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Keeping it together: Pulmonary alveoli are maintained by a hierarchy of cellular programs

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Abstract

The application of *in vivo* genetic lineage tracing has advanced our understanding of cellular mechanisms for tissue renewal in organs with slow turnover, like the lung. These studies have identified an adult stem cell with very different properties than classically understood ones that maintain continuously cycling tissues like the intestine. A portrait has emerged of an ensemble of cellular programs that replenish the cells that line the gas exchange (alveolar) surface, enabling a response tailored to the extent of cell loss. A capacity for differentiated cells to undergo direct lineage transitions allows for local restoration of proper cell balance at sites of injury. We present these recent findings as a paradigm for how a relatively quiescent tissue compartment can maintain homeostasis throughout a lifetime punctuated by injuries ranging from mild to life-threatening, and discuss how dysfunction or insufficiency of alveolar repair programs produce serious health consequences like cancer and fibrosis.

Keywords

lung; alveoli; AT2 cell; stem cell; renewal; repair; gas exchange; lineage tracing; transdifferentiation; pulmonary fibrosis

Introduction

The principal function of the lung is gas exchange, which takes place in a collection of delicate air sacs called alveoli¹. Loss or dysfunction of alveoli underlies acute and chronic respiratory failure from a variety of causes. In health, the cells lining the alveolar surface are renewed very infrequently, which is surprising given their direct exposure to the airborne environment^{2,3}. This slow cellular turnover made it difficult to identify alveolar progenitors using classical methods that mark proliferating cells⁴. The advent of genetic lineage tracing⁵ in the mouse removed this obstacle by allowing specific cell populations, even slowly proliferating ones, to be permanently and heritably marked *in vivo*. The behavior of the

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Conflict of Interest

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marked cells is inferred from the distribution and identity of cells that express the marker at a future point in time. This basic strategy has been refined to afford control over the timing and extent of cell marking. Strains have also been generated that allow stochastic marking of cells by one of several distinct tags, facilitating clonal analysis, in which the fate of an individual cell can be mapped.

Application of lineage tracing to investigate renewal and repair of pulmonary alveoli has yielded important insights into the cellular sources and mechanisms that maintain homeostasis in adult life. In this review, we summarize our current understanding of the cell types that populate the terminal airways and alveoli in the distal lung. We describe the unique features of a novel kind of stem cell that intermittently renews alveoli, and discuss whether and how it might differ from other cells within the same class. We integrate findings from various experimental injuries that collectively suggest a regulative model for cellular programs to maintain alveolar homeostasis. We summarize the transdifferentiation events observed throughout the lungs after injury and during the evolution of different lung cancer subtypes, and what is known about their molecular control. Finally, we discuss how insufficiency of these renewal and repair programs may be involved in the pathogenesis of chronic, progressive alveolar diseases.

The ‘business end’ of the lung: a collection of gas exchange sacs at the ends of the bronchial tree

The mammalian lung is comprised of proximal and distal compartments that form two spatially, structurally, and functionally distinct anatomical regions¹. The proximal compartment is a ‘tree’ of progressively branching airway tubules that accomplish ventilation, transmitting air into and out of the distal compartment, a densely packed aggregate of thin-walled air sacs termed ‘alveoli’ where gas exchange takes place (Figure 1). Each alveolus (literally, ‘small cavity’) consists of an epithelial lining exposed to ambient air that is tightly apposed to a dense underlying capillary meshwork. This ultra-thin barrier⁶ facilitates efficient release of carbon dioxide and uptake of oxygen by the continuous circulation of erythrocytes through the capillaries. Alveoli arise from the last generation of the bronchial tree, off of small airways called terminal bronchioles. The sharp anatomical boundary that separates the gas exchange region from the conducting airways is referred to as the bronchoalveolar junction.

