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Lung regeneration: mechanisms, applications and emerging stem cell populations

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Abstract

Recent studies have shown that the respiratory system has an extensive ability to respond to injury and regenerate lost or damaged cells. The unperturbed adult lung is remarkably quiescent, but after insult or injury progenitor populations can be activated or remaining cells can re-enter the cell cycle. Techniques including cell-lineage tracing and transcriptome analysis have provided novel and exciting insights into how the lungs and trachea regenerate in response to injury and have allowed the identification of pathways important in lung development and regeneration. These studies are now informing approaches for modulating the pathways that may promote endogenous regeneration as well as the generation of exogenous lung cell lineages from pluripotent stem cells. The emerging advances, highlighted in this Review, are providing new techniques and assays for basic mechanistic studies as well as generating new model systems for human disease and strategies for cell replacement.

The lung is a highly quiescent tissue, previously thought to have limited reparative capacity and a susceptibility to scarring[1]. It is now known that the lung has a remarkable reparative capacity, when needed, and scarring or fibrosis after lung injury may occur infrequently in scenarios where this regenerative potential is disrupted or limited[1,2]. Thus, the tissues of the lung may be categorized as having facultative progenitor cell populations that can be

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induced to proliferate in response to injury as well as differentiate into one or more cell types. This response is different from those of organs that show either high levels of cellular turnover and require a dedicated and well-defined undifferentiated stem cell population, such as the intestine and hematopoietic system, or organs where there is little capacity for regeneration even after injury, such as the heart and brain (Fig. 1). Classic stem cells, functionally defined as cells showing indefinite self-renewal as well as a clonal, multipotent differentiation repertoire within a cellular hierarchy, may not be necessary for either homeostasis or repair of the normally quiescent lung. In this way, the biology of lung maintenance may be more akin to that of other endodermally derived epithelia, such as the liver and pancreas, where mature, differentiated cells or facultative progenitor cells are the predominant regenerative cells in many *in vivo* growth or injury models[3].

The search for reparative cells that can contribute to the process of lung regeneration, whether called progenitors or stem cells, has been fueled by the need for improved clinical therapies to treat patients suffering from the burden of diseases that arise from injury or degeneration of lung tissue. Beyond supportive care or, in extreme cases, allogeneic lung transplantation, there are no effective treatments for acute damage to lung epithelia, as in acute respiratory distress syndrome, or chronic degeneration of airway and alveolar tissues, as in chronic obstructive pulmonary disease (COPD) or idiopathic pulmonary fibrosis (IPF). Therefore, a better understanding of the underlying mechanisms that promote self-renewal and differentiation of lung cells will be crucial in identifying new therapeutic approaches for lung disease.

Given the complexity of the respiratory system, a single lung stem cell capable of generating all of the various lineages within the lung is difficult to conceive. It is more likely that there are multiple spatially and temporally restricted stem or progenitor cell lineages that have varying abilities to respond to injury and disease. An alternative hypothesis is that many, if not most, lung epithelial cell lineages have the capacity to re-enter the cell cycle and replace lost cells through their ability to proliferate. Thus, the lung could respond to injury and stress by activating stem cell populations and/or by re-entering the cell cycle to repopulate lost cells.

Currently, little is understood about the cellular complexities involved in the process of human lung regeneration. In contrast, the use of lineage-tracing techniques for inducible markers and clonal cells (Table 1), high-density transcriptome analysis and advanced imaging techniques has provided an exquisite developmental map for the mouse lung in the past decade. In contrast to the quiescent adult lung, the developing lung features rapidly proliferating cells with broad multipotency that gradually becomes restricted as the organ develops. Because it is unclear whether any lung cells of comparably expansive proliferative potential or differentiation repertoire remain in postnatal life, we refer to these developing cells as progenitors rather than stem cells, as their self-renewal capacity may be transient. Regardless, the pathways identified that regulate these progenitors during lung development may be activated during adult lung repair. Importantly, such pathways have been used to generate lung epithelial lineages *in vitro* from pluripotent stem cells, providing a new source of material for research and for clinical studies.

Here we focus on the following concepts important in the field of lung regeneration. First, we discuss what is known about lung progenitor populations in the developing embryo. Second, we discuss the emerging understanding of adult lung cell lineages that can respond robustly to injury by re-entering the cell cycle and differentiating. Third, we focus on the *de novo* derivation of lung lineages from pluripotent stem cells *in vitro*. Finally, we discuss the increasing interest and controversies in the clinical application of 'lung stem cell research' for the future treatment of patients with lung disease.

Progenitor populations in lung development

The increasing understanding of the molecular pathways important for lung development provides a framework for exploring postnatal lung regeneration, as many of these pathways and processes are recapitulated during injury and regeneration. Thus, in any discussion of adult lung regeneration, it is important to understand the basics of how the lung is constructed during embryonic development, when rapid proliferation and differentiation are the rule rather than the exception.

The respiratory system comprises two branched structures: the respiratory tree and the adjacent cardiopulmonary vasculature. Basic developmental studies have shown that the epithelium of the trachea and lungs derives from the anterior foregut endoderm, which generates multiple other tissues, including the liver, esophagus and thyroid. The laryngotracheal groove is formed in this anterior foregut region, with the ventral portion of the gut tube generating the trachea and the dorsal portion generating the esophagus. The mesenchymal components of the lung, such as the vasculature, arise predominantly from a specialized region of lateral mesoderm contiguous with the developing cardiac mesoderm[4]. The interconnection and juxtaposition between these two components of the respiratory system provides the essential function of gas exchange between the external environment and circulating blood.

Endoderm-derived precursors

The endoderm of the respiratory system is specified on the ventral side of the anterior foregut endoderm at approximately embryonic day 9 (E9.0) in mice and at an unknown time in humans. This specification event is detected by expression of the earliest known marker of the lung epithelial lineage, the transcription factor Nkx2.1 (ref. 5), which is expressed in all epithelial lineages of the respiratory system during development. Genetic mouse models, canonical Wnt signaling, fibroblast growth factor (FGF) signaling and bone morphogenetic protein (BMP) signaling have all been demonstrated to mechanistically contribute to the lineage specification of lung from endodermal foregut precursor cells[6–10]. Following lineage specification, Nkx2.1-expressing (Nkx2.1$^+$) cells of the endodermal lung primordium evaginate (at E9.5 in mouse) from the ventral side of the anterior foregut to form the trachea and two primary lung buds, which subsequently branch into lobes, found in various numbers in the lungs of mammalian species[5] (Fig. 2). Following branching, the distal regions of the branch tips generate air sacs called alveoli, where gas exchange with the vasculature occurs. The more proximal regions of the airway tree comprise different epithelial lineages important for mucous secretion and particle clearance.

The proximal conducting airways and distal gas-exchanging alveoli have overlapping embryonic origins but diverge early during fetal lung development[8,11]. The transcription factors Sox2 and Sox9 mark the proximal and distal epithelial progenitors, respectively. Sox2 is required for differentiation of the proximal progenitors into secretory and ciliated epithelial lineages, and the correct concentration of Sox9 is important for terminal differentiation of alveolar lineages[12–17]. Additional markers of the distal progenitor $Sox9^+$ cells include surfactant proteins such as surfactant protein C (Sftpc), the secreted signaling factor Bmp4 and the transcription factor Id2. Lineage tracing studies have suggested that $Id2^+$ distal cells can generate both distal and proximal cell lineages[18]. This capacity for multipotent differentiation is lost by E16.5, after which $Id2^+$ progenitor cells can form only distal alveolar epithelia[18]. Thus, in early development, lung endoderm–derived progenitors have a broad multipotent capacity that is curtailed as the organ matures in preparation for birth.

Alveolar progenitors

Toward late gestation, the cells at the distal branch tips begin to differentiate into alveolar epithelial type 1 cells (AEC1s) and AEC2s (Fig. 2). AEC1s provide the thin interface overlying the vascular endothelium essential for gas exchange, and AEC2s secrete pulmonary surfactant to reduce surface tension so the alveoli do not collapse upon expiration. This differentiation process is poorly understood, but its interruption is a leading cause of respiratory deficiency in neonates born prematurely. AEC2s have been proposed to be the precursors of AEC1s on the basis of *in vitro* observations that most AEC2s in culture transdifferentiate into AEC1s[19–21] and *in vivo* findings from cytodynamic, alveolar epithelial thymidine-labeling studies in developing rodents[22] and injured adults[23,24]. Importantly, this AEC2-AEC1 relationship has been demonstrated using genetic lineage–tracing studies in adult mice[25]. A more recent report has suggested that, in developing mice, both AEC2s and AEC1s derive directly from a bipotent alveolar progenitor late in gestation, whereas in postnatal life AEC1s derive from self-renewing AEC2 precursors[26]. Additional studies, however, including the identification of new marker genes and additional cell lineage–tracing experiments, are required to further refine our understanding of the development and maintenance of the alveolar niche. Moreover, it remains unclear whether all AEC2s or only a rare subset are capable of functioning as alveolar progenitors. A recent study using single-cell RNA sequencing may provide additional clues on lung alveolar epithelial heterogeneity[27].

Mesoderm-derived progenitors

Little is understood about the mesoderm-derived progenitors in the developing lung. The lung mesoderm is a source of essential paracrine instructive signals that regulate endoderm progenitor proliferation and differentiation and also contributes to the various structures in the lung, including airway smooth muscle, vascular smooth muscle, endothelial cells, mesothelial cells and many less-understood mesodermal lineages, such as pericytes, alveolar fibroblasts and lipofibroblasts. The lung mesoderm is thought to arise from the early mesoderm that surrounds the ventral anterior foregut. Cell lineage–tracing experiments have demonstrated that this mesoderm contains a cardiopulmonary mesoderm progenitor (CPP) that can generate both cardiac and lung mesodermal derivatives, including cardiomyocytes,

endocardium, pulmonary vascular and airway smooth muscle and pulmonary pericyte-like cells expressing platelet-derived growth factor receptor-β (PDGFR-β)[4]. Single-cell clonal analysis shows that CPPs can clonally generate airway and vascular smooth muscle as well as proximal endothelium expressing the glycoprotein von Willebrand factor (vWF), demonstrating that these lineages are inter-related and that CPPs are multipotent in early lung development. CPPs are also able to coordinate the structural connection between the developing lung and heart through hedgehog signaling[4].

Vascular progenitors

Data have supported both angiogenic (growth from pre-existing vessels) and vasculogenic (*de novo* formation of new vessels) processes in generating the pulmonary vascular tree[28–31]. Lineage tracing techniques have now shown that CPPs in mice generate most of the lineages that comprise the pulmonary vasculature including smooth muscle and proximal vWF^+ endothelium[4]. However, CPPs lack the ability to generate the bulk of the distal capillary endothelium in the lung. These distal endothelial cells are generated from pre-existing embryonic vasculature and can be labeled using an inducible Cre expressed from the vascular endothelial cadherin locus[4]. Thus, the combined action of CPPs and early endothelium is required to form the cardiopulmonary vasculature.

