CALL FOR PAPERS | Bioengineering the Lung: Molecules, Materials, Matrix, Morphology, and Mechanics

Characterization of the platelet-derived growth factor receptor-α-positive cell lineage during murine late lung development

Aглаia Нtoku,1* Friederike Klein,1* Daria Dontiредdy,1 Sven Becker,1 Saverio Bellusci,2 William D. Richardson,3 Marten Szibor,4 Thomas Braun,4 Rory E. Morty,1,2 Werner Seeger,1,2 Robert Voswinckel,1* and Katrin Ahlbrecht1,2*

1Department of Lung Development and Remodelling, Max Planck Institute for Heart and Lung Research, Member of the German Center for Lung Research [Deutsches Zentrum für Lungenforschung (DZL)], Bad Nauheim, Germany; 2Department of Internal Medicine (Pulmonology), University of Giessen and Marburg Lung Center, Member of the German Center for Lung Research (DZL), Giessen, Germany; 3Wolfson Institute for Biomedical Research, University College London, London, United Kingdom; and 4Department of Cardiac Development and Remodelling, Max Planck Institute for Heart and Lung Research, Member of the German Center for Lung Research (DZL), Bad Nauheim, Germany

Submitted 19 September 2014; accepted in final form 20 August 2015

LUNG DISEASES THAT CAUSE IRREVERSIBLE destruction of the gas exchange regions, such as chronic obstructive pulmonary disease (COPD) (13) and fibrotic lung diseases (21), represent a significant medical burden. Similarly bronchopulmonary dysplasia (BPD), where alveolarization is stunted, causes long-term structural defects in the lung architecture, in affected neonates (4, 28, 53). Currently, curative therapeutic strategies are not available. To identify target molecules for new therapeutic strategies, knowledge of the basic mechanisms of septum formation during the process of alveolarization needs to be advanced.

Secondary septation represents a key event during alveolarization that occurs during late lung development (19). It would be desirable to stimulate secondary septation during arrested lung development associated with BPD, and to stimulate neo-alveolarization where alveolar destruction has occurred, for example, in emphysema associated with COPD. Different fibroblast subtypes have been demonstrated to be critically involved in secondary septation (8, 29, 54), particularly the platelet-derived growth factor receptor-α-positive (PDGFRα +) subset, and, as such, the spatiotemporal dynamics of the presence of this fibroblast subset during lung development was addressed in the present study. Myofibroblast progenitors of the septal tip myofibroblasts have been demonstrated to be committed in early lung mesoderm development, which involves Gli-1 and PDGFRα (25).

In the mesenchyme, three major fibroblast cell types have been described: lipo- and myofibroblasts (10) as well as fibroblasts that express PDGFRα (5). Myofibroblasts are defined as α-smooth muscle actin-positive (αSMA +) fibroblasts (24), which produce elastin and collagen, the key extracellular matrix components of the alveolus (37). Lipofibroblasts are defined by the presence of storage vesicles that contain neutral lipids (49) and are always located in direct cellular contact with alveolar type II cells (AEC II) (16). Lipofibroblasts support the surfactant phospholipid synthesis by AEC II through various mechanisms (46, 49).
Furthermore, lipofibroblasts have been demonstrated to produce retinoic acid (RA) (31). The abundance of RA increases before the period of maximal elastin production during alveolarization (31). Thus retinoic acid might represent a crucial factor, impacting on alveolarization. Lipofibroblasts express adipocyte differentiation-related protein (ADPR), an amphiphatic protein that coats the neutral lipid droplets (27). Therefore, ADRP may be used as a marker for lipofibroblasts in the alveolar septum.

Furthermore, the glycoprotein CD90 (Thy-1) is expressed in lipofibroblasts. The downstream signaling of CD90 leads to an increase in fatty acid uptake and esterification in lipofibroblasts by increased transcription of peroxisome proliferator-activated receptor (PPAR)-γ and ADRP, which promote the lipogenic phenotype (42, 51).

In contrast, loss of CD90 in vivo impaired alveolarization through increased transforming growth factor (TGF)-β signaling, increased fibroblast proliferation, and increased production of elastin and collagen (36). Moreover, CD90 belongs to a group of markers characterizing mesenchymal stem cells (MSC) (48). Further members of the CD90 phenotype (42, 51).

To better understand the contribution of PDGFRα+ precursors to the process of alveolarization, and the possible contribution to various fibroblast subtypes, the present study lineage-traced and characterized the spatiotemporal distribution of the PDGFRα+ cell population during the pseudoglandular, canalicular, saccular, and alveolar stages of lung development. To this end, a Cre-loxP cell lineage tracing approach was employed, using a tamoxifen-inducible PDGFRαCreERT2 (37) or constitutively active PDGFRαCre (38) to activate a fluorescent reporter transgene (mT/mG) (35). Endogenous PDGFRα expression was assessed in PDGFRαGFP knock-in mice (18).

