis, it is a dynamic population with some AT2s gaining or losing WNT-responsiveness. However, in the adult, it becomes a small, stable alveolar epithelial progenitor (AEP) population that is poised for regeneration based on transcriptome enrichment and chromatin architecture. After influenza injury, AEPs preferentially proliferate to replace AT2s and later differentiate to contribute to some AT1 regeneration [99, 100]. While AEPs appear to contribute significantly to AT2 regeneration surrounding areas of moderate injury following influenza infection, their contribution in other injury models is unclear.

Conclusion

The lung is not a quiescent organ and requires an orchestra of cellular components to interact and carry out the basic functions of respiration. To achieve this complexity,

progenitor cells must receive and integrate signals from their respective niches. We have outlined some of the major fundamental morphogen signaling systems involved in lung development to provide a foundation for understanding progenitor cell specification and maintenance. These pathways provide a roadmap for progenitor cell specification in lung regeneration. Technology continues to advance discoveries in biology and uncover novel signaling pathways conducting progenitor cell fate. Recent insights in the lung biology field have taken advantage of these tools to identify new lineages. Lineages that now will entice investigations to understand their ontogeny, morphogenesis, and contribution to lung homeostasis and regeneration. While most lung disease today has no cure, model organisms provide a blueprint for therapy. Whether we can genuinely replicate human disease is not known. However, our advances in imaging, single cell analysis, and computational trajectory mapping of lineages will help alleviate the constraints in human disease samples. At the same time, we must bridge knowledge unraveling regulatory mechanisms in fate decisions of other organ systems with prospective discovery in lung biology. It is only with integration of knowledge and novel tools we will be able to direct therapy in lung regeneration.

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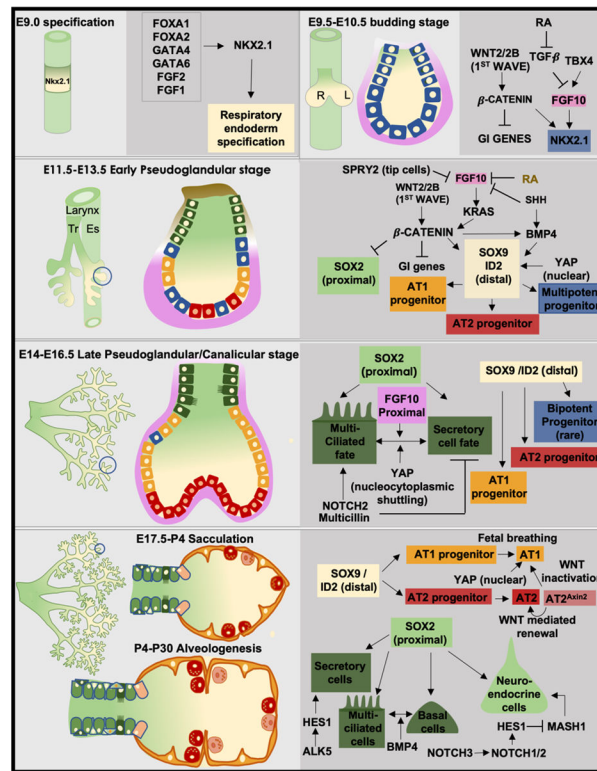


Figure 1: Epithelial progenitor specification and differentiation during lung development.

(A) Lung identity is established at E9.0 with the induction of the transcription factor Nkx2.1 expression (yellow) by a combination of morphogens and transcription factors shown in the dark grey box (B) This is subsequently followed by the outpouching of the respiratory endoderm to form trachea and primary lung buds. The whole mount view of the lung is shown in the left while the morphology of a single branch is shown in the right side. The network of genes involved in maintaining Nkx2.1 expression (blue cells) is shown to the right. (C) By E12.5, the pseudoglandular stage involves the separation of the trachea (Tr) from the esophagus (Es). Whole mount view of the lung is on the left and the morphology of a single branch is on the right. During this stage, reciprocal mesodermal–endodermal interactions that govern the specification of Nkx2.1+ multipotent progenitor cells into a Sox2+ proximal (green cells) and Sox9+/Id2+ distal fate with concurrent alveolar cell fate specification programs (blue, red, orange cells) are shown in dark grey. (D) By the end of the pseudoglandular and into the canalicular stage, the majority of branching is complete. Whole mount view of the lung is on the left and the morphology of a single branch is on the right. The proximal Sox2+ progenitors begin to give rise differentiated cell lineages such as multiciliated and secretory cells (dark green cells). At the distal end, the specification of the Sox9+ cells to the alveolar fate is complete. The corresponding gene network involved in the specification is shown. (E) The proximal Sox2+ progenitors give rise to all proximal cell types: club cells, multi-ciliated cells, basal cells and neuroendocrine cells (green cells). The distal alveolar progenitors give rise to AT1s and AT2s (orange and red cells). The signaling networks that contribute to the differentiation of the progenitors are shown in the side.

