Niche Cells and Signals that Regulate Lung Alveolar Stem Cells In Vivo

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The distal lung is a honeycomb-like collection of delicate gas exchange sacs called alveoli lined by two interspersed epithelial cell types: the cuboidal, surfactant-producing alveolar type II (AT2) and the flat, gas-exchanging alveolar type I (AT1) cell. During aging, a subset of AT2 cells expressing the canonical *Wnt* target gene, *Axin2*, function as stem cells, renewing themselves while generating new AT1 and AT2 cells. *Wnt* activity endows AT2 cells with proliferative competency, enabling them to respond to activating cues, and simultaneously blocks AT2 to AT1 cell transdifferentiation. Acute alveolar injury rapidly expands the AT2 stem cell pool by transiently inducing *Wnt* signaling activity in “bulk” AT2 cells, facilitating rapid epithelial repair. AT2 cell “stemness” is thus tightly regulated by access to *Wnts*, supplied by a specialized single-cell fibroblast niche during maintenance and by AT2 cells themselves during injury repair. Two non-AT2 “reserve” cell populations residing in the distal airways also contribute to alveolar repair, but only after widespread epithelial injury, when they rapidly proliferate, migrate, and differentiate into airway and alveolar lineages. Here, we review alveolar renewal and repair with a focus on the niches, rather than the stem cells, highlighting what is known about the cellular and molecular mechanisms by which they control stem cell activity in vivo.

The skin, gastrointestinal (GI) tract and lung are all large epithelial surfaces of the body that are constantly exposed to the environment. Unlike the skin and GI tract, however, the lung is characterized by slow cellular renewal (Spencer and Shorter 1962; Evans and Bils 1969). Continuous turnover of the skin and GI epithelium is accomplished by dedicated stem cells residing in physically sheltered niches. This anatomical sequestration of stem cells may be protective against the development of cancer, because their

progeny that will be the first to encounter mutagenic toxins are destined to be rapidly extruded. The alveolar epithelium, in contrast, undergoes slow turnover during aging by intermittent activation of a small subset of “bifunctional” alveolar epithelial type II (AT2) stem cells, so-called because they constitutively execute both physiologic and regenerative functions (Logan and Desai 2015). Unlike stem cells for the skin and gut, the AT2 stem cells that renew alveoli during aging are not physically shielded from

N.H. Juul et al.

the environment; however, they make up a small proportion of the overall epithelial population (Desai et al. 2014). Although maintaining a small number of AT2 stem cells limits the opportunities for toxin-induced oncogenic transformation, it also carries the risk of inadequate regenerative reserve for surviving acute alveolar injury. The lung has evolved an elegant solution to this existential threat, by rapidly harnessing “bulk” AT2 cells to transiently participate in alveolar repair (Evans et al. 1973; Adamson and Bowden 1974). Two non-AT2 “reserve” alveolar progenitors, so-called because they are only induced following major lung injury, reside in the distal-most bronchioles and can regenerate both airway and alveolar epithelial cells. These facultative populations have been identified by using a variety of acute lung injury models, including ablation of specific epithelial cell types (diphtheria toxin, naphthalene); more generalized cell killing (hyperoxia, bleomycin, acid instillation); infection (H1N1 influenza or bacterial pneumonia); and stimulation of compensatory lung regrowth (unilateral pneumonectomy, refeeding after severe caloric restriction). Despite fundamental differences between these models (e.g., infectious vs. noninfectious, diffuse vs. alveolar-restricted injury), some generalizable principles of alveolar progenitor regulation have emerged.

Our most comprehensive understanding of progenitor regulation at present is of the AT2 stem cell, for which it is apparent that the niche controls stem cell number, proliferative competency, and differentiation outcome. However, substantial progress has recently been made in mapping the activity of the “reserve” progenitors by combining in vivo lineage tracing with experimental lung injury. Although the cellular niches harboring non-AT2 “reserve” alveolar progenitors have not yet been delineated, targeted gene deletion followed by induction of acute injury has revealed some of the signals important for their activity. Here, we summarize the available literature to present a “niche-centric” view of these distinct stem and progenitor cell populations. Our review includes what is known and outstanding about how they are controlled in health and perspectives on how their dysregulation may contribute to alveolar disease.

