Resident interstitial lung fibroblasts and their role in alveolar stem cell niche development, homeostasis, injury, and regeneration

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Abstract
Developing, regenerating, and repairing a lung all require interstitial resident fibroblasts (iReFs) to direct the behavior of the epithelial stem cell niche. During lung development, distal lung fibroblasts, in the form of matrix-, myo-, and lipofibroblasts, form the extra cellular matrix (ECM), create tensile strength, and support distal epithelial differentiation, respectively. During de novo septation in a murine pneumonectomy lung regeneration model, developmental processes are reactivated within the iReFs, indicating progenitor function well into adulthood. In contrast to the regenerative activation of fibroblasts upon acute injury, chronic injury results in fibrotic activation. In murine lung fibrosis models, fibroblasts can pathologically differentiate into lineages beyond their normal commitment during homeostasis. In lung injury, recently defined alveolar niche cells support the expansion of alveolar epithelial progenitors to regenerate the epithelium. In human fibrotic lung diseases like bronchopulmonary dysplasia (BPD), idiopathic pulmonary fibrosis (IPF), and chronic obstructive pulmonary disease (COPD), dynamic changes in matrix-, myo-, lipofibroblasts, and alveolar niche cells suggest differential requirements for injury pathogenesis and repair. In this review, we summarize the role of alveolar fibroblasts and their activation stage in alveolar septation and regeneration and incorporate them into the context of human lung disease, discussing fibroblast activation stages and how they contribute to BPD, IPF, and COPD.

KEYWORDS
alveolar niche, bronchopulmonary dysplasia (BPD), chronic obstructive pulmonary disease (COPD), development, interstitial lung fibroblasts, idiopathic pulmonary fibrosis (IPF)

1 | INTRODUCTION

A diverse set of genes driven by various cell types are key to the regulation of alveolar septation and regeneration. Distal alveolar epithelial cells (AECs) (Alveolar Type 1 [AT1] and Type 2 [AT2]) facilitate proper gas exchange and maintain proper alveolar surface tension. An increase in alveolar surface area that occurs during the late phases of lung development is key to proper alveolar function. Throughout development, the epithelium requires constant and finely tuned molecular cues from neighboring fibroblasts to direct proper proliferation and differentiation.¹ Interruption of alveolar maturation by premature birth and exposure to hyperoxia can lead to...
become the focus of several studies. Alveolar fibroblasts: in directing alveolar epithelial proliferation and differentiation has Since the mid-1990s, the importance of the alveolar mesenchyme activation stages
| matrix fibroblasts create ECM components to stabilize the newly formed septa, whereas PDGFRα+ lipofibroblasts support AT2 cell function during homeostasis. Moreover, recently defined alveolar niche cells, marked by PDGFRα and Axin2/Wnt2/Lgr5 coexpression, support alveolar epithelial regeneration after injury. All four of these cell populations arrive at precisely the right time to provide both the scaffold and the paracrine signals the epithelium needs to proliferate and differentiate. The advent of single-cell RNA sequencing (scRNA-seq) and the refinement of inducible mouse lineage-tracing systems have yielded a plethora of data on the interstitial lung fibroblast during alveolarization, but individually analyzing these data can be overwhelming. In this review, we summarize the role of alveolar fibroblasts in alveolar septation and regeneration and incorporate them into context of how they are modified in and contribute to human lung diseases like BPD, IPF, and COPD.

1.1 | Fibroblast subpopulations or fibroblast activation stages

Since the mid-1990s, the importance of the alveolar mesenchyme in directing alveolar epithelial proliferation and differentiation has become the focus of several studies.13,14 Alveolar fibroblasts: (a) provide and modulate an ECM scaffold for epithelial cells to expand upon, (b) provide tensile forces to extend and thin the septal walls during secondary septation, and (c) provide paracrine cues to the surrounding epithelium and endothelium to initiate proliferation and differentiation. During development, alveolar fibroblasts can be defined as four functional populations: myofibroblasts, lipofibroblasts, matrixfibroblasts, and alveolar niche cells.15 These four populations cover all functions of the alveolar fibroblast but have been described as several fibroblast lineages that partially overlap. Based on individual localization and PDGFRα expression they have been called interstitial resident fibroblasts, “iRef,” or alveolar niche cells. We will discuss interstitial fibroblasts in the formation of the alveolus during development, their role during reseption after partial pneumonectomy (PNX), and their role in disease formation and progression.

