Endogenous Lung Regeneration
Potential and Limitations

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The exploration of the endogenous regenerative potential of the diseased adult human lung represents an innovative and exciting task. In this pulmonary perspective, we discuss three major components essential for endogenous lung repair and regeneration: epithelial progenitor populations, developmental signaling pathways that regulate their reparative and regenerative potential, and the surrounding extracellular matrix in the human diseased lung. Over the past years, several distinct epithelial progenitor populations have been discovered within the lung, all of which most likely respond to different injuries by varying degrees. It has become evident that several progenitor populations are mutually involved in maintenance and repair, which is highly regulated by developmental pathways, such as Wnt or Notch signaling. Third, endogenous progenitor cells and developmental signaling pathways act in close spatiotemporal synergy with the extracellular matrix. These three components define and refine the highly dynamic microenvironment of the lung, which is altered in a disease-specific fashion in several chronic lung diseases. The search for the right mixture to induce efficient and controlled repair and regeneration of the diseased lung is ongoing and will open completely novel avenues for the treatment of patients with chronic lung disease.

Keywords: lung restoration; stem cells; developmental pathways; emphysema; pulmonary fibrosis

Chronic lung diseases (CLD) are the second leading cause of death worldwide and thus represent a significant global health problem. Despite intensive research efforts, the underlying pathogenesis of the majority of CLD, such as chronic pulmonary disease (COPD) or pulmonary fibrosis, remains elusive (1, 2). Current therapies mainly target symptoms, and effective causal therapy has not been developed thus far. Lung transplantation is often the only option for patients with end-stage disease; however, this procedure has the lowest 5-year survival rate of any solid organ transplantation (3–5). Moreover, there is a large imbalance of the number of patients listed for lung transplantation compared with the number of available transplantable organs (5).

As one approach to address the issue of transplantable organ shortage, recent progress has been made toward the heroic feat of bioengineering lungs and airways for transplantation (6–8). However, many more obstacles have to be overcome, such as the composition of biomaterials, suitable and specific cellular sources, and proper vascularization of engineered tissues (9). Another approach will be the manipulation of endogenous lung cells to restore homeostasis and promote regeneration of diseased and damaged lungs (10). This would be a tremendous step forward, particularly in light of the fact that the *de novo* regeneration of the adult human diseased lung has not been demonstrated yet. Here, we will summarize recent findings on (*1*) endogenous epithelial progenitors, (*2*) the signaling pathways that regulate their reparative and regenerative potential, and (*3*) the modulating role of the extracellular matrix (ECM) present in human diseased lungs. We discuss the similarities and differences of these aspects over the whole life span, including pre- and postnatal stages through adolescence and adulthood, and how this might influence the progression and therapy of CLD.

STEM/PROGENITOR CELLS OF THE ADULT LUNG

The epithelial lining of the respiratory system varies along its proximo-distal axis (11). A pseudostratified epithelium lines conducting airways and performs mucociliary transport. This transitions to a simple columnar, then cuboidal, and eventually squamous epithelium in the alveoli across which gases can exchange. Evidence from *in vivo* and *in vitro* models suggests that distinct stem/progenitor cell populations maintain and repair these different epithelia (12) (Figure 1, Table 1).

Although ample evidence suggested that self-renewing epithelial cells exist in the conducting airways (11), the role of endogenous epithelial progenitor cells in the distal lung has long been debated. Early experiments provided evidence that alveolar epithelial type (AT) II cells serve as progenitors for the distal lung (13). Genetic lineage tracing in mice has confirmed that these cells do in fact generate ATI cells under steady state conditions and in response to acute lung injury induced by bleomycin (14, 15). Importantly, these recent studies have shown that in areas of extreme injury, there might exist another alveolar progenitor cell that does not express surfactant protein C (SFTPC), a common marker for ATII cells. There is evidence that a population of integrin (Itg)a6/b4$^+$ alveolar epithelial cells might represent a unique progenitor of alveolar lineages. Lineage tracing with a Secretoglobin 1A member 1 (Scgb1a1/CC10)-CreER allele has shown that a population of Scgb1a1$^+$ cells (also named Clara cells or Club cells) can give rise to alveolar lineages in response to some injuries, including bleomycin, but not under steady state conditions or in response to naphthalene or hyperoxia (14, 16). A population of Scgb1a1$^+$;Sftpc$^+$ dual positive cells resides at the bronchoalveolar duct junction of mouse lungs. These putative bronchioalveolar stem cells (BASCs) expand in response to oncogenic transformation and give rise to bronchiolar and alveolar lineages *in vitro* (17).