IDENTITIES, LOCATIONS, AND BEHAVIORS OF CELLS THAT RENEW AND REPAIR PULMONARY ALVEOLI

Anatomical Compartments and Cellular Microenvironments Harboring Alveolar Progenitors

There are at least three distinct cell populations that have been shown by in vivo fate mapping to generate new AT2 and AT1 cells (see Figs. 1 and 2, Legend panel). The originally identified and most studied is the AT2 cell (Kapanci et al. 1969; Adamson and Bowden 1974; Barkauskas et al. 2013; Desai et al. 2014), which also plays a critical physiological role in producing surfactant. AT2 cells express surfactant-associated protein C (*SftpC*) and are restricted to the distal gas-exchange (alveolar) region of the lung, where they are interspersed among flat AT1 cells in a monolayer epithelial sheet (Low 1953; Bertalanffy and Leblond 1955). In rat, AT2 cells have been estimated to be from 1.5 to 2 times as prevalent as AT1 cells, but the latter comprise 95% or more of the alveolar surface area because of their expansive morphology (Haies et al. 1981; Crapo et al. 1983). Although only a specialized subset of AT2 cells appears to act as stem cells during maintenance, “bulk” AT2 cells become transiently activated following acute alveolar injury, as described above.

A second population was originally shown to exhibit stem cell activity in vitro, capable of generating both alveolar and bronchiolar epithelial lineages in spheroid culture. This class, originally referred to as the bronchioalveolar stem cell (BASC) and now, as the EPCAM⁺/SCA1⁺ stem cell, coexpresses the AT2 marker *SftpC* and the club cell marker secretoglobin 1A1 (*Scgb1a1*) and resides at the end of the terminal bronchiole. It is estimated that between 25% and 50% of terminal bronchioles harbor at least one BASC (Kim et al. 2005). This *SftpC* and *Scgb1a1* double-positive population was recently specifically marked and lineage-traced in vivo and shown to contribute locally to both airway and alveolar lineages following severe acute lung injury (Liu et al. 2019).

A third alveolar progenitor class also resides outside the gas exchange compartment, in

Figure 1. Anatomic domains and cellular microenvironments for alveolar progenitor cells. The three best-characterized alveolar stem and progenitor cells are schematized within the distal lung (*left*) with a close-up of the microenvironment in which each resides (*right*). (AT2) Alveolar epithelial type II cell, (BASC) bronchioalveolar stem cell, (LNEP/DASC) lineage-negative epithelial progenitor/distal airway stem cell, (A) alveolar, (I) interstitial.

slightly more proximal bronchioles than the BASC. These progenitors were identified by two different research groups, and although it is not entirely clear whether they are the same population, we will consider them as such because they are similar in anatomical localization,

marker expression, and behavior (Kumar et al. 2011; Vaughan et al. 2015; Zuo et al. 2015). The lineage-negative epithelial progenitor (LNEP)/distal airway stem cell (DASC) comprises a minor population in distal bronchioles that resemble basal cells in morphology and by expression

N.H. Juul et al.

Figure 2. Signaling between alveolar stem and niche cells operative in specific experimental perturbations and contexts. (*Top left*) Panel summarizes each color-coded cell type and its position relative to the stem cells. Each additional panel schematizes the molecular signals and their sources that impact on the stem cell. The arrows are coded to indicate specific effects on the stem cell (legend *below* the panels), and the box outlining each panel is color-coded to indicate the experimental context in which the signaling is operative (*maintenance*; *repair*; *pneumonectomy*). (AT1) Alveolar epithelial type I, (AT2) alveolar epithelial type II, (BASC) bronchioalveolar stem cell, (Endo) endothelial cell, (FB) fibroblast, (LNEP/DASC) lineage-negative epithelial progenitor/distal airway stem cell, (L-cyte) lymphocyte, (AM) alveolar macrophage, (M2) M2 polarized macrophage, (Mono) monocyte, (PC) pericyte, (ASM) airway smooth muscle cell, (VSM) vascular smooth muscle, (?) unknown signal(s).

of keratin 5 (*Krt5*). Like basal stem cells that reside in more proximal airways, LNEP/DASCs can replenish club and ciliated lineages, but unlike basal cells they have also been shown to generate new AT2 cells (Rock et al. 2009). LNEP/DASC cells have not been comprehensively

mapped, but on average there is one LNEP per three small airways (Vaughan et al. 2015). Following widespread lung injury, LNEP/DASC cells extensively proliferate and spread into the alveolar compartment. Once there, a subset integrates into the epithelial monolayer, differen-

tiating into AT2 cells that can go on to generate new AT1 cells. The remainder curiously fail to either integrate or differentiate, remaining physically clustered as distinctive “pods” of cells that continue to express *Krt5* and presumably do not contribute to gas exchange (Ray et al. 2016).