**Materials and Methods**

Transgenic mice. All animal experiments were approved under approval number B2/304 by the Regierungspräsidium Darmstadt, which bears jurisdictional responsibility for animal studies conducted at the Max Planck Institute for Heart and Lung Research in Bad Nauheim, Germany.

PDGFRα-GFP mice bearing the PDGFRαGFP allele were generated by Dr. Philippe Soriano (Fred Hutchinson Cancer Research Center, Seattle, WA) and were obtained from The Jackson Laboratory (stock no. 007669). This mouse line carries a GFP allele fused to a histone 2B moiety introduced in the endogenous PDGFRα locus, placing nuclear GFP expression under PDGFRα gene control. Mice that were heterozygous for the PDGFRαGFP allele were employed in this study; thus, the mice still carry one functional PDGFRα allele (40). PDGFRαCre mice have been described previously (44) and were a kind gift from Botond Roska (Friedrich Miescher Institute for Biochemical Research, Basel, Switzerland). The PDGFRαCreERT2 mice also have been described previously (43). The mT/mG reporter mice, which express a membrane-associated tandem dimer tomato fluorescent protein (mT) constitutively in all cells, switching to membrane-bound green fluorescent protein (mg) following Cre-recombination, were employed in the present study. These mice were obtained from the Jackson Laboratory (stock no. 007676). Mice were housed in a barrier facility with purified air and water, supplied with food and water ad libitum, and exposed to a 12:12-h light-dark cycle. All animals were maintained on a C57Bl/6 background.

Tamoxifen administration. A tamoxifen stock solution of 16 mg/ml was produced by dissolving tamoxifen powder (T-5648; Sigma-Aldrich, St. Louis, MO) in Miglyol (Caesar & Loretz, Hilden, Germany) and shaking for 1 h at room temperature (RT). Neonatal mice received a single intraperitoneal injection of 0.4 mg tamoxifen/animal at either postnatal day (P) 1, P2, or P5.

Preparation of lung tissue for confocal microscopy. PDGFRα-GFP mice were killed with a dose of >5% isoflurane inhalation (Baxter, Deerfield, IL) with additional exsanguination. The thoracic cavity was opened to expose the lungs. The lungs were perfused transcardially with 1× phosphate-buffered saline (PBS: 0.145 M NaCl, 0.0015 M KCl, 0.027 M NaHCO3, 0.0086 M Na2HPO4, pH 7.4). Lungs were inflated to total lung capacity using 20 cmH2O hydrostatic pressure for 2 min with 50% Optimal Cutting Temperature Medium (Leica Biosystems, Wetzlar, Germany), removed from the thorax, and frozen in a bath of isomethylbutane. The frozen lung tissue was cut into 10-μm sections using a cryostat, mounted on glass slides, and stored at −20°C before fixation. Cryosections of the mT/mG mice were prepared as described previously (35).

Preparation of mouse lung homogenates for cytospins. Mice were killed by decapitation, and lungs were removed and manually homogenized using a scalpel. Lung tissue was digested in digestion buffer consisting of (total volume 20 ml, 3–4 ml/lung) 17.3 ml Dulbecco’s modified Eagle’s medium (DMEM, with glucose and pyruvate; PAA, Pasching, Austria), 1.5 ml 10× trypsin (≥4 U/mg protein; Invitrogen Life Technologies), 1 ml collagenase B (2% [mass/vol (m/v)] stock solution, >10 U/mg; Roche, Basel, Switzerland), and 200 μl DNase I (2,601 U/ml; 10 mg/ml; Serva, Heidelberg, Germany) for a total period of 30 min by shaking at 37°C, with resuspension after 25 min. The enzyme reaction was blocked with fetal calf serum (FCS; PAA), and cells were filtered through a 40-μm mesh. After being washed one time with 1× PBS, cells were resuspended in 2–3 ml 1× PBS for cytospin. An aliquot (2 × 100 μl) of the cell suspension was spun down on a glass slide using the cytospin equipment of the SORVALL, Heraeus 7502001 L centrifuge and stained for ADRP and αSMA using the same antibodies and protocol as for immunofluorescence (see below).

Analysis of cytospins. Cells of one animal were distributed to three microscope slides and stained as described below. Four optical fields per slide were assessed randomly by confocal microscopy. Thus, 12 optical fields for each animal per time point were analyzed. Per time point, three to five animals were analyzed (n = 3–5). Data were analyzed using the STEPanizer stereology tool (47).

