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Heterogeneity of Fibroblasts and Myofibroblasts in Pulmonary Fibrosis

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Abstract

Purpose of review—Idiopathic Pulmonary Fibrosis (IPF) is the most common form of interstitial lung diseases of unknown eathiopathogenesis, mean survival of 3-5 years and limited therapeutics. Characterized by a loss of alveolar type II epithelial cells and aberrant activation of stromal cells, considerable effort was undertaken to characterize the origin and activation mechanisms of fibroblasts and myofibroblasts in IPF lungs. In this review, the origin and contribution of fibroblast and myofibroblasts in lung fibrosis will be summarized.

Recent findings—Lineage tracing experiments suggested that interstitial lung fibroblasts and lipofibroblasts, pericytes and mesothelial cells differentiate into myofibroblasts. However, epithelial and bone marrow derived cells may give rise to collagen expressing fibroblasts but do not differentiate into myofibroblasts.

Summary—There is great heterogeneity in fibroblasts and myofibroblasts in fibrotic lungs. Further, there is evidence for the expansion of pericyte derived myofibroblasts and loss of lipofibroblasts and lipofibroblast derived myofibroblasts in IPF.

Keywords

Idiopathic Pulmonary Fibrosis; Lung Fibrosis; Lung Fibroblasts; Mesenchymal progenitors; Fibroblast progenitors; fibroblast heterogeneity

Introduction

Idiopathic Pulmonary Fibrosis (IPF) is the most common clinical form of Interstitial Lung Disease (ILD), with poor prognosis, median survival of 3–5 years after diagnosis, and limited pharmacological intervention[1–3]. IPF is characterized histologically by the presence of Usual Interstitial Pneumonia (UIP), containing fibroblastic foci, which are believed to be sites of active tissue remodeling. The fibrotic triggers in IPF are unknown but

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it is speculated that recurring micro-injury leads to alveolar epithelial cell death, epithelial progenitor exhaustion, and subsequent aberrant repair mechanism(s) ablates the alveolus. IPF patients present with variable progression rates, with some showing a slow decline and others showing a more rapid progressive decline of lung function[4]. In addition to the variable progression rates, some IPF patients experience acute exacerbations, where diffuse alveolar damage is apparent and subsequent rapid decline of lung function ensues[5]. Indeed, various studies have focused on identifying mechanisms behind the progressive decline of lung function in IPF patients, which have identified multiple pathways modulating epithelial regeneration, immune activation, and fibroblast differentiation and invasion. In this review, the heterogeneity of mesenchymal cells in normal and fibrotic murine lungs, mechanisms propagated by these cells in preclinical models of lung fibrosis will be summarized and their potential human counterparts in IPF will be discussed. Due to wording limitations, reference to all relevant studies pertaining to stromal cell function and heterogeneity in the lung is not possible, and the authors apologize for any omissions.

Mesodermal development in the lung

Mesodermal development in the lung is a complex but poorly understood process due to a lack of good mesodermal markers. However, with the advent of cell tracing techniques, various recent studies have provided some key insight into mesodermal development in the lung. Pulmonary mesoderm is thought to be derived from the lateral plate mesoderm[6], which gives rise to the splanchnic mesoderm and subsequently to the mesodermal lineages in the heart, lung, gut, connective tissues, and blood vessels. One of the earliest mesenchymal specific transcription factor to be activated in the developing murine respiratory system is TBX4, which is observed as early as E9.25 in lung buds and is expressed most mesenchymal cells by E11.5[7, 8]. Following *Tbx4* expression, *Gli1* (a direct Sonic Hedgehog (Shh) signaling transcriptional target) is detected at E10.5–11.5[9], and it remains activated for the development of bronchial and vascular smooth muscle cells and various stromal cell populations in murine lungs[9, 10]. Following Shh signaling, it is speculated that Wnt singling is required for further differentiation of mesodermal progenitors into pericytes, resident interstitial fibroblasts, and smooth muscle cells[9, 11, 12]. Pericytes appear to arise from *Foxn1*-expressing perivascular progenitor cells, which were shown to give rise to several distinct populations of PDGFRß$^{+}$ NG2^{+} pericytes, some of which co-express PDGFRα and collagen 1[13]. Pericyte heterogeneity is exemplified by the further differentiation of *Foxj1* expressing pericyte progenitors into mature perivascular *NG2*-expressing pericytes[14]. Interstitial Collagen 1-expressing fibroblasts arise from mesodermal progenitors in a Wnt-[9, 12], Shh-[9] and PDGFRα-[15–17] dependent manner. The differentiation of pulmonary vascular and bronchial smooth muscle cells is a complex process[18] requiring the activation of both Shh[9] and FGF10[19] signaling, and the inhibition of FGF9 signaling[20]. Further, a subset of resident peribronchial fibroblasts, bronchial and vascular smooth muscle cells arise from Wt1- expressing mesothelial cells[21, 22]. Finally, lipofibroblasts, a neutral lipid-rich fibroblast population that are required for alveolar type II epithelial cell proliferation, differentiation, and surfactant protein production[23] arise from mesodermal progenitor cells through the activation of FGF10[24, 19] and PPARγ[25] and the inhibition of ALK5[26]. Together, these various cell types