AT2 Cells Are the Primary Stem Cells in Steady State and during Aging, whereas Non-AT2 Alveolar Progenitors Are Activated by Widespread Epithelial Injury

Broad genetic marking of the AT2 cell population followed by a long-term “chase” revealed that the proportion of labeled AT2 cells did not dilute over time, suggesting that mouse AT2 cells replenish themselves in adult life and during aging (Desai et al. 2014). This result implies that BASCs and LNEP/DASCs do not generate new AT2 cells in the absence of injury, which is corroborated by lineage-tracing experiments showing their lack of contribution to alveolar maintenance (Rawlins et al. 2009; Vaughan et al. 2015; Liu et al. 2019). After recovery from severe lung injury involving endotracheal bleomycin administration or H1N1 influenza pneumonia, however, the proportion of marked AT2 cells was reduced (Chapman et al. 2011). Because AT2 cells were broadly tagged before the injury, most unmarked AT2 cells observed after recovery presumably derived from non-AT2 cells. Candidate non-AT2 alveolar progenitors include BASCs, LNEP/DASCs, a minor population of integrin $\alpha 6 \beta 4^+$ alveolar epithelial cells, and AT1 cells (Chapman et al. 2011; Jain et al. 2015). Lineage-tracing supports the contribution of each of these classes to new alveolar cells except for the integrin $\alpha 6 \beta 4^+$ alveolar population, which has not yet been specifically marked and lineage traced in vivo.

BASCs have been shown to contribute to a modest number of cells in alveoli bordering terminal bronchioles following severe lung injury, whereas LNEP/DASCs massively expand and infiltrate the alveolar compartment. During their proliferation and distal spread, LNEP/DASC cells appear to maintain their basal cell-like phenotype, with a subset giving rise to new club and ciliated cells along the way.

Although some progeny are found to have generated AT2 cells, others remain physically clustered as nonphysiologic “keratin pods” described above. Whether or not LNEP/DASCs give rise to BASCs or vice versa following severe injuries involving the distal airways has not yet been specifically examined.

THE AT2 STEM CELL NICHE IS THE ALVEOLAR AGING NICHE

The AT2 Stem Cell–Fibroblast Connection: A Molecular Basis for an Intimate Physical Association

In the 1950s, when the emergence of electron microscopy (EM) enabled visualization of alveolar architecture at subcellular resolution, researchers noted that some AT2 cells appeared to physically contact underlying fibroblasts via protrusions across the intervening basal lamina (Sirianni et al. 2003). By ultrastructure, there appeared to be at least three distinct alveolar fibroblast classes. One of these, the lipofibroblast, was typically juxtaposed to AT2 cells and believed to supply them with lipid for the synthesis of surfactant, constituting a physiological fibroblast niche (Brody and Kaplan 1983; Endale et al. 2017). Subsequently, coculturing AT2 cells with $PDGFR\alpha^+$ alveolar fibroblasts (that were found to contain lipid droplets) was shown to enhance their expansion in vitro, suggesting that lipofibroblasts also produce factors that promote AT2 cell proliferation (Barkauskas et al. 2013).

We recently discovered that AT2 cell-adjacent $PDGFR\alpha^+$ fibroblasts constitute single-cell *Wnt* niches for AT2 stem cells, which correspondingly express the canonical *Wnt* target gene, *Axin2*, and comprise ~10% of the AT2 population (Nabhan et al. 2018). The niche fibroblast produces WNT5A (and possibly other *Wnts*) that signals in a juxtacrine manner to the adjacent AT2 stem cell and typically in an autocrine manner to itself (see Fig. 2, Fibroblast-AT2 panel). Although WNT5A was initially described as a noncanonical *Wnt* ligand, it was subsequently shown capable of signaling either canonically or noncanonically, depending on which receptor is expressed on the receiving

N.H. Juul et al.

cell (Mikels and Nusse 2006; van Amerongen et al. 2012). In vitro culture of purified AT2 cells confirmed that WNT5A induces expression of *Axin2* and enables AT2 cell proliferation, reproducing the effects of *Wnts* that only signal canonically. An important generalized property of WNT proteins is that they are hydrophobic, which restricts their ability to diffuse away from the cell that produces them. Thus, the observed intimate physical association between an AT2 stem cell and adjacent *Wnt*-producing *PDGFRα*⁺ fibroblast is essential for maintaining a functional *Wnt* signaling niche.