Immunofluorescence, confocal and fluorescent microscopy. Tissue sections and cytospins of lung cells were fixed with methanol-acetone (1:1) for 10 min at −20°C. Nonspecific antibody binding was minimized by blocking with normal goat serum (PAA; Austria) for 1 h. The following antibodies were used: ADRP 1:200 rabbit polyclonal antibody to ADRP (ab52356; abcam, Cambridge, UK); PDGFRα 1:100 (abcam; ab90867), both with secondary antibody Alexa Fluor 488- or 647-conjugated goat antirabbit IgG (Invitrogen); αSMA 1:500 monoclonal αSMA antibody clone 1A4 Cy3 conjugated (C 6198; Sigma-Aldrich, Hamburg, Germany) or 1:500 monoclonal αSMA antibody clone 1A4 (A 5228; Sigma-Aldrich) followed by secondary antibody Alexa Fluor 488- or 647-conjugated goat antirabbit IgG (Invitrogen); CD31 1:200 purified rat antimouse CD31 antibody (550274; BD Bioscience, San Jose, CA) with secondary antibody Alexa Fluor 647-conjugated goat antirat IgG (Invitrogen); GFP-FITC 1:300 goat
anti-GFP FITC conjugated (NB100-1771; Novus Biologicals, Littleton, CO); CD45 1:500 monoclonal rat anti-CD45 antibody (550539; BD Biosciences); cytokeratin 1:500 polyclonal rabbit anticytokeratin antibody (Z0622; Dako, Glostrup, Denmark), and collagen I 1:500 polyclonal rabbit anticollagen I antibody (T 40777R; Rockland, Gilbertsville, PA) followed by secondary antibody Alexa Fluor 555-conjugated goat IgG (Invitrogen); CD44 (ab41478; abcam); CD105 (ab81456; abcam); CD73 (13160; Cell Signaling, Leiden, Netherlands); CD146 (04–1147; Millipore, Vienna, Austria); and CD90 (sc-73161; Santa Cruz, Heidelberg, Germany). These antibodies were
diluted 1:100. Secondary antibodies employed were Alexa 555 goat antimouse IgG (H+L, A21422; Invitrogen); Alexa 555 goat antirat (A21434; Invitrogen); Alexa 555 goat antirabbit IgG (H+L, A21428; Invitrogen); and Alexa 647 goat antirabbit (A21244; Invitrogen). All antibodies were diluted in histobuffer (3% BSA in PBS, 0.2% Triton X-100). Images of the stained sections were acquired using a Zeiss LSM710 Laser Scanning Confocal Microscope. The Z-stack images were taken using a Leica DFC360 FX Fluorescent Microscope with Leica Application Suite AF microscope software.

**Primary cell isolation.** Primary mouse lung cells from wild-type mice were isolated from homogenized tissues (n = 2–6 lungs) in serum-free DMEM + GlutaMax containing 0.2% collagenase IV (261 U/mg), 0.5% trypsin (≤4 U/mg protein) (all purchased from Invitrogen Life Technologies), and 0.5% DNase (4,065.6 U/mg; Serva) by shaking for 30–40 min at 37°C. The enzymatic activity was stopped by adding DMEM-F-12 medium containing 20% FBS. The cell suspension was filtered through a 70-μm cell strainer (BD Biosciences) to remove cells and tissue fragments. The nonadherent cell fraction was isolated by gentle centrifugation (500 g for 5 min) and the cell pellet was resuspended in DMEM-F-12 medium containing 20% FBS. Adherent cells were dispersed enzymatically (0.05% trypsin (≤4 U/mg protein) for 20 min at 37°C) and nonadherent cells were not removed to demonstrate PDGFRα+ cells did not coexpress cytokeratin or CD45. E–H: primary fibroblasts were isolated from wild-type mouse lungs at P6 and maintained in cell culture for 1 day (d1), or isolated at P40 (adult) and kept in culture for d1, d2, and d3. Some fibroblasts isolated at P6 coexpressed αSMA (red) and ADRP (green) after d1. The αSMA-positive (αSMA+) cells isolated at P40 did not coexpress ADRP (green) at d1 but gained ADRP coexpression after d2 and d3. Nuclear stain DAPI (blue). A–H illustrate representative images of n = 3 experiments. Scale bar = 30 μm.

**Fig. 1.** Localization of platelet-derived growth factor receptor (PDGFR)-α expression at different time points during mouse lung development. A–F: lung tissue from PDGFRα-green fluorescence protein (GFP) mice (which bear the PDGFRαGFP allele) was stained for platelet endothelial cell adhesion molecule (PECAM)-1 (orange) and α-smooth muscle actin (αSMA; red) followed by a Sytoxblue nuclear counterstain. A: at embryonic day (E) 14.5, PDGFRα expression was restricted to large bronchi that already expressed αSMA. B: at E16.5, PDGFRα expression was detected throughout the mesenchyme of the canaliculi. C: from E18.5 on, PDGFRα signal was detected in the mesenchyme and around the bronchi, and no expression in the perivascular mesenchyme was seen. D: at postnatal day (P) 3, the PDGFRα signal in the mesenchyme was still present. E: at P5, the septal tip cells of the secondary septa exhibited coexpression of PDGFRα and αSMA. F: at P40, PDGFRα-positive (PDGFRα+) cells were abundant. G–L: lung tissue from PDGFRαGFP mice was stained for adipocyte differentiation-related protein (ADRP, orange) and αSMA (red) followed by Sytoxblue nuclear counterstain. ADRP-positive cells were abundant before birth (E18.5) and less abundant in adulthood (P40). Scale bar = 50 μm.