1.2 | Fibroblasts in development

Interstitial fibroblast function during alveolarization

Pulmonary interstitial fibroblasts are critical for the formation and extension of alveolar septa postnatally but already prepare for septation prenatally. The alveolus is architecturally conserved between the human and murine peripheral lung. Mice are born in the saccular phase, and alveolarization is observed postnatally, whereas human alveolarization starts at around 36 weeks of gestation. Babies born before 36 weeks of gestation are therefore born in the saccular stage of lung development and are susceptible to barotrauma and hyperoxia-induced damage, contributing to the chronic lung injury seen in BPD. As alveolarization occurs postnatally in mice, and interventions like hyperoxia and pharmacological treatments are amenable in neonatal mice, the murine system is highly valuable to study alveolarization and BPD. Here, we summarize the temporal role of myo-, matrix, lipo-, and alveolar niche cell fibroblasts in the process of alveolarization.

A temporal comparison of human and murine alveolarization and the functional roles of interstitial fibroblasts are illustrated in Figure 1. During sacculation, alveolar ducts that end in simple primary alveoli are formed. At the onset of alveolarization (murine: PN2-PN3; human: 36 weeks of gestation until 1 month after birth), secondary crests/ridges bulge out from the walls of the primary septa (Figure 1.1). The secondary crests are elongated by the contractile force of secondary-crest myofibroblasts (Figure 1.2) (murine: PN3-PN14, human: 1-18 months after birth). These myofibroblasts also produce a framework of elastin and tenascin, supporting the newly forming secondary crest. As the myofibroblast contracts, it is assumed to pull the ECM that the matrixfibroblast beneath is actively making. As secondary crests form and elongate, endothelial cells

Significance statement

This concise review summarizes the existing literature on alveolar fibroblasts and compares and contrasts findings in murine development and fibrosis resolution and human fibrotic lung disease. This article summarizes the fibroblast stages during lung development, regeneration, and repair, with a focus on their role in supporting the alveolar stem cell niche and their potential to function as stromal progenitor cells. The murine findings were extended and incorporated into the context of human disease, and the article also reports on the fibroblast activation stages and how they contribute to bronchopulmonary dysplasia, idiopathic pulmonary fibrosis, and chronic obstructive pulmonary disease.
proliferate and form a double-capillary bed. During secondary septa maturation, the double-capillary bed fuses to a single capillary (Figure 1.3) (murine: PN4-PN21, human: 1-36 months after birth).

**Secondary Crest Formation**
- ECM production
- Elastin deposition

**Secondary Crest Maturation**
- Single Capillary

**Secondary Crest Elongation**
- Extension of ECM
- Double Capillary

**Alveolarization**
- Collagen production and remodeling

**FIGURE 1** Interstitial fibroblasts and their role in alveolar septation and epithelial niche formation. Illustrations of mesenchymal subtypes in their spatial and temporal contributions to alveolarization. Three key stages of alveolarization are arranged in a clockwise fashion in the center of the illustration. (1) Secondary crest formation occurs in mice between PN2 and PN3 and in humans between 36 weeks’ gestational age (GA) and 1 month. (2) Secondary crest elongation occurs in mice between PN3 and PN14 and in humans between 1 and 18 months. (3) Secondary crest maturation occurs in mice between PN4 and PN14 and in humans between 1 and 36 months. Major events, such as elastin deposition during primary septum formation, are labeled in the respective panels. The spatial location of each cell type is reflected, such as the myofibroblast that resides at the septal tip and the lipofibroblast sitting adjacent to the AT2 cell. Signature gene expression of each cell type, identified by developmental studies and scRNA-seq, are given in the figure legend.