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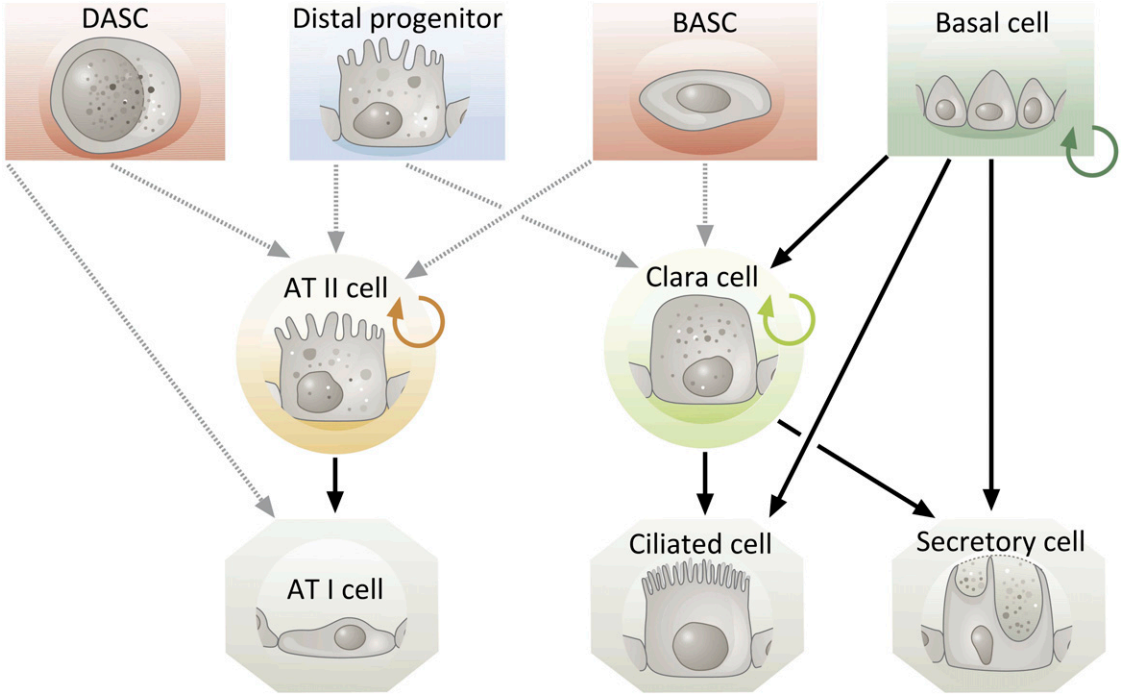

Figure 1. Endogenous epithelial progenitor cells of the lung. Detailed characteristics of identified epithelial progenitor cells are given in Table 1. Dashed lines indicate that definitive *in vivo* evidence of lineage data is missing. AT = alveolar epithelial type; BASC = bronchioalveolar stem cell; DASC = distal airway stem cell.

However, precise knowledge about the initial localization and behaviors of Scgb1a1$^+$ progenitors, including BASCs, awaits the identification of more discriminating markers. Importantly, because functional studies are difficult in humans, many of the data related to stem cells of the lung come from animal models. There are significant differences between rodent and human lungs, so hypotheses must be validated with respect to significance (11). For example, Sftpc$^+$;Scgb1a1$^+$ dual positive cells have not yet been reported in human lungs.