comprise the pulmonary mesenchyme and considerable effort has focused on characterizing their roles in lung remodeling and regeneration.

Fibroblast and myofibroblasts heterogeneity in injured murine lungs

Due to a heightened interest in developing anti-fibrotic therapeutics, considerable effort has been directed at characterizing the cellular origin of fibroblasts and myofibroblasts during organ fibrosis. In this section, a summary of findings from various studies characterizing the origin of collagen - and/or αSMA-expressing fibroblasts and myofibroblasts in experimental lung injury in mice is provided. A simplified pictorial summary of key mesenchymal and hematopoietic lineages present in the lung during fibrosis and myofibroblast differentiation is shown in Figure 1 and summarized in Table 1. For additional review on this topic, the reader is referred to[27].

Pericytes

Pericytes are heterogeneous perivascular, mesenchymal-like cells that are known to regulate blood vessel formation and maintenance under physiologic conditions, and have an emerging role in lung remodeling (reviewed in[27]). In a recent study, NG2 (a cell surface pericyte marker) and Foxj1 (a pericyte and club cell specific transcription factor) were used as lineage tracers for pericytes in a mouse model of bleomycin-induced pulmonary fibrosis. In this study, no significant contribution of lineage-labeled pericytes to αSMA expressing myofibroblasts, present at day 21 after intratracheal bleomycin instillation, was observed[14]; however, the contribution of lineage-labeled cells to collagen 1-expressing fibroblasts was not assessed. In another study that employed both Foxn1 and collagen 1 as a lineage markers[13], Foxn1 labeled two distinct PDGFRß$^{+}$ NG2^{+} pericyte lineages, one of which expressed collagen 1 (PDGFRβ^{+} PDGFRα^{+} Collagen 1^{+}; Figure 1). At days 7 and 14 after bleomycin-induced lung injury, Foxd1-labeled cells accounted for ~45% and ~68% of αSMA expressing myofibroblasts in the lungs, respectively. Consistent with these findings, when Foxd1 and Collagen 1 chimeric reporter mice were studied, approximately 47% of Collagen 1 lineage-labeled cells were also lineage labeled for Foxd1 at day 7 after bleomycin challenge. However, this finding also highlights that approximately 53% of collagen 1 lineage-labeled cells were not labeled with the Foxd1 lineage tracer suggesting that the majority of collagen 1-expressing cells come from other sources in experimental fibrosis. In a third study using ABCG2 as a lineage tracer for a subset of NG2^{-} pericytes[28], ABCG2^{+} cells gave rise to αSMA^{+} myofibroblasts at day 14 after intrathecal bleomycin delivery. Further, *in vitro* analysis indicated that AGCG2^{+} cells were phenotypically, genotypically, and functionally distinct from NG2^{+} pericytes. Collectively, these studies provide evidence for the heterogeneity of pericytes in the lung and suggest that there are several populations of NG2/Foxj1-negative pericytes, which give rise to myofibroblasts in lung fibrosis.

Resident fibroblasts and lipofibroblasts

Resident fibroblasts are defined as fibroblasts that are localized in the interstitium of uninjured lungs immediately adjacent to alveolar epithelial cells. Lipofibroblasts are resident lung fibroblasts rich in neutral lipids that have been shown to be important for alveolar

