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Basal-like Progenitor Cells: A Review of Dysplastic Alveolar Regeneration and Remodeling in Lung Repair

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Despite the central importance of the respiratory system, the exact mechanisms governing lung repair after severe injury remain unclear. The notion that alveolar type 2 cells (AT2s) self-renew and differentiate into alveolar type 1 cells (AT1s) does not fully encompass scenarios where these progenitors are severely affected by disease, e.g., H1N1 influenza or SARS-CoV-2 (COVID-19). Intrapulmonary p63$^+$ progenitor cells, a rare cell type in mice but potentially encompassing more numerous classic basal cells in humans, are activated in such severe injury settings, proliferating and migrating into the injured alveolar parenchyma, providing a short-term "emergency" benefit. While the fate of these cells is controversial, most studies indicate that they represent a maladaptive repair pathway with a fate restriction toward airway cell types, rarely differentiating into AT2 or AT1 cells. Here, we discuss the role of intrapulmonary basal-like p63$^+$ cells in alveolar regeneration and suggest a unified model to guide future studies.

Introduction

The lung is unique among mammalian vital organs; while it exhibits low cellular turnover during homeostasis, the lung can regenerate through widespread proliferation and differentiation of resident progenitor cell populations upon injury. Epithelial insults and respiratory infections such as influenza can disrupt the gas exchange units of the lung, the alveoli, destroying alveolar epithelial type 2 (AT2) and type 1 (AT1) populations, which in extreme cases results in diffuse alveolar damage (DAD) and acute respiratory distress syndrome (ARDS). While likely an oversimplification, speaking generally two distinct regenerative responses to lung injury can occur in parallel within the alveoli: functionally beneficial regeneration that typically follows mild-to-moderate pulmonary injury, and maladaptive epithelial remodeling that follows severe lung injury exemplified by pandemic strains of H1N1 influenza and likely SARS-CoV-2 (COVID-19). During functionally beneficial regeneration, surviving AT2 cells self-renew and differentiate into oxygen-exchanging AT1 cells (Barkauskas et al., 2013; Evans et al., 1973), providing the damaged lung with a source of essential surfactant and gas-exchanging epithelial surface. In more severely injured regions of alveolar parenchyma, maladaptive repair occurs in which injury-associated hypoxia activates a population of rare, airway-resident *intrapulmonary p63$^+$ progenitors* that migrate into and proliferate within the alveoli (Kumar et al., 2011; Vaughan et al., 2015; Zuo et al., 2015), forming an ectopic bronchiolar-like epithelium expressing markers of basal stem cells, such as cytokeratin 5 (*Krt5*) and *Trp63* (referred to hereafter as p63, indicating both protein and gene expression) and, later, differentiated airway epithelial markers, including *Scgb1a1* and *Scgb3a2* (Kanegai et al., 2016; Vaughan et al., 2015) (Figure 1). Notably these intrapulmonary progenitor cells in humans likely encompass the much more abundant "classic" basal cells, already expressing both *Krt5* and p63, present throughout the airways. Considering the ongoing COVID-19 pandemic, which similarly induces ARDS accompanied by DAD and a sustained decrease in pulmonary function in some individuals (Cheung, 2020; Salehi et al., 2020), defining the response of intrapulmonary p63$^+$ progenitors to injury and the mechanisms underlying their sustained activation, persistence, and differentiation is critical in understanding and treating severe pulmonary insults. Here, we attempt to provide a comprehensive and unified overview of this phenomenon, which, we posit, ultimately represents maladaptive epithelial remodeling founded upon a short-term benefit.

Foundations of Basal-like Cell Expansion

While injury-induced expansion of intrapulmonary p63$^+$ cells has now been best characterized in mouse models, to our knowledge the first actual demonstration of this phenomenon was in humans. After the 1957 influenza pandemic, it was observed in explanted patient tissue that the lung epithelium was able to apparently repair starting 5 to 7 days after infection by replacing the damaged cells with a "stratified pseudo-metaplastic layer" in the respiratory bronchioles and alveolar ducts and a monolayer of epithelial cells in alveolar regions, "giving the impression of neoplastic growth" (Hers et al., 1958). Almost two decades later, this finding was confirmed in mice: after experimental infection of mice, Loosli et al. (1975) demonstrated that H1N1 (PR8) influenza partially destroyed alveolar epithelial cells and profoundly disrupted lung function, followed later by the onset of fibrosis. Provocatively, the

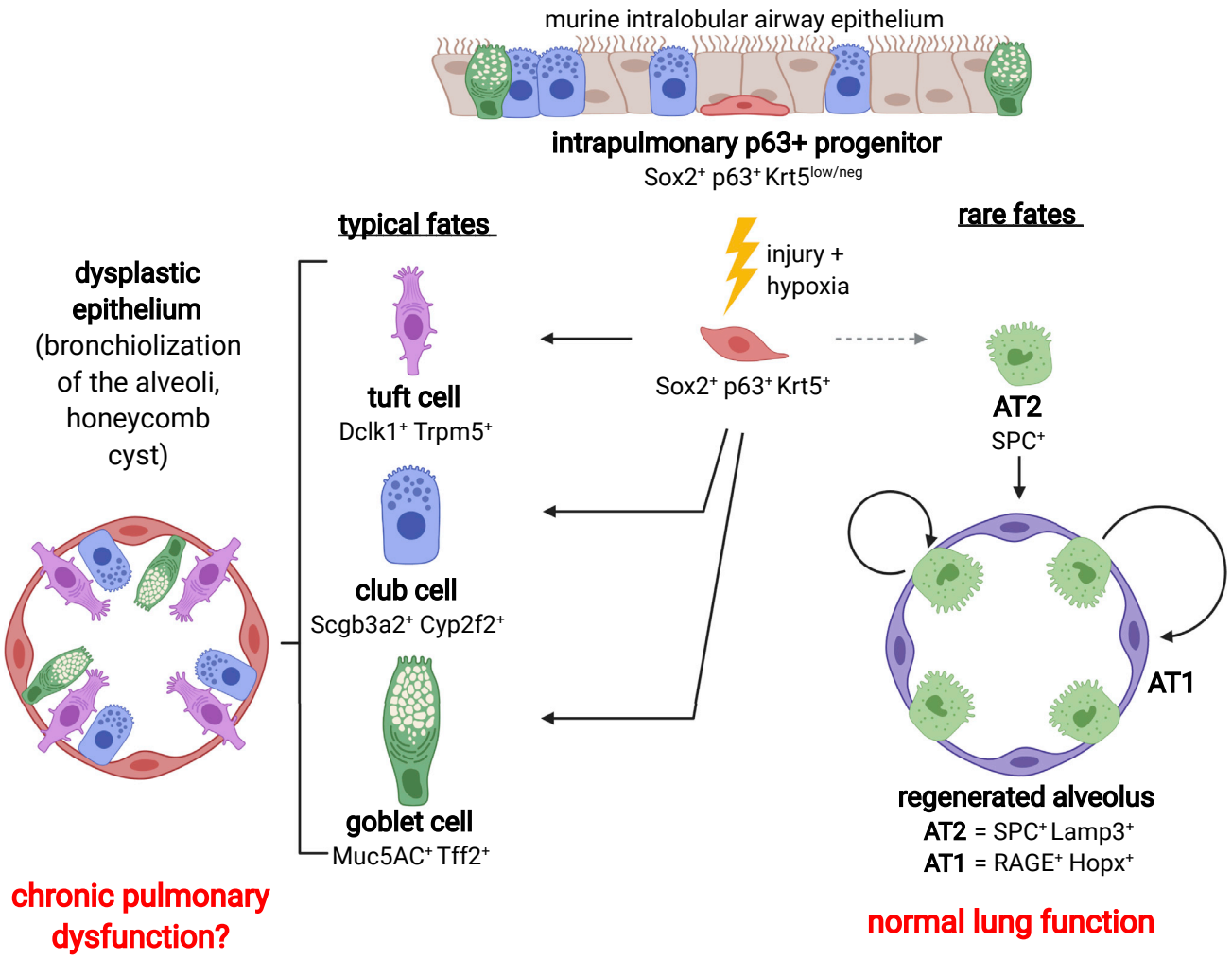

Figure 1. Overview of Activation, Expansion, and Differentiation of Intrapulmonary p63+ Progenitor Cells Upon Lung Injury