The alveolar compartment has a seamless honeycomb-like pattern on cross-section, which is remarkable given that it represents a packing together of alveoli emanating in clusters from different terminal bronchioles like grapes off a stalk. Because of this seamless appearance and the incredible density of alveoli, it is difficult to distinguish where one cluster (acinus) ends and another begins. As a result, neither the absolute nor relative position of a given alveolar sac along the proximo-distal axis can be accurately inferred. This ability to see only the ‘trees’ and not the ‘forest’ makes it difficult to superimpose molecular (or lineage) markers onto an anatomical map. Therefore, specific spatial patterns that correspond to anatomical boundaries might not be appreciated, for instance distinct classes of alveoli or the presence of compartments analogous to intestinal crypts.

Despite the topographic complexity of the distal lung, the monolayer epithelial sheet lining the alveolar surface is composed primarily of only two differentiated cell types, each of which performs a specialized function that is essential for survival⁷. The squamous alveolar epithelial type I (AT1) cell⁸ facilitates gas exchange. These cells are exquisitely flat and expansive, covering over 95% of the alveolar lining and thereby maximizing the surface area for passive gas diffusion⁹. The cuboidal AT2 cell produces and secretes surfactant phospholipid to minimize surface tension and thereby prevent alveolar collapse during breathing¹⁰. AT2 cells reside in alveolar ‘corners’ (where three or more inter-alveolar walls converge) and are marked by distinctive cytoplasmic lamellar bodies that store surfactant prior to release.

Along with AT1 and AT2 cells, other less abundant populations have also been observed in the distal airways and alveoli. One such example is the ‘brush’ cell, a morphologically distinctive cell with apical microvilli identified in alveoli of rat and several other species, but not reported in mouse or human^{11,12}. More recently, a cuboidal cell that is marked by integrin $\alpha 6\beta 4$ but lacks the canonical AT2 cell marker, Surfactant Protein C (SftpC), has been identified and estimated to make up 8% of the alveolar epithelium¹³. These are presumed to be the same population as the low-lying cells found in very low numbers in terminal bronchioles, termed ‘lineage-negative epithelial progenitors’ (LNEPs) because they lack markers of differentiated lung cells¹⁴. A similar population of rare, low-lying cells in terminal bronchioles has been identified by another group, which they have termed the ‘distal airway stem cell’ (DASC)¹⁵. While it has not been directly shown that these two populations are the same, they overlap in marker expression and behavior. At least a subset of DASCs that reside in terminal bronchioles resembles basal cells, which are stem cells in the upper (proximal) airways¹⁶. Another molecularly distinct population called the ‘bronchioalveolar stem cell’ (BASC)¹⁷ also resides in terminal bronchioles near the alveolar junction, and co-expresses an alveolar (AT2 cell, SftpC) and bronchiolar (Club cell, Secretoglobin 1a1/Scgb1a1) marker.

Other minor populations have been identified by the use of flow cytometry to isolate lung cells expressing surface markers that characterize stem cells in other tissues^{17,18}. However, these populations have not been localized in the lung based on expression of the markers used for their isolation, so it is unknown where they reside. The major epithelial cell types populating the terminal bronchioles and alveoli, their distinctive features, and common markers used to mark them are shown in Figure 1. Additional rare alveolar cell types may yet exist, whose identification will be facilitated by the recent availability of powerful, single-cell molecular profiling techniques¹⁹.

A novel ‘bifunctional’ stem cell renews alveoli throughout the lifespan

A variety of classical experiments have revealed some unique features of the alveolar compartment and have shown that AT2 cells are stem cells for this region of the lung. First, alveolar epithelial proliferation is infrequent and occurs diffusely throughout the compartment²⁰. When experiments involving acute hyperoxic injury were performed to accelerate cellular turnover, it appeared that AT2 cells were a progenitor capable of simple duplication and transdifferentiation into an AT1 cell phenotype²¹. Modern day, bulk-cell

lineage tracing of AT2 cells has confirmed this model, demonstrating the appearance of the AT2 cell lineage tag in AT1 cells during aging and after injury with bleomycin²². Single cell (clonal) analysis of AT2 cells using a stochastic, multi-color lineage reporter further showed that renewal is accomplished by rare AT2 cells that generate AT1 cells in discrete ‘renewal foci’ that slowly enlarge over the lifespan²³. The ‘founder’ AT2 cell forming each renewal focus was present even at advanced ages, confirming its long-term self-renewal potential. Together, these findings indicate that rare AT2 cells function *in vivo* as bona fide stem cells²⁴.