Candidate stem cells of the respiratory system

Because the unperturbed adult lung is a remarkably quiescent organ, the detection of proliferative postnatal cells that might serve as candidate stem or progenitor cells has been challenging. Hence, many studies attempting to identify adult stem or progenitor candidates in the lung have used injury and disease models primarily in rodents. Early studies of human and animal lung morphology defined subsets of lung epithelia with proliferative capacity, including basal, secretory or club cells (formerly known as Clara cells) of the proximal airway, and AEC2s of the alveolus. All of these lineages have the capacity to enter the cell cycle in response to lung injury[23,32–39]. More recent techniques, including cell-lineage tracing, have suggested that most types of lung epithelial cells, except for airway ciliated cells, can proliferate and expand after injury to promote repair[40–44]. Importantly, identifying these cell populations provides key information about how to harness the endogenous abilities of the lung to regenerate.

Tracheal and proximal bronchial stem cell candidates

The trachea and main stem bronchi (upper airways) of both humans and mice are lined with a pseudostratified epithelium composed of basal and luminal cells (Fig. 3). Luminal airway cells include secretory (club), ciliated and neuroendocrine cells. In the lungs of humans, as well as other mammals, this region of the airway also contains goblet cells, which are marked by expression of the transcription factor SPDEF and mucin-5ac (Muc5ac). Cell lineage–tracing studies in mice have shown that goblet cells, which arise in mice only after injury or in disease states, are derived from secretoglobin family 1a member 1–expressing ($Scgb1a1^+$) secretory cells and not ciliated epithelial cells in the postnatal lung after injury[45]. Studies grafting basal and luminal cell populations into tracheas denuded of epithelium in mice followed by implantation of the reseeded tracheal xenografts into immunodeficient

mice suggest that either basal or luminal cells can regenerate the tracheal epithe-lium[40,46,47]. However, other studies suggest that only luminal[48] or basal cells[49] can restore all the tracheal epithelial subtypes.

Investigators have found subsets of basal cells in mice and humans that have extensive proliferative potential, self-renewal capacity and the ability to differentiate into basal, secretory and ciliated lung epithelial cells *in vivo*[50,51]. Modern flow cytometry allows the cells to be sorted to purity and has enabled the cataloging of multiple gene and protein markers selectively expressed in these basal cells, including the basal cell–restricted transcription factor Trp63, the keratins Krt5 and Krt14 and the cell-surface markers Pdpn, NGFR and GS1 lectin B4. The global transcriptome of these cells has also been examined by microarray analysis[50,52], and these studies have also highlighted the heterogeneity and diversity within the basal cell lineage[51–55]. Importantly, mouse basal cells can expand almost indefinitely in culture while retaining multipotent differentiation capacity, similar to classically studied stem cells of the skin[50,55]. This body of work, along with the prevailing view that basal cells have no other known function in the lung, supports the concept that basal cells can function as tissue-specific stem cells of the conducting airway epithelium. However, there is still much that is not known about basal cell self-renewal and differentiation and whether it involves asymmetric cell division as do other stem cells. The use of clonal multicolor cell lineage–tracing experiments should help to better define basal cell heterogeneity. The molecular mechanisms for basal cell self-renewal and differentiation are poorly understood, but recent evidence points to an important role for Notch signaling in promoting secretory cell fate over ciliated epithelial cell fate[50,56] (Fig. 3). Importantly, determining whether the differentiation repertoire of basal cells of the airways includes the potential to reconstitute injured alveolar epithelial cells, as has been suggested[55], will require confirmatory lineage-tracing studies.

Distal airway stem cell candidates

The more distal bronchiolar region of mouse lungs are lined with a monolayered epithelium consisting of secretory, ciliated and neuroendocrine epithelial lineages (Fig. 4). $Krt5^{+}Trp63^{+}$ basal cells also line this region of the airway tract in humans, but their presence has not been well documented in the rodent lung. Ciliated epithelium is marked by expression of the transcription factor Foxj1 and the cytoskeletal protein Tubb4, and the secretory epithelium in this region is marked by expression of the secretoglobins Scgb1a1 and Scgb3a2 (ref. 57). Neuroendocrine cells are often clustered in neuroendocrine bodies (NEBs) and are marked by expression of ubiquitin carboxyl terminal esterase-L1 (UCHL1; also known as Pgp9.5), calcitonin gene–related peptide (CGRP) and the transcription factor Ascl1.

The bronchiolar epithelium is quiescent until injured. Using naphthalene-induced secretory epithelial cell depletion, several reports have shown that a subset of secretory cells expressing Scgb1a1 but not cytochrome p450 (Cyp2f2) is spared from naphthalene toxicity and functions as a facultative progenitor, expanding rapidly to regenerate the damaged airways by forming both secretory and ciliated cell progeny[58–60]. Cell lineage–tracing experiments have shown that $Scgb1a1^{+}Cyp2f2^{-}$ cells can self-renew and differentiate into $Tubb4^{+}Foxj1^{+}$ ciliated epithelium during normal homeostatic turnover as well as after

naphthalene-induced injury[61]. This has prompted some to name them variant club cells. Cell proliferation has an important role in this injury response, but it remains unclear whether Scgb1a1^{+}Cyp2f2^{-} cells go through a process of dedifferentiation to re-enter the cell cycle and then differentiate again after expansion. Their region-specific location adjacent to NEBs[62] of the airways or at the bronchoalveolar duct junctions (BADJs)[63] are compelling evidence of the existence of microenvironmental progenitor cell niches in the airways[62,64–66]. In addition to lacking expression of Cyp2f2, variant club cells express uroplakin 3a (Upk3a), a marker of unknown function, and in some cases have lower Scgb1a1 and higher Scgb3a2 expression than the bulk of nonvariant club cells[67]. A role for localized Notch signaling in the NEB microenvironment was also recently demonstrated[67,68]. Parabronchial smooth muscle cells, which line most of the proximal airways, were proposed to help make up a niche capable of activating variant club cells following naphthalene exposure, as Fgf10 secreted by parabronchial smooth muscle seems to be necessary for regeneration of variant club cells[69].

An intriguing recent study showed that the modality of regeneration of the secretory epithelium after injury depends on the severity of injury[70]. Mild injury is repaired by proliferation and expansion of surviving club cells, which in turn can also differentiate into ciliated epithelium. In contrast, severe injury by naphthalene results in a more regionally restricted mode of regeneration mediated through variant club cells located around NEBs and within the BADJ. These studies suggest that the type and severity of injury is likely to contribute to the cell sources and degrees of regeneration observed. Taken together, this growing body of literature demonstrates that region-specific variant club cells of the NEB and BADJ niches preferentially contribute to bronchiolar repair and regeneration after significant depletion of secretory cells, and this provides strong support for calling these cells facultative airway progenitors of the adult lung.

Candidate stem cells for the alveolar compartment

So far, the AEC2 remains the best candidate progenitor of the adult lung alveolus[24] (Fig. 5). Thymidine labeling studies, immunostaining for proliferative markers and transgenic lineage-tagging studies have suggested that during late development, or after various postnatal alveolar injuries, some AEC2s proliferate, with coincident labeling in adjacent AEC1s[11,22,23,34,71]. Importantly, an inducible Cre recombinase targeted to the endogenous Sftpc locus has been used to detect proliferation and clonal expansion of AEC2s after adult lung injury as well as lineage-tagged AEC1s deriving from AEC2 *in vivo*[25,72].

Methods have been developed for the clonal expansion *in vitro* of AEC2s by culturing them with Pdgfrα^{+} lung mesenchymal cells that are hypothesized to serve as alveolar ‘niche cells’ juxtaposed to AEC2s (ref. 25). The ability to grow and expand alveolar epithelium *ex vivo* could provide a ready source of cells for cell-based regenerative medicine therapies in the future. Follow-up studies have also shown that AEC2s in adults can self-renew and form AEC1s[26]. A recent study has identified several AEC2 self-renewal signals, including epidermal growth factor receptor (EGFR) and the GTPase KRAS[26]. These pathways are important for increases in AEC2s and AEC2-derived AEC1s during aging or after hyperoxic injury. Both these lineage-tracing studies provide evidence interpreted by their authors as

suggesting that AEC2s are 'stem cells' of the alveolus. Alternatively, although AEC2s in these mouse studies have been convincingly shown to retain lifelong self-renewal potential as well as the capacity to differentiate into AEC1s, their well-known differentiated state and functional role in normal alveolar biology (for example, surfactant production) suggests that they may have additional attributes not shared by more traditionally defined stem cell lineages such as basal cells.

Several reports have recently suggested additional alveolar progenitor candidates beyond AEC2s. For example, $Trp63^{+}Krt14^{+}$ basal cells in mice can generate alveolar cells expressing the AEC1 marker gene, *Pdpn*, after severe influenza injury[55], a finding that prompted the authors to refer to this population as alveolar stem cells (Fig. 5). Importantly, these $Trp63^{+}Krt14^{+}$ lung cells seemed to emerge in regions of distal lung alveoli, an area of the lung that does not normally harbor cells expressing Trp63 or Krt14. However, the investigators chose to induce lineage tracing in $Krt14^{+}$ cells following the initiation of injury, thus limiting interpretation of the *in vivo* differentiation repertoire of their cells, and the tracing did not demonstrate that the $Krt14^{+}$ cells generate AEC2s, a crucial epithelial lineage in the alveolus. Other recent studies have used flow cytometry to isolate new candidate alveolar progenitors, identified by coexpression of α_6 and β_4 integrins, but lacking expression of either Scgb1a1 or Sftpc[73]. The authors of the study, seeking to study epithelial integrins that bind to the lung matrix component laminin, unexpectedly identified a novel distal lung epithelial candidate progenitor. This population seemed to proliferate in response to lung injury, and after purification from mouse lungs displayed remarkable potential for multipotent airway and alveolar differentiation.

BADJ cells

At the transition from the bronchiolar region of the lung to the alveolar region is the BADJ, a region known to harbor variant club cells possessing airway epithelial regenerative potential after naphthalene-induced lung injury. In the mouse, there are cells at this junction that coexpress markers of both the secretory epithelium (Scgb1a1) and AEC2 (Sftpc) lineages, which have been referred to as bronchioalveolar stem cells (BASCs). The original description of BASCs was founded on an observation that dual-positive ($Scgb1a1^{+}Sftpc^{+}$) cells at the BADJ could expand *in vivo* after bleomycin-induced lung injury, and cells proposed to be the same population could be purified from lung digests using a defined flow-sorting algorithm (including $CD45^{-}CD31^{-}Sca1^{+}CD34^{+}$ gating)[74]. These sorted cells could be clonally expanded *in vitro*, where they show multipotent differentiation into bronchiolar and alveolar lineages[74,75].

The existence of BASCs *in vivo* has been contested by work using Scgb1a1-restricted lineage tagging to trace the progeny of club cells expressing this secretory cell marker, which should include the BASC population. During development or after hyperoxic alveolar injury, these studies found little evidence in support of an Scgb1a1 lineage–tagged population contributing to alveolar reconstitution[61]. In contrast, after bleomycin-induced lung injury, $Scgb1a1^{+}$ lineage-tagged cells are capable of generating AEC2s, although their contribution is limited, and they do not seem to expand substantially[76,77]. Creating further uncertainty about the *in vivo* differentiation repertoire of club cells and putative BASCs is

the observation that, *in vivo*, the BASC population at the BADJ is not unique in coexpressing Scgb1a1 and Sftpc. For example, occasional AEC2s throughout mouse alveolar regions have been shown to express both Scgb1a1 and Sftpc[56]. Consequently, Scgb1a1 lineage tracing does not entirely distinguish airway secretory cells (including putative BASCs and club cells) from rare AEC2s that also lineage trace with Scgb1a1-driven Cre tagging[61,78].