Upon injury and subsequent hypoxia, rare intrapulmonary p63+ progenitor cells are activated, upregulate other basal cell markers, including *Krt5*, and proliferate and migrate distally into the small airways and alveoli. These cells have been demonstrated to readily generate airway cell types (left), but only rarely give rise to normal alveolar cell types (right), thus representing a dysplastic alveolar repair response. This may result in persistent loss of lung function where a significant fraction of the alveolar epithelium has been replaced by dysplastic, "bronchiolized" epithelium. Figure created with BioRender.com.

authors also described the formation of cystic lesions that replaced AT2s, were adjacent to fibrotic areas, and did not resolve with time, ultimately proposing that these "epithelial nodules" had a cellular origin in the bronchiolar epithelium (Loosli et al., 1975). Interpretation of these early studies is nonetheless limited by the lack of immunohistochemical staining and lineage-tracing analysis, and these reports were largely forgotten for nearly 40 years.

Many years later, alveolar epithelial injury after murine infection with the same H1N1 strain used by Loosli et al. was shown to induce the dramatic appearance of proliferating epithelial cells expressing cytokeratin 5 (*Krt5*) and the transcription factor p63 (*Trp63*), bearing markers of trachea basal cells but appearing ectopically in small airways and alveoli. Given that basal cells are normally restricted to the trachea in mice, the development of what appeared to be airway basal cells in the alveoli was an overtly dramatic and seemingly bizarre observation, capturing the immediate attention of the pulmonary research community. These cell clusters, often called "pods," eventually organized into structures resembling alveoli (Kumar et al., 2011), similar to the cysts noted by Loosli et al. (1975). It has been suggested that pod formation represents a bona fide regenerative response to lung injury, i.e., that *Krt5*+ p63+ cells ultimately differentiate into AT2s and AT1s, although the initial report did not definitively demonstrate this

transdifferentiation via Cre-mediated fate mapping (Kumar et al., 2011). As discussed later, there is in fact scant evidence that these *Krt5*$^+$ pods contribute significantly to normal alveolar cells types in most contexts (Kanegai et al., 2016; Ray et al., 2016; Vaughan et al., 2015; Xi et al., 2017; Yang et al., 2018). Nonetheless, many subsequent reports confirmed the appearance of ectopic *Krt5*$^+$ cell expansion into the alveoli (Ray et al., 2016; Xi et al., 2017; Yang et al., 2018; Zuo et al., 2015), and the occurrence of this phenomenon is no longer considered controversial.

Spurred by these studies in mice, recent work has confirmed that basal cell-like expansion occurs readily in human cases of severe lung injury (Taylor et al., 2018). The authors of this study specifically examined biopsies and autopsy samples of ARDS patients diagnosed with DAD, finding that 39% of these cases exhibited peribronchiolar basaloid pods staining for KRT5 and p63, which the authors remarked "mirrored data from murine models of lung injury/regeneration." These data also seem to mirror the original 1958 report of Hers et al., acknowledging of course that without immunohistochemistry one cannot be certain they are describing the same phenomenon. While bronchiolization/honeycomb cysts featuring basal-like cells is a well-described histopathological feature of interstitial lung disease (Plantier et al., 2011; Seibold et al., 2013), it appears that, as in mice, it is also a common feature of more acute lung injury. Importantly, DAD has been frequently observed in COVID-19-related ARDS (Xu et al., 2020), suggesting KRT5$^+$ basal-like cell expansion may play an important role in that setting as well, although this has not yet been directly demonstrated.

The ectopic appearance of *Krt5*$^+$ pods in the small airways and alveoli after lung injury seems to be correlated to the severity of the disease. It has been exhaustively demonstrated that mild-to-moderate injury induces AT2 proliferation and differentiation into AT1s (Barkauskas et al., 2013; Evans et al., 1975), re-establishing the gas-exchanging alveolar epithelial surface without the formation of bronchiolization/dysplasia. However, there appears to be a threshold for depletion of the alveolar epithelium after which barrier restitution can no longer be achieved solely by the surviving AT2s. One very informative report took advantage of a relatively mild H3N2 influenza virus, HK-x31, which causes airway injury but only minor AT2 loss and no *Krt5*$^+$ cell expansion. Only by first ablating ~50% of AT2s (via diphtheria toxin) and then infecting with HK-x31 was *Krt5*$^+$ cell expansion observed (Yee et al., 2017). Although the numerical threshold of alveolar epithelial cell death required to observe *Krt5*$^+$ expansion is unclear, it appears that in extreme cases of AT2 loss an alternate epithelial repair pathway must be engaged to maintain barrier function and prevent death of the organism (Vaughan et al., 2015). It is important for future studies to assess whether *Krt5*$^+$ dysplastic expansion is a function *only* of a threshold loss of alveolar epithelium or whether injury-associated cues, perhaps particular inflammatory cytokines, might be required for basal-like cell expansion to occur.

The Dysplastic Cell-of-Origin in Mice and Humans

While expansion of *Krt5*$^+$ cells after severe injury is dramatic, there are nonetheless no pre-existing *Krt5*$^+$ or p63$^+$ cells present in the alveoli in either mice or humans, so the origin of these cells was not immediately obvious. Extensive lineage-tracing studies have since elucidated the cell-of-origin of post-injury *Krt5*$^+$ pods in the murine lung. Utilizing the p63-CreERT2 mouse (Lee et al., 2014), it has been established that virtually all *Krt5*$^+$ pods arise from pre-existing intrapulmonary p63$^+$ progenitors (Xi et al., 2017; Yang et al., 2018). These cells are absent in the alveoli at baseline and instead reside in the intrapulmonary airways before their injury-induced activation and migration (Vaughan et al., 2015; Yang et al., 2018; Zuo et al., 2015). Despite their localization to the airways, similarities in cellular markers, and common embryonic origin (Yang et al., 2018), intrapulmonary p63$^+$ progenitors in mice constitute a distinct stem cell population from tracheal basal cells, as bona fide basal cells from the trachea do not appear to migrate into the alveoli after injury as demonstrated by transplant of fluorescent tracheas into non-fluorescent isogenic mice followed by influenza infection (Vaughan et al., 2015). Instead, these rare intrapulmonary p63$^+$ cells become activated by injury and proliferate and migrate significantly, to result in the expansive "blooms" of *Krt5*$^+$ pods in injured alveoli.