epithelial type II cell homeostasis. These cells are known to express high levels of the Adipose Differentiation-Related Protein (ADRP), PPARγ and PTHrP receptor (PTH1R). Several investigators have embarked on studies to identify cell surface fibroblast specific markers but to date success has been limited. These studies have culminated on the identification of a few markers including Sca-1[29], CD248[29–32], PDGFRα[17, 33, 15, 16, 34] as good candidate markers. Among them, PDGFRα seems to best distinguish interstitial resident fibroblasts and lipofibroblasts from pericytes, where resident fibroblasts are lineage negative (Lin^-; $CD45^-$ $EpCAM^-$ & $CD31^-$), $PDGFR\alpha^+$, $PDGFR\beta^-$, $CD90^-$, lipofibroblasts are Lin^-, $PDGFR\alpha^+$, $PDGFR\beta^-$, $CD90^+$ and $LipidTOX^+$ (A Neutral lipid stain) and a small subset of pericytes are Lin^-, $PDGFR\alpha^+$ & $PDGFR\beta^+$[13, 17, 29]. Collagen 1 lineage-labeled $PDGFR\alpha^+$ $PDGFR\beta^-$ cells (which are distinct from lineage-labeled pericytes, see above), contributed to ~53% of collagen 1-expressing cells at day 7 after bleomycin-induced lung injury and remodeling[13]. In another study utilizing PDGFRα as a lineage tracer and pneumonectomy as a model of lung injury, two major subtypes of PDGFRα-expressing cells were identified in uninjured lungs - a more abundant $PDGFR\alpha^{hi}$ and a less abundant $PDGFR\alpha^{low}$ cell types[17]. Via transcriptomic characterization, $PDGFR\alpha^{low}$ cells were enriched for various transmembrane collagens and other transcripts typically observed in myofibroblasts. However, a few reports have suggested that αSMA expression was confined to vascular and bronchial smooth muscle cells in uninjured murine lungs[14] and that these cells did not contribute to interstitial αSMA^+ myofibroblasts after bleomycin injury[35], suggesting that that the $PDGFR\alpha^{low}$ population might represent a population of smooth muscle cells. $PDGFR\alpha^{hi}$ cells were enriched for fibrillar collagens, matrix remodeling enzymes (Loxl2, Loxl4, MMP3, MMP17 & MMP17) consistent with interstitial fibroblasts. Flow cytometric analysis of PDGFRα lineage-labeled cells indicated that ~60% of these cells expressed Sca-1, ~50% expressed CD34, ~30% expressed CD29, <10% expressed Thy-1, CD44 and/or CD49, and a small subset (<5%) expressed CD40. After pneumonectomy, CD29-expressing, PDGFRα lineage-labeled cells were more proliferative, and expressed the highest level of αSMA, whereas CD34 and Sca1 expressing lineage-labeled cells were less abundant. Interestingly, $CD34^+$ PDGFRα lineage-labeled cells showed the highest levels of neutral lipid content indicating that these cells might represent the PDGFRα-expressing lipofibroblast population. In addition, there were various subsets of αSMA-expressing cells, including $PDGFR\alpha^+$ $CD34^+$, $PDGFR\alpha^+$ $CD34^+$ $CD29^+$ and $PDGFR\alpha^+$ $CD34^-$ $CD29^+$ suggesting considerable heterogeneity in αSMA-expressing cells at day 5 after pneumonectomy. Nevertheless, this study did not address the relative contribution of PDGFRα-expressing pericytes co-expressing PDGFRβ to the αSMA-expressing myofibroblast population in this model.

To better characterize the role of lipofibroblasts in lung remodeling and repair, the lipofibroblast specific transcript *adrp* has been utilized as a lineage tracer. In a recent study, a subset of *adrp* lineage-labeled lipofibroblasts transdifferentiated into αSMA-expressing myofibroblasts in response to bleomycin injury. Interestingly, FGF10 expression and neutral lipid content was observed to increase in αSMA-expressing myofibroblasts from days 14 to 28 after bleomycin challenge. This was followed by a loss of αSMA expression and the reappearance of lipofibroblasts, suggesting that myofibroblast to lipofibroblast trans-differentiation occurs during the resolution of bleomycin-induced lung remodeling[35].

Coincidently, the PPAR-γ agonist, Rosiglitazone, which have been shown to induce lipofibroblast differentiation[25], markedly reduced αSMA expression in interstitial fibroblasts after pneumonectomy[36] and attenuated TGFß-induced human lung fibroblast to myofibroblast differentiation[35], further supporting the hypothesis that a subset of lipofibroblasts give rise to myofibroblasts after lung injury and that lipofibroblast reconstitution is required for lung regeneration. Collectively, these studies suggest that PDGFRα-expressing interstitial fibroblasts and/or lipofibroblasts respond to lung injury and differentiate into myofibroblasts, and that myofibroblast to lipofibroblasts trans-differentiation is required for the resolution of fibrosis in the mouse lung.