Wnt activity in AT2 stem cells appears to have two distinct functions: The first is enabling proliferation, without directly driving it, and the second is inhibiting transdifferentiation into an AT1 cell. Together, these effects of the fibroblast-produced WNT on AT2 cells can be considered as conferring and maintaining “stemness.” The role of *Wnt* activity in *PDGFRα*⁺ fibroblast niche cells that are often also *Axin2*⁺ has not yet been specifically examined, so it is unknown whether the autocrine *Wnt* signaling is integral to its AT2 stem cell niche function. As discussed below, alveolar fibroblasts have been shown to also receive signals from nearby cells that modulate their impact on neighboring AT2 stem cells. Overall, considering it also plays a role in supplying lipid substrate for surfactant biogenesis, the AT2 stem cell–associated alveolar niche fibroblast constitutes an integrated “hub” for alveolar physiology.

Two Mechanisms for Actively Restraining AT2 Cell Proliferation

In some tissues, like the intestine, continuous stem cell activity is a feature of normal physiology, whereas in others, like the *Drosophila* ovary, stem cell proliferation is tonically inhibited (Losick et al. 2011). Classical radiolabeled thymidine and stathmokinetic experiments revealed that the alveolar epithelium is remarkably quiescent—meaning AT2 stem cells spend most of their time in an inactive state (Spencer and Shorter 1962; Evans and Bils 1969). Recently, two mechanisms for active suppression of AT2 stem cell activity have been identified (see Fig. 2,

“Antiproliferation” symbols). One is mediated by claudin 18 (*Cldn18*), a tight junction protein whose genetic deletion results in the spontaneous development of multifocal lung adenomas, revealing its role in restraining AT2 cell proliferation (Zhou et al. 2018). The second involves an unidentified factor produced by *BMPRIα*-activated alveolar fibroblasts, inferred from studies using experimental unilateral pneumonectomy (Chung et al. 2018). This fibroblast-mediated inhibition of AT2 stem cell activation counterbalances the conferral of stemness by the provision of WNT ligand, providing a safeguard against inappropriate proliferation of *Axin2*⁺ AT2 stem cells. Altogether, the incorporation of multiple distinct anti- and proproliferative cues for AT2 stem cells confers resiliency to their renewal program, because acquired dysfunction of any given one may not be sufficient to disrupt their regulation.

Growth Factors that Drive AT2 Stem Cell Proliferation

Epidermal growth factor receptor (*EGFR*) activation is presumed to be an important trigger for AT2 stem cell proliferation in vivo, inferred from its implication as an oncogene for lung adenocarcinoma of AT2 cell origin (Lin et al. 2012; Xu et al. 2012; Desai et al. 2014). The provision of EGF ligands to cultured AT2 cells induces their proliferation, supporting this idea, although the source(s) and identity of the EGF ligand(s) within the AT2 stem cell niche remains unknown. One research group has shown *EGFR* activation to be important for alveolar regeneration after unilateral pneumonectomy (Ding et al. 2011). They propose a model in which vascular endothelial cells initiate production of *MMP14* whose proteolytic activity cleaves HBEGF, liberating the protein to signal to nearby AT2 cells and BASCs (see Fig. 2, Endothelial-BASC/AT2 panel). The cellular source of the *HbEGF* is unknown, as is the identity and source of the putative endothelial cell–activating signal. It is also not known whether this mechanism is operative during alveolar maintenance or after injury, which presumably activates different signals. Is the autocrine (injury) *Wnt* program activated in

“bulk” AT2 cells with pneumonectomy, and are endothelial cells activated by sporadic epithelial cell loss during aging? It will be important to uncover how niche factors regulating independent properties like stemness (*Wnt*) and proliferation (*EGFR*) are dynamically integrated by AT2 cells during maintenance and after injury.