Several recent reports challenge the idea of regionally distinct epithelial stem cells of the adult lung. For example, one study provided evidence that Keratin (Krt)5$^+$ basal cells (distal airway stem cells) can give rise to regenerative alveolar pods in response to lung injury induced by a murinized H1N1 virus (18). Where these cells initially reside and whether their descendants go on to express markers of ATI and ATII cells awaits more rigorous lineage-tracing experiments. Intriguingly, basal cells are commonly observed in areas of bronchiolization within

TABLE 1. SUMMARY OF CHARACTERISTICS OF ENDOGENOUS EPITHELIAL PROGENITOR POPULATIONS OF THE LUNG

Cell Type	Markers	Behaviors	References
Basal cells	Trp63, Krt5 (and variably Krt14), NGFR, Pdpn	Self renew and generate ciliated and secretory cells *in vivo* and *in vitro*	75, 76
ATII cells	Sftpc	Self renew and give rise to ATI under steady-state conditions and in response to injury	13–15
Clara cells (club cells, nonciliated secretory cells)	Scgb1a1, electron-dense secretory granules	Self renew and give rise to ciliated and secretory (including mucus-producing) cells *in vivo* over the long term in intralobar airways	16, 77
Variant Clara cells	Same as above. Might also express Upka3 and Scgb3a2. Resistant to injury by naphthalene.	Survive and generate ciliated and secretory cells after injury by systemic administration of naphthalene	24, 78
Distal lung progenitors	ITGA6B4 (negative for Sftpc, Scgb1a1, and Krt5)	These parenchymal cells generate Scgb1a1$^+$ and Sftpc$^+$ structures when grafted under the kidney capsule with dissociated embryonic lung	15
DASCs	Krt5, p63	Only reported after injury. Give rise to potentially regenerative alveolar "pods" after sublethal H1N1 lung infection in mice.	18
BASCs	Scgb1a1, Sftpc, localized to the bronchoalveolar duct junction	Nonclonal cultures of isolated cells give rise to Sftpc$^+$ and Scgb1a1$^+$ cells *in vitro*. BASCs have not been reported in human lungs. Note that Sftpc$^+$/Scgb1a1$^+$ cells are also found in the alveoli of mice; how these relate to BASCs is not currently known.	17
c-kit$^+$	c-kit (Kit)	Cells isolated and expanded from human lungs were reported to engraft into mouse lungs after cryoinjury and give rise to all epithelial and mesenchymal lineages	21

Definition of abbreviations: ATII cells = alveolar epithelial type II cells; BASC = bronchioalveolar stem cell; DASC = distal airway stem cell.

the parenchyma of lungs from patients with idiopathic pulmonary fibrosis (IPF) (19); however, these $Krt5^+$ pods were not reported in bleomycin model of pulmonary fibrosis in mice (18). Even more contrary to the paradigm of lineage-restricted epithelial stem cells of the lung, another group recently reported a population of $c\text{-}kit^+$ cells in human lungs that, when grafted into injured mouse lungs, gave rise to epithelial and mesenchymal (i.e., fibroblasts, smooth muscle, and vascular) lineages (20, 21) (Table 1). Although such a cell would have great therapeutic potential, these findings will have to be confirmed and await independent validation (22, 23).

These emerging concepts have stimulated discussion in the field. One important idea is that different populations probably survive and respond to various injuries by varying degrees. For example, $Scb1a1^+$ secretory cells are particularly susceptible to injury by systemic administration of naphthalene, an injury that is restricted to $Scgb1a1^+$ cells and repaired by surviving basal cells and a subpopulation of variant Clara cells near neuroepithelial bodies (24). In contrast, $Scgb1a1^+$ cells survive the injury induced by bleomycin. Bleomycin is a cytostatic drug, which on intratracheal application affects the whole epithelium, including bronchial and alveolar epithelial cells. Subsequently, fibrosis development is observed in peribronchiolar regions as well as in the alveolar space (25). In this model, $Scgb1a1^+$ cells give rise to alveolar epithelial cells to repair this injury (14).