Epithelial and mesothelial cells

The role of epithelial to mesenchymal transition (EMT) in pulmonary fibrosis remains controversial. *In vitro* studies have demonstrated that EMT occurs in TGFß treated alveolar type II epithelial cells[37] and pulmonary mesothelial cells[38]. To determine the contribution of alveolar type II epithelial cells to generation of lung fibroblasts and myofibroblasts *in vivo, sftpc* (the gene encoding for Pro-Surfactant protein C) was used as a cell lineage tracer. Utilizing an active TGFß adenoviral expression model of pulmonary fibrosis in a triple transgenic reporter mouse (*sftpc-rtTA, tetO-CMV-Cre, Floxed ROSA26*) to drive the permanent expression of ß-gal in *sftpc*-expressing cells after doxycycline treatment, *sftpc* lineage-traced cells were observed to express vimentin at 3 weeks after intranasal delivery of adenovirus[39]. Further, these cells made up of <20% of total vimentin-expressing cells in this model. However, there were few lineage-labeled cells that expressed αSMA (<5%), suggesting that these cells were not a major source of myofibroblasts in this study. Further, in two other studies using *sftpc-Cre, R26Rosa.stop.LacZ* reporter mice (which express ß-gal in all *sftpc*-expressing cells in the lung) *sftpc* lineage-labeled cells were observed to give rise to S100A4-expressing fibroblast-like cells, in response to a single dose[37] or a repetitive dosing regimen[40] of bleomycin. Also in both studies, *sftpc*-labeled epithelial cells were not observed to express αSMA after lung injury and remodeling. In another study using an *sftpc-CreER^{T2},ROSA-tomato* double heterozygous mice to lineage label *sftpc*-expressing cells after tamoxifen treatment, lineage-labeled cells were not observed to give rise to αSMA, vimentin or S100A4 expressing cells at 10 and 21 days after bleomycin challenge[14]. This was confirmed via cell sorting of lineage-labeled cells followed by qPCR analysis of the sorted cells relative to lineage-negative cell. Unlike these negative cells, lineage-labeled cells expressed negligible amounts of *acta2, vim, s100a4*, and *col1a1* transcripts. In the same study, using a club cell specific *scgb1a1-CreER; ROSA Tomato* lineage tracer to label *scgb1a1* expressing club cells upon tamoxifen treatment, lineage-labeled cells did not give rise to αSMA, vimentin or S100A4 expressing cells at day 21 after bleomycin challenge. Interestingly, in another study using an *Acta2-Cre, R26Rosa.stop.LacZ* lineage driver to label β-gal in all *acta2* expressing cells, scattered bronchial epithelial cells were lineage-labeled, suggesting that *acta2* expression does not readily occur in these cells[41]. Unfortunately, the relative contribution of bronchial epithelial cells to *acta2* expressing myofibroblasts was not systematically examined in this study.

Discrepancies between these studies might be due to differences in the reporter system(s) or the fibrotic models utilized. The mesenchymal transition of alveolar type II epithelial cells has been observed to be dependent on the presence of deposited fibrin and/or fibronectin[39], suggesting that potential differences in fibronectin and/or fibrin deposition in different murine models of pulmonary fibrosis could potentially contribute to the divergent observations in these studies. A seminal study employing single cell RNAseq analysis of epithelial cells isolated from normal and IPF lung samples demonstrated the co-expression of basal cell markers (i.e. KRT5 and TP63) and the mesenchymal marker, vimentin, in IPF but not normal epithelial cells[42]. Collectively, these studies raise the possibility that alveolar type II and basal epithelial cells might give rise to collagen 1, vimentin and/or S100A4 expressing fibroblasts, but there is no conclusive evidence for these cells also giving rise to αSMA-expressing myofibroblasts. Future studies are warranted to better characterize the transition of *Sftpc*-expressing alveolar epithelial and *Krt5 Tp63*-expressing cells into mesenchymal cells *in vivo*.