One point of contention surrounding the cell-of-origin of *Krt5*$^+$ pods concerns their markers during homeostasis aside from p63. It was originally thought that intrapulmonary p63$^+$ progenitors expressed the basal cell marker *Krt5* and its heterodimerization partner *Krt14* before injury, similarly to basal cells in other epithelia (e.g., the trachea, skin, mammary gland). As such, two of the original studies documenting the origin of *Krt5*$^+$ pods utilized both Krt5-CreERT2 and Krt14-CreERT2 lineage-tracing methods to isolate and mark intrapulmonary p63$^+$ progenitors (originally called distal alveolar stem cells [DASCs]) before and after injury (Kumar et al., 2011; Zuo et al., 2015). Later studies indicated that the majority of intrapulmonary p63$^+$ progenitors do not express appreciable *Krt5* at baseline, and that the abundance of Krt5/Krt14-traced p63$^+$ progenitors originally noted was due to tamoxifen persistence (Vaughan et al., 2015). Indeed, tamoxifen persistence and prolonged recombination have been reported in other systems and can severely confound interpretations of lineage-tracing experiment results (Reinert et al., 2012). In this scenario, inadequate chase periods between administration of the final tamoxifen dose and subsequent injury

results in residual tamoxifen inducing recombination in intrapulmonary p63$^+$ progenitors as they respond to injury and upregulate *Krt5*, explaining initial reports that *Krt5*$^+$ pods are derived from pre-existing *Krt5*$^+$ cells. Instead, lineage tracing indicates that most pods are derived from non-*Krt5*-expressing progenitor cells and that only 15%–20% of post-injury *Krt5*$^+$ pods arise from cells expressing appreciable *Krt5* (Ray et al., 2016; Vaughan et al., 2015). Considering that most *Krt5*$^+$ pods come from a *Krt5*^{negative} source and all *Krt5*$^+$ pods are derived from intrapulmonary p63$^+$ progenitors, it was surmised that most of these cells adopt *Krt5* expression in response to injury (Vaughan et al., 2015). These findings have been confirmed through reports that pre-existing *Krt5*-expressing cells make minimal contributions to post-injury *Krt5*$^+$ cell expansion (Ray et al., 2016; Yang et al., 2018; Zheng et al., 2014).

There is also debate as to whether intrapulmonary p63$^+$ progenitors express the club cell marker *Scgb1a1* (also known as CC10). Club cells themselves exhibit stem/progenitor properties in the airways, demonstrated by their ability to dedifferentiate into basal cells (Tata et al., 2013) and their ability to regenerate the distal airway epithelium after chemical injury (Rawlins et al., 2009). Indeed, one report suggested that *Krt5*$^+$ cells arise from transdifferentiated club cells (Zheng et al., 2014). In Vaughan et al., 2015, the CC10-CreERT2 (aka Scgb1a1-CreERT2) mouse was used to label club cells before injury to determine if they contributed to alveolar regeneration. As long as proper chase time was used, regenerated cells in the alveoli that arose from pre-existing club cells were found to be entirely separate from post-injury *Krt5*$^+$ pods, suggesting that pre-existing CC10$^+$ club cells do not give rise to *Krt5*$^+$ dysplastic cysts (Vaughan et al., 2015). This too has been confirmed by reports of contribution of club cells to $\leq$1% of *Krt5*$^+$ pods after injury (Ray et al., 2016). However, others refute this finding, reporting variable contribution of pre-existing CC10-labeled cells to post-injury *Krt5*$^+$ pods (Yang et al., 2018; Yuan et al., 2019; Zheng et al., 2014). It should be noted that the tamoxifen washout periods used in these publications differs. Tamoxifen persistence from the relatively short chase period of 1 week could explain the higher percentage of CC10-labeled *Krt5*$^+$ pods described in some studies (Zheng et al., 2014) as opposed to lower percentages found with a longer chase period of 21 days (Yang et al., 2018). Possibly contributing to this ambiguity, the CC10-CreERT2 line is notable for labeling cells outside of the club cell lineage, exhibiting recombination in up to 8% of AT2s (especially those residing near terminal airways) (Rawlins et al., 2009) and a significant number of ciliated cells (Kathiriya et al., 2020). Other reasons for the discrepancies in the reported contribution of club cells to post-injury *Krt5*$^+$ areas remain unclear, especially since all aforementioned publications utilize the same CC10-CreERT2 mouse (Rawlins et al., 2009). Still, the uncertainty of CC10 expression in some intrapulmonary p63$^+$ progenitors does not alter their fundamental identity as an intrapulmonary basal-like progenitor cell which can be uniformly characterized by p63 expression.

Importantly, bronchiolization/dysplastic remodeling appears histologically quite similar in both mice and humans (Taylor et al., 2018), indicating that this response is likely evolutionarily conserved among mammals. That said, recognizing that important cellular and anatomical differences exist across species (nicely reviewed in Basil and Morrisey, 2020), the cell-of-origin for alveolar *Krt5*$^+$ expansion may not itself be conserved. While classic basal cells (*Krt5*$^+$ p63$^+$) persist only as far distally as the mainstem bronchi in mice, larger mammals, including humans, possess basal cells persisting distally all the way to the terminal bronchioles (Evans et al., 2001). It therefore seems quite possible that post-injury bronchiolization in humans is derived from classic basal cells rather than the rare intrapulmonary p63$^+$ *KRT5*^{negative} progenitors present in mice. However, several groups have also observed p63$^+$ cells lacking KRT5 and KRT14 staining present in distal airways of human lungs (Smirnova et al., 2016; Vaughan et al., 2015), which could possibly preferentially expand into the alveoli upon injury akin to the intrapulmonary p63$^+$ cells in mice. Further complicating this question is the recent identification of additional "basaloid" cells present in bronchiolized epithelium identified by single-cell RNA sequencing of human idiopathic pulmonary fibrosis samples. These aberrant basal-like cells express some basal cell markers (p63, KRT17) but lack KRT5 (Adams et al., 2020; Habermann et al., 2020). Whether this KRT5^{negative} basaloid expansion occurs after DAD, especially that induced by respiratory viruses, is unknown. Finally, it is also possible that, in humans, AT2s upregulate basal cell genes to give rise to KRT5$^+$ dysplasia, as has been observed in *in vitro* hypoxia experiments (Xi et al., 2017), although this does not occur in mice (Vaughan et al., 2015). While lineage-tracing studies cannot be performed in humans, it may be possible to begin to interrogate the cell-of-origin of human lung bronchiolization utilizing cell barcoding in human precision-cut lung slices *ex vivo* or to at least make inferences from pseudotime analysis of single-cell transcriptomic analyses. Ultimately, while the cell-of-origin may differ, dysplastic expansion of basal-like cells occurs quite similarly across species, or at least in mice and humans.