**Signaling pathways specify fibroblast subpopulations and control fibroblast function**

Several conserved developmental signaling pathways are important for both fibroblast differentiation and signaling to the epithelium. PDGFRα signaling is critical for the activation of myofibroblasts in the septa as pdgfrα-mutant mice fail to form septa during the initial phase of alveologenesis. In addition to platelet-derived growth factor A (PDGFA), other signaling pathways such as retinoic acid (RA), fibroblast growth factor (FGF), sonic hedgehog (SHH), bone morphogenetic protein (BMP), and wingless-related integration site (WNT) are indispensable for proper fibroblast differentiation and activation of their functional stages. RA induces PDGFA-PDGFRα autocrine signaling in fibroblasts, and interruption during development by the genetic ablation of RA in mice causes alveolar simplification. FGFR10, produced in lipofibroblasts, directs epithelial proliferation and differentiation into AT1 cells during sacculcation and alveolarization. FGF18 marks a myofibroblast lineage, providing evidence that distinct FGF signaling is necessary to induce certain functional fibroblast stages. The SHH ligand from the distal epithelium is required for the formation of Gli1+ secondary-crest...
myofibroblasts as a lack of SHH signaling blocks myofibroblast differentiation.\textsuperscript{3,32,85-87} The BMP/SMAD signaling pathway is reported to regulate alveolar stem cell proliferation and differentiation. PDGFRα+ myofibroblasts produce BMP4, which acts antagonistically to WNT and regulates AT2 cell renewal, differentiation, and regeneration.\textsuperscript{69} Alveolar niche cells produce WNT ligands, which act mainly through canonical WNT signaling to replenish the AXIN2+ epithelial progenitor pool during development and repair.\textsuperscript{70,71,88} Thus, all these fibroblast stages are integrated to form a fully functional alveolar niche. To aid in visualization, many of these signaling ligands that are specific to certain fibroblast subtypes during development are pictured in the legend of Figure 1. Further information about pulmonary fibroblast lineages during development can be found in a recent review that details the pathways and mice used for lineage-tracing studies in the mesenchyme.\textsuperscript{15}

Besides these common and developmentally conserved pathways, hormones and steroids are emerging as significant modulators of fibroblast differentiation in the lung.\textsuperscript{89} Recent findings identified that glucocorticoid receptor expression in pulmonary fibroblasts modulates alveolar maturation.\textsuperscript{9} Glucocorticoid enhanced the differentiation of proliferative mesenchymal progenitor cells into matrixfibroblasts by a mechanism involving extracellular matrix-associated target gene expression (including Fn1, Col16a4, and Eln) and by modulating vascular endothelial growth factor (VEGF). Janus kinase-signal transducer and activator of transcription proteins (JAK-STAT), and WNT signaling.

Based on scRNA-seq data, there are two distinct types of matrixfibroblasts characterized by the expression of cell-selective markers: MatrixFB-1 and MatrixFB-2. MatrixFB-1 is known by the expression of molecular regulators such as WNT, FGF signaling, and T-box binding domains. MatrixFB-2 is significantly enriched for Sfrp2, an inhibitor of WNT signaling, and a family of insulin-like growth factors. Although these matrixfibroblasts differ largely in their signature genes and main signaling pathways, some shared gene expression profiles suggest overlapping physiological functions such as ECM organization and collagen formation. Their spatial location, however, has not been defined thus far and might explain differences in paracrine signaling profiles.\textsuperscript{11} Future scRNA-seq studies and validation of their spatial and temporal localization will aid in understanding the differential and overlapping functions of various populations and cell stages of fibroblasts. Although interstitial fibroblasts play an important role in developmental alveolarization, they are equally important in injury and repair.

### 1.3 Fibroblasts in murine models of reseptation and repair

#### Reseptation

Although adult compensatory lung growth is restricted in humans,\textsuperscript{90} unilateral left lobe PNX in mice initiates realveolarization in the remaining right lobes. PNX is an excellent model system to study molecular and cellular mechanisms of alveolar regeneration but does not recapitulate injury response. During realveolarization, myo- fibroblasts are critical to extend new septa (reseptation), and matrixfibroblasts are required for the production of new ECM to stabilize the septa.\textsuperscript{29} The different fibroblast stages in reseptation after PNX are visualized in Figure 2A, along with their signature gene expression profiles. After PNX, septal tip myofibroblasts, which are PDGFRα-GFP\textsuperscript{dim} and alpha smooth muscle actin (αSMA) positive, increase in total number. PDGFRα-GFP\textsuperscript{bright} matrixfibroblasts subsequently expand and produce ECM required for the newly forming septal tip, defining the alveolar entry ring. A second wave of myofibroblast activation is observed toward the end of reseptation.\textsuperscript{2,26,29,35} Taken together, these data demonstrate that dynamic PDGFRα activity controls temporal switches between myofibroblast and matrixfibroblast stages.\textsuperscript{2,29,35}