Thus, although putative BASCs could represent a true stem cell lineage existing in a unique niche between the airways and alveoli and capable of *in vivo* self-renewal and differentiation into multiple lineages, dual lineage-tagging approaches unique to the proposed BASCs will need to be used to rigorously define their potential *in vivo*.

Cellular plasticity in the regenerating lung

One important concept that affects our understanding of epithelial cell–lineage fates during lung regeneration is epithelial cell plasticity. There are now several instances of apparent and dramatic epithelial cell–lineage switching upon injury in the lung. As discussed above, one example is the $Trp63^+$ or $Krt14^+$ clusters of cells that seem to migrate from large airways toward the alveolar region, where they are thought to differentiate into alveolar epithelium, although there are serious limitations in this study owing to the lack of convincing evidence that these cells generate AEC2s[55]. Additional studies, including more specific lineage tracing of $Trp63^+Krt14^+$ basal cells at different times during the regeneration process, will be required to understand the capacity of these cells to regenerate distant lineages such as alveolar epithelium.

Further emphasizing lung epithelial cell plasticity, a recent study showed that differentiated secretory epithelial cells can revert to a basal cell fate in a stable fashion *in vivo*. Building on earlier reports of secretory cells differentiating into basal cells in tracheal xenograft models[46] and after sulfur dioxide tracheal injury[61], it was recently shown that upon depletion of basal cells, $Scgb1a1^+$ secretory cells can dedifferentiate into $Trp63^+Krt5^+$ basal cells[79]. These ‘dedifferenti-ated’ cells have the full capability of other basal cells to redifferentiate into secretory as well as ciliated epithelial lineages. An additional study showed that $Scgb1a1^+$ cells can generate $Trp63^+$ cells *in vivo* after bleomycin- or influenza-based lung injury[80]. These studies raise several important questions. First, do other epithelial lineages in the lung show similar phenotypic switches? Second, how is this dedifferentiation normally restricted during lung homeostasis? Finally, what are the mechanisms that drive this process? Such a dramatic acquisition of a different cellular fate is likely to be controlled by powerful mechanisms to avoid deleterious cellular transformation that would occur during tumorigenesis. For lung regeneration, such findings suggest that cell fate is not permanently locked in adult lung epithelial cells and that manipulating such fate decisions could promote repair and regeneration.

Are most lung epithelial cells ‘stem cells’?

Because stem cells are classically defined on the basis of their potential to indefinitely self-renew and differentiate, an argument could be made in support of referring to basal cells, secretory or club cells, variant club cells, submucosal gland duct cells, AEC2s, BASCs and

Itga6$^+$Itgb4$^+$ cells as lung 'stem cells'. Indeed, all these cell types have potential to proliferate as well as differentiate into one or more distinct lineages. Mouse p63$^+$ basal cells, Scgb1a1$^+$ airway cells, Itga6$^+$Itgb4$^+$ cells, BASCs and AEC2s are also capable of growing extensively in culture while retaining the capacity to differentiate[25,50,55,73,79,81]. These cell types together represent >50% of all currently described epithelial cells in the adult lung, raising the unusual possibility of referring to the majority of cells in the adult organ as stem cells.

Rather than considering the majority of lung epithelial cells to be stem cells, an alternative concept for organs such as the lung is a model of a highly plastic and 'democratic' tissue in which a broad diversity of quiescent cell lineages can be induced to proliferate, dedifferentiate or redifferentiate, and even change phenotype (for example, secretory cells become basal) to repair an injured region. The cell lineage responsible for reconstitution of specific tissues can differ depending on the type and severity of injury, model system or *in vitro* culture assay studied. Indeed, a particularly robust *in vivo* lung regeneration stimulus that fits this model occurs when the entire organ responds during compensatory growth of the remaining lung after pneumonectomy (PNX)[82,83]. This postnatal lung-growth model has been optimized in a variety of species, including rat, mouse and dog[84–87], and was recently demonstrated to occur in a selected human case[88]. Most studies so far have suggested that lung growth after PNX involves rapid, coordinated proliferation of a broad diversity of lung lineages, including most epithelial and endothelial cells, and only a minority of reports suggest that specialized rare stem or progenitor cells, such as putative BASCs, have a specialized or initiating role in the process[85,89,90]. In marked contrast, when a highly selective injury model, such as naphthalene-induced ablation of mature club cells, is used, a rare subset of epithelial cells, variant club cells, that have survived the injury then reconstitute the airway in a process that shows features of classically defined facultative progenitors, including reactivation of proliferation. With so many cell types in the lung capable of proliferating and differentiating, it is therefore likely that the lung employs multiple mechanisms for regeneration, including activation of many facultative regenerative cell populations[40]. Development of an *in vivo* functional engraftment assay would be helpful in assessing which lineages have classic stem cell characteristics.

Mechanisms underlying lung regeneration

Previous studies have demonstrated that the increase in proliferation after PNX is regulated by signal transduction pathways and matrix composition[84–86,89]. Elastin content, for example, is known to have an important role, possibly by guiding the correct growth and structure of the alveolar compartment[86,91]; EGF, HGF, retinoic acid and FGF signaling have been demonstrated to promote post-PNX alveolar regeneration[89,92,93]. Despite these insights, little is understood about the signaling or transcriptional pathways that underlie such a substantial regeneration of lost tissue that occurs after PNX. Greater focus on the pathways that can promote endogenous lung regeneration would move the field closer to the identification of new pharmacological approaches for human lung repair and treatments for chronic diseases.

Wnt signaling

Previous reports have demonstrated activation of canonical Wnt signaling in several compartments in the lung using various regeneration models. In Wnt reporter mouse lines, increased Wnt signaling activity occurs in developing lung epithelium deficient for the crucial transcription factor Gata6 and in the postnatal airway epithelium after naphthalene-based depletion of secretory cell[94]. Interestingly, secretory cell–restricted deletion of β-catenin, an essential effector of canonical Wnt signaling, does not alter secretory cell regeneration in mice, indicating that although the pathway is activated, it is not essential for the regenerative response in the adult secretory epithelium of the lung[95]. Loss of β-catenin in postnatal AEC2s results in fibrosis after bleomycin-induced lung injury, but whether Wnt signaling has an important role in alveolar epithelial regeneration will require further study[96]. This finding correlates with previous studies showing that pharmacological blockade of Wnt signaling reduces bleomycin-induced fibrosis, although whether this is a direct effect on the epithelium or mesenchyme is unclear[97]. Furthermore, increased activation of Wnt signaling has been associated with IPF lesions, but whether hyperactivation of Wnt signaling in lung mesenchymal lineages directly promotes pulmonary fibrosis is unknown[98–100]. Such results offer a note of caution in the use of potent pathways such as Wnt signaling in promoting lung regeneration. Activation may promote a regenerative response under specific conditions, and too much Wnt activity can lead to fibrosis and other deleterious effects.

Notch signaling

Notch signaling has been shown to play an important part in airway epithelial regeneration. During lung development, Notch signaling is essential for differentiation of the secretory epithelium from proximal lung endoderm[101,102] and for induction of the Upk3a^{+}Scgb3a2^{+} secretory cell subset juxtaposed to NEBs[67]. In the adult lung, Notch signaling is essential for differentiation of basal cells into secretory cells after severe injury[56]. Further, ectopic Notch activation can promote mucous metaplasia as defined by hyperactivation of the goblet cell program in the developing and postnatal lung[56,103] and can broadly activate the Upk3a variant club cell marker in the developing airway epithelium[67]. The ability of Notch to promote a specific cell fate such as the secretory cell lineage may provide a useful approach in promoting airway epithelial regeneration after acute lung injury.

Histone deacetylases

Histone deacetylases (HDACs) are enzymes that remove acetyl groups on histones as well as other proteins. One report has shown that people who have COPD and are smokers have a substantial decrease in HDAC activity in their lungs and an almost complete loss of HDAC2 expression[104]. Moreover, deletion of HDAC1 and HDAC2 in postnatal club cells in mice inhibits their regeneration after naphthalene-based depletion[105]. This effect is due primarily to the direct regulation by HDAC1 and HDAC2 of a set of tumor suppressors including Rb1, p21 (Cdkn1a) and p16 (Ink4a), which results in reduced proliferation of club cells after naphthalene injury. Because HDAC inhibitors are approved for therapeutic use in some cancers, the use of these drugs or their counterparts, which may increase histone acetylation and gene expression, could have therapeutic value in promoting lung regeneration.

Regenerative therapies

Deriving lung stem and progenitor cells *de novo*

Whereas the studies discussed above have emphasized the search for endogenous lung stem or progenitor cells, other teams have sought to exogenously derive stem or progenitor cells that might allow the *de novo* generation *in vitro* of lung epithelial, endothelial or mesenchymal lineages for basic studies or future cell-based therapies. Early attempts to derive lung epithelial lineages from mouse or human embryonic stem cells (ESCs) were inefficient or ineffective until it was discovered that stimulation of nodal signaling using activin A could drive ESCs to recapitulate early embryonic development, yielding efficient definitive endoderm[106]. Since then, other investigators have had varying degrees of success using growth factors to direct the differentiation of ESCs and induced pluripotent stem cells (iPSCs) to lung epithelial cells[107–109]. For example, methods were initially described for patterning endoderm-derived from pluripotent stem cells (PSCs) into anterior foregut-like endoderm using activin stimulation followed by brief inhibition of transforming growth factor-β (TGF-β) and BMP signaling pathways, rendering this population competent for lung endoderm specification and differentiation[110]. Methods were then optimized for the induction of the lung cell fate decision in PSC-derived endoderm, evidenced by expression of Nkx2.1 in response to combinatorial FGF, BMP and Wnt signaling pathways. It is thus clear that known signaling pathways involved in *in vivo* lung development can be harnessed for the directed differentiation of PSCs into lung epithelium[111,112]. Most markers of differentiated lung epithelia, including Sftpc, Sftpb, Scgb1a1, Foxj1, Cftr, Trp63, mucins and T1-α (podoplanin), can now be induced in PSCs undergoing directed differentiation, including in iPSCs from people with cystic fibrosis[111–113] (Box 1).

The current challenge is achieving a maturation state of ESC- or iPSC-derived lung epithelial lineages similar to their *in vivo* counterparts. Without a reliable engraftment assay for the study of exogenous candidate lung cells, a rigorous comparison of PSC-derived lung lineages to their *in vivo* counterparts remains challenging. Investigators have used air-liquid interface models[113], three-dimensional (3D) recellularization model systems[111] and classical ectopic grafting assays (subcutaneous or kidney capsule transplantation)[110,112] to begin to assess the function of the new cell types engineered from PSCs. Additional information, such as global transcriptome profiling[111], will probably help in the full characterization of lung epithelium generated *in vitro*.

Tissue engineering

Despite the rapid advances in deriving lung epithelial lineages from ESCs and iPSCs, the generation of complex 3D tissue structures, or even functional organs, from these cells remains a high hurdle. A rapidly emerging area of research aiming to address this challenge is the use of tissue-engineering approaches to generate complex, multicellular structures that might mimic functional lung tissues. Tubes shaped like upper airways, such as trachea and bronchi, have already been engineered *in vitro*, coated with various cell preparations such as bone marrow derivatives and surgically grafted into patients who have regions of tracheal or bronchial atresia[114,115]. So far, there has been a tendency to refer to the bone marrow or

other cell preparations used to coat these airway grafts as stem cells without clear evidence that they are true stem cells.