Interestingly, the ‘founder’ AT2 stem cells express the same profile of molecular markers as other AT2 cells, including proteins involved in the surfactant pathway, suggesting that they also execute a secretory function. Indeed, studies of AT2 cell proliferation *in vivo* following injury have shown that surfactant-containing lamellar bodies are present throughout mitosis²⁵. To describe this novel property in which a single cell constitutively executes both a stem cell and a specialized physiologic function characteristic of differentiated cells *in vivo*, we use the modifier ‘bifunctional.’

The bifunctionality of AT2 stem cells is noteworthy because it contrasts with the traditional conception of stem cells as being ‘undifferentiated,’ i.e. lacking a specialized function outside of cellular renewal. The idea of the ‘stem cell’ originated in the 1800s when it was observed that some individual cells had the capacity to function as precursors and generate multiple lineages over time²⁶. A modern definition was established in the 1960s when hematopoietic stem cells were identified²⁷. These studies described stem cells functionally, as cells that can proliferate indefinitely and generate at least one other cell type. However, because hematopoietic and several other stem cell populations shared additional features such as being relatively rare, expressing surface markers not found on mature cells, lacking specialized ultrastructural features and markers of differentiated cells, and proliferating infrequently, these properties also became associated with stem cells²⁴. Exceptions to many of these features have since been observed in adult cells that nevertheless are multipotent, self-renewing, and active throughout the lifespan, meeting the functional definition of a stem cell²⁸. Therefore, beyond their specialized progenitor role, adult stem cells from different tissues may be quite diverse. For the lung, the dual participation of AT2 cells in the surfactant and cellular renewal programs may reflect the specific demands of alveoli, which must simultaneously and continuously execute essential barrier, gas exchange, and surfactant functions. These demands differ significantly from those of other tissues, such as the rapidly cycling intestinal epithelium, where a segregated stem cell pool that is restricted to the base of each crypt continuously generates ‘transit amplifying’ progeny that extensively proliferate and differentiate as they flow upwards towards the villus²⁹ (Figure 2).

Are all AT2 cells equal, or are some ‘more equal’ than others?

When AT2 cells were pulse-labeled in bulk, the percentage of marked AT2 cells did not decrease over one year, suggesting that new AT2 cells derive from pre-existing ones during aging³⁰. Despite a low frequency of AT2 cell proliferation during renewal, a robust response is generated by acute AT1 cell injury³¹. This broad induction of AT2 cell proliferation following injury, or in response to genetic targeting of constitutively activated KrasG12D to

AT2 cells³², suggests that many AT2 cells are capable of proliferating. This possibility is supported by the observation that AT2 ‘founder’ stem cells express the same limited profile of mature cell type markers as other AT2 cells, and that mitotically active AT2 cells contain lamellar bodies, which are a hallmark of differentiated AT2 cells²⁵. AT2 stem cells thus do not appear to constitute a dedicated subpopulation that exclusively participates in cellular replacement. On the other hand, when AT2 cells were labeled in bulk using a multicolor lineage reporter, and then a significant subset was acutely ablated, coarse foci of monochromatic AT2 cell clusters resulted³⁰. This finding suggests that local replacement of lost AT2 cells was accomplished by repeated proliferation of a single AT2 cell, which could be either because it was the only surviving AT2 cell in that region, or that it was intrinsically more potent than nearby survivors. The latter model of preferential proliferation of a specialized AT2 cell is supported by clonal analysis of AT2 cells during aging²³, which demonstrated large ‘renewal foci’ spanning multiple alveoli that were monoclonal, despite the local availability of multiple other healthy AT2 cells.