AT2 cells have also been shown to proliferate when exposed to growth factors activating other receptor families, including insulin-like growth factor receptor (*IGFR*) and fibroblast growth factor receptor (*FGFR*). What specific roles these may play in driving AT2 stem cell proliferation in vivo remains to be elucidated. In a spontaneous emphysema mouse model due to a heterozygous mutation in the gene fibrillin 1, which results in dysfunctional collagen, exogenous provision of hepatocyte growth factor (*HGF*) attenuated airspace enlargement (Calvi et al. 2013). Although the operative mechanism has not been precisely established, genetic deletion in developing lung epithelial cells of the *HGF* receptor, *Met*, resulted in airspace enlargement. Together, these findings suggest *HGF* may play a role in alveolar maintenance by promoting epithelial cell survival or proliferation. The cellular sources of *HGF* and other candidate growth factors involved in aging and injury have not yet been spatially mapped in vivo in relation to AT2 stem cells.

Regional Aspects of Renewal by AT2 Stem Cells and Implications for Alveolar Diseases

During aging, AT1 cell renewal by AT2 stem cells takes place intermittently in patchy foci located in peripheral (submesothelial) and perivascular alveoli. Upon targeted induction of constitutively active *KrasG12D* in AT2 cells throughout the lungs, the ones residing in these same regions preferentially activate an adenomatous response (Desai et al. 2014). This enhanced responsiveness implies that AT2 cells in these specific regions are either intrinsically more stem-like, enjoy privileged access to mesothelial and vasculature produced factors that promote *Kras*-driven proliferation, or both. Submesothelial alveoli are also the site of the greatest AT2 cell proliferation during alveolar regenera-

tion after experimental unilateral pneumonectomy and upon refeeding after severe caloric restriction induced emphysema (Massaro et al. 2004; Voswinckel et al. 2004). Intriguingly, peripheral alveoli not only are enriched for age-related turnover, *Kras*-responsiveness, and neo-alveolarization but also are the initial site of disease in idiopathic pulmonary fibrosis (IPF) (Martinez et al. 2017). IPF, a disease in which stem cell dysfunction has been implicated, is characterized by distinctive patchy foci of submesothelial alveolar fibrosis and epithelial abnormalities (Carrington et al. 1978). In another disease of elderly smokers, emphysema, patchy foci of alveolar loss may be observed in the lung periphery, referred to as paraseptal emphysema. However, the pathologic alveolar destruction in emphysema is concentrated at the center of the pulmonary lobule, correlating with the initial entry site of inhaled pollutants into the gas exchange compartment via the terminal bronchioles.

INDUCIBLE ALVEOLAR AND AIRWAY NICHE SIGNALS

A Dynamic *Wnt*-Niche Switch: Deputizing “Bulk” AT2 Cells for Rapid Alveolar Repair

Despite its slow turnover in health, the alveolar compartment of the lung has a tremendous capacity to rapidly repair acute injury. We found that in the setting of hyperoxic lung injury and moderate lung epithelial ablation, in which the subset of *Axin2*⁺ AT2 stem cells may have insufficient capacity, “bulk” (i.e., *Axin2*-negative) AT2 cells are recruited as progenitors (Nabhan et al. 2018). This broad expansion of the alveolar stem cell pool is associated with induction of *Axin2* expression in the surviving AT2 cells. Remarkably, the source of *Wnt* for injury-activated AT2 cells is the AT2 cells themselves, which rapidly initiate expression of a panel of *Wnts* that signal in an autocrine manner (see Fig. 2, AT2 intrinsic panel). This switch from a juxtacrine to autocrine *Wnt* stem cell niche is short-lived, spontaneously resolving once the alveolar epithelium has been repaired. The injury-associated trigger for broad *Wnt* expression by AT2 cells

N.H. Juul et al.

is unknown, but induction of Wnts following damage has also been noted in other tissues, so it may be a generalized response to acute injury (Whyte et al. 2012). Interestingly, systemic inhibition of WNT signaling by administration of the porcupine inhibitor C59 results in impaired reepithelialization up to 5 days after injury, by which time much of the alveolar epithelium in control lungs has already been renewed (Nabhan et al. 2018). This notable lack of efficient repair by non-AT2 alveolar progenitors when AT2 cell proliferation has been abrogated by WNT inhibition suggests either that BASCs and LNEP/DASCs were not activated, respond more slowly, also depend on WNT, or are somehow insufficient in the diphtheria toxin-mediated lung epithelial ablation model that was used. Another group independently identified *Axin2*⁺ AT2 cells as making up ~20% of the AT2 cell population and functioning as alveolar stem cells for repair of H1N1 influenza pneumonia (Zacharias et al. 2018).