Pulmonary mesothelial cells are cells lining the pulmonary pleura. Since lung remodeling in IPF is first observed in the distal subpleural regions in the lung, there is considerable interest in determining the role of these cells in this disease. Most studies characterizing pulmonary mesothelial cells in lung fibrosis utilize Wilms' tumor suppressor gene, *wt1*, as a marker for these cells. *Wt1* is exclusively expressed in embryonic mesothelial cells as early as embryonic day E10.5 and by E18.5 its expression levels significantly declines such that this gene is not expressed in adult lungs[21, 43]. Importantly, several studies have shown that this tumor suppressor is induced after lung injury and/or remodeling[44, 45, 43]. Two studies utilizing $Wt1^{tm1EGFP/Cre)wtp/}J$ to label all cells with an active *wt1* promoter with EGFP have showed that lineage-labeled cells invaded into the pulmonary parenchyma and a subset of these cells expressed αSMA at 4 to 24 h after intratracheal delivery of active TGFß[44, 45]. This was confirmed using a different *wt1* lineage tracing mouse strain, $Wt1^{Tm2(Cre/ERT2)Wtp}/J$; B6.129S4-Gt(ROSA)-26Sor^{tm1Sor}/J to label *wt1*-expressing cells irreversibly with ß-gal at 4 h after intratracheal TGFß administration[44]. Further, in a CCSP/TGFα transgenic mouse model of lung fibrosis, Wt1-immunostained cells appeared to invade into the pulmonary parenchyma and express vimentin by co-immunofluorescent analysis[43]. Interestingly, utilizing a $Wt1^{CreERT1/+}ROSA^{mTmG/+}$ strain to label all cells with tdTomato and *wt1*-expressing cells with GFP, intradermal bleomycin administration induced pleural thickening and increased *wt1* lineage-labeled cells in the pulmonary pleura but these cells were not observed to invade into the pulmonary parenchyma[43]. Indeed, a similar observation was made in another report, utilizing $Wt1^{CreERT2/+}ROSA26^{mTmG/}$ strain in which tamoxifen was administered at postnatal day P4 to label *wt1*-expressing cells, and lung remodeling was induced via 8 biweekly intratracheal doses of bleomycin[22]. Surprisingly, *wt1* lineage labeled cell proliferation and pleural thickening at 4 weeks after intratracheal active TGFß adenoviral overexpression did not lead to vimentin, or αSMA expression by lineage-labeled cells.

Differences observed in these studies might be due to the lineage tracing methods employed and/or the variable changes in WT1 expression after the distinct forms of pulmonary injury. It is also possible that *de novo* WT1 expression in invading mesothelial cells might represent a population of cells undergoing mesenchymal transition. Transient lineage labeling of *wt1*

expressing cells prior to lung injury might not lineage label *de novo* WT1 -expressing cells thereby explaining why cells invaded into the parenchyma and expressed stromal markers were observed in studies in which constitutive *wt1* labeling reporters were utilized. Finally, it has been observed that pleural thickening in IPF is associated with the parenchymal localization of Wt1- or calretinin- (another marker of pleural mesothelial cells) expressing cells [44, 43, 45, 46], and the co-localization of vimentin in parenchymal Wt1-immunostained cells[43]. Collectively, these studies suggest that pulmonary mesothelial cells might contribute to the sub-pleural fibrotic response in various murine models of fibrosis and in IPF via their proliferation and mesenchymal transition to generate fibroblasts and myofibroblasts. Future studies are warranted to better characterize the *in vivo* mesenchymal transition of mesothelial cells using other more stably-expressed cell markers after lung injury and remodeling.

Bone marrow derived cells

The role of bone marrow derived cells in pulmonary fibrosis remains controversial albeit frequently reviewed[47–52]. With the advent of GFP transgenic mice, adoptive transfer of GFP^+ bone marrow into GFP^- mice to create chimeras has been a popular approach to characterize the role of bone marrow-derived cells in pulmonary fibrosis. One study showed a large influx of GFP^+ bone marrow derived cells in the lungs of bleomycin-challenged mice and these cells co-expressed collagen 1[53]. Specifically, flow cytometric analysis of lung cellular suspensions suggested that approximately 80% of collagen 1 expressing cells co-expressed GFP. However, this percentage is likely inflated since it is possible that GFP^+ immune cells might stain positively for collagen due to the propensity of soluble collagen fragments to bind to activated integrins. *In vitro* culture of GFP^+ fibroblasts from the fibrotic lungs of bleomycin-challenged, bone marrow chimeric mice indicated that these fibroblasts expressed collagen but lacked αSMA after stimulation with TGFß. In another study employing a lung irradiation-induced fibrosis model, GFP^+ $vimentin^+$ bone marrow-derived cells were detected in fibrotic areas of the irradiated lungs[54]. Thus, these studies suggest that bone marrow-derived cells can differentiate into collagen 1-expressing fibroblasts, but these cells fail to differentiate into myofibroblasts during fibrosis.

There are two major bone marrow-derived cell types that have been implicated in lung fibrosis, mesenchymal stem cells (MSCs), and fibrocytes. The role of MSCs in bleomycin induced lung fibrosis is controversial with some studies claiming that MSCs are protective in mice challenged with bleomycin whereas others indicating that these cells are protective only when given preventatively prior to bleomycin instillation. Still other studies have not reported any beneficial effect of these cells (reviewed in [55]). Further, a Phase 1b study addressing the safety of the infusion of placental derived mesenchymal stromal cells in IPF patients failed to show any significant changes in Forced Vital Capacity (FVC), Diffusing Capacity of the lungs for Carbon Monoxide (DLCO), 6-minute walk distance or CT fibrosis score, at 6 months after a single infusion of 1–2 million cells intravenously[56]. While it remains to be further investigated, these current studies suggest that MSC might not modulate or contribute to fibrotic remodeling in mouse or human lung.