Lipofibroblasts transfer neutral lipids to AT2 cells for surfactant phospholipid synthesis with the help of adipose differentiation-related protein (ADRP)\textsuperscript{91} and support AT2 cell proliferation and differentiation into new AT1 cells.\textsuperscript{2,53,92} At a transcriptional level, activation of the transcription factor PPARγ via rosiglitazone treatment has been shown to induce lipofibroblast differentiation.\textsuperscript{93} In the context of injury response, rosiglitazone treatment significantly reduced transforming growth factor-β (TGFβ)-mediated myofibroblast differentiation. During regeneration following PNX, rosiglitazone treatment inhibited reseptation as myofibroblast activation in PDGFRα+ fibroblasts was blocked.\textsuperscript{29} These data suggest PPARγ signaling as both an inhibitor of myofibroblast differentiation and an activator of lipofibroblast differentiation and support the theory that lipofibroblast differentiation is necessary to drive new septa formation.\textsuperscript{94} These regeneration studies revealed molecular drivers and cell type-specific roles of fibroblasts during reseptation. In the future, the use of transgenic mice with gene activation and inactivation before PNX surgery or during regeneration has great potential for discerning the molecular regulation of regeneration.

Age-specific decline in alveolar regeneration has been investigated in murine models after PNX. Decreased fibroblast clonality and increased myofibroblast differentiation impair reseptation in aged mice compared with young mice.\textsuperscript{95-97} In addition, perinatal hyperoxia exposure has recently been linked to the induction of senescence in the mesenchyme and is demonstrated to be a cause of BPD.\textsuperscript{98} Priming aged mice with epigenetic modifiers to study gene silencing in the context of failed lung regeneration has translational application for initiating regeneration in human lungs. Although the murine PNX model gives insight into the role of fibroblasts during reseptation, a plethora of murine lung fibrosis models has been used to study the contribution of fibroblasts in chronic lung disease.

#### Injury and nonfibrotic repair

Bleomycin injury has been extensively used to study acute lung fibrosis and the contribution of various cell types to the fibrotic response. Mechanical and paracrine cues from the site of injury regulate
FIGURE 2  A, Role of resident interstitial fibroblasts in bleomycin-induced fibrosis and realveolarization. Illustrations of spatial location and gene expression of the major fibroblast subtypes/stages in regeneration after partial pneumonectomy (PNX) and fibrosis/repair after bleomycin lung injury. To highlight critical epithelial-mesenchymal crosstalk during repation or injury repair, signature ligands and receptors of epithelial and fibroblast subtypes are listed in tables next to the illustrations. Differences between the myofibroblast niche and epithelial repair niche in fibrosis are given within the schematic illustration of fibrosis. The bleomycin illustration follows a chronological progression from fibrosis to repair/fibrosis resolution to homeostasis (left to right). Similar illustration of alveolar regeneration after PNX. The figure legend shows each cell type and extracellular matrix (ECM) components depicted in the figure. B, Context-dependent interstitial fibroblast cell stage in IPF and BPD. Although lineage-tracing experiments in murine injury models revealed some insight of the origin of the fibrotic fibroblast, we are limited to drawing any parallels to human diseases by reviewing single-cell RNA sequencing, bulk RNA sequencing, flow cytometry, and immunofluorescence from fibrotic human lung tissue. On the left are illustrations of altered morphology of the lung in idiopathic pulmonary fibrosis (IPF) and bronchopulmonary dysplasia (BPD). IPF has extensive regions of fibrosis, parenchymal honeycombing, and bronchiolization of the epithelium. BPD has alveolar simplification and septal wall thinning. Arrows point to hallmark morphological changes, described in the central portion of the figure. Signature genes and their expression changes for each fibroblast population in both diseases are listed on the right.
controlled recruitment of fibroblasts to initiate repair in the bleomycin models. As each fibroblast population/stage has unique functions, murine lung models of fibrosis demonstrate that not all fibroblasts have the same injury response. Different stages of fibroblasts contribute to either fibrosis or regeneration after injury and are visualized, along with their signature gene expression, in Figure 2A. A subset of myofibroblasts identified by Axin2+ responsiveness (AMPs) become pathologically deleterious myofibroblasts in the alveolar niche that proliferate and create extensive ECM components. MANCs, identified by scRNA-seq and spatial mapping analyses, are Axin2+ PDGFRα+ fibroblast subtypes and contribute to regeneration. MANCs initiate reciprocal paracrine signaling with AT2 cells and AEPs to promote alveolar growth and regeneration after bleomycin injury. The contribution of lipofibroblasts to bleomycin injury has been studied using ADRP as a lineage tracer. In response to bleomycin injury, lipofibroblasts transdifferentiate into αSMA-expressing myofibroblasts and revert to lipofibroblasts as injury resolves. These data support the hypothesis that a subset of lipofibroblasts give rise to myofibroblasts after lung injury and that lipofibroblast reconstitution is required for lung regeneration. However, the existence of lipofibroblasts in human lungs remains to be further investigated. Recent scRNA-seq analysis on human lungs discerns a lipofibroblast signature that resembles the murine lipofibroblast but, in comparison with other stromal cells within the dataset, is a small population. Single-dose bleomycin and flu injury enable lung recovery to occur, whereas multidose bleomycin, surfactant protein C (SPC) mutant epithelium, and diphtheria toxin receptor transgenic mice are models for chronic lung fibrosis. These chronic fibrosis models have been used to study epithelial injury and repair; future studies focusing on the fibroblast response will shed more light on the epithelial-mesenchymal crosstalk during injury and fibrosis initiation.