Two basic models might explain how a single AT2 cell from a field of many undergoes repeated proliferation during renewal, whereas multiple AT2 cells are recruited to proliferate during repair, as schematized in Figure 3. The first model proposes that a rare, specialized AT2 stem cell ‘subtype’ is repeatedly activated to accomplish the slow local cellular renewal. In the second model, all AT2 cells are equivalent but one is stochastically selected to renew a lost cell, and once this occurs, it maintains local clonal dominance by suppressing nearby AT2 cells. In both models, acute alveolar injury induces ‘ancillary’ AT2 cell recruitment, either in response to an injury signal (Model 1), release of local inhibition (Model 2), or a combination. Regardless of the operative molecular mechanism, it is clear that AT2 cells are tightly regulated to restrain the number that proliferate under basal conditions, and yet to maximize rapid restoration of barrier integrity following acute injury. Limiting the number of AT2 cells that enter a proliferative program may be adaptive at the organismal level by minimizing the chances of activating an AT2 cell harboring an oncogenic mutation that requires cellular proliferation to exert its tumorigenic effect.

What happens if a severe alveolar injury locally depletes AT2 cells?

Exposure to gases like oxygen³³ and nitrogen dioxide³⁴, which are toxic to AT1 but not AT2 cells, results in rapid restoration of normal alveolar epithelium by diffuse proliferation of AT2 stem and ancillary cells. With bulk labeling of AT2 cells followed by injury with bleomycin, however, investigators noticed some repaired alveolar regions were populated by AT2 and AT1 cells that *lack* the AT2 lineage tag, suggesting that non-AT2 cells contributed to repair¹³. They proposed that these un-marked AT1 and AT2 cells derived from rare integrin $\alpha 6\beta 4$ alveolar and bronchiolar populations they had identified by immunostaining (the LNEPs, see Figure 1). In a follow up study, they isolated LNEPs then transplanted them endotracheally into a mouse whose lungs had been injured by influenza, observing engraftment with clonal proliferation and apparent differentiation into either bronchiolar or alveolar epithelial lineages¹⁴. As mentioned earlier, these LNEPs may be the same population as the DASCs that have similarly been shown to be alveolar progenitors following influenza pneumonia^{15,35}. The DASCs likewise appear to proliferate extensively in the terminal bronchioles and then spread distally to repopulate the alveoli. These cells

appear to be quiescent during aging and after selective AT1 cell injury, so they may be considered ‘facultative’ progenitors, meaning they are only active under certain conditions³⁶. In fact, the LNEP/DASC may represent the most significant facultative progenitor described in any tissue to date, given the lack of consensus over the contribution or importance of ductal epithelial cells in the pancreas³⁷ and liver³⁸ to generate beta cells and hepatocytes, respectively, following specific injuries.

Taken altogether, these lineage studies during aging and injury suggest a hierarchical model of cellular programs for replenishing alveolar cells that operate along a continuum ranging from a low rate of basal turnover to acute, life-threatening lung injuries that deplete local progenitors. The discrete programs are schematized in sequence of their deployment relative to the severity of injury in Figure 4, and presumably all three may be simultaneously active in different regions of the lung depending on the distribution of a particular insult.

Despite the remarkable ability of the alveoli to be repaired by a hierarchy of cells that are recruited to restore missing AT1 and AT2 cells, it must be noted that alveolar regeneration is not unlimited. Following experimental injury by agents that damage AT1 and AT2 cells, classical studies found that some alveolar regions are repaired but others demonstrate residual structural abnormalities even after clinical recovery. With bleomycin, for instance, AT2 cells initially replace AT1 cells, but at later stages morphologically abnormal AT2 cells and inappropriately localized ciliated and squamous cells lining abnormal tubular structures in alveolar regions are found^{39,40}. Infection with influenza PR8-A, which is toxic to AT1, AT2, and bronchiolar epithelial cells, also results in alveolar regions demonstrating persistent pathology⁴¹. The cellular dynamics of the influenza response involve broad epithelial denudation with subsequent proliferation and migration of bronchiolar cells into contiguous alveoli, resulting in large alveolar ‘nodules’ that obstruct the airway lumen⁴². Recent studies examining the LNEPs found that the persistent airway nodules in some alveolar regions appeared to include LNEPs that did not successfully complete an alveolar differentiation program. These data indicate that although the alveoli possess a remarkable regenerative capacity to repair acute environmental injuries by harnessing different pools of cells, this ability is not unlimited. In cases of severe injury, areas of failed repair may result from damage to the microenvironment with loss of instructive signals that drive proper differentiation and integration of these progenitors.