The Acute Role and Ultimate Fate of Intrapulmonary p63$^+$ Progenitors

In light of the apparent destruction of the native alveolar epithelium, it has been assumed that intrapulmonary p63$^+$ progenitors are important immediately after alveolar injury to re-epithelialize the denuded alveolar epithelium

Table 1. Differentiation of Intrapulmonary p63$^+$ Basal-like Cells after Influenza Injury

Intrapulmonary p63$^+$ Basal-like Cell Fate after Influenza Injury	Endogenous Differentiation Frequency	Notes and Evidence
AT2 cells	very rare*	*Differentiation potential of intrapulmonary p63$^+$ cells into AT2 cells is higher after bleomycin injury, up to ~30% of Krt5-traced cells.
AT1 cells	very rare	PDPN expression is shared by basal cells, so reports of Krt5-traced AT1 cells are inconclusive
Club cells	common	CC10 and SCGB3A2 staining
Goblet cell	common	MUC5AC and TFF2 staining
Ciliated cells	unknown	
Tuft cells	common	DCLK1 staining and Trpm5-GFP reporter
Neuroendocrine cells	unknown	
Ionocytes	unknown	

and restore barrier function. Perhaps the best evidence for this is the demonstration that diphtheria toxin-mediated ablation of expanding *Krt5*$^+$ cells results in mice with permanent, dramatic reduction in oxygen saturation and the development of profound, non-resolving immune infiltrates and fibrosis (Zuo et al., 2015). Moreover, as discussed earlier, mice in which AT2s are experimentally depleted have increased levels of *Krt5*$^+$ pod expansion after influenza infection, suggesting that intrapulmonary p63$^+$ progenitors can "sense" disturbances in the alveolar epithelium and act quickly to "remedy" epithelial defects (Yee et al., 2017). These studies imply that expansion of p63$^+$ cells into *Krt5*$^+$ dysplastic epithelium serves to rapidly restore epithelial barriers in acute injury settings. But what then becomes of these alveolar *Krt5*$^+$ cells?

Despite the growing consensus that intrapulmonary p63$^+$ progenitors primarily give rise to *Krt5*$^+$ pods and ultimately bronchiolized epithelium as opposed to alveolar cell types (Table 1), some studies disagree, instead indicating that they can readily differentiate into AT1s and AT2s (Kumar et al., 2011; Zuo et al., 2015). For example, some reports utilize podoplanin (Pdpn) expression as a specific marker of AT1s and observe Krt5 lineage-traced PDPN$^+$ cells after recovery from injury. This is interpreted as *Krt5*$^+$ cells differentiating into AT1 cells to regenerate normal alveolar epithelial structures (Zuo et al., 2015). However, *Pdpn* is also expressed by basal cells, thus providing no definitive distinction between dysplastic tissue and regenerated AT1s (Rock et al., 2011; Van de Laar et al., 2014). This same study indicated that ~70% of *Krt5* "lineage-labeled cells ultimately express type I (1H8$^+$ and PDPN$^+$) or type II (SPC$^+$) pneumocyte markers," but the relative proportion of cells stained by each marker are not indicated (Zuo et al., 2015). Earlier studies used proprietary monoclonal antibodies that marked alveoli in humans and rats to identify supposed alveolar cells derived from intrapulmonary p63$^+$ progenitor precursors (Kumar et al., 2011). While these antibodies appear to label regenerated alveolar regions, the ambiguity of which antigens are recognized by these antibodies makes precise interpretation of intrapulmonary p63$^+$ progenitor contribution to alveolar epithelial regeneration difficult (Kumar et al., 2011).

All this said, several studies have observed some, albeit quite rare (typically less than 10%), differentiation of *Krt5*$^+$ cells into pro-surfactant protein C$^+$ (SPC$^+$) AT2s after influenza utilizing well-validated rabbit anti-sera. This appears to be dependent on the nature of injury, as ~30% of Krt5 lineage-traced cells exhibit AT2 differentiation upon resolution of bleomycin injury (Vaughan et al., 2015), although many fewer *Krt5*$^+$ cells arise in the first place with bleomycin. So, while most studies dispute the idea that intrapulmonary p63$^+$ progenitors *readily* differentiate into alveolar cell types, many agree that some plasticity is possible. Manipulating this plasticity may prove useful for future strategies aimed at enhancing functional lung regeneration.

Signals Controlling Intrapulmonary p63$^+$ Basal-like Cell Activation and Fate

The signaling pathways upstream of intrapulmonary p63$^+$ progenitors' activation, mobilization, and differentiation offer critical insights into how cell fate trajectories might be experimentally modulated, especially to promote transdifferentiation into AT2 and AT1 cells. Many studies into perturbations of these pathways utilize the Sox2-CreERT2 mouse to induce signaling changes *before* injury, so *Sox2* expression in the adult lung warrants careful description here. *Sox2* is expressed in all airway cells, including intrapulmonary p63$^+$ progenitors, which give rise to *Krt5*$^+$ pods, but also p63^{negative} *Sox2*$^+$ progenitors that appear

more capable of AT2 differentiation (Kathiriya et al., 2020; Vaughan et al., 2015; Xi et al., 2017). Both intrapulmonary p63$^+$ progenitors and AT2-biased *Sox2*$^+$ progenitors have been grouped under the catch-all term lineage-negative epithelial progenitors (LNEPs), and studies using the Sox2-CreERT2 mouse effectively target this entire heterogeneous LNEP population (Xi et al., 2017). Moreover, so-called bronchoalveolar stem cells present in distal airways are almost certainly Sox2 lineage labeled as well (Kim et al., 2005; Liu et al., 2019), not to mention the possibility that Sox2-labeled club cells may also differentiate into AT2s (Guha et al., 2017; Yuan et al., 2019). While these additional progenitor lineages are of great interest, existing reviews provide an excellent overview of these other cell types, and further discussion here is outside the scope of our focus on intrapulmonary p63$^+$ progenitors.

Accordingly, studies utilizing Sox2-CreERT2 mice can be difficult to interpret since changes to signaling pathways will occur in all airway cells, making it impossible to deconvolute which specific cell type was primarily affected by the signal modulation. When studying pathway modulation *after* the onset of injury, many groups use Krt5-CreERT2 mouse, which specifically targets post-injury intrapulmonary p63$^+$ progenitors and is far easier to interpret since *Krt5* specifically marks injury-activated intrapulmonary p63$^+$ progenitors (see "Cell-of-Origin" section).

Severe pulmonary insults cause widespread alveolar epithelial cell death, stripping some of the alveoli of their critical oxygen-exchanging cells and promoting edema, generating sporadic hypoxic microenvironments within injured lungs. Intriguingly, post-injury *Krt5*$^+$ pods are found only in hypoxic regions of the damaged lung, which suggested hypoxia-inducible factor (HIF1α) as a likely mediator of cellular responses to severe injury (Xi et al., 2017). When the Sox2-CreERT2 mouse is used to both label airway progenitors and delete HIF1α (*Hif1a*) before injury, *Krt5*$^+$ pod expansion overall is not only significantly reduced, but the Sox2-labeled *Hif1a*$^{-/-}$ cells that do expand differentiate into highly proliferative AT2s instead (Xi et al., 2017). Again, it is unknown whether intrapulmonary p63$^+$ progenitors, p63^{negative} *Sox2*$^+$ progenitors, or both are reacting to *Hif1a* deletion in this study due to the broad labeling of the Sox2-CreERT2 mouse. At the molecular level, Notch signaling and the basal-like differentiation program are highly induced in hypoxic *in vitro* culture of *Sox2*-enriched populations; both are abolished with *Hif1a* deletion, suggesting that HIF1α-dependent Notch signaling is a driver of the basal-like phenotype, either promoting this fate pathway in intrapulmonary p63$^+$ progenitors or AT2-biased *Sox2*$^+$ progenitors (Xi et al., 2017).