**Fig. 2.** A–D: primary fibroblasts were isolated from lungs of PDGFRα-GFP mice (which bear the PDGFRαGFP allele) at P5 and maintained in cell culture for 3 days (d3). The PDGFRα+ cells (green) expressed αSMA (red) and collagen I (red), but not cytokeratin (red) or CD45 (red). Nuclear stain 4',6-di-amidino-2-phenylindole (DAPI; blue). Scale bar = 100 μm. For experiments presented in A, nonadherent cells were not removed to demonstrate that PDGFRα+ cells did not coexpress cytokeratin or CD45. E–H: primary fibroblasts were isolated from wild-type mouse lungs at P6 and maintained in cell culture for 1 day (d1), or isolated at P40 (adult) and kept in culture for d1, d2, and d3. Some fibroblasts isolated at P6 coexpressed αSMA (red) and ADRP (green) after d1. The αSMA-positive (αSMA+) cells isolated at P40 did not coexpress ADRP (green) at d1 but gained ADRP coexpression after d2 and d3. Nuclear stain DAPI (blue). A–H illustrate representative images of n = 3 experiments. Scale bar = 30 μm.
medium (GIBCO, Darmstadt, Germany) containing 10% FCS and 1% penicillin/streptomycin (both purchased from PAA). Undigested remains were eliminated by filtering the cell suspension through a cell strainer with a 100-µm nylon mesh (BD Bioscience, Heidelberg, Germany). The single-cell suspension was centrifuged at 128 g for 10 min at RT. Red blood cells were lysed using lysis buffer (pH 7.4; 0.15 M NH₄Cl, 10 mM KHCO₃, and 0.1 mM EDTA; all from Roth, Karlsruhe, Germany) for 5 min at RT and pelleted at 450 g for 5 min at RT. The cell suspension was washed successively with Dulbecco’s PBS (PAA) and centrifuged at 128 g for 5 min at RT. Cell number was established microscopically using a Neubauer hemocytometer chamber (Marienfeld, Lauda Königshofen, Germany). The cell pellets were frozen at −80°C for protein and RNA isolation, or further cell cultured.

Primary cell culture. Lung primary cells were cultured in DMEM-F-12 medium (GIBCO) supplemented with 10% FCS and 1% penicillin/streptomycin (both purchased from PAA). The cells

---

**Fig. 3.** Platelet-derived growth factor receptor-α-positive (PDGFRα⁺) cells expressed the fibroblast marker collagen I and the mesenchymal stem cell markers CD44 and CD105 at certain time points during lung development. A–C: lung tissue of PDGFRα-GFP mice (which bear the PDGFRα⁺ allele) was stained for collagen I (orange) and vimentin (red). A: E18.5. B: P5. C: P40. D–I: lung tissue of prenatal, postnatal, and adult PDGFRα⁺ mice was stained for the mesenchymal stem cell markers CD44 (orange) and CD105 (red) (scale bar = 50 µm). D and E: at E14.5 and E16.5 the PDGFRα⁺ signal did not colocalize with CD44 and CD105. F: at E18.5, some PDGFRα⁺ cells exhibited CD44 and CD105 expression (arrows). G: at P3, some PDGFRα⁺ cells revealed CD105 expression (arrows) but did not exhibit signal for CD44. H: at P5, PDGFRα⁺ cells within the primary septa revealed expression of CD44 (arrows). Some tip cells of the secondary crests revealed CD105 expression (arrows with asterisk). I: in the adult lung, PDGFRα⁺ cells did not exhibit CD44 and CD105 expression. b, Bronchus; v, vessel.
were seeded on chamber slides (1.3 × 10^4 cells) (BD Bioscience) at 37°C under a 5% CO₂ atmosphere, for 40–60 min, to allow for differential adherence separation of lung fibroblasts. After 60–120 min the medium was aspirated, and cells were washed with PBS (PAA) and supplemented with fresh complete medium. For experiments demonstrated in Fig. 2A, nonadherent cells were not removed, to demonstrate that PDGFRα+ cells do not coexpress cytokeratin or CD45. The cells were maintained for further cell expansion for up to 1, 2, or 3 days at 37°C under a 5% CO₂ atmosphere.

**Statistical analysis.** Results are presented as means ± SE for each condition. The significance of parametric differences was evaluated with two-way ANOVA with Bonferroni’s post hoc test using GraphPad Prism 5 (GraphPad Software, San Diego, CA). Differences were considered statistically significant at *P* < 0.05.