Cells undergo surprising phenotypic transitions that are ‘off the beaten path’ after severe injury

The mainstay of dissecting lineage hierarchies and contributions of specific cell types to alveolar repair involves conditional cell type marking in mice using a tamoxifen-inducible Cre allele to ‘pulse’ label the desired population⁴³. One of the major caveats to this approach is the lack of absolute specificity for marker genes (which are used to drive the Cre alleles) that distinguish specific cell types. This caveat becomes particularly acute when a rare outcome results, and the bulk of the targeted population does not demonstrate the observed behavior. For pulmonary alveoli, it has been particularly challenging to map the contribution of Scgb1a1-expressing cells, since this marker is expressed by three different populations

that reside in alveoli and terminal bronchioles: a subset of AT2 cells⁴⁴, Club cells⁴⁵ and BASCs¹⁷ (see Figure 1). A recent discovery has complicated matters further, namely that activated bronchiolar LNEPs transiently express high levels of *Scgb1a1* following an injury¹⁴. Therefore, accurately inferring which specific populations contribute to repair may require characterizing *de novo* gene expression with injury, an awareness of tamoxifen perdurance⁴⁶, and perhaps new strategies to mark cells with greater specificity.

Aside from this caveat, the induction of *Scgb1a1* by LNEPs is intriguing because it provides a molecular snapshot of a cell transitioning from an LNEP to an AT2 cell phenotype. That the intermediate expresses high levels of *Scgb1a1* is surprising, since this is not a molecular feature of precursors of AT2 cells either in embryonic development⁴⁷ or during aging⁴⁴. This observation reveals that phenotypic transitions during repair of severe injury do not appear to be restricted to routes taken during development or programmed renewal. Similar ‘inappropriate’ lineage transitions have been observed in other lung regions after various injuries, suggesting a capacity for reprogramming from one differentiated lung cell type to another beyond what is observed during renewal. These lineage ‘switches’ and additional ones observed in lung cancer (discussed below) are summarized in Figure 5. This flexibility or ‘plasticity’ of differentiated cells in the lung to adopt the phenotype of nearby cells from distinct sub-lineages may be adaptive for enabling rapid restoration of an intact epithelial barrier with the appropriate cell type composition after severe injury. Regulation of these transitions presumably involves exposure of surviving cells to instructive signals from the niches vacated by the lost cells that the survivors then occupy.

Insights from cancer into the molecular regulation of lung cell phenotypic transitions

The lung cell transdifferentiation programs after severe injury are not yet well understood with regard to the regulators and pathways of reprogramming. However, human lung cancer may offer some hints into how such switches are regulated, since lineage transitions in several different subtypes have been characterized. Perhaps the best-known cancer ‘switch’ occurs in some patients with lung adenocarcinoma resulting from activating mutations in the Epidermal Growth Factor Receptor (EGFR). In these instances, tumor cells autonomously transform from an AT2 to a neuroendocrine cell phenotype, with concurrent histological conversion of the tumor from adenocarcinoma to small cell lung cancer⁴⁸. Remarkably, execution of this AT2 to neuroendocrine phenotypic switch apparently requires only the loss of Retinoblastoma (Rb) in the EGFR mutant tumor cells⁴⁹. Another example is the clinical observation that patients can present with lung cancer of mixed histological type, so-called adenosquamous cell carcinoma, in which all cells carry the identical ‘driver’ mutation⁵⁰. It was shown in mice that deletion of a single gene was sufficient to drive progressive histological transformation of lung adenocarcinoma into squamous cell carcinoma, with an accompanying partial molecular reprogramming of tumor cells from AT2 cell-like to basal cell-like⁵¹.