1.4 Fibroblasts in human disease

The functional significance of fibroblasts is not limited to development and regeneration in animal models but extends to human lung pathology. A significant increase, decrease, or shift in fibroblast functional stages/phenotypes changes homeostasis. Change in fibroblast activation stages affects the functionality of neighboring cells resulting in IPF, BPD, and COPD.

1.4.1 Idiopathic pulmonary fibrosis

IPF is characterized by extensive fibrosis, causing progressive respiratory decline and mortality, usually within 5 years of diagnosis. Although the pathogenesis of IPF remains unclear, chronic alveolar epithelial cell injury and chronic fibrotic activation of fibroblasts are linked to the disorder. Treatment regimens using pirfenidone and nintedanib showed effectiveness in reducing morbidity but not mortality. Considerable effort has been taken to study the role and origin of fibroblasts as they are promising targets of antifibrotic therapy. At the tissue level, IPF is defined by a fibroblastic focus with an immuno-hyaluronic acid-rich matrix underneath the epithelial layer, loss of alveolar type 1 cell differentiation, and increased αSMA+ myofibroblasts. The presence of epithelial basal-like cells that coexpress epithelial and mesenchymal markers has been reported in IPF lungs by scRNA-seq. These indeterminate alveolar type 2 cells were found to be located at the edge of myofibroblast foci in the IPF lung. Despite its limitations, bleomycin injury in mice has been used to model and study IPF. In both human IPF samples and murine models of pulmonary fibrosis, the myofibroblast population expands considerably. Myofibroblasts arise from both resident myofibroblasts and resident lipofibroblasts, suggesting aberrant fibrotic activation in a variety of fibroblast populations. In the bleomycin injury model, interstitial lung fibroblasts, pericytes, and mesothelial cells are known to differentiate into myofibroblasts. Partial epithelial-mesenchymal transition has also been reported using multiple reported systems and injury models.