Fibrocytes are bone marrow-derived, monocyte-like cells expressing CD45-RO, CD34, and collagen 1[57]. These cells have been extensively studied in fibrosis and cancer, and have been shown to express a plethora of general markers including CD11b, CD80, CD86, CCR2, CCR7, and CXCR4[57–59]. Further, two studies have suggested that fibrocytes may differentiate from Myeloid Derived Suppressor Cells (MDSCs) in a KLF4-dependent manner[60, 61]. Interestingly, it was recently reported that there is an expansion of circulating monocyte-like MDSCs and a positive correlation between MDSC numbers and the decline of pulmonary function was noted [62]. Two predominant populations of fibrocytes have been described and these include $CD45^+$ Collagen 1^+ $CXCR4^+$ $CCR7^-$ and $CD45^+$ Collagen 1^+ $CXCR4^-$ $CCR7^+$[59], but the CXCR4-expressing fibrocytes appear to be the predominant population of cells recruited to the fibrotic lungs. This is supported by another study utilizing CCR7-deficient mice, in which there were no apparent changes in fibrocyte recruitment into the lungs of CCR7-deficient lungs compared with their wildtype counterparts after bleomycin[63]. Indeed, it appears that $CXCR4^+$ fibrocytes are the predominant population of circulating fibrocytes in IPF patients[64, 65]. Finally, it was recently reported that adoptively transferred fibrocytes enhanced the accumulation and/or proliferation of $WT1^+$ mesothelial cells in a club cell-driven TGFα overexpression model of lung fibrosis[43]. Collectively, these studies suggest that bone marrow-derived fibrocytes might contribute to lung fibrosis and remodeling through the expression of collagens and the modulation of mesothelial cell activation but these cells do not seem to be a source of myofibroblasts in experimental fibrosis in the mouse lung.

Heterogeneity of fibroblasts and myofibroblasts in IPF

As described above, considerable effort has been undertaken to characterize mesenchymal populations in remodeled murine lungs. Collectively, these studies indicate that both fibroblasts and myofibroblasts arise from multiple cell types. Further, transcriptomic analysis on different fibroblasts and myofibroblasts lineages indicates that these cells show many variations in their response(s) to the fibrotic milieu in the lung. Because many of these cells lose the expression of their lineage identifying marker(s) after fibroblast and myofibroblast differentiation, it has been particularly difficult to translate these findings into clinical fibrotic lung diseases such as IPF. Compared with their normal counterparts, IPF lung fibroblasts and myofibroblasts exhibit key alterations in epigenetic regulation[66–71], genetic polymorphisms[72], resistance to apoptosis[73–76], elevated microbial sensing pathways[77–79], and increased invasiveness[80–86] causing IPF cells to be more pro-inflammatory, profibrotic and injurious than normal cells.

Evidence for the expansion of pericyte-derived fibroblasts and myofibroblasts in IPF

Given the heterogeneous sources of murine fibroblasts and myofibroblasts during fibrosis in the lung, it is likely that the changes observed in IPF might be due, in part, to an expansion and/or loss of comparable cell populations. Accordingly, there is a loss of ABCG2-expressing pericytes in IPF patients, which is a process also observed in experimental fibrosis characterized by a loss of ABCG2 expression in pericytes and the differentiation of

these cells into myofibroblasts [87, 28], suggesting that pericytes might be giving rise to myofibroblasts in IPF lungs. Further, in the same report, an expansion of PDGFRα$^+$ PDGFRβ$^+$ stromal cells in IPF was noted and these cells may represent a population of collagen 1-expressing pericytes that differentiate into myofibroblasts[13]. Indeed, our mining of RNAseq datasets from lung fibroblasts derived from slow and rapid progressing IPF patient lung samples, and normal lung samples supports an expansion of a PDGFRß- and PDGFRα-expressing stromal population in IPF. Further, various studies have suggested that pericytes express higher levels of innate immune microbial sensors[13, 88] and IPF lung fibroblasts, notably from progressive IPF patients, express TLR9[78, 79] and TLR4[77], and the activation of these microbial sensors leads to myofibroblast differentiation[77–79]. Together, these studies suggest that pericyte-derived fibroblasts and myofibroblasts are present in IPF, and these cells might contribute to fibrotic progression through various mechanisms including microbial sensing pathways.