Notch signaling is critical for *Krt5*$^+$ expansion both during the course of injury and after its resolution, and influenza-injured lungs harbor intense expression of the canonical Notch target *Hes1* in *Krt5*$^+$ cysts (Vaughan et al., 2015). Notch inhibition shortly after influenza injury (5 days post-infection) significantly attenuates *Krt5*$^+$ expansion, while Notch inhibition both *in vitro* and at later time points *in vivo* (31 days post-infection) increases the differentiation of *Krt5*$^+$ cells into SPC$^+$ AT2s, again demonstrating that some plasticity toward alveolar cell types is present in these cells (Vaughan et al., 2015). This was foreshadowed by developmental studies years earlier demonstrating that persistent Notch signaling is incompatible with alveolar differentiation (Guseh et al., 2009). It should be noted, however, that high-dose γ-secretase inhibition combined with known alveolar differentiation factors IBMX and dexamethasone still only resulted in ~10% of *Krt5*$^+$ cells transdifferentiating into AT2s, indicating that Notch inhibition alone is insufficient to induce widespread alveolar conversion.

Recognizing that intrapulmonary p63$^+$ progenitors can differentiate into AT2s (albeit rarely) under certain conditions, the role of Wnt signaling, a potent pro-differentiation and proliferation signal for AT2s (Nabhan et al., 2018; Zacharias et al., 2018), was investigated. β-Catenin stabilization *in vivo* before injury using Sox2-CreERT2 increases the percentage of Sox2-traced SPC$^+$ cells, while the amount of *Krt5*$^+$ cells decreases, indicating that increased Wnt signaling is sufficient to drive an airway cell-to-AT2 transition (Xi et al., 2017). However, as with HIF1α deletion, it is unclear *which Sox2*$^+$ progenitor responds to β-catenin stabilization to promote AT2 differentiation (Xi et al., 2017).

Importantly, "activated" intrapulmonary p63$^+$ progenitors post-influenza are refractory to many of these pro-alveolar differentiation signals. When the Krt5-CreERT2 mouse was used to delete *Hif1a* or stabilize β-catenin in injured intrapulmonary p63$^+$ progenitors, the fraction of Krt5-traced cells that differentiate into AT2s remains unchanged (Xi et al., 2017). This finding suggests at least two possibilities: (1) a change—possibly transcriptional, epigenetic, or both—takes place in intrapulmonary p63$^+$ progenitors after injury that narrows their fate potential dramatically, rendering them unaffected by signaling pathways that could have otherwise altered their fate trajectory, or (2) intrapulmonary p63$^+$ progenitors cannot be induced to differentiate into AT2s via the HIF1α or Wnt signaling pathways at all, and the changes in Sox2-traced AT2s observed using the Sox2-CreERT2 mouse to delete *Hif1a* or stabilize β-catenin primarily affected the *Sox2*$^+$ p63^{negative} progenitor subpopulation and not intrapulmonary p63$^+$ progenitors. The outcome of increased AT2 generation in these experiments can therefore be interpreted as competition between heterogeneous progenitor cell types (p63$^+$ and p63^{negative}) as opposed to true fate conversion of intrapulmonary p63$^+$ progenitors.

Akin to Wnt signaling, FGF10, which signals through the receptor FGFR2B, is known to be an important niche-specifying signal for AT2s and was investigated as a potential fate-modifying signal in the airway progenitor population. *Fgfr2b* deletion before bleomycin injury using the Sox2-CreERT2 mouse abrogates both *Krt5*$^+$ pod and AT2 expansion from the *Sox2*$^+$ progenitors, implicating a role for FGF signaling in airway contribution to alveolar regeneration as a whole (Yuan et al., 2019). Conversely, sustained FGF10 signaling in all Sox2-traced cells beginning 2 weeks after bleomycin injury resulted in a significant shift favoring AT2 fate over *Krt5*$^+$ fate (Yuan et al., 2019). Again, it is unclear in which *Sox2*$^+$ cell type this FGF10-induced AT2 fate promotion occurs. To this end, the Krt5-CreERT2 mouse was utilized to delete *Fgfr2b* after injury, resulting in similarly lowered *Krt5*$^+$ area outgrowth (Yuan et al., 2019). These data, along with reports that FGF10 activation *in vitro* augments intrapulmonary p63$^+$ progenitor proliferation and that administration of recombinant FGF10 to influenza-injured mice results in more *Krt5*$^+$ pod areas (Quantius et al., 2016), confirms that FGF10 signaling indeed promotes intrapulmonary p63$^+$ progenitor expansion after injury.

In addition to external signals that influence intrapulmonary p63$^+$ cell fate, an understanding of cell-autonomous transcriptional control underlying cell fate and activation is essential toward modulating lung repair responses. The essential basal cell transcriptional regulator p63 (specifically the ΔN isoform) has been implied as an important player in intrapulmonary p63$^+$ progenitor maintenance largely due to the finding that p63-CreERT2 mice have fewer intrapulmonary p63$^+$ progenitors before injury and exhibit a dampened *Krt5*$^+$ outgrowth response to influenza (Yang et al., 2018). The authors attribute this attenuated response to p63 haploinsufficiency due to the CreERT2 knock-in allele at the *Trp63* locus, lending credence to the possibility that loss of even a single p63 allele can disrupt maintenance of the this cell population at baseline and impede dysplastic outgrowth. These data suggests that dysplasia and the maintenance of resting and activated intrapulmonary p63$^+$ progenitors relies on p63 expression. Similarly, *Sox2* has been implicated as exerting transcriptional control over the basal-like program of intrapulmonary p63$^+$ cells. As with p63-CreERT2, attenuated *Krt5*$^+$ pod expansion after influenza has been noted in Sox2-CreERT2 mice (in which CreERT2 replaces the *Sox2* coding region) compared with non-transgenic controls, suggesting that functional copies of both *Sox2* alleles are necessary for normal intrapulmonary p63$^+$ cell response to injury (Ray et al., 2016). It has not been determined if Sox2-CreERT2 mice have fewer intrapulmonary p63$^+$ progenitors at baseline, and so it is unknown if *Sox2* haploinsufficiency results in a defective response to injury or if *Sox2* regulates intrapulmonary p63$^+$ progenitor maintenance as well.

Tuft Cells—an Elusive Cell with a "Sense" of Purpose

While much focus has been placed on AT2/AT1 differentiation (or lack thereof) from intrapulmonary p63$^+$ progenitors, less attention has been directed to the airway-related cell types they more readily give rise to. One such cell arising within dysplastic regions of the lung post-influenza are tuft cells (Rane et al., 2019), also called brush or solitary chemosensory cells, that were shown to be derived from intrapulmonary p63$^+$ progenitors (Rane et al., 2019). The function of these cells and their downstream effects in the lungs are currently being investigated, but it has been demonstrated that tuft cells are responsible for activation of Th2-polarized immune response through the secretion of interleukin-25 in the intestines (Gerbe et al., 2016), as well as the trachea (Bankova et al., 2018; McGinty et al., 2020) and nasal epithelium (Kohanski et al., 2018). Given their clear role in epithelial remodeling in the intestine, it is possible that crosstalk between *Krt5*$^+$ cells and tuft cells contributes to chronic inflammation and persistent pathological changes in the lung parenchyma (Keeler et al., 2018; Rane et al., 2019). Stimulation of tuft cells in the mouse lung results in increased permeability and edema (Rane et al., 2019) and promotes generation of even more tuft cells in a "feedforward" loop, as has been described for intestinal tuft cells. The physiological impact of ectopic tuft cells in post-influenza lungs and their ultimate relationship to intrapulmonary p63$^+$ progenitor-derived dysplastic epithelium remains to be elucidated.