Beyond the successful generation of functional tubes to conduct airflow, the engineering of functional alveolar tissue for use *in vivo* remains an unmet challenge. One exciting approach is the use in animal models of 'decellularization' by perfusion of detergents through excised whole lungs to prepare 3D scaffolds comprising solely lung extracellular matrix[116,117].

Recellularization of this scaffold with epithelial cell lines (A549 or C10)[116,117], endothelial cells[116,117], mesenchymal stem cells (MSCs)[118,119], whole lung cell–suspension digests[116] or differentiated ESCs or iPSCs[111,120,121] has been used in proof-of-concept studies. The resulting recellularized lungs can be ventilated and perfused with blood and their partial function, including capacity for gas exchange, has been demonstrated through a variety of physiological measurements. Some investigators have even accomplished orthotopic transplantation of these bioartificial lung grafts into pneumonectomized rodents and demonstrated partial function *in vivo* for short time periods[116,117].

Current therapuetic approaches for lung regeneration

Given that safe clinical translation of most stem cell advances remains years away, the American Thoracic Society has warned patients that many stem cell trials charging fees for claimed 'treatments' have not been substantiated or reviewed by experts (http://patients.thoracic.org/materials/stem-cells.php). Currently, a number of clinical trials are listed at ClinicalTrials.gov for evaluating various cell-based therapies, including infusions of cell types referred to as stem cells, for several lung diseases[53]. For example, infusions of stromal cells isolated from human bone marrow, adipose tissue, placental tissue or cord blood (referred to either as MSCs or as marrow stromal cells) to treat patients with COPD, bronchopulmonary dysplasia, bronchiolitis obliterans, asthma or acute lung injury are under way or being planned. Additional trials are listed to test treatments for pulmonary hypertension (using infusions of endothelial progenitor cells or monocytic bone marrow progenitors) and to assess treatment of pulmonary silicosis using intrabronchial instillations of bone marrow cells[53]. Clinical investigations in Europe have also targeted IPF with cell-based therapy using MSCs[122].

It is too early to determine whether any of these trials will prove efficacious, but accumulating data from more than 100 MSC clinical trials (mostly phase 1 or 2) registered on ClinicalTrials.gov for treating diseases affecting other organs suggests that, at least for MSCs, there seems to be little safety risk to participants. However, there is also little reason to believe that these trials will result in regeneration of lung tissue for participants, as the bulk of basic mechanistic studies suggest the infused cells, such as MSCs, work mostly through paracrine or immunomodulatory effects on recipient lung tissue[53]. Most basic biological studies so far indicate that MSCs function as bone marrow stromal cells, modulating the marrow microenvironment and serving as precursors to differentiated skeletal lineages, such as cartilage or bone, *in vivo*[123]. There is little evidence suggesting that endogenous MSCs are recruited from the bone marrow to lung tissue[124]; consequently, injection of these cells into lung tissue should be viewed as an artificial, engineered therapy

rather than an augmentation of naturally occurring mechanisms for lung repair. Thus, these trials might be viewed as evaluations of cell-based immunomodulatory drug delivery rather than as attempts to regenerate or reconstitute lung tissue[125].

Infused endothelial progenitor cells also seem to have paracrine, and perhaps angiogenic, effects on recipient tissue[53]. However, their capacity to form replacement endothelial cells directly in the lung remains unproven[126]. With these results in mind, we believe that referring to the cells being infused in trials so far as ‘stem or progenitor cells’ risks misleading clinicians and patients attracted by the promise that these terms imply.

Conclusions

Lung disease is a major cause of morbidity and mortality worldwide, surpassed only by cardiovascular disease and cancer. The lung’s ability to regenerate extensively after injury suggests that this capability could be promoted in diseases in which loss of lung tissue occurs. However, understanding of the cell types involved and the underlying mechanisms that control the proper regeneration of lung tissue is in its infancy. There are several open questions related to the basic understanding of how the lung responds to injury and regenerates lost cells. How many true stem or progenitor cell types are there in the lung? Is reactivation of cell proliferation in the normally quiescent lung a characteristic shared by many, or even most, lung cell lineages? The challenge ahead will be to rigorously define the roles of various cell types and the precise signaling pathways in regulating lung repair and regeneration *in vivo*. Drug therapies designed to modulate these pathways may thus be a more immediate, realistic goal for promoting lung repair in patients, given the very high hurdle of *in vivo* delivery of reparative or regenerative cell therapies.

The dream of delivering truly regenerative or reconstituting cells, such as endogenous lung progenitors or iPSCs, to the lung will require additional investigation in animal models before human application can be attempted. In the case of ESCs or iPSCs, their potent differentiation and proliferation potential makes them risky and potentially teratogenic in human trials, if they were to be deployed before their biology is more fully understood. Much-needed studies defining the genetic and epigenetic programs of these and other stem populations are now under way and should help to better define these cell populations and their regenerative potential.

The dramatic progress being made in the laboratory makes the idea of ‘translating’ these findings into therapies all the more tempting. The pressure to bring stem cell and regenerative therapies to clinical trials continues to build from multiple directions, including funding agencies and the public. Despite such great potential, it would be wise to remember that many, if not most, advances in clinical medicine are based on seminal insights made by researchers in the basic sciences with little or no practical clinical use in mind[127]. Loss of such insights would be disastrous over the long term and could seriously curtail the identification of new therapeutic strategies for treating lung diseases. Researchers, drug developers and clinicians should realize that this work resides on a continuum that relies on the success of each step. Only by working together, with an emphasis on rigorous scientific

and clinical approaches, can we fully realize the promise of regenerative biology in the lung in the years ahead.

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References

1. Beers MF, Morrisey EE. The three R's of lung health and disease: repair, remodeling, and regeneration. J. Clin. Invest. 2011; 121:2065–2073. [PubMed: 21633173]
2. Wansleeben C, Barkauskas CE, Rock JR, Hogan BL. Stem cells of the adult lung: their development and role in homeostasis, regeneration, and disease. Wiley Interdiscip. Rev. Dev. Biol. 2013; 2:131–148. [PubMed: 23799633]
3. Zaret KS, Grompe M. Generation and regeneration of cells of the liver and pancreas. Science. 2008; 322:1490–1494. [PubMed: 19056973]
4. Peng T, et al. Coordination of heart and lung co-development by a multipotent cardiopulmonary progenitor. Nature. 2013; 500:589–592. [PubMed: 23873040]
5. Cardoso WV, Lu J. Regulation of early lung morphogenesis: questions, facts and controversies. Development. 2006; 133:1611–1624. [PubMed: 16613830]
6. Goss AM, et al. Wnt2/2b and β-catenin signaling are necessary and sufficient to specify lung progenitors in the foregut. Dev. Cell. 2009; 17:290–298. [PubMed: 19686689]
7. Harris-Johnson KS, Domyan ET, Vezina CM, Sun X. β-catenin promotes respiratory progenitor identity in mouse foregut. Proc. Natl. Acad. Sci. USA. 2009; 106:16287–16292. [PubMed: 19805295]
8. Domyan ET, et al. Signaling through BMP receptors promotes respiratory identity in the foregut via repression of Sox2. Development. 2011; 138:971–981. [PubMed: 21303850]
9. Sekine K, et al. Fgf10 is essential for limb and lung formation. Nat. Genet. 1999; 21:138–141. [PubMed: 9916808]
10. Serls AE, Doherty S, Parvatiyar P, Wells JM, Deutsch GH. Different thresholds of fibroblast growth factors pattern the ventral foregut into liver and lung. Development. 2005; 132:35–47. [PubMed: 15576401]
11. Perl AK, Wert SE, Nagy A, Lobe CG, Whitsett JA. Early restriction of peripheral and proximal cell lineages during formation of the lung. Proc. Natl. Acad. Sci. USA. 2002; 99:10482–10487. [PubMed: 12145322]
12. Metzger RJ, Klein OD, Martin GR, Krasnow MA. The branching programme of mouse lung development. Nature. 2008; 453:745–750. [PubMed: 18463632]
13. Tompkins DH, et al. Sox2 activates cell proliferation and differentiation in the respiratory epithelium. Am. J. Respir. Cell Mol. Biol. 2011; 45:101–110. [PubMed: 20855650]
14. Tompkins DH, et al. Sox2 is required for maintenance and differentiation of bronchiolar Clara, ciliated, and goblet cells. PLoS ONE. 2009; 4:e8248. [PubMed: 20011520]
15. Que J, Luo X, Schwartz RJ, Hogan BL. Multiple roles for Sox2 in the developing and adult mouse trachea. Development. 2009; 136:1899–1907. [PubMed: 19403656]
16. Que J, et al. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development. 2007; 134:2521–2531. [PubMed: 17522155]
17. Rockich BE, et al. Sox9 plays multiple roles in the lung epithelium during branching morphogenesis. Proc. Natl. Acad. Sci. USA. 2013; 110:E4456–E4464. [PubMed: 24191021]

18. Rawlins EL, Clark CP, Xue Y, Hogan BL. The Id2^{+} distal tip lung epithelium contains individual multipotent embryonic progenitor cells. Development. 2009; 136:3741–3745. [PubMed: 19855016]
19. Mason R, Williams MC, Clements JA. Isolation and identification of type 2 alveolar epithelial cells. Chest. 1975; 67:36S–37S. [PubMed: 46191]
20. Wang J, et al. Differentiated human alveolar epithelial cells and reversibility of their phenotype *in vitro* . Am. J. Respir. Cell Mol. Biol. 2007; 36:661–668. [PubMed: 17255555]
21. Dobbs LG, Williams MC, Brandt AE. Changes in biochemical characteristics and pattern of lectin binding of alveolar type II cells with time in culture. Biochim. Biophys. Acta. 1985; 846:155–166. [PubMed: 3839418]
22. Adamson IY, Bowden DH. Derivation of type 1 epithelium from type 2 cells in the developing rat lung. Lab. Invest. 1975; 32:736–745. [PubMed: 1171339]
23. Adamson IY, Bowden DH. The type 2 cell as progenitor of alveolar epithelial regeneration. A cytodynamic study in mice after exposure to oxygen. Lab. Invest. 1974; 30:35–42. [PubMed: 4812806]
24. Mason RJ, Williams MC. Type II alveolar cell. Defender of the alveolus. Am. Rev. Respir. Dis. 1977; 115:81–91. [PubMed: 326115]
25. Barkauskas CE, et al. Type 2 alveolar cells are stem cells in adult lung. J. Clin. Invest. 2013; 123:3025–3036. [PubMed: 23921127]
26. Desai TJ, Brownfield DG, Krasnow MA. Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature. 2014; 507:190–194. [PubMed: 24499815]
27. Treutlein B, et al. Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. Nature. 2014; 509:371–375. [PubMed: 24739965]
28. Schachtner SK, Wang Y, Scott Baldwin H. Qualitative and quantitative analysis of embryonic pulmonary vessel formation. Am. J. Respir. Cell Mol. Biol. 2000; 22:157–165. [PubMed: 10657936]
29. Anderson-Berry A, et al. Vasculogenesis drives pulmonary vascular growth in the developing chick embryo. Dev. Dyn. 2005; 233:145–153. [PubMed: 15765515]
30. deMello DE, Sawyer D, Galvin N, Reid LM. Early fetal development of lung vasculature. Am. J. Respir. Cell Mol. Biol. 1997; 16:568–581. [PubMed: 9160839]
31. Gebb SA, Shannon JM. Tissue interactions mediate early events in pulmonary vasculogenesis. Dev. Dyn. 2000; 217:159–169. [PubMed: 10706140]
32. Adamson IY, Bowden DH. Origin of ciliated alveolar epithelial cells in bleomycin-induced lung injury. Am. J. Pathol. 1977; 87:569–580. [PubMed: 68683]
33. Adamson IY, Bowden DH. Bleomycin-induced injury and metaplasia of alveolar type 2 cells. Relationship of cellular responses to drug presence in the lung. Am. J. Pathol. 1979; 96:531–544. [PubMed: 89815]
34. Bowden DH, Adamson IY, Wyatt JP. Reaction of the lung cells to a high concentration of oxygen. Arch. Pathol. 1968; 86:671–675. [PubMed: 5701641]
35. Cabral-Anderson LJ, Evans MJ, Freeman G. Effects of NO_2 on the lungs of rats I. Morphology. Exp. Mol. Pathol. 1977; 27:353–365. [PubMed: 923749]
36. Evans MJ, Cabral LC, Stephens RJ, Freeman G. Acute kinetic response and renewal of the alveolar epithelium following injury by nitrogen dioxide. Chest. 1974; 65:62S–65S. [PubMed: 4819237]
37. Evans MJ, Cabral LJ, Stephens RJ, Freeman G. Renewal of alveolar epithelium in the rat following exposure to NO_2. Am. J. Pathol. 1973; 70:175–198. [PubMed: 4566990]
38. Evans MJ, Cabral LJ, Stephens RJ, Freeman G. Transformation of alveolar type 2 cells to type 1 cells following exposure to NO_2. Exp. Mol. Pathol. 1975; 22:142–150. [PubMed: 163758]
39. Evans MJ, Dekker NP, Cabral-Anderson LJ, Freeman G. Quantitation of damage to the alveolar epithelium by means of type 2 cell proliferation. Am. Rev. Respir. Dis. 1978; 118:787–790. [PubMed: 707897]
40. Rawlins EL, Hogan BL. Epithelial stem cells of the lung: privileged few or opportunities for many? Development. 2006; 133:2455–2465. [PubMed: 16735479]