Table 1.

Functional epithelial cells of the lung

Location	Cell Type	Cell Markers	Function
Conducting Airway	Secretory (Club) Cells	SCGB3A1, SCGB1A1	<ul style="list-style-type: none"> • Secrete glycoproteins, uteroglobin, lysozymes, lipids • Detoxification
	Multiciliated Cells	FOXJ1, TUBB4	<ul style="list-style-type: none"> • Mucociliary clearance
	Basal Cells	KRT5, TRP63	<ul style="list-style-type: none"> • Anchor/insulate epithelium-stroma • Progenitor cell
	Neuroendocrine Cells	UCLH1, CALCA, ASCL1	<ul style="list-style-type: none"> • Innervated/stimulated to produce CGRP, GRP, 5-HT, somatostatin, substance P, etc. • Oxygen sensing • Airway sensor for immune function • Bronchial tone
	Tuft (Brush) Cells	IL25, TSLP, DCLK1	<ul style="list-style-type: none"> • Chemosensory cells • Innate immunity
	Ionocytes	FOXI1, ATP6V1C2	<ul style="list-style-type: none"> • Possible fluid regulation to maintain airway surface physiology
	Goblet Cells	MUC5AC, TFF2, SPDEF	<ul style="list-style-type: none"> • Mucin production • Innate immune defense
Alveolar	Type 1 Cells	HOPX, AQP5, PDPN, AGER	<ul style="list-style-type: none"> • Gas exchange surface • Barrier function
	Type 2 Cells	SFTPC, SFTPB, SFTPA, SFTPD, ABCA3	<ul style="list-style-type: none"> • Surfactant production & secretion • Ion transport • Immune function

Table 2.

Adult progenitor cells and progeny at homeostasis and after injury.

Location	Progenitor cell type	Context	Progeny cell type	
Submucosal glands of airway	Myoepithelial cell	Naphthalene, sulfur dioxide, influenza injury	Myoepithelial, transitional basal cell to secretory and multiciliated cells	
		Homeostasis	Basal, secretory, tuft, neuroendocrine, and ionocytes	
		Sulfur dioxide, naphthalene	Basal, secretory, multiciliated cells	
	Basal Cells	Influenza	SOX2+/KRT5+/TRP63+ basal transition cell into AT2s upon inhibition of NOTCH, prevention of hypoxia-induced events, and WNT activation	
		Homeostasis	Secretory, multiciliated cells	
		Basal cell depletion, sulfur dioxide, influenza	Dedifferentiated basal, secretory, multiciliated cells	
	Conducting airway	Secretory Cells	Naphthalene, sulfur dioxide	Basal, secretory, tuft, neuroendocrine, and ionocytes
			Homeostasis or naphthalene injury	Secretory, multiciliated cells
			Bleomycin	AT2s, AT1s
		Variant Secretory (NEB niche)	Homeostasis	Secretory and AT2, (rare AT1)
Naphthalene			Secretory, multiciliated cells	
Bleomycin			AT2, AT1	
Influenza			Secretory, AT2	
Alveolar		Type 1	Homeostasis,	AT1
			Pneumonectomy	AT1, AT2
		Type 2	Homeostasis, bleomycin, AT2 depletion model, pneumonectomy	AT2, AT1
	Homeostasis		AT2 (rare AT1)	
	AEP	Influenza, lung epithelial deletion model	AT2, AT1	
		a4b6/SFTPC-	Bleomycin	AT2s