Functional Characterization of Post-injury Keratinization/Dysplasia—"*Band-Aid*" or Permanent Maladaptation?

Because intrapulmonary p63$^+$ progenitor cells and their progeny occupy alveolar space where AT2/AT1 restoration and functional regeneration might otherwise occur, and given that differentiation of these cells into AT2/AT1s is infrequent in most cases (Kanegai et al., 2016; Vaughan et al., 2015), it must be considered whether p63$^+$ progenitors and their derivatives are potential contributors to the long-term compromise of pulmonary function seen in some human survivors of severe pulmonary injury (Koppe et al., 2016; Liu et al., 2015). The development of chronic inflammation (Keeler et al., 2018; Rane et al., 2019) and bronchiolized epithelium within previously gas-exchanging areas of the lung almost certainly have deleterious effects on long-term lung health. Physiological impairment and decreased oxygen saturation would be one of the most obvious and immediate results of the substitution of AT2/AT1 cells by *Krt5*$^+$ dysplastic epithelia in the distal airspace, assuming that this dysplastic

epithelium does not perform effective gas exchange. In addition to the *Krt5*$^{+}$ dysplasia itself, in mice chronic inflammation persists in the lung in areas where viral remnants are detectable (Keeler et al., 2018). Moreover, some club cells appear capable of surviving infection but exhibit transcriptomic alterations (likely epigenetic) that enable broad, temporary antiviral immunity but may also contribute to long-term dysfunction (Hamilton et al., 2016). Indeed, several smaller studies and case reports in influenza survivors demonstrate long-term dysfunction, which may be at least partially attributable to epithelial dysplasia. For instance, nearly 50% of patients who survived infection with an avian-origin H7N9 influenza A strain in 2013 exhibited long-term ($\geq$2 years) lung pathology as judged by imaging (fibrosis, ground-glass opacities) and lung function defects (carbon monoxide diffuse capacity, FEV) (Chen et al., 2017). Similar but more minor chronic pathology has been observed in survivors of H1N1-associated ARDS (Luyt et al., 2012). All this said, it is true that most ARDS patients do seemingly show full recovery over time, although even this does not necessarily mean *Krt5*$^{+}$ dysplasia has resolved. Instead, less-injured regions of the lung may undergo compensatory regrowth, similar to what occurs after pneumonectomy, essentially resulting in the generation of new alveoli via expansion and neo-alveolarization of remaining (or in this case, uninjured) lung lobes to compensate for the loss of the gas exchange area (Buhain and Brody, 1973). Still, if the persistence of intrapulmonary p63^{+} progenitor-derived dysplasia accounts for even a fraction of observed long-term dysfunction, future studies focused on resolving dysplasia would be expected to have significant impacts on the restoration of lung function.

Blind Men and an Elephant

Despite the many studies focused on injury-induced *Krt5*$^{+}$ cell expansion, there is a conspicuous lack of shared terminology, making a unified model of this unique regenerative phenomenon difficult. Adoption of a naming convention would unify the disparate terminology of DASCs and the p63^{+} subset of LNEPs. While there is some disagreement as to whether these intrapulmonary p63^{+} basal-like cells express additional markers (*Krt5*, *Scgb1a1*) at baseline, many other characteristics are agreed upon: their scarcity in mice, presence in intrapulmonary airways, p63 expression, and the fact that these cells are the sole source of post-influenza *Krt5*$^{+}$ dysplastic cell expansion. Although not unanimous, most studies reviewed here agree that intrapulmonary p63^{+} progenitors' inherent capacity to generate alveolar cell types is limited. Nonetheless, other p63^{negative} airway cell types seem quite capable of transdifferentiating into AT2s, as demonstrated by both Sox2- and CC10-lineage tracing, recent studies utilizing dual SPC- and CC10-lineage tracing, and orthotopic transplantation of LNEPs. In retrospect, the fact that LNEPs are a heterogeneous pool of both intrapulmonary p63^{+} progenitors and less well-defined *Sox2*$^{+}$ p63^{negative}/*H2-K1*^{high} (Kathiriya et al., 2020) cells has only confused the issue of p63^{+} cell fate potency. For the time being, referring to these cells explicitly as *intrapulmonary p63*$^{+}$ *progenitors* should help clarify that these emergency responders are fundamentally distinct from other airway progenitor cells which, unlike intrapulmonary p63^{+} progenitors, exhibit higher potential for AT2/AT1 differentiation.

CONCLUSIONS—IS SOMETHING BETTER THAN NOTHING?

While the appearance of dysplastic, keratinized areas after severe influenza has been described extensively, it remains an entity of much debate. Though some authors argue that p63^{+} *Krt5*$^{+}$ cells that compose these "epithelial scars" can resolve and differentiate into AT2s and AT1s, this view is not supported by most subsequent studies. Most studies instead support the view that honeycomb cysts, or keratinized pods, are a near-permanent and indelible result of alveolar progenitor cell (AT2) depletion and, while beneficial in the acute phase of injury, are maladaptive in the long term (Kanegai et al., 2016; Rane et al., 2019).

Careful analysis and interpretation of these studies might lead to the conclusion that, in the absence of able progenitors, any kind of epithelial repair is better than none. The general recruitment of airway cells into the alveoli for the purposes of repair (Guha et al., 2017; Kathiriya et al., 2020; Liu et al., 2019; Ray et al., 2016; Strunz et al., 2020; Yee et al., 2017) further supports this hypothesis. In this view, albeit simplistic, the propagation of p63^{+} *Krt5*$^{+}$ cells in the immediate aftermath of distal lung injury with significant loss of AT2 progenitors represents a much-needed temporary solution to a grave problem. The initial presence and proliferation of intrapulmonary p63^{+} progenitors would not be the problem, per se, but rather their persistence. Still, the fact that these cells seem to possess some, if limited, plasticity to generate alveolar cell types presents a strong rationale for further investigation into the signaling and epigenetic status of intrapulmonary p63^{+} progenitors. We posit that identification of means to reprogram/transdifferentiate intrapulmonary p63^{+} progenitors into AT2s and AT1s represents a promising therapeutic target, possibly converting detrimentally remodeled tissue into truly regenerated, functional lung.

AUTHOR CONTRIBUTIONS

A.I.W. and M.F.d.M.C. contributed equally to the writing of the manuscript, under the supervision of A.E.V.

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REFERENCES

Adams, T.S., Schupp, J.C., Poli, S., Ayaub, E.A., Neumark, N., Ahangari, F., Chu, S.G., Raby, B.A., DeIuliis, G., Januszyk, M., et al. (2020). Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. Sci. Adv. *6*, eaba1983.

Bankova, L.G., Dwyer, D.F., Yoshimoto, E., Ualiyeva, S., McGinty, J.W., Raff, H., von Moltke, J., Kanaoka, Y., Frank Austen, K., and Barrett, N.A. (2018). The cysteinyl leukotriene 3 receptor regulates expansion of IL-25-producing airway brush cells leading to type 2 inflammation. Sci. Immunol. *3*, eaat9453.