41. Rawlins EL, Ostrowski LE, Randell SH, Hogan BL. Lung development and repair: contribution of the ciliated lineage. Proc. Natl. Acad. Sci. USA. 2007; 104:410–417. [PubMed: 17194755]
42. Rawlins EL, Hogan BL. Ciliated epithelial cell lifespan in the mouse trachea and lung. Am. J. Physiol. Lung Cell. Mol. Physiol. 2008; 295:L231–L234. [PubMed: 18487354]
43. Rawlins EL, et al. Epithelial stem/progenitor cells in lung postnatal growth, maintenance, and repair. Cold Spring Harb. Symp. Quant. Biol. 2008; 73:291–295. [PubMed: 19028985]
44. Borthwick DW, Shahbazian M, Krantz QT, Dorin JR, Randell SH. Evidence for stem-cell niches in the tracheal epithelium. Am. J. Respir. Cell Mol. Biol. 2001; 24:662–670. [PubMed: 11415930]
45. Pardo-Saganta A, Law BM, Gonzalez-Celeiro M, Vinarsky V, Rajagopal J. Ciliated cells of pseudostratified airway epithelium do not become mucous cells after ovalbumin challenge. Am. J. Respir. Cell Mol. Biol. 2013; 48:364–373. [PubMed: 23239495]
46. Liu JY, Nettesheim P, Randell SH. Growth and differentiation of tracheal epithelial progenitor cells. Am. J. Physiol. 1994; 266:L296–L307. [PubMed: 8166299]
47. Avril-Delplanque A, et al. Aquaporin-3 expression in human fetal airway epithelial progenitor cells. Stem Cells. 2005; 23:992–1001. [PubMed: 16043462]
48. Johnson NF, Hubbs AF. Epithelial progenitor cells in the rat trachea. Am. J. Respir. Cell Mol. Biol. 1990; 3:579–585. [PubMed: 1701305]
49. Ford JR, Terzaghi-Howe M. Basal cells are the progenitors of primary tracheal epithelial cell cultures. Exp. Cell Res. 1992; 198:69–77. [PubMed: 1727059]
50. Rock JR, et al. Basal cells as stem cells of the mouse trachea and human airway epithelium. Proc. Natl. Acad. Sci. USA. 2009; 106:12771–12775. [PubMed: 19625615]
51. Hong KU, Reynolds SD, Watkins S, Fuchs E, Stripp BR. *In vivo* differentiation potential of tracheal basal cells: evidence for multipotent and unipotent subpopulations. Am. J. Physiol. Lung Cell. Mol. Physiol. 2004; 286:L643–L649. [PubMed: 12871857]
52. Cole BB, et al. Tracheal basal cells: a facultative progenitor cell pool. Am. J. Pathol. 2010; 177:362–376. [PubMed: 20522644]
53. Weiss DJ, et al. Stem cells and cell therapies in lung biology and lung diseases. Proc. Am. Thorac. Soc. 2011; 8:223–272. [PubMed: 21653527]
54. Borok Z, et al. Cell plasticity in lung injury and repair: report from an NHLBI workshop, April 19–20, 2010. Proc. Am. Thorac. Soc. 2011; 8:215–222. [PubMed: 21653526]
55. Kumar PA, et al. Distal airway stem cells yield alveoli *in vitro* and during lung regeneration following H1N1 influenza infection. Cell. 2011; 147:525–538. [PubMed: 22036562]
56. Rock JR, et al. Notch-dependent differentiation of adult airway basal stem cells. Cell Stem Cell. 2011; 8:639–648. [PubMed: 21624809]
57. Reynolds SD, Reynolds PR, Pryhuber GS, Finder JD, Stripp BR. Secretoglobins SCGB3A1 and SCGB3A2 define secretory cell subsets in mouse and human airways. Am. J. Respir. Crit. Care Med. 2002; 166:1498–1509. [PubMed: 12406855]
58. Reynolds SD, Reynolds PR, Pryhuber GS, Finder JD, Stripp BR. Secretoglobins SCGB3A1 and SCGB3A2 define secretory cell subsets in mouse and human airways. Am. J. Respir. Crit. Care Med. 2002; 166:1498–1509. [PubMed: 12406855]
59. Giangreco A, Reynolds SD, Stripp BR. Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. Am. J. Pathol. 2002; 161:173–182. [PubMed: 12107102]
60. Reynolds SD, et al. Conditional Clara cell ablation reveals a self-renewing progenitor function of pulmonary neuroendocrine cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 2000; 278:L1256–L1263. [PubMed: 10835332]
61. Rawlins EL, et al. The role of Scgb1a1^{+}Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. Cell Stem Cell. 2009; 4:525–534. [PubMed: 19497281]
62. Hong KU, Reynolds SD, Giangreco A, Hurley CM, Stripp BR. 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. 2001; 24:671–681. [PubMed: 11415931]

63. Giangreco A, Reynolds SD, Stripp BR. Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. Am. J. Pathol. 2002; 161:173–182. [PubMed: 12107102]
64. Peake JL, Reynolds SD, Stripp BR, Stephens KE, Pinkerton KE. Alteration of pulmonary neuroendocrine cells during epithelial repair of naphthalene-induced airway injury. Am. J. Pathol. 2000; 156:279–286. [PubMed: 10623676]
65. Reynolds SD, Giangreco A, Power JH, Stripp BR. Neuroepithelial bodies of pulmonary airways serve as a reservoir of progenitor cells capable of epithelial regeneration. Am. J. Pathol. 2000; 156:269–278. [PubMed: 10623675]
66. Reynolds SD, et al. Conditional Clara cell ablation reveals a self-renewing progenitor function of pulmonary neuroendocrine cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 2000; 278:L1256–L1263. [PubMed: 10835332]
67. Guha A, et al. Neuroepithelial body microenvironment is a niche for a distinct subset of Clara-like precursors in the developing airways. Proc. Natl. Acad. Sci. USA. 2012; 109:12592–12597. [PubMed: 22797898]
68. Morimoto M, Nishinakamura R, Saga Y, Kopan R. Different assemblies of Notch receptors coordinate the distribution of the major bronchial Clara, ciliated and neuroendocrine cells. Development. 2012; 139:4365–4373. [PubMed: 23132245]
69. Volckaert T, et al. Parabronchial smooth muscle constitutes an airway epithelial stem cell niche in the mouse lung after injury. J. Clin. Invest. 2011; 121:4409–4419. [PubMed: 21985786]
70. Giangreco A, et al. Stem cells are dispensable for lung homeostasis but restore airways after injury. Proc. Natl. Acad. Sci. USA. 2009; 106:9286–9291. [PubMed: 19478060]
71. Adamson IY, Bowden DH, Wyatt JP. Oxygen poisoning in mice. Ultrastructural and surfactant studies during exposure and recovery. Arch. Pathol. 1970; 90:463–472. [PubMed: 5476243]
72. Xu X, et al. Evidence for type II cells as cells of origin of K-Ras-induced distal lung adenocarcinoma. Proc. Natl. Acad. Sci. USA. 2012; 109:4910–4915. [PubMed: 22411819]
73. Chapman HA, et al. Integrin α6β4 identifies an adult distal lung epithelial population with regenerative potential in mice. J. Clin. Invest. 2011; 121:2855–2862. [PubMed: 21701069]
74. Kim CF, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell. 2005; 121:823–835. [PubMed: 15960971]
75. Lee JH, et al. Lung stem cell differentiation in mice directed by endothelial cells via a BMP4-NFATc1-thrombospondin-1 axis. Cell. 2014; 156:440–455. [PubMed: 24485453]
76. Rock JR, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. Proc. Natl. Acad. Sci. USA. 2011; 108:E1475–E1483. [PubMed: 22123957]
77. Zheng D, et al. Regeneration of alveolar type I and II cells from Scgb1a1-expressing cells following severe pulmonary damage induced by bleomycin and influenza. PLoS ONE. 2012; 7:e48451. [PubMed: 23119022]
78. Rock JR, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. Proc. Natl. Acad. Sci. USA. 2011; 108:E1475–E1483. [PubMed: 22123957]
79. Tata PR, et al. Dedifferentiation of committed epithelial cells into stem cells *in vivo* . Nature. 2013; 503:218–223. [PubMed: 24196716]
80. Zheng D, Yin L, Chen J. Evidence for Scgb1a1^{+} cells in the generation of p63^{+} cells in the damaged lung parenchyma. Am. J. Respir. Cell Mol. Biol. 2014; 50:595–604. [PubMed: 24134540]
81. McQualter JL, Yuen K, Williams B, Bertoncello I. Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. Proc. Natl. Acad. Sci. USA. 2010; 107:1414–1419. [PubMed: 20080639]
82. Buhain WJ, Brody JS. Compensatory growth of the lung following pneumonectomy. J. Appl. Physiol. 1973; 35:898–902. [PubMed: 4765830]
83. Brody JS. Time course of and stimuli to compensatory growth of the lung after pneumonectomy. J. Clin. Invest. 1975; 56:897–904. [PubMed: 1159093]

84. Voswinckel R, et al. Characterisation of post-pneumonectomy lung growth in adult mice. Eur. Respir. J. 2004; 24:524–532. [PubMed: 15459128]

85. Eisenhauer P, et al. Endogenous distal airway progenitor cells, lung mechanics, and disproportionate lobar growth following long-term postpneumonectomy in mice. Stem Cells. 2013; 31:1330–1339. [PubMed: 23533195]