**RESULTS**

**Localization and phenotype of PDGFRα-expressing cells during embryonic and postnatal lung development.** To investigate the localization and phenotype in terms of expression of cell type-specific markers of PDGFRα-expressing cells during lung development, the lungs of PDGFRαGFP knock-in mice were analyzed by confocal immunofluorescence microscopy. Nuclear GFP expression reflected endogenous PDGFRα expression. Due to the prolonged stability of the GFP protein, GFP remained expressed in the PDGFRα-negative (PDGFRα−) progeny of PDGFRα+ progenitor cells.

At embryonic day (E) 14.5 (Fig. 1, A and G), bronchial smooth muscle cells, but not vascular smooth muscle cells, expressed PDGFRα. Moreover, interstitial cells adjacent to bronchial smooth muscle cells exhibited PDGFRα expression. At E14.5, PDGFRα-expressing cells were not yet detected in the distal lung. At E16.5, PDGFRα-expressing cells appeared to be less prominent in the peribronchial region and appeared in interstitial parts of the canalliculi region (Fig. 1B). At E18.5, some PDGFRα-expressing cells in the peribronchial region were still evident, while additional interstitial cells of the sacculi started to display PDGFRα expression (Fig. 1, C and I). The perivascular region remained devoid of PDGFRα+ cells. At P3, PDGFRα+ cells were dispersed in the primary septa (Fig. 1, D and J). At P5, the time of a burst in secondary septa formation, PDGFRα+ cells were located at the tip of secondary septa, and exhibited coexpression of PDGFRα and αSMA (Fig. 1E), strongly suggesting transdifferentiation of PDGFRα+ cells to alveolar myofibroblasts. At P40, PDGFRα+ cells were abundant in the alveolar interstitial cell compartment as well as around the conducting airways and large vessels (Fig. 1, F and L). The lipofibroblast marker ADRP was abundantly expressed at E18.5 and colocalized in some interstitial cells with PDGFRα (Fig. 1I). In the adult lung at P40, some ADRP-expressing cells were present (Fig. 1L).

In vitro, GFP+ cells isolated from the lungs of PDGFRαGFP mice at P5 exhibited expression of αSMA and collagen I, but did not express CD45 or cytokeratin after 3 days in cell culture (Fig. 2, A–D). Staining for CD45 and cytokeratin was performed to demonstrate that PDGFRα+ cells do not obtain phenotypic markers of epithelial cells and leukocytes and do not contribute to these cell types. Fibroblasts isolated from wild-type mice at P6 revealed colocalization of ADRP and αSMA in some cells when cultured for 1 day (Fig. 2E). Other cells expressed ADRP alone (Fig. 2E). Isolated fibroblasts from adult wild-type mice did not exhibit colocalization of ADRP and αSMA after 1 day in culture (Fig. 1F), but contained ADRP-positive cells. However, after 2 and 3 days in culture, colocalization of ADRP and αSMA was detectable (Fig. 2, G and H). To further characterize the PDGFRα+ cell population, lung tissue of PDGFRαGFP mice was stained for the phenotypic fibroblast markers collagen I and vimentin and the MSC markers CD44, CD105, CD73, CD146, and CD90. Lungs were stained at different time points of lung development analyzing the late pseudoglandular (E14.5), the canalicular (E16.5), the saccular (E18.5), the alveolar (P3, P5), and the adult (P40) stage.

At E18.5, P5, and in adult lung tissue PDGFRα+ cells expressed the fibroblast marker collagen I (Fig. 3, A–C), providing evidence that peribronchial and perivascular PDGFRα+ cells obtain fibroblast characteristics (Fig. 3, A–C). The MSC markers CD44 and CD105 were transiently and differentially expressed in PDGFRα+ cells. During the late pseudoglandular and canalicular stage, PDGFRα+ cells did not express CD44 and CD105 (Fig. 3, D and E). At E18.5 of the saccular stage, some PDGFRα+ cells coexpressed CD44 and CD105 (Fig. 3F). The expression of CD44 at P3 remained restricted to the epithelial tubes without giving a signal in mesenchymal PDGFRα+ cells (Fig. 3G). In contrast some PDGFRα+ cells of the primary septa expressed CD105 (Fig. 3G). At P5, PDGFRα appeared to be expressed in the tip cells of the secondary crests and colocalized with CD105 (Fig. 3H). In the primary septa, PDGFRα+ cells expressed CD44 (Fig. 3H). In adulthood, PDGFRα+ cells did not coexpress CD44 and CD105 (Fig. 3I).