As with the *Scgb1a1*-expressing LNEP intermediates transitioning into AT2 cells, the reprogramming in cancer appears to ‘bypass’ developmental pathways. Specifically, the AT2

to neuroendocrine or basal cell lineage relationship observed in cancer does not appear to exist in embryonic or adult life. Even more extreme or ‘long-range’ lineage transformations have also been observed, in one case from AT2-like lung epithelial into gastric epithelial identity, two organs that originate from adjacent portions of the embryonic foregut^{52,53}. As in severe injury, these transformations involve lineage relationships that are not observed in development or renewal, and suggest that reprogramming between lung cell states may not involve simple de-differentiation to a more primitive state followed by ‘re-differentiation’ (Figure 5).

Given that cancer cells are highly dysregulated, can reprogramming of healthy lung cells also be induced by alterations in a single gene? Several *in vivo* examples support this idea. For instance, forced expression of the transcription factor Sox2 in AT2 cells induced both proliferation and aberrant gene expression, including initiation of Scgb1a1 and the basal cell marker, p63⁵⁴. Although the molecular signals that regulate the lineage transitions between AT1, AT2 and other cell types are not known, one prediction from these examples is that they may be driven by only a few key signals. Elucidating the mechanisms for facultative conversions in the context of both injury and cancer will provide insights into the molecular regulation of lung cell identity. In turn, these discoveries may inform strategies to either therapeutically manipulate cell phenotypes to restore proper cellular balance in chronic lung diseases or facilitate the use of abundant populations for cell-based therapies.

Does impaired cellular renewal produce specific alveolar diseases?

What is the relationship of cellular renewal programs to chronic diseases that progress slowly and are not the direct consequence of an acute injury? Presumably, in a healthy individual, the AT2 stem cell program (Figure 4) is sufficient to meet the low demands of cellular renewal during aging. This cellular replacement does not occur uniformly throughout the lung, but rather in patchy, slowly enlarging foci preferentially located in peripheral (subpleural) and peri-vascular regions²³. The reason for the inhomogeneity and peripheral localization of alveolar cell turnover is unknown. However, it has also been shown that following experimental, unilateral pneumonectomy in rodents, the most rapid and substantial alveolar re-growth also localizes to subpleural regions, where angiogenesis⁵⁵ and AT2 stem cell activity⁵⁶ have been mapped. It is thus intriguing that the spatial distribution of disease in Idiopathic Pulmonary Fibrosis (IPF), a chronic progressive alveolar disease, mimics this pattern⁵⁷. In IPF, patchy foci of fibrosis localize to subpleural regions throughout the lungs. The scars relentlessly progress despite the lack of any obvious ongoing injury, and histology demonstrates areas of alveolar epithelial denudation and abnormal clusters of cuboidal cells believed to represent AT2 cell hyperplasia⁵⁸. Studies in human cohorts with familial forms of IPF have implicated inactivating mutations in telomerase, which causes stem cell ‘burnout’ in the hematopoietic system⁵⁹, suggesting that IPF may involve dysregulated AT2 renewal. There is also evidence implicating the alveolar epithelium as a driver of fibrosis in childhood interstitial lung disease⁶⁰, in which a mutation selectively impacting AT2 cells produces fibrosis in neonates. Together, these observations suggest that impaired alveolar renewal or repair by AT2 stem cells may underlie the pathogenesis or progression of IPF⁶¹.

What role might LNEP/DASCs play in IPF? Clinically, it is known that patients with IPF often suffer a stepwise decline in lung function, with intermittent exacerbations of uncertain etiology⁶². Perhaps LNEP/DASCs are normally quiescent but proliferate following an injury in patients with IPF. If so, and if their proliferative capacity is intrinsically impaired from telomerase dysfunction like AT2 cells, they could potentially execute a defective repair program results in additional pathology. Indeed, honeycomb lesions in fibrotic lungs of patients have been noted to structurally and molecularly resemble the abnormal alveolar structures seen in mice after influenza that represented incomplete differentiation of LNEPs¹⁴.

Chronic obstructive pulmonary disease (COPD) is another significant disease but is associated primarily with alveolar destruction rather than fibrosis⁶³. COPD may occur in conjunction with IPF⁶⁴ and also shares an association with mutations in telomerase⁶⁵, but why the same genetic defect would manifest clinically as two different diseases is uncertain. Further research will be required to examine AT2 cell renewal and behavior during progression of these diseases and determine the potential for cell-based or regenerative therapies.