This has fueled a debate over terminology: should these lineage-restricted populations be called "facultative progenitor cells"? The classical definition of a stem cell is one that can both self-renew and generate differentiated progeny. By these criteria, basal cells, secretory cells, and ATII cells are all stem-cell populations. Similar models, in which more than one progenitor population coordinately fuels maintenance and repair, have recently come to light in other systems, including the intestinal and mammary epithelia (26, 27). These emerging models are different from the classical stem cell hierarchy of the hematopoietic system, in which a single cell can give rise to every lineage in a linear progression of differentiation and restricted potential (28). Regardless of nomenclature, the cells with long-term potential for self-renewal and differentiation represent ideal targets for genetic and molecular therapy for lung disease.

DEVELOPMENTAL PATHWAYS IN THE ADULT LUNG

Developmental studies have emphasized the importance of a tight spatiotemporal interplay between initiating and differentiating factors for proper organ formation. It is well accepted today that instead of a few organ-specific master genes, several molecules must mutually act as a finely tuned orchestra to control organ development (29, 30). For example, the Wnt signaling pathway is expressed in a cell-specific manner in the developing lung, tightly regulating epithelial and mesenchymal cell behavior (31, 32).

Importantly, in the human adult lung, the identification of initiating and/or differentiating factors essential for regeneration of damaged lungs remains largely unexplored. Recent findings, however, have shed light on the relevance of developmental pathways in CLD. Unbiased screening approaches analyzing the mRNA/miRNA profile within several different CLD revealed that classical developmental pathways, among them Wnt, Notch, or sonic hedgehog, might be involved in disease pathogenesis (33–37). Several recent studies suggested that developmental pathways are silenced in COPD. Here, reduced Wnt/β-catenin and Notch signaling has been particularly observed in several lung epithelial cells (38–40), such as airway epithelial cells of healthy smokers or smokers with COPD (39). Furthermore, reduced nuclear (i.e., active) β-catenin has been observed in patients with stage IV COPD as well as in animal models of COPD/emphysema (38). Intriguingly, these pathways are not only critically involved in lung development but also known to regulate progenitor cell maintenance (41). Activation of Wnt/β-catenin signaling in the animal model led to attenuation of emphysema, suggesting that Wnt/β-catenin activation promotes lung restoration. The mechanism behind this has not been elucidated thus far; however, it can be speculated that Wnt/β-catenin activation may target epithelial progenitor cell niches. Indeed, it has recently been described that mouse embryonic stem cells are able to differentiate into airway progenitor cells on fine-tuned recapitulation of relevant developmental signaling pathways, among them Wnt signaling (42). Moreover, Wnt proteins have been implicated in endogenous lung epithelial progenitor cell maintenance and activation. In BASC, loss of the transcription factor GATA6 led to increased Wnt signaling and concurrent BASC expansion (43).

Similar to Wnt/β-catenin, altered Notch signaling has been linked to COPD. This pathway has been implicated in the regulation of airway epithelial differentiation, and down-regulation of Notch pathway genes in the adult airway epithelium of smokers and patients with COPD has been observed (40, 44). In line with this, recent data suggest that active Notch signaling sustains $Scgb1a1^+$ cells and protects from goblet cell metaplasia, a feature commonly found in patients with COPD (45).

Given this accumulating evidence, reactivation of developmental pathways represents a suitable therapeutic strategy to combat CLD. This conclusion, however, is challenged by observations made in other CLD, such as pulmonary fibrosis. Pulmonary fibrosis is characterized by epithelial cell injury and activation concomitant with a gain of mesenchymal cells, and aberrant reactivation of a multitude of developmental pathways has been described (2, 35, 46). Several groups demonstrated that Wnt/β-catenin signaling is activated in IPF, and inhibition of this pathway led to amelioration of experimental fibrosis in animal models (33, 47–51). In addition, noncanonical Wnt signaling has been also described to exert profibrotic effects (52, 53). Most interestingly, recent studies suggest that β-catenin signal activity in IPF not only is regulated by Wnt proteins but also participates in profibrotic transforming growth factor (TGF)-β signaling. TGF-β–mediated Smad3/β-catenin signaling has been observed in alveolar epithelial cells in IPF tissue specimens and has been linked to epithelial-to-mesenchymal transition, thereby contributing to fibrosis (49, 54). Moreover, there is increasing evidence that several developmental pathways are altered in pulmonary fibrosis. For example, in addition to TGF-β and Wnt/β-catenin, Notch receptor expression colocalized with myofibroblasts in IPF tissue and has also been implicated in epithelial cell plasticity (55, 56).