YAP1 Activity in AT2 Cells Drives Proliferation and Attenuates Alveolar Inflammation

Following unilateral pneumonectomy in rodents, transpulmonary pressures are increased in the remaining lung lobes that undergo compensatory growth with regenerative alveolarization (Hoffman et al. 2010). It was shown almost half a century ago that regeneration was blocked if a wax cast was placed into the evacuated thorax, implicating the alveolar distending force in driving this process (Brody et al. 1978). More recently, it has been reported that AT2 cells and BASCs proliferate postpneumonectomy in response to elevated mechanical tension (see Fig. 2, AT2 intrinsic panel). The Hippo signaling pathway effector, Yes-associated protein 1 (*Yap1*), was found to be required for AT2 cell proliferation and the process of neo-alveolarization (Liu et al. 2016). In another context, *Yap1* was implicated as the driver of spontaneous AT2 cell proliferation resulting from genetic deletion of *Cldn18*, caused by loss of sequestration of YAP1 protein at the cell junction (see Fig. 2, AT2 intrinsic panel) (Zhou et al. 2018). As discussed below, *Yap1* and *Taz* activity in AT2 cells

has also been shown to facilitate alveolar repair by suppressing inflammation in the setting of bacterial pneumonia (LaCanna et al. 2019). *Yap1* activity in AT2 cells has thus been implicated as an important promoter of alveolar repair by two different mechanisms, driving AT2 stem cell proliferation and attenuating the immune response after injury.

Resident and Recruited Immune Cells Modulate AT2 Cell Activation in Repair and Regeneration

There is evidence that immune cells have important noncanonical effects on alveolar epithelial stem cell activity, and sessile macrophages have even been reported to share direct physical connections with AT2 cells (Westphalen et al. 2014). During homeostasis, in addition to patrolling for pathogens, alveolar macrophages cooperate with AT2 cells in surfactant homeostasis. During infection, both AT2 cells and macrophages produce factors that recruit immune cells like neutrophils and T-effector cells to the site of injury. After cessation of recruitment, neutrophils and some macrophages undergo apoptosis, although the remaining cells modulate alveolar epithelial restoration (Aggarwal et al. 2014). Some genetic studies have examined communication between AT2 and immune cells in vivo during infection. In one recent report, conditional deletion of *Yap1* and *Taz* in AT2 cells in the setting of bacterial pneumonia resulted in excessive proinflammatory cytokine production, resulting in delayed alveolar repair (LaCanna et al. 2019).

In other settings, immune cells have been found to facilitate, rather than impair, alveolar regeneration (see Fig. 2, Immune-AT2 panel). One such mechanism following experimental unilateral pneumonectomy has been well-delineated. Here, secretion of *CCL2* by AT2 cells recruits *CCR2*⁺ monocytes to the lung where they mature and become alternately activated via innate lymphoid cell 2 (ILC2)-produced interleukin (IL)-13, whereupon they promote AT2 cell proliferation through an unknown mediator (Lechner et al. 2017). In another study, *IL-1β* and tumor necrosis factor α (*TNF-α*) were in-

duced by H1N1 influenza pneumonia and found to signal directly to AT2 cells to promote their proliferation (Katsura et al. 2019). Future studies will be important for dissecting the particular immune cell populations and specific signals that mediate these at times opposing effects on alveolar repair in infectious and noninfectious states.

Summoning “Reserve” Alveolar Progenitors and an Airway Smooth Muscle FGF Niche

How are progenitor cells residing in airways able to remotely sense and respond to cell loss in the alveolar compartment? One possibility is that they receive activating signals via an intermediary—for instance, mobile immune cells broadly infiltrating an injured lung. In this regard, it is interesting that monocytes are recruited to the lung following unilateral pneumonectomy, despite the absence of injured or dying cells. Another possibility is that the injury directly involves the airway progenitor cell microenvironment, which would explain why reserve progenitors have been found to be activated when the damage is not limited to the alveolar compartment—for instance, with bleomycin or H1N1 influenza. To properly generate new alveolar cells, LNEP/DASC and BASC cell progeny must migrate distally along the bronchiolar surface to reach the gas exchange compartment. This ingress might be facilitated by damaged terminal airways along which progenitors could move without having to dynamically remodel epithelial cell–cell junctions. Loss of epithelial integrity might also derepress “reserve” airway progenitors if, like AT2 cells, their proliferation is inhibited by sequestration of proteins like YAP1 at cell–cell junctions. It currently remains unknown which, if any, of these potential mechanisms for alerting the airway “reserve” progenitors to alveolar perturbations is operative in vivo.