Lipofibroblasts, whose existence was once questioned in the adult human lung, have recently been identified as a stable cell population during homeostasis using scRNA-seq. Studies of lipofibroblast function in the murine lung indicate a role in fibrosis. As previously mentioned, PDGFα-expressing lipofibroblasts differentiate into myofibroblasts upon injury and transdifferentiate back to lipofibroblasts during fibrosis resolution in the mouse lung. In IPF, lipofibroblasts are prominent; however, the relative mRNA expression of canonical lipofibroblast markers PLIN2, PPARγ, and TCF21 is reduced. Another study using freshly isolated PDGFα-expressing fibroblasts from IPF lungs showed that PDGFα+ lipofibroblasts shift to a PDGFα+ myofibroblast stage/activation. Moreover, PDGFα+ matrixfibroblasts are significantly reduced in IPF lungs. In a transgenic mouse model, expression of a constitutively active PDGFα+ kinase mutation in PDGFα+ cells significantly increased matrixfibroblast over myofibroblast differentiation. The dynamic gene expression changes of myofibroblasts, lipofibroblasts, and matrixfibroblasts in IPF and mouse models of fibrosis are displayed in Figure 2B. Recent in vitro organoid studies demonstrated that fibroblasts from aged mice or adult human donors do not support alveolar type 2 to type 1 differentiation. In organoid cultures, young fibroblasts support alveolar type 1 cell differentiation, even in “indetermined” epithelial cells isolated from human IPF lungs. PDGF-A treatment of human lung organoids, administered with aged and IPF fibroblasts, restored epithelial alveolar type 1 cell differentiation. These results suggest that restoration of the PDGFα-high matrixfibroblast stage may be beneficial for restoring epithelial differentiation to alveolar type 1 cells, which is lost in IPF. “Activated myofibroblasts” identified by scRNA-seq studies of IPF and other fibrotic lung diseases often share signature genes normally associated with the developmental matrixfibroblast. These studies suggest that, in IPF, not only epithelial cells but also fibroblasts can take on an indeterminate form, such as a “myo/matrix fibroblast.” As myofibroblasts are the driver of IPF, and beneficial matrix function is lost, it is
important for future studies to identify key pathways that trigger the activation of fibrotic matrix phenotypes over the beneficial regenerative matrix phenotype. Future studies using both in vivo lineage labeling and in vitro organoid cultures will shed more light on the plasticity of fibroblasts and their limitations to contribute to nonfibrotic repair after proinflammatory stimulus.

1.4.2 | Bronchopulmonary dysplasia

Hyperoxic and hyperbaric conditions due to supplemental oxygen in association with premature birth cause disruption of alveologenesis, resulting in permanent alveolar simplification (BPD).124 Because of medical advances like antenatal steroid treatment and neonatal surfactant therapy, the fibrotic pathophysiology of “Old” BPD as described by Northway is rarely seen.125 The “New” BPD, constituting alveolar simplification via arrest of lung development, remains a prominent comorbidity of premature birth today.126,127 The injury is further characterized by damage to the lung epithelial cells that normally facilitate gas exchange and disruption in vascular development, particularly alveolar capillaries.128 Unraveling the molecular mechanisms of fibroblast response to hyperoxia is essential to develop new strategies for the prevention of BPD. As previously mentioned, PDGFRα signaling is necessary for the normal functioning myofibroblasts that drive alveolar septation during distal lung development.129-131 In mice, pharmacological or genetic ablation of PDGFRα signaling results in the loss of alveolar myofibroblasts and failure of alveolar septation.132,133 In vitro studies suggest that failed alveolarization could be attributed in part to the loss of beneficial lipofibroblasts and activation of myofibroblasts.131 Upon in vitro exposure to hyperoxia, lipofibroblasts rapidly transdifferentiate into a myogenic phenotype.100 A lack of lipofibroblasts causes a decrease in the production of surfactants as they are the major providers of triglycerols to alveolar type 2 cells. Loss of lipofibroblasts and poor alveolar epithelial cell growth and differentiation result in failed alveolarization and BPD.100 These preclinical observations implicate a critical role for PDGFRα fibroblasts in BPD. In humans, reduced PDGFRα expression was reported in neonatal mesenchymal stromal cells obtained from infants with BPD.132 Recent scRNA-seq data denote an extensive loss of PDGFRα+ fibroblasts in BPD and an increase of immature fibroblast subtypes accompanied by a reduction of mature matrix, lipofibroblasts, and myofibroblasts.133,134 The dynamic gene expression changes of fibroblasts in BPD and in vivo animal hyperoxia models are summarized in Figure 2B. BPD has recently been identified as a risk factor for COPD and severity of Coronavirus disease 2019 in both murine models and epidemiological studies, highlighting the importance of understanding the long-term consequences of BPD.135-138

Limitations and alternative models for BPD

In vitro studies are a reductionist approach that is inherently limited because of a lack of cellular microenvironmental context. On the other hand, the in vivo murine hyperoxia model is limited as mice are naturally born in the saccular phase.139 To interrupt lung development during lung sacculation and simultaneously recapitulate BPD, rabbit kits can be delivered via c-section and subjected to mechanical ventilation.140,141 Modeling of BPD in sheep and nonhuman primates is expensive but provides excellent alternatives to better understand human lung development and BPD.142-146