Altogether, IPF presents as a disease characterized by a loss of spatiotemporal fine tuning of developmental pathways. This idea has stimulated a lively discussion: Is it possible to correct and retrieve control to shift impaired repair and remodeling to successful repair and regeneration in pulmonary fibrosis? How can we further advance reactivation of developmental pathways as a therapeutic target in COPD given the risk of inducing fibrogenic or oncogenic processes? Clearly, the search for the right balance of pathways will be an essential part of future studies.

THE ECM OF THE ADULT LUNG

In the lung, the ECM surrounds the conducting airways, alveolar cells, and vascular system. The ECM has a major impact on lung architecture and function, such as gas exchange, by facilitating cell signaling via adhesive molecules, surface receptors, or growth factors. The pulmonary ECM is subjected to a continuous

turnover of greater than 10% of the total ECM per day (57). Thus, a dynamic equilibrium between synthesis and degradation of the pulmonary ECM is maintaining the physiological balance, and disturbances in ECM turnover represent a key feature of several CLD. Several factors are involved in (dys)balancing the ECM, such as *de novo* synthesis and ECM deposition by structural cells, proteolytic degradation by matrix metalloproteinases (MMPs), and inhibition of MMP activity by tissue inhibitors of metalloproteinases (58). In COPD, ample evidence demonstrated an increased activity of proteolytic enzymes that may subsequently lead to impaired ECM turnover (59, 60). Similarly, altered activity of MMPs and tissue inhibitors of metalloproteinases, along with increased collagen deposition in the lung parenchyma, has also been demonstrated in pulmonary fibrosis (46, 61). The detailed scenario finally resulting in apparent disease-specific pathological differences warrants further investigation.

The impact of cell–matrix interaction in the context of CLD has become an area of significant research. Substantial evidence suggests that several signal molecules and pathways shape