86. Hoffman AM, et al. Matrix modulation of compensatory lung regrowth and progenitor cell proliferation in mice. Am. J. Physiol. Lung Cell. Mol. Physiol. 2010; 298:L158–L168. [PubMed: 19915155]

87. Hsia CC, Herazo LF, Fryder-Doffey F, Weibel ER. Compensatory lung growth occurs in adult dogs after right pneumonectomy. J. Clin. Invest. 1994; 94:405–412. [PubMed: 8040282]

88. Butler JP, et al. Evidence for adult lung growth in humans. N. Engl. J. Med. 2012; 367:244–247. [PubMed: 22808959]

89. Ding BS, et al. Endothelial-derived angiocrine signals induce and sustain regenerative lung alveolarization. Cell. 2011; 147:539–553. [PubMed: 22036563]

90. Nolen-Walston RD, et al. Cellular kinetics and modeling of bronchioalveolar stem cell response during lung regeneration. Am. J. Physiol. Lung Cell. Mol. Physiol. 2008; 294:L1158–L1165. [PubMed: 18375744]

91. Koh DW, Roby JD, Starcher B, Senior RM, Pierce RA. Postpneumonectomy lung growth: a model of reinitiation of tropoelastin and type I collagen production in a normal pattern in adult rat lung. Am. J. Respir. Cell Mol. Biol. 1996; 15:611–623. [PubMed: 8918368]

92. Kaza AK, Kron IL, Leuwerke SM, Tribble CG, Laubach VE. Keratinocyte growth factor enhances post-pneumonectomy lung growth by alveolar proliferation. Circulation. 2002; 106:I120–I124. [PubMed: 12354720]

93. Kaza AK, et al. Epidermal growth factor augments postpneumonectomy lung growth. J. Thorac. Cardiovasc. Surg. 2000; 120:916–922. [PubMed: 11044318]

94. Zhang Y, et al. A Gata6-Wnt pathway required for epithelial stem cell development and airway regeneration. Nat. Genet. 2008; 40:862–870. [PubMed: 18536717]

95. Zemke AC, et al. β-catenin is not necessary for maintenance or repair of the bronchiolar epithelium. Am. J. Respir. Cell Mol. Biol. 2009; 41:535–543. [PubMed: 19213872]

96. Tanjore H, et al. β-catenin in the alveolar epithelium protects from lung fibrosis after intratracheal bleomycin. Am. J. Respir. Crit. Care Med. 2013; 187:630–639. [PubMed: 23306543]

97. Henderson WR Jr, et al. Inhibition of Wnt/β-catenin/CREB binding protein (CBP) signaling reverses pulmonary fibrosis. Proc. Natl. Acad. Sci. USA. 2010; 107:14309–14314. [PubMed: 20660310]

98. Levänen B, Wheelock AM, Eklund A, Grunewald J, Nord M. Increased pulmonary Wnt (wingless/integrated)-signaling in patients with sarcoidosis. Respir Med. 2011; 105:282–291. [PubMed: 21146388]

99. Königshoff M, et al. Functional Wnt signaling is increased in idiopathic pulmonary fibrosis. PLoS ONE. 2008; 3:e2142. [PubMed: 18478089]

100. Chilosi M, et al. Aberrant Wnt/β-catenin pathway activation in idiopathic pulmonary fibrosis. Am. J. Pathol. 2003; 162:1495–1502. [PubMed: 12707032]

101. Morimoto M, et al. Canonical Notch signaling in the developing lung is required for determination of arterial smooth muscle cells and selection of Clara versus ciliated cell fate. J. Cell Sci. 2010; 123:213–224. [PubMed: 20048339]

102. Tsao PN, et al. Notch signaling controls the balance of ciliated and secretory cell fates in developing airways. Development. 2009; 136:2297–2307. [PubMed: 19502490]

103. Tsao PN, et al. Notch signaling prevents mucous metaplasia in mouse conducting airways during postnatal development. Development. 2011; 138:3533–3543. [PubMed: 21791528]

104. Ito K, et al. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. N. Engl. J. Med. 2005; 352:1967–1976. [PubMed: 15888697]

105. Wang Y, et al. Development and regeneration of $Sox2^{+}$ endoderm progenitors are regulated by a Hdac1/2-Bmp4/Rb1 regulatory pathway. Dev. Cell. 2013; 24:345–358. [PubMed: 23449471]

106. Kubo A, et al. Development of definitive endoderm from embryonic stem cells in culture. Development. 2004; 131:1651–1662. [PubMed: 14998924]

107. Christodoulou C, et al. Mouse ES and iPS cells can form similar definitive endoderm despite differences in imprinted genes. J. Clin. Invest. 2011; 121:2313–2325. [PubMed: 21537085]

108. Gouon-Evans V, et al. BMP-4 is required for hepatic specification of mouse embryonic stem cell-derived definitive endoderm. Nat. Biotechnol. 2006; 24:1402–1411. [PubMed: 17086172]

109. Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell. 2008; 132:661–680. [PubMed: 18295582]

110. Green MD, et al. Generation of anterior foregut endoderm from human embryonic and induced pluripotent stem cells. Nat. Biotechnol. 2011; 29:267–272. [PubMed: 21358635]

111. Longmire TA, et al. Efficient derivation of purified lung and thyroid progenitors from embryonic stem cells. Cell Stem Cell. 2012; 10:398–411. [PubMed: 22482505]

112. Mou H, et al. Generation of multipotent lung and airway progenitors from mouse ESCs and patient-specific cystic fibrosis iPSCs. Cell Stem Cell. 2012; 10:385–397. [PubMed: 22482504]

113. Wong AP, et al. Directed differentiation of human pluripotent stem cells into mature airway epithelia expressing functional CFTR protein. Nat. Biotechnol. 2012; 30:876–882. [PubMed: 22922672]

114. Baiguera S, et al. Tissue engineered human tracheas for *in vivo* implantation. Biomaterials. 2010; 31:8931–8938. [PubMed: 20800273]

115. Macchiarini P, et al. Clinical transplantation of a tissue-engineered airway. Lancet. 2008; 372:2023–2030. [PubMed: 19022496]

116. Ott HC, et al. Regeneration and orthotopic transplantation of a bioartificial lung. Nat. Med. 2010; 16:927–933. [PubMed: 20628374]

117. Petersen TH, et al. Tissue-engineered lungs for *in vivo* implantation. Science. 2010; 329:538–541. [PubMed: 20576850]

118. Daly AB, et al. Initial binding and recellularization of decellularized mouse lung scaffolds with bone marrow-derived mesenchymal stromal cells. Tissue Eng. Part A. 2012; 18:1–16. [PubMed: 21756220]

119. Wallis JM, et al. Comparative assessment of detergent-based protocols for mouse lung de-cellularization and re-cellularization. Tissue Eng. Part C Methods. 2012; 18:420–432. [PubMed: 22165818]

120. Cortiella J, et al. Influence of acellular natural lung matrix on murine embryonic stem cell differentiation and tissue formation. Tissue Eng. Part A. 2010; 16:2565–2580. [PubMed: 20408765]

121. Ghaedi M, et al. Human iPS cell-derived alveolar epithelium repopulates lung extracellular matrix. J. Clin. Invest. 2013; 123:4950–4962. [PubMed: 24135142]

122. Weiss DJ, Ortiz LA. Cell therapy trials for lung diseases: progress and cautions. Am. J. Respir. Crit. Care Med. 2013; 188:123–125. [PubMed: 23855686]

123. Bianco P, et al. The meaning, the sense and the significance: translating the science of mesenchymal stem cells into medicine. Nat. Med. 2013; 19:35–42. [PubMed: 23296015]

124. Lama VN, et al. Evidence for tissue-resident mesenchymal stem cells in human adult lung from studies of transplanted allografts. J. Clin. Invest. 2007; 117:989–996. [PubMed: 17347686]

125. Caplan AI. What's in a name? Tissue Eng. Part A. 2010; 16:2415–2417. [PubMed: 20412005]

126. Ohle SJ, Anandaiah A, Fabian AJ, Fine A, Kotton DN. Maintenance and repair of the lung endothelium does not involve contributions from marrow-derived endothelial precursor cells. Am. J. Respir. Cell Mol. Biol. 2012; 47:11–19. [PubMed: 22323363]

127. Comroe JH Jr, Dripps RD. Scientific basis for the support of biomedical science. Science. 1976; 192:105–111. [PubMed: 769161]

128. Perl AK, et al. Conditional recombination reveals distinct subsets of epithelial cells in trachea, bronchi, and alveoli. Am. J. Respir. Cell Mol. Biol. 2005; 33:455–462. [PubMed: 16055670]

129. Flodby P, et al. Directed expression of Cre in alveolar epithelial type 1 cells. Am. J. Respir. Cell Mol. Biol. 2010; 43:173–178. [PubMed: 19767448]

130. Somers A, et al. Generation of transgene-free lung disease-specific human iPS cells using a single excisable lentiviral stem cell cassette. Stem Cells. 2010; 28:1728–1740. [PubMed: 20715179]

131. Rashid ST, et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J. Clin. Invest. 2010; 120:3127–3136. [PubMed: 20739751]

132. Yusa K, et al. Targeted gene correction of α1-antitrypsin deficiency in induced pluripotent stem cells. Nature. 2011; 478:391–394. [PubMed: 21993621]

133. Lachmann N, et al. Gene correction of human induced pluripotent stem cells repairs the cellular phenotype in pulmonary alveolar proteinosis. Am. J. Respir Crit. Care Med. 2014; 189:167–182. [PubMed: 24279725]

134. Suzuki T, et al. Use of induced pluripotent stem cells to recapitulate pulmonary alveolar proteinosis pathogenesis. Am. J. Respir. Crit. Care Med. 2014; 189:183–193. [PubMed: 24279752]

Box 1 Modeling lung disease in pluripotent stem cells

The discovery of pluripotent reprogramming was a seminal event for lung researchers, as it has made possible the derivation of an inexhaustible supply of patient-specific cells through the generation of iPSCs from individuals with any lung disease[111]. In 2010 the first 100 iPSC lines were generated from individuals with genetic lung diseases, including the two most common inherited monogenic lung diseases, cystic fibrosis and α1 antitrypsin deficiency–related emphysema[130]. Shortly thereafter, patient-specific and disease-specific lines were used to model the intracellular protein misfolding of mutant α1 antitrypsin protein[131,132] and the aberrant intracellular trafficking of mutant cystic fibrosis transmembrane conductance regulator[113] in differentiated epithelial lineages derived from patient-specific iPSCs. In addition, the monogenic inherited form of pulmonary alveolar proteinosis was recently modeled *in vitro* using macrophages derived from iPSCs generated from pediatric patients with this rare disease[133,134]. iPSCs from individuals with α1 antitrypsin deficiency have even undergone successful gene correction *in vitro* followed by functional hepatic transplantation into rodents[132]. Thus, in the years ahead, iPSCs from patients will undoubtedly provide unprecedented opportunities to model human lung disease *in vitro* and predict person-to-person differences in disease severity and drug toxicity.