At E16.5, E18.5, and P3, some PDGFRα+ cells showed expression of CD73 (Fig. 4, B–D). The PDGFRα+ tip cells of the secondary crests and some PDGFRα+ cells in the primary septa expressed CD73 at P5 (Fig. 4E). In adulthood, only few PDGFRα+ cells of the lung expressed CD73 (Fig. 4F). Similarly, CD146 was not expressed in PDGFRα+ cells at E14.5 of the pseudoglandular stage but was expressed in PDGFRα+ cells at E16.5 and E18.5 of the canalicular and saccular stage (Fig. 4, G–I). At P3 some PDGFRα+ cells expressed CD146, and peribronchial PDGFRα+ cells remained without CD146 expression (Fig. 4J). Like CD73, CD146 was expressed in PDGFRα+ tip cells of the secondary crests and in some PDGFRα+ cells of the primary septa (Fig. 4K). In the adult lung only scattered PDGFRα+ cells expressed CD146 (Fig. 4L).

During the late pseudoglandular and the canalicular stage CD90 was not expressed in PDGFRα+ cells (Fig. 5, A and B). At E18.5 most of the ADRP-positive (ADRP+) cells exhibited CD90 expression, including some PDGFRα+ cells (Fig. 5C). At P3, single cells expressing CD90 alone were present in the developing primary septa (Fig. 5D). Some PDGFRα+ cells of the primary septa expressed ADRP (Fig. 5D). In contrast at P5 some PDGFRα+ cells of the primary septa coexpressed CD90 and ADRP (Fig. 5E). The PDGFRα+ tip cells of the secondary crests did express CD90 but not ADRP at P5 (Fig. 5E). Cells at the base of the secondary septa coexpressed CD90 and ADRP (Fig. 5E). In the adult lung single PDGFRα+ cells coexpressed CD90 and ADRP (Fig. 5F). Some PDGFRα+ cells expressed ADRP solely, whereas some CD90-expressing cells that were negative for PDGFRα and ADRP were also detected (Fig. 5F). To further characterize PDGFRα+ cells, which express CD90, lung sections of the same developmental stages as illustrated in Fig. 5, A–F, were co stained for αSMA and CD90. During the late pseudoglandular and canalicular stage, CD90 was not expressed in PDGFRα+ cells (Fig. 5, G.
and $H$). Peribronchial PDGFR$\alpha^+$ cells demonstrated $\alpha$SMA expression as already demonstrated in Fig. 1 (Fig. 5, $G$ and $H$). Some PDGFR$\alpha^+$ cells in the mesenchyme between the alveolar ducts demonstrated coexpression of $\alpha$SMA and CD90 at E18.5 (Fig. 5). Double-positive cells for PDGFR$\alpha$ and $\alpha$SMA were detectable surrounding the alveolar ducts at E18.5 (Fig. 5). At P3, PDGFR$\alpha^+$ cells did not express CD90 or $\alpha$SMA. Cells expressing CD90 alone were also present (Fig. 5). Some PDGFR$\alpha^+$ cells within the primary septa expressed CD90 at P5 (Fig. 5). Some PDGFR$\alpha^-$ cells of the primary septa
Quantification of fibroblast subsets and phenotypic markers during lung development. To quantify fibroblast subsets during lung development, all cells from digested lungs of PDGFrαcreGFP mice were isolated, subjected to cytopsins, and stained for the phenotypic markers ADRP and αSMA (Fig. 6A). The abundance of cells expressing specific markers were quantified using the STEPanizer stereology tool (Fig. 6, B and C). Relative numbers of cells are presented as percentages of whole lung cells (means ± SE). The proportion of all cells that were PDGFrα+ was stable throughout the canalicular, saccular, and alveolar stage (E16.5: 7.3 ± 1.7%, E18.5: 7.5 ± 1.2%, P2: 7.5 ± 1.5%, P5: 4.2 ± 0.5%, and P14: 8.9 ± 0.3%; Fig. 4B). However, the relative number of PDGFrα+ cells (8.9 ± 0.3%) was significantly higher compared with the relative numbers of ADRP+ cells (2.1 ± 0.5%, P < 0.05) and αSMA+ cells (2.0 ± 0.3%, P < 0.01) at P14 (Fig. 6B). The proportion of ADRP+ cells peaked at E16.5 (9.8 ± 2.7%) and significantly decreased at P14 (2.1 ± 0.5%, P < 0.01) (Fig. 6B). Meanwhile, during the saccular and alveolar stage, the relative numbers of ADRP+ cells did not change (E18.5: 8.3 ± 0.9%, P2: 4.4 ± 1.1%, and P5: 5.0 ± 1.7%; Fig. 6B). The proportion of αSMA+ cells did not significantly change among the time points analyzed (E16.5: 3.0 ± 0.3%, E18.5: 5.7 ± 0.9%, P2: 3.3 ± 0.8%, P5: 3.9 ± 0.3%, and P14: 2.0 ± 0.3%; Fig. 6B). Only at E16.5 was the relative number of αSMA+ cells (3.0 ± 0.3%) significantly lower compared with the number of ADRP+ cells (9.8 ± 2.7%, P < 0.05) (Fig. 6B).