Barkauskas, C.E., Cronce, M.J., Rackley, C.R., Bowie, E.J., Keene, D.R., Stripp, B.R., Randell, S.H., Noble, P.W., and Hogan, B.L. (2013). Type 2 alveolar cells are stem cells in adult lung. J. Clin. Invest. *123*, 3025–3036.

Basil, M.C., and Morrisey, E.E. (2020). Lung regeneration: a tale of mice and men. Semin. Cell Dev. Biol. *100*, 88–100.

Buhain, W.J., and Brody, J.S. (1973). Compensatory growth of the lung following pneumonectomy. J. Appl. Physiol. *35*, 898–902.

Chen, J., Wu, J., Hao, S., Yang, M., Lu, X., Chen, X., and Li, L. (2017). Long term outcomes in survivors of epidemic influenza A (H7N9) virus infection. Scientific Rep. *7*, 17275.

Cheung, E. (2020). Coronavirus: some recovered patients may have reduced lung function and are left gasping for air while walking briskly, Hong Kong doctors find. In South China Morning Post (Hong Kong, China: South China Morning Post), https://www.scmp.com/news/hong-kong/health-environment/article/3074988/coronavirus-some-recovered-patients-may-have.

Evans, M.J., Cabral, L.J., Stephens, R.J., and Freeman, G. (1973). Renewal of alveolar epithelium in the rat following exposure to NO_2. Am. J. Pathol. *70*, 175–198.

Evans, M.J., Cabral, L.J., Stephens, R.J., and Freeman, G. (1975). Transformation of alveolar type 2 cells to type 1 cells following exposure to NO_2. Exp. Mol. Pathol. *22*, 142–150.

Evans, M.J., Van Winkle, L.S., Fanucchi, M.V., and Plopper, C.G. (2001). Cellular and molecular characteristics of basal cells in airway epithelium. Exp. Lung Res. *27*, 401–415.

Gerbe, F., Sidot, E., Smyth, D.J., Ohmoto, M., Matsumoto, I., Dardalhon, V., Cesses, P., Garnier, L., Pouzolles, M., Brulin, B., et al. (2016). Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. Nature *529*, 226.

Guha, A., Deshpande, A., Jain, A., Sebastiani, P., and Cardoso, W.V. (2017). Uroplakin 3a(+) cells are a distinctive population of epithelial progenitors that contribute to airway maintenance and post-injury repair. Cell Rep. *19*, 246–254.

Guseh, J.S., Bores, S.A., Stanger, B.Z., Zhou, Q., Anderson, W.J., Melton, D.A., and Rajagopal, J. (2009). Notch signaling promotes airway mucous metaplasia and inhibits alveolar development. Development *136*, 1751–1759.

Habermann, A.C., Gutierrez, A.J., Bui, L.T., Yahn, S.L., Winters, N.I., Calvi, C.L., Peter, L., Chung, M.-I., Taylor, C.J., Jetter, C., et al. (2020). Single-cell RNA sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. Sci. Adv. *6*, eaba1972.

Hamilton, J.R., Sachs, D., Lim, J.K., Langlois, R.A., Palese, P., and Heaton, N.S. (2016). Club cells surviving influenza A virus infection induce temporary nonspecific antiviral immunity. Proc. Natl. Acad. Sci. U S A *113*, 3861–3866.

Hers, J.F.P., Masurel, N., and Mulder, J. (1958). Bacteriology and histopathology of the respiratory tract and lungs in fatal Asian influenza. Lancet *272*, 1141–1143.

Kanegai, C.M., Xi, Y., Donne, M.L., Gotts, J.E., Driver, I.H., Amidzic, G., Lechner, A.J., Jones, K.D., Vaughan, A.E., Chapman, H.A., and Rock, J.R. (2016). Persistent pathology in influenza-infected mouse lungs. Am. J. Respir. Cell Mol. Biol. *55*, 613–615.

Kathiriya, J.J., Brumwell, A.N., Jackson, J.R., Tang, X., and Chapman, H.A. (2020). Distinct airway epithelial stem cells hide among club cells but mobilize to promote alveolar regeneration. Cell Stem Cell *26*, 346–358.e4.

Keeler, S.P., Agapov, E.V., Hinojosa, M.E., Letvin, A.N., Wu, K., and Holtzman, M.J. (2018). Influenza A virus infection causes chronic lung disease linked to sites of active viral RNA remnants. J. Immunol. *201*, 2354–2368.

Kim, C.F.B., Jackson, E.L., Woolfenden, A.E., Lawrence, S., Babar, I., Vogel, S., Crowley, D., Bronson, R.T., and Jacks, T. (2005). Identification of bronchioalveolar stem cells in normal lung and lung cancer. Cell *121*, 823–835.

Kohanski, M.A., Workman, A.D., Patel, N.N., Hung, L.-Y., Shtraks, J.P., Chen, B., Blasetti, M., Doghramji, L., Kennedy, D.W., Adappa, N.D., et al. (2018). Solitary chemosensory cells are a primary epithelial source of IL-25 in patients with chronic rhinosinusitis with nasal polyps. J. Allergy Clin. Immunol. *142*, 460–469.e7.

Koppe, S., Túlio, A.I.B., Villegas, I.L.P., and Motter, A.A. (2016). Pulmonary function in patients with pandemic H1N1. Fisioterapia em Movimento *29*, 805–815.

Kumar, P.A., Hu, Y., Yamamoto, Y., Hoe, N.B., Wei, T.S., Mu, D., Sun, Y., Joo, L.S., Dagher, R., Zielonka, E.M., et al. (2011). Distal airway stem cells yield alveoli in vitro and during lung regeneration following H1N1 influenza infection. Cell *147*, 525–538.

Lee, D.-K., Liu, Y., Liao, L., Wang, F., and Xu, J. (2014). The prostate basal cell (BC) heterogeneity and the p63-positive BC differentiation spectrum in mice. Int. J. Biol. Sci. *10*, 1007–1017.

Liu, Q., Liu, K., Cui, G., Huang, X., Yao, S., Guo, W., Qin, Z., Li, Y., Yang, R., Pu, W., et al. (2019). Lung regeneration by multipotent stem cells residing at the bronchioalveolar-duct junction. Nat. Genet. *51*, 728–738.

Liu, W., Peng, L., Liu, H., and Hua, S. (2015). Pulmonary function and clinical manifestations of patients infected with mild

influenza A virus subtype H1N1: a one-year follow-up. PLoS One *10*, e0133698.

Loosli, C.G., Stinson, S.F., Ryan, D.P., Hertweck, M.S., Hardy, J.D., and Serebrin, R. (1975). The destruction of type 2 pneumocytes by airborne influenza PR8-A virus; its effect on surfactant and lecithin content of the pneumonic lesions of mice. Chest *67*, 7S–14S.

Luyt, C.E., Combes, A., Becquemin, M.H., Beigelman-Aubry, C., Hatem, S., Brun, A.L., Zraik, N., Carrat, F., Grenier, P.A., Richard, J.M., et al. (2012). Long-term outcomes of pandemic 2009 influenza A(H1N1)-associated severe ARDS. Chest *142*, 583–592.

McGinty, J.W., Ting, H.-A., Billipp, T.E., Nadjsombati, M.S., Khan, D.M., Barrett, N.A., Liang, H.-E., Matsumoto, I., and von Moltke, J. (2020). Tuft-cell-derived leukotrienes drive rapid anti-helminth immunity in the small intestine but are dispensable for anti-protist immunity. Immunity *52*, 528–541.e7.