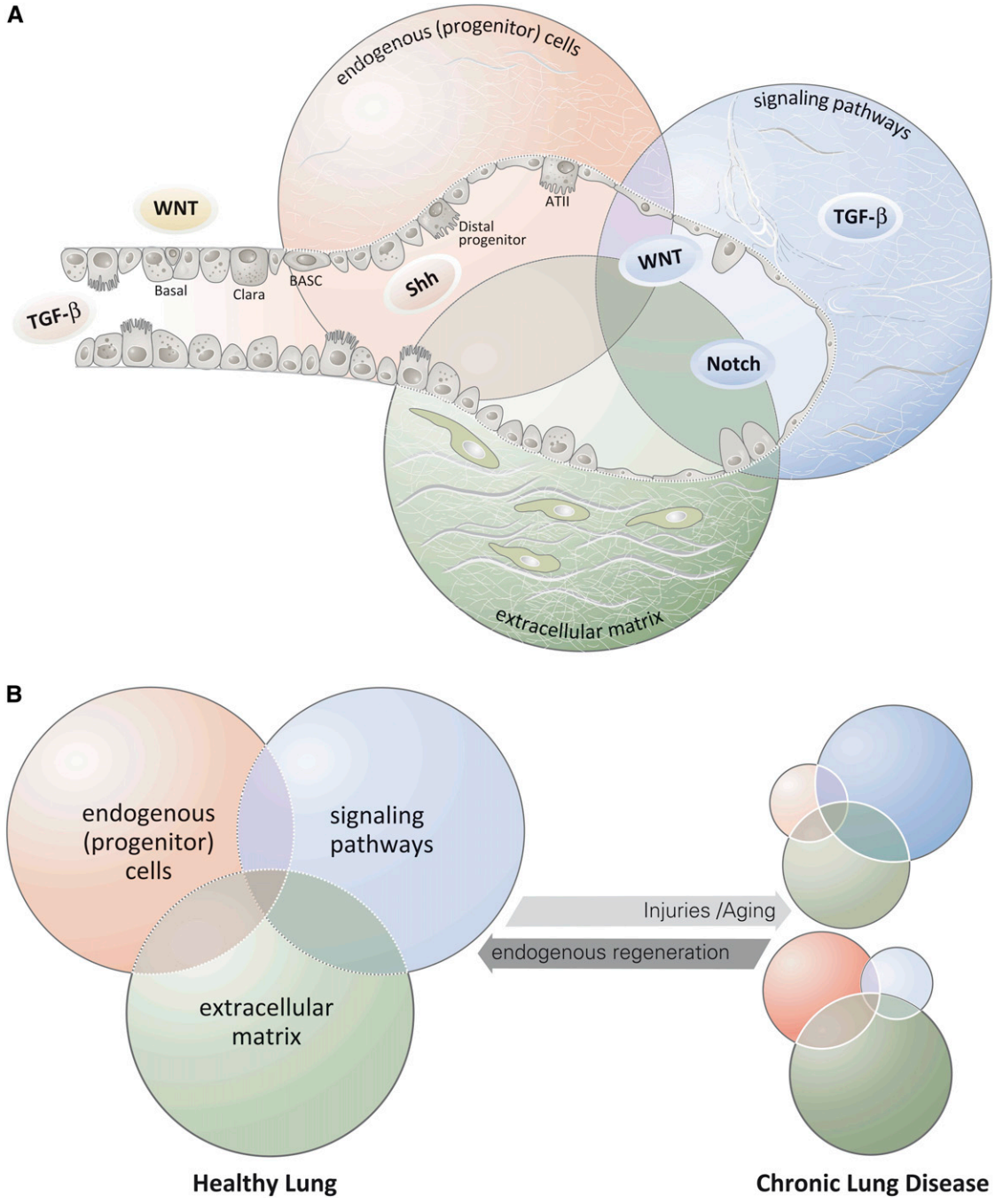

Figure 2. The microenvironment of the lung. (*A*) Lung homeostasis is maintained by endogenous (progenitor) cells, developmental signaling systems, and the extracellular matrix within the microenvironment. (*B*) Various lung injuries lead to alterations of these components, resulting in distinct chronic lung diseases. Therapeutic strategies that aim to restore the initial balance of endogenous (progenitor) cells, developmental signaling systems, and extracellular matrix are required to achieve lung regeneration. AT = alveolar epithelial type; BASC = bronchioalveolar stem cell; Shh = sonic hedgehog; TGF = transforming growth factor.

matrix deposition and turnover; however, recent studies pointed out that the lung matrix exerts a stable priming effect on cell behavior, such as myofibroblast activation (62). Furthermore, alterations in matrix biology ultimately lead to changes in lung mechanics and subsequently signal transduction and cellular phenotypes (63).

One of the major questions that were raised in the initial tissue engineering studies addresses the relevance and impact of the surrounding matrix in a diseased lung. Thus far, studies applying exogenous progenitor cells have mainly been successful to restore lung architecture and function within a nondiseased matrix (64, 65). These studies suggest that the decellularized matrix is capable of dictating the fate of exogenous progenitor cells (7).

Importantly, studies elucidating how these cells behave in a diseased matrix are currently being performed and are essential to reveal the impact of the existing matrix on the capacity of the human lung to repair and regenerate. Finally, we need to understand how the diseased matrix can be modified, along with targeting progenitor cells using the right composition of signaling pathways to identify suitable therapeutic options for patients with CLD (Figures 2A and 2B).