Loss of lipofibroblasts in IPF

Given the important role of lipofibroblasts in alveolar type II epithelial homeostasis and function, recent studies have addressed the state of these cells in IPF patients. There is a loss of lipofibroblast associated ADRP and PPARγ transcript expression in IPF relative to normal lungs[35]. Transcriptomic and immunohistochemical analysis suggested that FGF10 protein expression is elevated in IPF compared with normal lungs and localized to αSMA expressing cells present in heavily fibrotic areas. These findings suggest that FGF10-expressing myofibroblasts in IPF may be potentially derived from lipofibroblasts. Interestingly, a recent report characterizing mesenchymal cells in bronco-alveolar lavage (BAL) from progressive versus stable IPF showed a loss of FGF10 transcript and protein expression in progressive IPF mesenchymal cells compared with stable IPF mesenchymal cells[89], indicating a loss of lipofibroblasts or myofibroblasts derived from lipofibroblasts in these patients. Mining of RNAseq datasets from our cultured rapid IPF, slow IPF, and normal lung fibroblasts indicate that there are no significant changes in *ADRP* transcript but a marked reduction in *PPARG* and *FGF10* transcript expression in both IPF groups compared with normal lung fibroblasts. Thus, FGF10 expression might predict progression in IPF, in which patients with a loss of FGF10 progress more rapidly relative to patients with high FGF10 expression due, in part, to the loss of lipofibroblasts or lipofibroblast-potent myofibroblasts. Given the role of these cells in alveolar type II epithelial cell homeostasis and in the efficient repair of bleomycin-induced lung fibrosis[35, 23], loss of lipofibroblasts in IPF further supports the hypothesis that a lack of appropriate epithelial-mesenchymal cell cross talk might lead to the chronic disrepair observed in lungs.

Concluding remarks

Lung remodeling and repair is a complex process driven by the activation and interplay of multiple stromal populations. Murine studies have shed light on the putative origin of highly synthetic myofibroblasts. Specifically, lung myofibroblasts predominately arise from resident fibroblasts, lipofibroblasts, a subset of pericytes, and mesothelial cells. However, other collagen-producing cells including fibrocytes and epithelial cell-derived fibroblasts might also play a role in pulmonary fibrosis. Interestingly, transcriptome sequencing studies

in various populations of murine fibroblasts and myofibroblasts has elucidated the identity of the pathways elevated in fibrotic lung diseases, and demonstrated a plethora of different populations of cells with distinct activation pathway, migratory, and invasive characteristics. Further, the loss of lipofibroblast or lipofibroblast- potent myofibroblasts and an expansion of PDGFRβ$^+$ PDGFRα$^+$ pericyte-derived myofibroblasts might be two key cellular processes leading to progressive remodeling in IPF. Future work is warranted to better characterize the stromal populations in IPF and other fibrotic/remodeling lung diseases. It is likely that there are beneficial fibroblast and myofibroblast populations, which are required for alveolar epithelial support and lung regeneration. Thus, therapeutics specifically targeting “pathologic” populations such as pericyte-derived myofibroblasts while concomitantly promoting the expansion of “reparative/regenerative” populations of fibroblasts such as lipofibroblasts might provide beneficial effects in IPF patients.