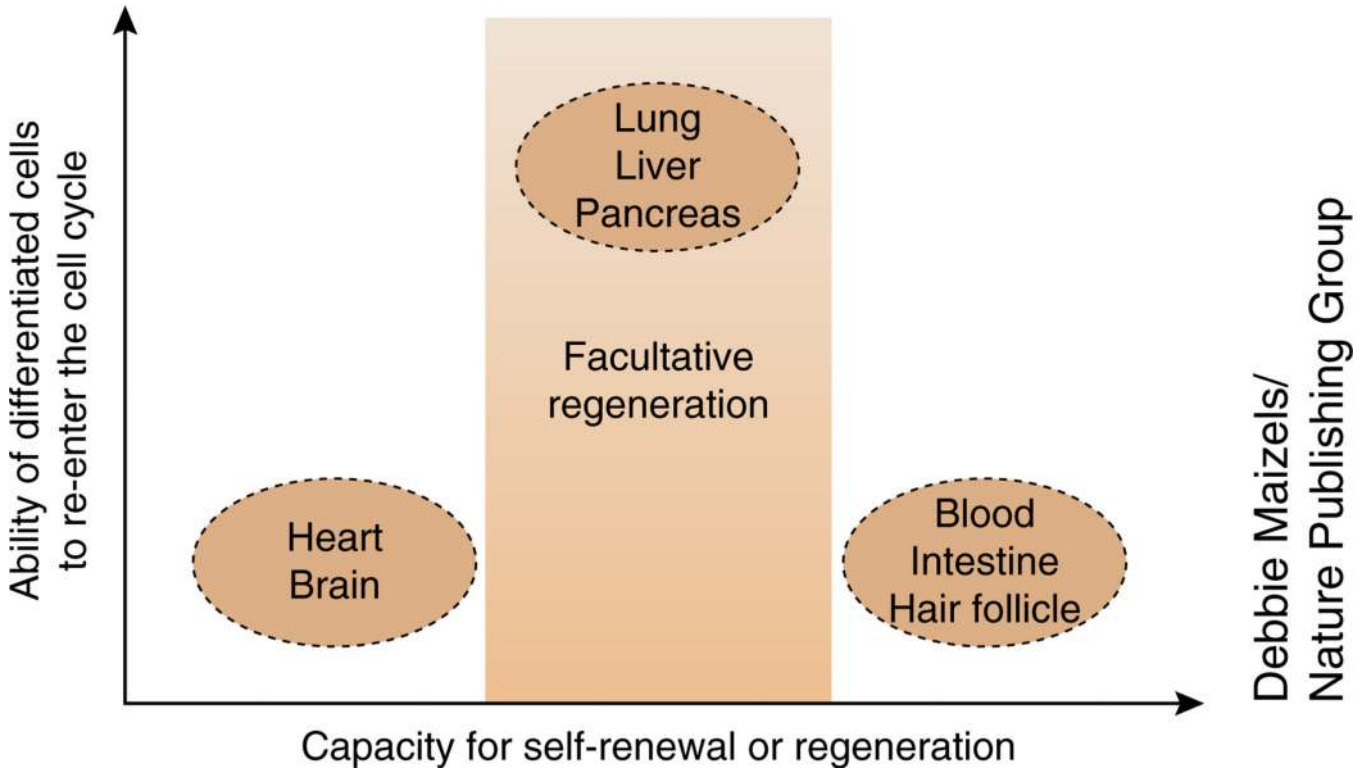

Figure 1.

Relationship between the regenerative capacity of different tissues and the existence of resident tissue-specific stem cells. Tissues such as the hematopoietic system and the intestine undergo rapid turnover assisted by well-documented stem cell lineages. Other tissues, such as the lung, can respond robustly after injury to replace lost cells but are normally quiescent in the adult. A third group of tissues, including the heart and brain, does not regenerate well after injury and generally forms scar tissue. Differentiated cells in tissues that undergo rapid turnover do not exhibit the robust ability to re-enter the cell cycle, whereas facultative regenerative tissues, such as the lung, do.

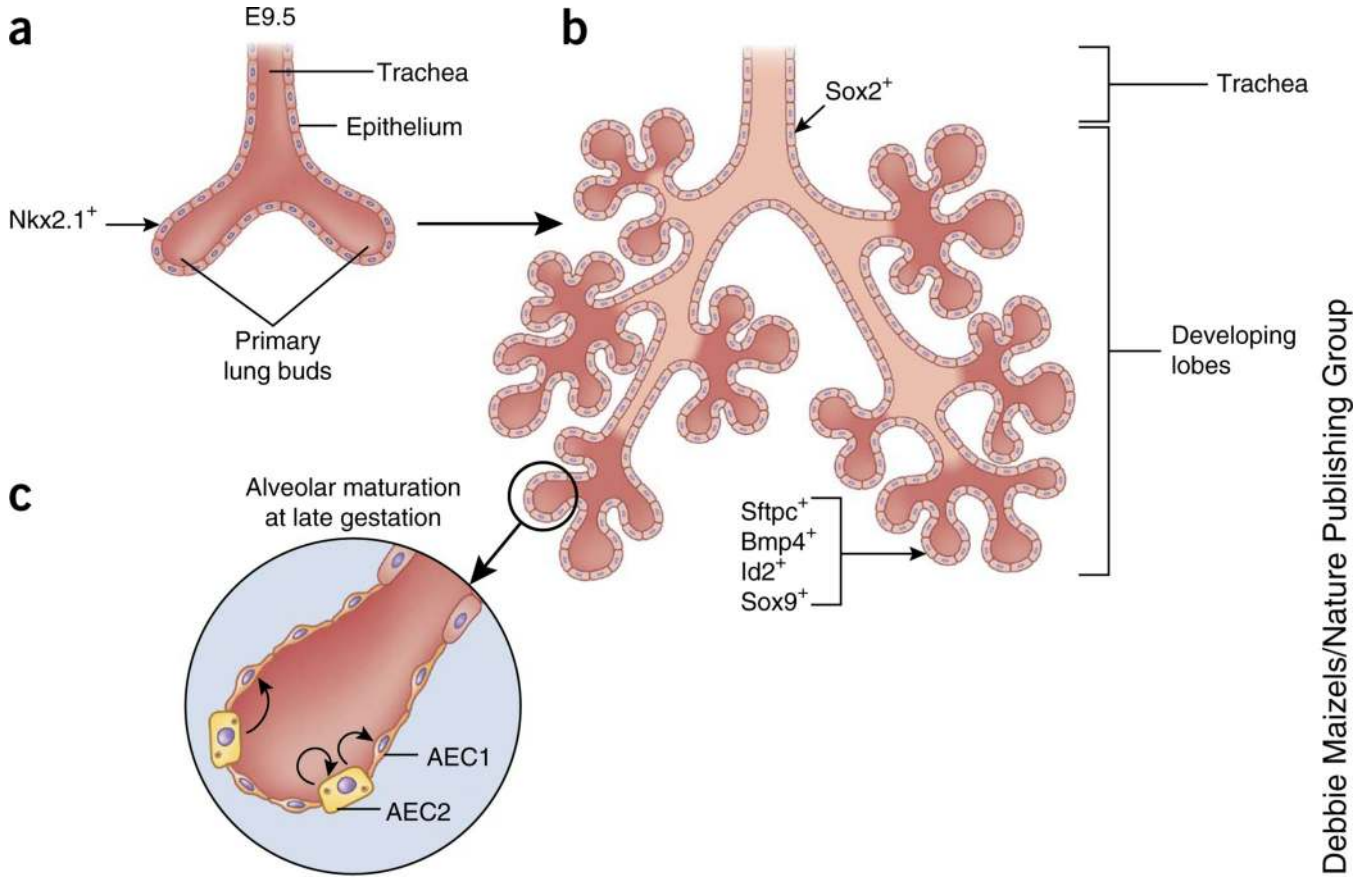

Figure 2.

Cell lineages in early lung development in mouse. **a**. At E9.5 in mouse, the trachea and two primary lung buds are formed. **b**. These buds subsequently branch into lobes, which develop into alveoli. Proximal and distal developing epithelia of the developing lungs express different markers. At this early stage, $Id2^+$ cells can generate proximal and distal epithelia. **c**. Toward late gestation, AEC1s and AEC2s are formed.

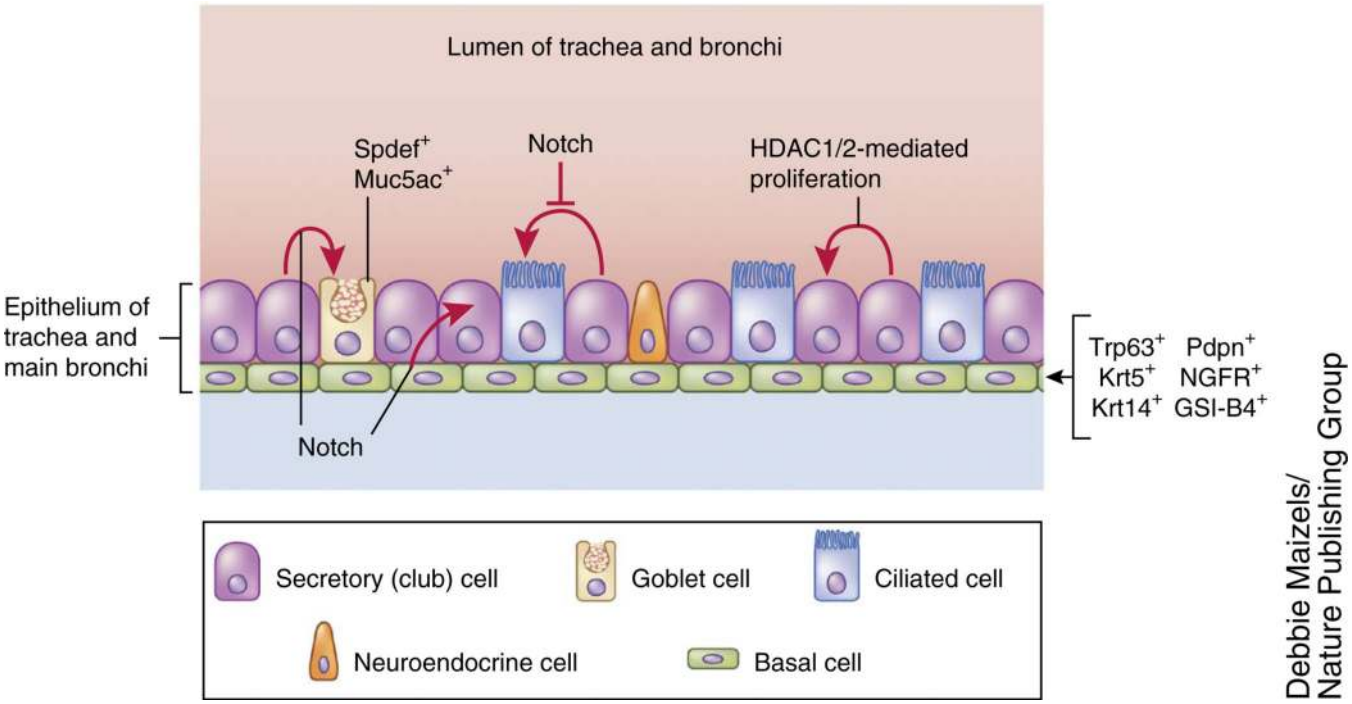

Figure 3.

Stem cell and differentiated epithelia ineages in the trachea and main stem bronch of the lung. The trachea and most proxima airways of the rodent and human lung are lined with multiple epithelial lineages. Basal cells are located in this region and can generate secretory and ciliated cell lineages. Cell signaling pathways such as Notch are crucial for differentiation of basal cells and also suppress the ciliated epithelial–cell fate. HDAC1 and HDAC2 (HDAC1/2) are essential for secretory epithelial regeneration. Red arrows indicate cells that have been shown by lineage-tracing techniques to generate the indicated lineages after injury or during homeostasis.