The recent development of organoid and precision-cut lung slice (PCLS) models recapitulate certain aspects of hyperoxia exposure during alveolarization. Exposure of three-dimensional organotypic coculture to hyperoxia mimics aspects of BPD pathogenesis, including activation of ACTA2 and COL1A1 expression in fibroblasts.147 Ex vivo PCLS exposed to hyperoxia maintain some features of pulmonary architecture and facilitate live imaging studies to assess cellular migration, proliferation, and differentiation.148 To advance the field of BPD, we will have to integrate findings from old and newly developing BPD model systems, with transcriptomic and proteomic analysis.

1.4.3 | Chronic obstructive pulmonary disease

Irreversible airway obstruction148 with emphysematous changes, such as loss of elastic fibers in the alveolar walls and subsequent destruction of the alveoli, define the pathology of COPD.149 Reduced fibroblast proliferation and altered repair mechanisms contribute to the emphysematous lung.150 A role for TGFβ1 has been reported in COPD patients compared with healthy control patients151 and showed that COPD fibroblasts are less responsive to TGFβ1 in terms of proliferation and elastin production compared with normal fibroblasts.152 Fibroblasts from moderate to severe COPD subjects show a secretory phenotype with upregulation of inflammatory molecules and increased soluble elastin. The formation of soluble elastin was inhibited by versican, an inflammatory matrix proteoglycan, which is predominately expressed in myofibroblasts.153 Furthermore, studies on COPD fibroblasts also show less chemotactic activity and collagen contraction.154 Although interstitial fibroblasts are important for septal tip formation and elastin deposition during development and repair, their role in COPD has not been studied. Investigating mechanisms and consequences to understand the association of functional fibroblast stages and ECM damage may pave the way for better outcomes.

2 | CONCLUSION

The advent of scRNA-seq has greatly strengthened the understanding of fibroblast heterogeneity within the lung field but, at the same time, has added to confusion over fibroblast nomenclature, fibroblast populations, and functional fibroblast stages. Although it is now possible to cluster similar cells based on gene expression in a total and unbiased manner, it becomes difficult to determine if fibroblasts exist as unique lineages or rather reside on a spectrum of activation and cell stages. Certain fibroblasts, like the myo- and lipofibroblasts, demonstrate clear functional, morphological, and lineage differences in development and disease. The matrixfibroblast and alveolar niche cell, however, remain mysterious and may be overlapping, nondistinct stages of the same fibroblast subtype. Both cell “types” aid in alveolar
organoid formation and express genes required for ECM production, suggesting functionality somewhere between the contractile myofibroblast and secretory matrixfibroblast. Further studies on the origin of the alveolar niche cell, as it is currently defined, will clarify whether the niche cell is a quiescent stem cell or just a quiet matrixfibroblast. If lineage relationships exist, they might be revealed with the help of pseudo-time analysis on developmental scRNA-seq studies in fibroblasts. A recent study identified both the transcriptional signatures and locations of 58 cell types in the human lung, including nine stromal subtypes.47 Pursing apart the heterogeneity and plasticity of the mesenchyme in the lung is necessary to develop effective therapies for BPD, IPF, COPD, and other human lung diseases.

As reflected in PNX studies, activation of proper fibroblast subtypes occurs in both a spatial and temporal manner, suggesting that treatment with pan-inhibitors of signaling pathways to reduce fibrotic activation of one fibroblast's function would impede the regenerative function of another. This might be why anti-fibrotic drugs like nintedanib and pirfenidone are not remarkably effective IPF therapies as they antagonize regenerative fibroblast activation. Future studies in murine models with combinatorial and time-restricted treatment of multiple drugs, to target different aspects of fibrosis and subsequent repair, will likely yield a better understanding of how to treat human lung diseases. The fibroblast will remain a critical mediator in any of these processes.

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CONFLICT OF INTEREST
The authors declared no potential conflicts of interest.

AUTHOR CONTRIBUTIONS
A.K.P., M.G.U. and M.R. wrote the manuscript. M.R. created the figures.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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