Nabhan, A.N., Brownfield, D.G., Harbury, P.B., Krasnow, M.A., and Desai, T.J. (2018). Single-cell Wnt signaling niches maintain stemness of alveolar type 2 cells. Science *359*, 1118–1123.

Plantier, L., Crestani, B., Wert, S.E., Dehoux, M., Zweytick, B., Guenther, A., and Whitsett, J.A. (2011). Ectopic respiratory epithelial cell differentiation in bronchiolised distal airspaces in idiopathic pulmonary fibrosis. Thorax *66*, 651–657.

Quantius, J., Schmoldt, C., Vazquez-Armendariz, A.I., Becker, C., El Agha, E., Wilhelm, J., Morty, R.E., Vadász, I., Mayer, K., Gattenloehner, S., et al. (2016). Influenza virus infects epithelial stem/progenitor cells of the distal lung: impact on fgfr2b-driven epithelial repair. PLoS Pathog. *12*, e1005544.

Rane, C.K., Jackson, S.R., Pastore, C.F., Zhao, G., Weiner, A.I., Patel, N.N., Herbert, D.B.R., Cohen, N.A., and Vaughan, A.E. (2019). Development of solitary chemosensory cells in the distal lung after severe influenza injury. Am. J. Physiol. Lung Cell Mol. Physiol. *316*, L1141–L1149.

Rawlins, E.L., Okubo, T., Xue, Y., Brass, D.M., Auten, R.L., Hasegawa, H., Wang, F., and Hogan, B.L. (2009). The role of Scgb1a1+ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. Cell Stem Cell *4*, 525–534.

Ray, S., Chiba, N., Yao, C., Guan, X., McConnell, A.M., Brockway, B., Que, L., McQualter, J.L., and Stripp, B.R. (2016). Rare SOX2+ airway progenitor cells generate KRT5+ cells that repopulate damaged alveolar parenchyma following influenza virus infection. Stem Cell Reports *7*, 817–825.

Reinert, R.B., Kantz, J., Misfeldt, A.A., Poffenberger, G., Gannon, M., Brissova, M., and Powers, A.C. (2012). Tamoxifen-induced cre-loxP recombination is prolonged in pancreatic islets of adult mice. PLoS One *7*, e33529.

Rock, J.R., Gao, X., Xue, Y., Randell, S.H., Kong, Y.Y., and Hogan, B.L. (2011). Notch-dependent differentiation of adult airway basal stem cells. Cell Stem Cell *8*, 639–648.

Salehi, S., Reddy, S., and Gholamrezanezhad, A. (2020). Long-term pulmonary consequences of coronavirus disease 2019 (COVID-19): what we know and what to expect. J. Thorac. Imaging *35*, W87–W89.

Seibold, M.A., Smith, R.W., Urbanek, C., Groshong, S.D., Cosgrove, G.P., Brown, K.K., Schwarz, M.I., Schwartz, D.A., and Reynolds, S.D. (2013). The idiopathic pulmonary fibrosis honeycomb cyst contains a mucocilary pseudostratified epithelium. PLoS One *8*, e58658.

Smirnova, N., Schamberger, A., Nayakanti, S., Hatz, R., Behr, J., and Eickelberg, O. (2016). Detection and quantification of epithelial progenitor cell populations in human healthy and IPF lungs. Respir. Res. *17*, 83.

Strunz, M., Simon, L.M., Ansari, M., Kathiriya, J.J., Angelidis, I., Mayr, C.H., Tsidiridis, G., Lange, M., Mattner, L.F., Yee, M., et al. (2020). Alveolar regeneration through a Krt8+ transitional stem cell state that persists in human lung fibrosis. Nat. Commun. *11*, 3559.

Tata, P.R., Mou, H., Pardo-Saganta, A., Zhao, R., Prabhu, M., Law, B.M., Vinarsky, V., Cho, J.L., Breton, S., Sahay, A., et al. (2013). Dedifferentiation of committed epithelial cells into stem cells in vivo. Nature *503*, 218–223.

Taylor, M.S., Chivukula, R.R., Myers, L.C., Jeck, W.R., Waghray, A., Tata, P.R., Selig, M.K., O'Donnell, W.J., Farver, C.F., Thompson, B.T., et al. (2018). A conserved distal lung regenerative pathway in acute lung injury. Am. J. Pathol. *188*, 1149–1160.

Van de Laar, E., Clifford, M., Hasenoeder, S., Kim, B.R., Wang, D., Lee, S., Paterson, J., Vu, N.M., Waddell, T.K., Keshavjee, S., et al. (2014). Cell surface marker profiling of human tracheal basal cells reveals distinct subpopulations, identifies MST1/MSP as a mitogenic signal, and identifies new biomarkers for lung squamous cell carcinomas. Respir. Res. *15*, 160.

Vaughan, A.E., Brumwell, A.N., Xi, Y., Gotts, J.E., Brownfield, D.G., Treutlein, B., Tan, K., Tan, V., Liu, F.C., Looney, M.R., et al. (2015). Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. Nature *517*, 621–625.

Xi, Y., Kim, T., Brumwell, A.N., Driver, I.H., Wei, Y., Tan, V., Jackson, J.R., Xu, J., Lee, D.K., Gotts, J.E., et al. (2017). Local lung hypoxia determines epithelial fate decisions during alveolar regeneration. Nat. Cell Biol. *19*, 904–914.

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., Zhu, L., et al. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir. Med. *8*, 420–422.

Yang, Y., Riccio, P., Schotsaert, M., Mori, M., Lu, J., Lee, D.-K., García-Sastre, A., Xu, J., and Cardoso, W.V. (2018). Spatial-temporal lineage restrictions of embryonic p63+ progenitors establish distinct stem cell pools in adult airways. Dev. Cell *44*, 752–761.e4.

Yee, M., Domm, W., Gelein, R., Bentley, K.L., Kottmann, R.M., Sime, P.J., Lawrence, B.P., and O'Reilly, M.A. (2017). Alternative progenitor lineages regenerate the adult lung depleted of alveolar epithelial type 2 cells. Am. J. Respir. Cell Mol. Biol. *56*, 453–464.

Yuan, T., Volckaert, T., Redente, E.F., Hopkins, S., Klinkhammer, K., Wasnick, R., Chao, C.-M., Yuan, J., Zhang, J.-S., Yao, C., et al. (2019). FGF10-FGFR2B signaling generates basal cells

and drives alveolar epithelial regeneration by bronchial epithelial stem cells after lung injury. Stem Cell Reports *12*, 1041–1055.

Zacharias, W.J., Frank, D.B., Zepp, J.A., Morley, M.P., Alkhaleel, F.A., Kong, J., Zhou, S., Cantu, E., and Morrisey, E.E. (2018). Regeneration of the lung alveolus by an evolutionarily conserved epithelial progenitor. Nature *555*, 251.

Zheng, D., Yin, L., and Chen, J. (2014). Evidence for Scgb1a1(+) cells in the generation of p63(+) cells in the damaged lung parenchyma. Am. J. Respir. Cell Mol. Biol. *50*, 595–604.

Zuo, W., Zhang, T., Wu, D.Z., Guan, S.P., Liew, A.A., Yamamoto, Y., Wang, X., Lim, S.J., Vincent, M., Lessard, M., et al. (2015). p63(+) Krt5(+) distal airway stem cells are essential for lung regeneration. Nature *517*, 616–620.