The proportion of cells that coexpressed two phenotypic markers exhibited a different temporal pattern of expression (Fig. 6C). The population of PDGFrα+ cells at the base of the primary septa and at the tip of the secondary crests (arrows with asterisk). In contrast, the abundance of PDGFrα+ and αSMA+ cells at P5 (3.7 ± 0.9%) significantly changed compared with P2 (1.1 ± 0.5%, P < 0.01) and P14 (0.1 ± 0.0%, P < 0.01) during the alveolar stage (Fig. 6C). During the canalicular and saccular stage the relative number of the PDGFrα+ and αSMA+ cells did not change (E16.5: 2.5 ± 0.8% and E18.5: 1.7 ± 0.5%; Fig. 6C). Comparison of the abundance of PDGFrα+ and αSMA+ population (2.5 ± 0.8%) with the PDGFrα+ and ADRP+ population (13.5 ± 1.5%) at E16.5 demonstrated a significant difference (P < 0.0001) (Fig. 6C). Quantification of ADRP+ and αSMA+ dual-positive cells, possibly reflecting transdifferentiation of lipo- to myofibroblasts, revealed very low relative numbers at all time points (E16.5: 0.5 ± 0.2%, E18.5: 0.3 ± 0.1%, P2: 0.1 ± 0.0%, P5: 0.1 ± 0.0%, and P14: 0%).

The proportion of all cells that were triple positive for PDGFrα, ADRP, and αSMA was also low and did not change over the course of the analyzed time points (E16.5: 1.3 ± 0.4%, E18.5: 0.4 ± 0.2%, P2: 0.1 ± 0.1%, P5: 0.2 ± 0.0%, and P14: 0%; Fig. 6C).

Lineage tracing of PDGFrα+ cells during early and late lung development. To perform lineage tracing of PDGFrα+ cells, two approaches were used in this study. First, a constitutively expressing PDGFrαCre line and mT/mG reporter mice were used to label PDGFrα+ cells throughout organ development. Lungs were analyzed during alveolarization before secondary septation at P3 (Fig. 7A), during secondary septation at P5 (Fig. 7B–D), and at the peak of alveolarization at P7 (Fig. 7E–G). At P3, GFP-labeled PDGFrα+ progenitor-derived cells were located in the smooth muscle layer of the bronchi. In primary septa, GFP-positive cells were found in the septal tips and in the septal (Fig. 7A). At P5, some interstitial cells were labeled by constitutive PDGFrα-driven Cre recombination. Additional ADRP staining revealed colocalization of this lipofibroblast marker with the GFP signal in some interstitial cells (Fig. 7B), documenting the contribution of PDGFrα+expressing cells to the lipofibroblast pool. Septal tip myofibroblasts were derived from PDGFrα+ precursors as revealed by colocalization with αSMA in these cells (Fig. 7C). At P7, GFP+ cells within the primary septa and at the base of the secondary crests expressed ADRP (Fig. 7E). Some GFP+ cells of the primary septa and the GFP+ tip cells of the secondary crests expressed αSMA at P7 (Fig. 7F). To quantify the relative numbers of fibroblasts in fibroblast subsets at P5 and P7, lungs were analyzed at P5 and P7 after cytopsins (Fig. 7D and G). At P5, the abundance of GFP+ cells, ADRP+ cells, and αSMA+ cells did not differ appreciably (GFP: 8.4 ± 1.7%, ADRP: 7.1 ± 1.4%, αSMA: 7.5 ± 0.8%). The relative number of cells is presented as percentage of whole lung cells. However, few ADRP+ cells were labeled by the constitutive PDGFrα-driven Cre-mediated recombination (Fig. 7D) at this stage of alveolarization (1.3 ± 0.4%). Furthermore, αSMA+ cells were labeled by the PDGFrα lineage (2.4 ± 0.6%) (Fig. 7D). At P7, the abundance of the different fibroblast populations also did not reveal differences (GFP: 7.9 ± 0.6%, ADRP: 5.4 ± 0.2%, αSMA: 4.9 ± 0.4%). Similarly to P5, ADRP+ cells (3.3 ± 0.6%) and αSMA+ cells (2.0 ± 0.1%) were labeled by the PDGFrα-driven Cre-mediated recombination at...
P7 (Fig. 7G). This constitutive Cre recombinase approach confirmed the expression pattern of PDGFRA in pulmonary cell types that has been demonstrated using the PDGFRA\textsuperscript{GFP} line. To perform true PDGFRA lineage tracing induced at defined time points, a conditional tamoxifen-inducible PDGFRA\textsuperscript{CreERT2} line was employed and crossed with mT/mG reporter mice. The Cre-mediated recombination...
was induced at P1 before the onset of secondary septation, and lungs were analyzed at P7 (Fig. 8, A–C) and P9 (Fig. 8, D–F). In separate experiments, recombination was induced at P2 (Fig. 9, A–D) and at P5 (Fig. 9, E and F). All lungs were analyzed at P14. Induction of Cre-mediated recombination at P1 led to labeling of ADRP+ cells of the primary septa and at the base of the secondary crests at P7 (Fig. 8A). Tip cells of the secondary crests were also labeled and expressed αSMA at P7 (Fig. 8B). Some GFP+ cells in the primary septa expressed αSMA at P7 (Fig. 8B). Lungs analyzed at P9 exhibited the same labeling pattern (Fig. 8, D and E). Quantification of the relative numbers of each fibroblast subpopulation at P7 (GFP: 5.6 ± 0.5%, ADRP: 9.2 ± 0.5%, αSMA: 4.8 ± 0.7%, GFP+, ADRP+ cells: 4.1 ± 1.0%, and GFP+, αSMA+ cells: 1.9 ± 0.3%) and P9 (GFP: 6.2 ± 0.7%, ADRP: 4.0 ± 0.4%, αSMA: 3.2 ± 0.4%, GFP+, αSMA+ cells: 3.2 ± 0.5%, and GFP+, αSMA+ cells: 2.8 ± 0.6%) again revealed nearly similar proportions with the exception of the ADRP+ cell population at P7 (ADRP: 9.2 ± 0.5%) (Fig. 8, C and F).