REBUILDING A DISEASED LUNG OVER THE LIFE SPAN

CLD in general lead to progressive distortion of normal lung architecture, loss of functional gas exchange, and impairment of lung function. Importantly, CLD occur over the entire life span, including a variety of disease phenotypes, such as bronchopulmonary dysplasia, asthma, cystic fibrosis, COPD, or interstitial pneumonias, including IPF. The endogenous ability of the human lung to induce repair is highly dependent on the "age" of the lung, which impacts its potential to respond to injuries and challenges (32, 66). It is well known that lung development proceeds into adolescence, and it is likely that stem/progenitor cells in young postnatal lungs are phenotypically distinct from adult epithelial cells. For example, the proportion of ciliated cells derived from adult secretory cells increases throughout life (16). In contrast, the proportion of ciliated cells derived from the population of secretory cells present at embryonic day 18.5 plateaus in adulthood. This suggests that the progenitor potential of young secretory cells is different from adult secretory cells.

In contrast to adult CLD, profound knowledge about endogenous epithelial progenitor cell niches or alterations of developmental pathways in newborn or childhood CLD is still missing and gave rise to an emerging research area (67). As an extension of this idea, it is likely that respiratory epithelial stem cell function declines with age and that this could contribute to the normal age-related decline in lung function. Moreover, ineffective repair by aging stem cells or reduced capability of activating signal pathways that promote repair might exacerbate these effects in older individuals (68). In some cases, aging stem cells might themselves cause lung disease. For example, short telomeres, characteristic of older cells, have recently been implicated in the progression of human lung pathology (69). In other cases, endoplasmic reticulum stress, perhaps owing to genetic mutations, could lead to a premature exhaustion of the stem cell pool and an inability to maintain the lung against the damages of everyday life (70). Moreover, structural cells, such as fibroblasts, have been reported to develop a progressive myofibroblast phenotype during age-related decline in lung regeneration in mice (71).

An important implication of this idea is how genetic and environmental insults to the progenitor cell population early in life affect lung homeostasis in adulthood. A number of studies suggest that hyperoxic injury in neonates can predispose individuals to lung disease later in life (72). Similarly, it has been demonstrated that maternal smoking during pregnancy affects the developmental pathways, such as Wnt/β-catenin signaling, in the lungs of neonatal offspring (73). Altogether, this may potentiate the risk of impaired lung development in early life and elevated risk of developing CLD.

An emerging concept is the influence of a stem cell's microenvironment, or niche, on its progenitor behaviors (74). In an increasing number of contexts, including the hematopoietic and nervous systems, there is evidence to suggest that the microenvironment, including structural cells, is changed by age, and this influences a stem cell's capacity for repair. Even in the context of lung transplant or bioengineered lung replacements, systemic responses or an inhospitable microenvironment might limit its long-term outcome.

OUTLOOK

The assessment of the regenerative potential of the adult diseased lung represents a challenging task, in particular because of the potential development of novel therapies for CLD. The capacity of endogenous progenitors to restore lung architecture and function is influenced by several factors that are susceptible to considerable variation over the whole life span: endogenous progenitor cell populations, activity of developmental pathways, and the modified pulmonary extracellular matrix. Recent findings have led to tremendous progress in our understanding of these components and provide hope that endogenous lung repair and regeneration capacity will emerge as a suitable approach for the treatment of CLD.

Relevant open questions emphasized throughout this perspective target the validation of endogenous progenitor sources and their lineage specificity in the human lung. Furthermore, it is unclear how developmental pathways influence progenitor cell behavior in distinct CLD and whether alterations in developmental pathways reflect a cause or consequence of disease pathogenesis. This will impact therapeutic strategies aiming to restore the spatiotemporal balance of signaling pathways and repair the diseased lung. Finally, we need to understand the interaction of the existing diseased matrix with endogenous (progenitor) cells and signaling pathways and whether this matrix can potentially be modified. Future studies addressing these questions will not only provide novel therapeutic strategies but also identify the appropriate progenitor cells, signaling pathways, and microenvironmental cues to facilitate bioengineering of the lung.

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