Conclusion and Outlook

There has been tremendous recent progress in elucidating the cellular sources and mechanisms of alveolar epithelial maintenance and repair through the application of *in vivo* genetic lineage tracing. Important outstanding tasks include definitively identifying the full panoply of epithelial cell types and sub-types populating alveoli and the adjacent terminal bronchioles, which will be facilitated by single cell genomics. Once all the cell classes have been identified and characterized, new tools can be made to allow for their *in vivo* mapping and manipulation to dissect their function and full potential in alveolar renewal, repair, and re-growth. In parallel, lung transplantation assays can be used to directly test the *in vivo* regenerative potential of candidate stem and progenitor cells that have already been identified, some of which demonstrate robust *in vitro* proliferative and differentiative capacity. As suggested by the surprising phenotypic transitions observed with severe injury and in cancer, differentiated lung cells may generally possess a latent potential for direct transdifferentiation that exceeds their activity in the healthy state. The molecular regulation of these lineage 'switches' should be more comprehensively elucidated. All of this information may yield insight into disease pathogenesis, progression, or the engineering of cell-based treatment for serious alveolar conditions. Comprehensively characterizing the cellular mechanisms of alveolar epithelial cell renewal and repair also sets the stage for identifying the signals that drive this behavior, and their local sources. Indeed, damage to non-epithelial 'niche' cells is likely to underlie the inability of LNEP/DASCs to fully restore normal alveolar structure following severe injury, and perhaps in chronic lung diseases. Ultimately, once we achieve a comprehensive understanding of every alveolar population including its full physiologic and pathologic potential, and the molecular factors that regulate these behaviors, we can move forward to exploiting this knowledge for therapeutic gain.

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Abbreviations

AT2	alveolar epithelial type II
BASC	bronchoalveolar stem cell
DASC	distal airway stem cell
LB	lamellar body
LNEP	lineage negative epithelial progenitor
SftpC	surfactant protein C
Scgb1a1	secretoglobin 1a1
SV	secretory vesicle

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Figure 1. Epithelial cell populations of the distal lung

Cartoons that sequentially zoom into the major airway structures and epithelial cell types lining the junction between the airways and alveoli are shown. (A) The mouse lung consists of five lobes, each of which contains an elaborate branched structure of cylindrical airways that conduct air into and out of the gas exchange region. (B) Each terminal conducting airway (terminal bronchiole) gives rise to a grape-like cluster (acinus) of alveolar sacs. (C) The major cell types populating terminal bronchioles and alveoli are schematized, and the boundary that separates these compartments is called the bronchioalveolar junction (BAJ). Each cell type is color-coded and shown below the schematic along with its distinguishing ultrastructural or morphologic feature and, in parentheses, canonical markers used to identify them. Alveolar epithelial type I (AT1) and AT2 cells are the major differentiated cells of the alveoli. AT2 cells synthesize surfactant phospholipid that is stored in cytoplasmic lamellar bodies (LB) prior to secretion, while AT1 cells are flat and facilitate gas exchange. A minor cell population termed the LNEP/DASC resides in alveoli and terminal bronchioles (see text for details). Ciliated cells populate the terminal bronchioles, as do Club cells that contain prominent secretory vesicles (SV). BASCs reside near the BAJ and simultaneously express a Club and AT2 cell marker.

SftpC, surfactant protein C; Pdpn, podoplanin; LNEP, lineage negative epithelial progenitor; DASC, distal airway stem cell; $\beta 4$, integrin $\alpha 6\beta 4$; p63, Trp63; CK5, cytokeratin 5; Foxj1, forkhead homeobox protein J1; Scgb1a1, secretoglobin 1a1; BASC, bronchioalveolar stem cell; ? indicates ultrastructural features have not been characterized.