For LNEP/DASC and BASC cells, it has recently been shown that with severe acute injury, airway smooth muscle cells produce *FGF10*, which induces proliferation and migration of nearby epithelial progenitors (see Fig. 2, SMC-LNEP/BASC panel). This study found that de-

letion of either *FGF10* in smooth muscle cells or its receptor, *FGFR2b*, in airway epithelial cells markedly reduced the number of *Krt5*⁺ LNEP/DASCs found in the terminal bronchioles and alveoli following bleomycin administration (Yuan et al. 2019). There was also an accompanying lack of AT2 cell differentiation, although whether this resulted from a failure of the cells to reach the alveoli or directly from the absence of *FGFR* activation is unclear.

The niche cells and signals that control LNEP/DASC and BASC stemness in vivo remain unknown. More proximally in the trachea, however, *FGF* signaling has been shown to maintain basal cell “stemness.” One research group reported that haploinsufficiency of *FGFR2* in *Krt5*⁺ basal cells resulted in stem cell depletion caused by differentiation without self-renewal (Balasooriya et al. 2017). Another study identified thrombospondin 1 (*TSP1*) produced by vascular endothelial cells in a *BMP4*-dependent manner as favoring BASC differentiation toward an AT2 cell phenotype, as opposed to bronchiolar lineages, on airway and alveolar injury (see Fig. 2, Endothelial-BASC/AT2 panel; Lee et al. 2014).

LEAVING THE NICHE: SEQUENTIAL SIGNALING ORCHESTRATES REGENERATIVE OUTCOMES

WNTs not only endow AT2 cells with proliferative capacity but also impact their differentiation status. Either deletion of *β-catenin* in vivo or inhibition of *Wnt* in vitro triggers AT1 transdifferentiation of a subset of AT2 cells, presumably *Axin2*⁺ stem cells because of abrogation of *Wnt* signaling. In contrast, experimental induction of stabilized *β-catenin* in AT2 cells does not drive proliferation but blocks AT1 transdifferentiation (Nabhan et al. 2018). In vivo, following physiologic *Axin2*⁺ AT2 stem cell duplication, one daughter cell is by default physically excluded from proximity to the WNT-producing fibroblast niche. Yet, lineage tracing of *Axin2*⁺ AT2 cells reveals that they generate both AT1 and AT2 cells, indicating that abrogation of active *Wnt* signaling is not always sufficient to drive AT1 transdifferentiation. Indeed, a recent

N.H. Juul et al.

report using bleomycin injury and AT2 cell spheroid culture found that the Hippo signaling pathway effector, *Taz*, is required for AT2 to AT1 cell conversion (Sun et al. 2019).

The temporal sequence and hierarchy of signals mediating proper execution of the proliferation, migration, and differentiation steps of the alveolar progenitor programs are beginning to be elucidated (see Fig. 3). For AT2 cells, it appears multistep signaling via *BMPR1α* is important for generating new AT1 cells. *BMP* signaling is initially down-regulated in AT2 cells during proliferation, and then it increases during AT1 cell transdifferentiation (Chung et al.

2018). *Notch* signaling, in contrast, is induced during AT2 cell activation and then is down-regulated during conversion into an AT1 cell (Finn et al. 2019). Interestingly, *Notch* is also important for LNEP/DASC activation, but subsequent AT2 cell differentiation is promoted by inhibition of *Notch* and induction of *Wnt* signaling (Vaughan et al. 2015). Thus, down-regulation of *Notch* can be associated with either loss or acquisition of the AT2 cell phenotype, depending on context. This divergent response to the identical signal suggests a hysteresis, in which the differentiation outcome of a lung progenitor is affected by preceding steps in the cell's

Figure 3. Dynamic signaling in alveolar stem cells that sequentially drive proliferation and differentiation. Signaling pathway activity (dark shade indicates active) in alveolar type II (AT2) cells (*top*) and LNEP/DASC cells (*bottom*) during sequential steps of their regenerative programs. “Stem” indicates *Axin2*⁺ and “Bulk” indicates *Axin2*[−] AT2 cells. Dashed outlines represent dying cells.

history. Additional work is needed to fully unravel the extrinsic signals that orchestrate the proper sequence and timing of each step in the distinct alveolar regenerative programs.