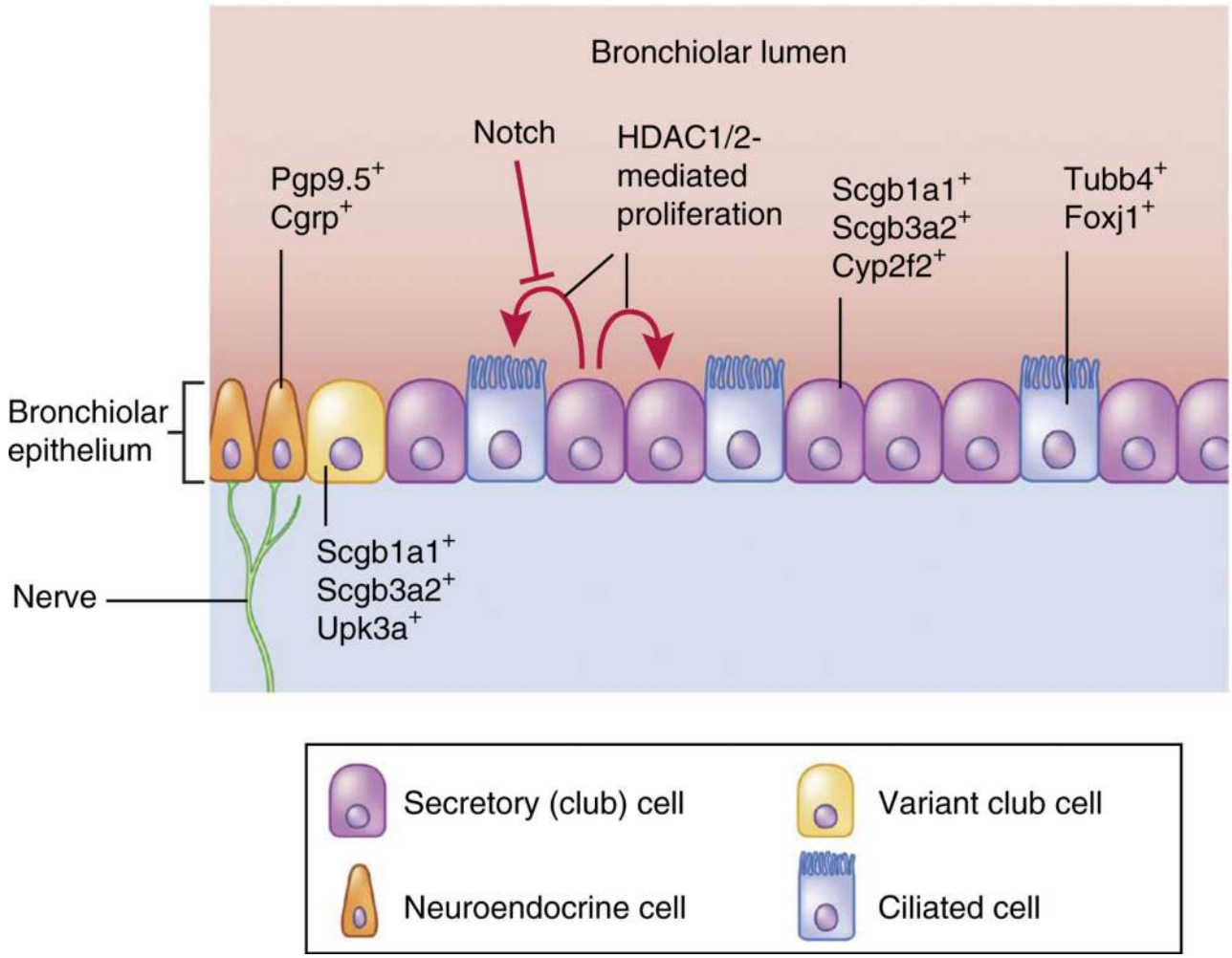

Figure 4.

Stem cell and differentiated epithelial lineages in the bronchiolar airways of the lung. The rodent lung contains a bronchiolar region that acks basal cells but is lined with a simple cuboidal epithelium. Notch suppresses the ciliated epithelial cell fate in this region of the airways. HDAC1 and HDAC2 (HDAC1/2) are essential for regeneration of secretory epithelial lineages, such as club cells. Red arrows indicate cells that have been shown by lineage-tracing techniques to generate the ndicated lineages after injury or during homeostasis.

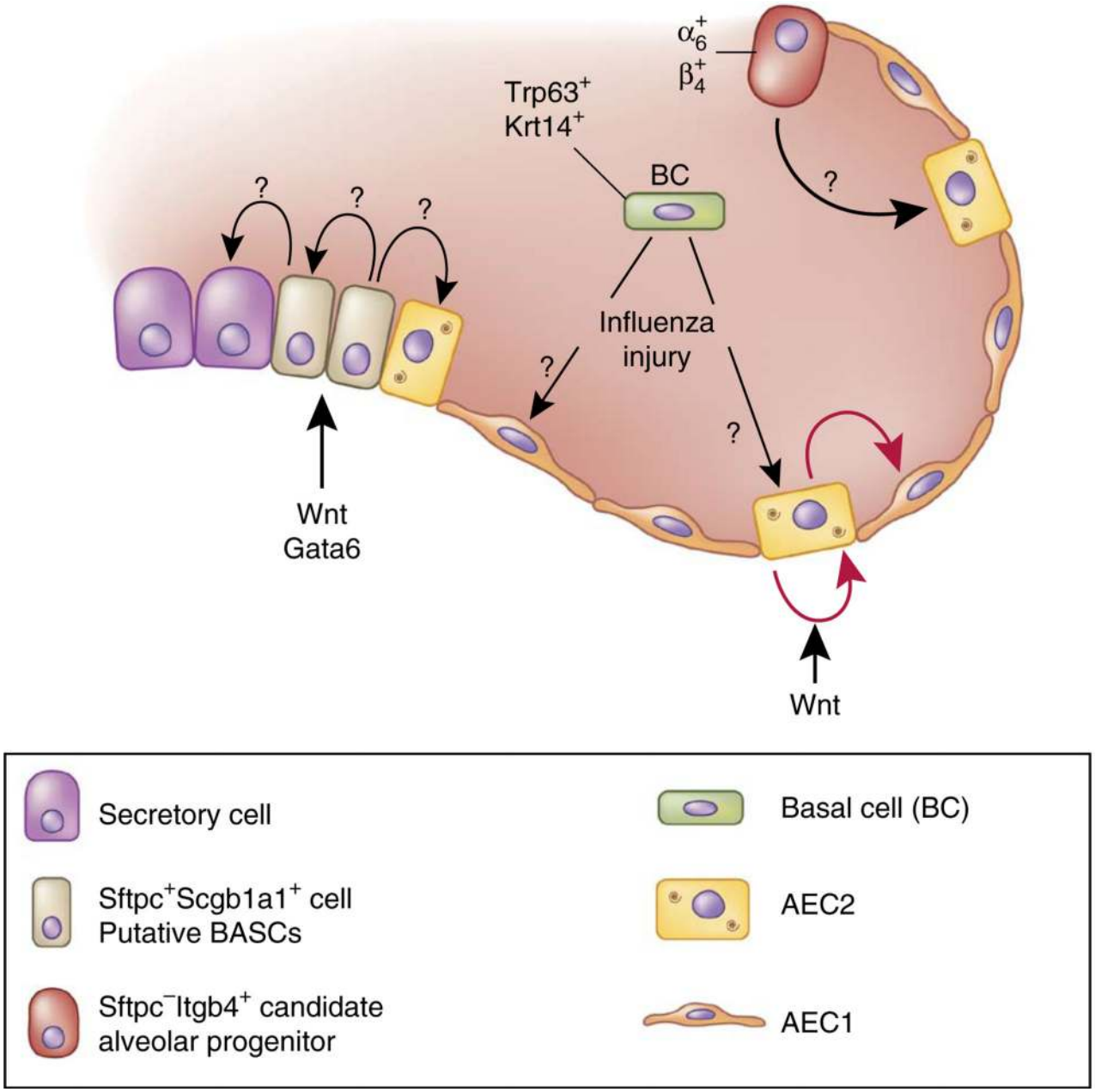

Figure 5.

Progenitor cell populations and their differentiated progeny in the lung alveolus. The alveolar epithelium consists of AEC1s and AEC2s. Lineage-tracing techniques indicate that AEC2s can generate AEC1s during homeostasis and after injury (red arrow). Generation of alveolar epithelium by other cells, such as $Sftpc^{+}Scgb1a1^{+}$ (putative BASCs) and $Itgb4^{+}$cells, has yet to be supported by lineage tracing (black arrows). One study also suggests that basal cells can generate alveolar epithelium, although additional lineage tracing is required to support this hypothesis. Red arrows indicate cells that have been shown by lineage-tracing techniques to generate the indicated lineages after injury or during homeostasis. In the case of the $Sftpc^{-}Itgb4+$ population, direct lineage tracing has not yet

been reported. Wnt signaling is thought to be important in regulating alveolar epithelial homeostasis after injury, and Wnt signaling and Gata6 are important for BASC expansion and differentiation. a6, Itga6; β4, Itgb4.

Table 1

Relationship between putative stem and progenitor populations and their differentiated progeny in the adult lung

Stem and progenitor Populations	Marker genes	Proliferative	Differentiation repertoire	Cell-lineage labeling	Reference
Proposed multipotent cells					
Alveolar Itga6^{+}Itgb4^{+}Sftpc^{-}	*Itga6, Itgb4*	Yes	Self, AEC1, AEC2, club, ciliated	Excluded Sftpc^{+} cells (using Sftpc^{-}CreERT2)[a]	73
BASCs	*Sftpc, Scgb1a1*	Yes	Self, club, AEC2	Scgb1a1-CreERT2[a] (not BASC specific)	74
Basal cell	*Trp63, Krt5, Krt14, Ngfr, Pdpn*	Yes	Self, club, ciliated, AEC1, AEC2	Krt14-CreERT2 after injury	55
Airway progenitors					
Basal cell	*Trp63, Krt5, Krt14, Ngfr, Pdpn*	Yes	Self, ciliated, basal	Krt5-CreERT2, Krt14-CreERT2	50–52,56
Club cell	*Scgb1a1, Cyp2f2*	Yes	Self, ciliated, basal	Scgb1a1-CreERT2[a]	61,79,128
Variant club cell	*Scgb1a1, Cyp2f2*	Yes	Self, club, ciliated	Scgb1a1-CreERT2[a], Upk3a–CreERT2[a]	61,67
Alveolar progenitors					
AEC2	*Sftpc*	Yes	Self, AEC1	Sftpc-CreERT2[a]	11,25,26,73,76
Nonprogenitor lineages					
AEC1	*Aqp5, Pdpn, Hopx*	No	NK	Aqp5-Cre[a]	129
Ciliated epithelium	*Foxj1, Tubb4a*	No	NK	Foxj1-CreERT2	42

[a] Mouse lines that contain knock-in alleles may provide greater fidelity to the expression patterns of the marker genes of interest. NK, none known; CreERT2, Cre recombinase–mutant estrogen receptor fusion protein; Aqp5, aquaporin 5; Krt5, keratin 5; Krt14, keratin 14.