Stem cell feature	AT2 stem cell	Intestinal stem cell
Localization	alveolar 'corners'	crypt base
Proliferative activity	infrequent	frequent (majority), infrequent (subset)
Differentiation capacity	bipotent	multipotent
Regenerative compartment	unknown	single crypt
Function(s)	cellular renewal, surfactant secretion	cellular renewal
Oncogenic suceptibility	adenocarcinoma	adenocarcinoma

Figure 2. Properties and behaviors of stem cells that renew the alveoli and intestine
Stem cell features and renewal activity are compared between the alveolar and small intestinal epithelium. The schematic shows the bifunctional nature of the AT2 cell compared to the relatively ‘undifferentiated’ intestinal stem cell, and the table below contrasts other features of alveolar and intestinal stem cells. In the alveoli, AT2 stem cells (bright red) self-duplicate and transdifferentiate into flat AT1 cells (faint red) intermittently during aging. They also constitutively produce and secrete surfactant phospholipid into the alveolar airspace to reduce surface tension. In the intestine, there are two populations of intestinal stem cells (both shown as bright red). A major population regularly proliferates and generates ‘transit amplifying’ cells that flow upwards while continuing to proliferate and differentiate (faint red). The minor population is quiescent during renewal, serving as a back-up pool to regenerate primary stem cells that may be lost with injury.

Figure 3. Two models for the cellular mechanism of alveolar renewal and repair by AT2 cells
 Rare AT2 cells (about 1% of the population) generate slowly expanding alveolar renewal foci during aging. Whether they are a molecularly distinct subset is unknown, but it has been shown that renewal foci are monoclonal, deriving from a single AT2 cell out of a pool of many. Two models are proposed to explain this monoclonal dominance. In one scenario (Model 1), a specialized AT2 stem cell intermittently proliferates to replace nearby dying cells (dashed circle) during aging. In the second scenario (Model 2), an AT2 cell is initially stochastically selected (thick green circle) then remains the dominant stem cell during aging by suppressing nearby AT2 cells (inhibitory arrow). In both models, acute injury with substantial alveolar cell loss results in activation of quiescent AT2 cells as ancillary progenitors (thick light green circles) to replace lost cells. Because they are quiescent during aging, their activation may involve either *de novo* initiation of an alveolar ‘injury’ signal that is not expressed during aging, downregulation of a suppressive signal, or a combination.

Figure 4. A hierarchy of cellular programs for local replacement of alveolar cells

A summary of the cellular mechanisms for renewing and repairing alveolar epithelium. (A) The cartoon indicates the relevant cell types depicted below. (B) Three distinct cellular programs for renewal and repair are schematized in order of deployment. Cells are represented as follows: solid grey fill, resting cells; dotted grey outlines, dead or dying cells; dark colors, proliferating cells; faint colors, progeny of proliferating cells. Top row: during aging, the sporadic loss of an alveolar cell (e.g. an AT1 cell shown here) triggers proliferation of an AT2 stem cell (red). In this example, a daughter cell transdifferentiates into an AT1 cell to replace the one that was lost. Middle row: if an injury locally depletes more alveolar cells than the AT2 stem cell can rapidly replace, ancillary AT2 cells (green) are induced to proliferate and transdifferentiate. Bottom row: if an injury locally depletes all AT2 cells, the LNEP/DASC population (blue) is activated. These cells extensively proliferate, migrate, and differentiate into Club and AT2 cells, which presumably generate other bronchiolar lineages and AT1 cells, respectively.

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Figure 5. Lineage transitions between lung cell types observed during aging, after injury, and in cancer

A summary of reported *in vivo* lineage transitions between lung cell types in different contexts. Lineage transitions observed during renewal and following mild injury are classified as ‘constitutive’ (curved black arrows). Phenotypic switches only observed during repair or regeneration are classified as ‘facultative’ (curved colored arrows). Arrows are color-coded to indicate the specific cells targeted and experimental manipulations that elicited the indicated lineage conversion, described in text box. Straight black arrows indicate cell types of origin for specific cancer subtypes, and histologic and molecular transformations observed in these cancers. Grey filled boxes indicate cell populations that

are self-maintaining during renewal. ‘?’ indicates uncertainty whether the indicated lineage switch occurs due to the inability to specifically mark and trace these cell types (see text for details).

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