AT2 STEM CELL NICHES AND SIGNALS IN LUNG ADENOCARCINOMA

The appreciation that every adenocarcinoma harbors molecularly heterogeneous tumor cells ushered in an experimental era of prospectively isolating distinct epithelial subsets and comparing their cancer-propagating activity when transplanted into immunocompromised mice. This approach is based on the idea that the most efficient subset may be selectively endowed with stem cell-like capacity that makes it essential for continued growth of the primary tumor and, thus, an attractive therapeutic target (Kreso and Dick 2014). However, a recent study showed in a mouse model of colon cancer that targeted elimination of a tumor propagating subset in vivo resulted in its replenishment by other cells that altered their phenotype, along with a failure to eradicate the primary tumor (de Sousa e Melo et al. 2017). This result illustrates how cells in the tumor microenvironment can exert profound control over epithelial cells in cancer, as they do on stem cells in normal physiology. Although some niche interactions in cancer may mediate susceptibility or resistance to elimination by infiltrating immune cells, others recapitulate their normal physiologic function in the specific epithelial cell type that generated the tumor.

In the lung, AT2 cells are susceptible to adenomatous transformation by constitutive activation of *EGFR* or the downstream *Kras* pathway, which appears to normally drive their proliferation in health. Intriguingly, *Wnt* activity in adenocarcinoma is important for cancer progression even though *Kras* is constitutively active, suggesting that its physiologic role in conferring proliferative competence and blocking AT1 transdifferentiation continues to be required (Tammela et al. 2017). Another example is M2 polarized alveolar macrophages, which have long been known to promote lung adenoma progression, mirroring their physiologic

Signals that Regulate Lung Alveolar Stem Cells

effect in enhancing regenerative neo-alveolarization by AT2 cells after pneumonectomy (Zaynagetdinov et al. 2011). Recently, a study showed attenuation of *Kras*-driven lung adenoma growth with inhibition of $\gamma\delta$ T-cell activation, implicating a different immune-mediated pathway that promotes epithelial cell proliferation (Jin et al. 2019). As with the M2 macrophage effect, $\gamma\delta$ T cells augment proliferation in epithelial cells that already have constitutive *Kras* activity, suggesting they drive proliferation through a distinct (non-*Kras*) pathway and/or somehow augment “stemness.” Figure 4 schematizes what is currently known about the lung adenocarcinoma “tumor niche” interactions. Future work is needed to identify and characterize additional niche cells and signals that regulate healthy alveolar regeneration and may be conserved in cancer, including interactions mediating more complex processes like neo-angiogenesis.

Figure 4. Lung adenocarcinoma “tumor niche.” Tumor-associated cells and the signals they produce that have been shown to confer proliferative competence (yellow arrows) and augment proliferation (green arrow) of epithelial tumor cells in *Kras*-driven lung adenocarcinoma. (FB) Fibroblast, ($\gamma\delta$) $\gamma\delta$ T cell, (M2 Φ) alternately activated macrophage, (?) unknown signal(s).

N.H. Juul et al.

CONCLUDING REMARKS

Mouse genetics has enabled the identification of distinct niche cells and signals that influence alveolar stem cell behavior in vivo under different conditions. One regulatory mode involves tonic provision of a single factor to maintain a specific state, exemplified by *Wnt* activity in AT2 stem cells mediating proliferative competency and differentiation inhibition. More complex behaviors involve the orchestrated execution of discrete stem cell activities—for instance, abrogation of quiescence, proliferation, migration, and differentiation of LNEP/DASCs after severe lung injury. Such stereotyped stem cell “programs” necessitate precise coordination of signaling arcs involving multiple pathways and may involve hysteresis, making it difficult to reconcile apparently disparate effects of the same factor in different physiologic contexts. Integrating diverse experimental findings will bring us closer to a holistic understanding of how alveolar stem cells are regulated in vivo. A critical next step is to translate these findings from mouse into human lung, which has been facilitated by the development of organoid assays, cocultures, and intact lung slice cultures (Nikolić and Rawlins 2017). The latter approach is particularly important because it enables manipulation of alveolar stem cell signaling within the context of a mostly intact niche, essential for testing candidate therapeutics (Lehmann et al. 2017). The comprehensive identification of the diverse signals and their effects on human alveolar stem cells and how they are specifically perturbed in disease will ultimately provide a blueprint for developing new regenerative and anticancer therapies.

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Signals that Regulate Lung Alveolar Stem Cells

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N.H. Juul et al.

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