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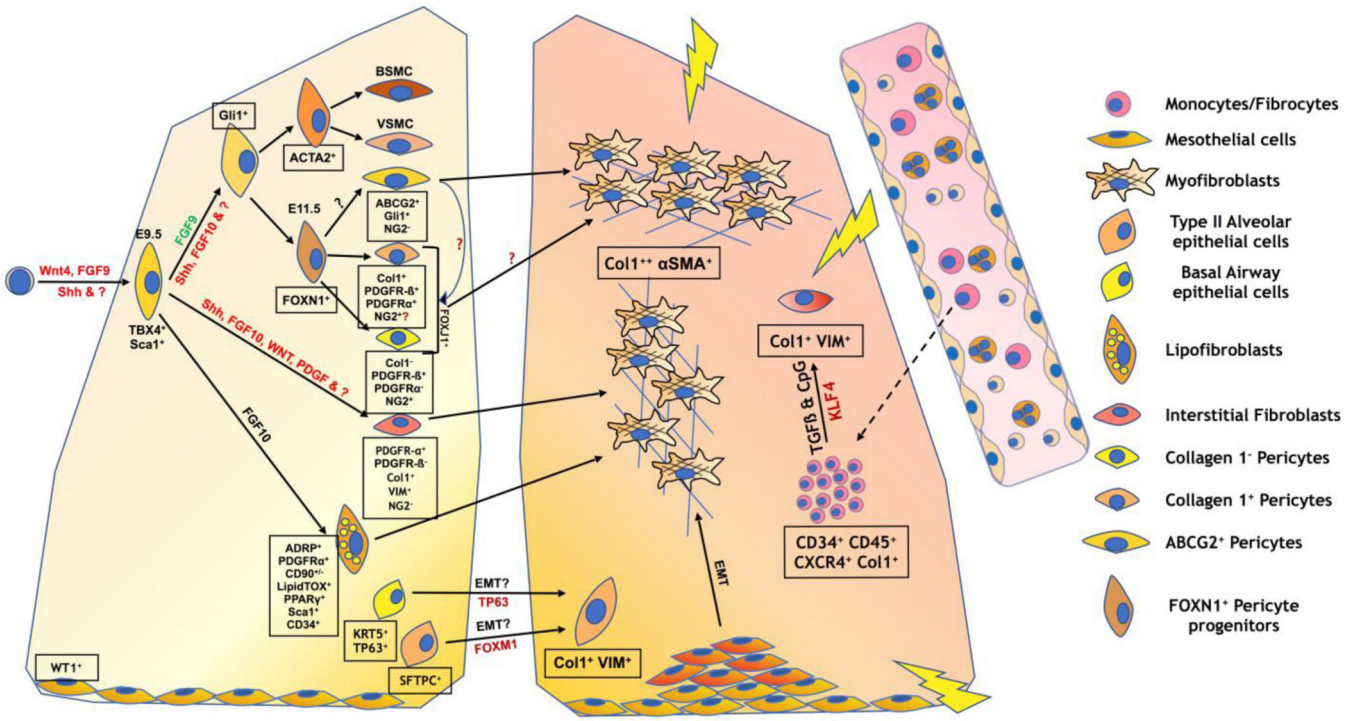

Figure 1.

Mesodermal development in the lung and heterogeneity of fibroblasts and myofibroblasts in fibrotic lungs. Depicted is a simplified summary of mesodermal development in normal lungs (left) and potential lineages leading to the generation of fibroblasts and myofibroblasts in remodeled lungs (right).

Table 1

Summary of Mesodermal lineages and their contribution to myofibroblasts in injured lungs

Cell type	Potential Markers	Myofibroblast differentiation potential	Location	References
Lipofibroblasts	$CD45^-$, $EpCAM^-$, $ADRP^+$, $PDGFR\alpha^+$ $PDGFR\beta^-$, $CD90^{+/-}$ $PPAR\gamma^+$, $Sca1^+$ $CD34^+$, $NG2^-$	Yes	Interstitium, near Alveolar Type II Epithelial cells.	[29, 24, 35, 34, 25]
Interstitial fibroblasts	$CD45^-$, $EpCAM^-$ $ADRP^-$, $PDGFR\alpha^+$ $PDGFR\beta^-$, $Col1^+$ VIM^+, $NG2^-$	Yes	Interstitium	[29, 34, 27, 13]
Pericytes ($FoxJ1^+$)	$CD45^-$, $EpCAM^-$, $PDGFR\beta^+$, $PDGFR\alpha^-$?, $NG2^+$, $Col1^-$?, $Foxn1^+$	No	Perivascular	[14, 27, 13]
Pericytes ($FoxJ1^-$)	$CD45^-$, $EpCAM^-$, $PDGFR\beta^+$, $PDGFR\alpha^+$ $Col1^+$, $NG2^-$?, $ABCG2^+$?, $Foxn1^+$?	Yes	Perivascular	[27, 13, 28]
Fibrocytes	$CD45^+$, $EpCAM^-$, $CD34^+$, $CXCR4^+$ $Col1^+$	No	Bone Marrow & Circulation	[53, 47, 48, 54, 49, 51]
Mesothelial cells	$Wt1^+$, $Calretinin^+$	Yes?	Pulmonary pleura	[44, 43, 45, 46]
Basal epithelial cells	$CD45^-$, $EpCAM^+$ $KRT5^+$, $TP63^+$	No	Pulmonary airways	[42]
Alveolar type II epithelial cells	$CD24^-$, $Sca1^-$, $CD45^-$, $EpCAM^+$, $SFTPC^+$	No	Interstitium	[40, 37, 39, 41, 14, 90]
Smooth muscle cells	$ACTA2^+$, $Desmin^+$	No	Peribronchial and Perivascular	[14, 35]