Labeling of PDGFRα-expressing cells at P2 revealed positive cells in the interstitial alveolar compartment when analyzed at P14. Some of those cells also expressed the lipofibroblast phenotypic marker ADRP (Fig. 9, A and B). In contrast to constitutive PDGFRαCre, induction of CreERT2 at P2 generated no labeling of septal tip myofibroblasts (Fig. 9C). Recombination at P2 leads to a lower number of PDGFRα+ cells (3.9 ± 0.7%) compared with the number of ADRP+ cells (10.4 ± 2.5%) when analyzed at P14 (Fig. 9D). At P14, 3.4 ± 0.6% αSMA+ cells were detected. When tamoxifen was administered at P2 and lungs were analyzed at P14, the PDGFRα lineage did not give rise to αSMA+ cells (relative no. of 0.1 ± 0% αSMA+ cells), but gave rise to 1.3 ± 0.5% ADRP+ cells (Fig. 9D). Lineage tracing of PDGFRα+ cells labeled at P5 did not give rise to αSMA+ septal tip cells (Fig. 9, E and F). Quantification revealed no contribution of the PDGFRα lineage to any αSMA+ cells and ADRP+ cells (GFP: 8.5 ± 0.8%, ADRP: 3.6 ± 1.2%, αSMA: 8.0 ± 0.8%, GFP + ADRP: 0.2 ± 0.1%, and GFP + αSMA: 0.1 ± 0.1%) (Fig. 9F).

**DISCUSSION**

The aim of the present study was to characterize the PDGFRα cell population in terms of localization and plasticity during the course of the pseudoglandular, canalicular, saccular, and the alveolar stages of lung development, and in the adult lung. The PDGFRα cell population has been chosen for the present study based on previous findings that demonstrated that the PDGFRα+ fibroblast population is critically involved in the process of septum formation, most likely by impacting myofibroblast differentiation and elastin production (7, 8, 26). The knowledge of cell function and differentiation during septum formation is essential for the development of new therapeutic strategies for pulmonary diseases that disturb the lung structure, such as COPD or fibrotic lung diseases in adults, and BPD, a disease of the preterm neonates.

New therapeutic strategies for diseases that disturb lung structure might be based on the induction of neoalveolarization of the diseased lung. Further detailed studies elucidating the features of PDGFRα+ fibroblasts using the same PDGFRαGFP knock-in mice employed in the present study demonstrated that PDGFRα+ cells accumulate at the alveolar entry ring during the process of secondary septation over the period P4-P12 (30).

Furthermore, PDGFRα+ cells at the alveolar entry ring express αSMA and elastin, in contrast to PDGFRα+ fibroblasts at the alveolar base, which exhibit lipofibroblast features during secondary septation (P2-P8) (30). These findings are consistent with the observations presented that demonstrate αSMA+, PDGFRα+ cells at P5 at the tips of the secondary crests and ADRP+, PDGFRα+ cells in the primary septa and at the base of the secondary crests.

Furthermore, McGowan and coworkers demonstrated that PDGF-A and sonic hedgehog could increase cytokinesis and chemotaxis of lung fibroblasts in vitro (33). Observations made in the present study demonstrate a localization of PDGFRα+ cells around the developing airways, the epithelial tubes of the pseudoglandular phase. During the canalicular stage, the peribronchial PDGFRα+ cells appeared to gradually decrease in number, and appeared in the more distal walls of the developing air sacs. Furthermore, these data demonstrate that, during secondary septation, PDGFRα+ cells appeared in the primary septa and in the tip cells of the secondary crests. The change in localization between the different developmental stages might be caused by cytokinesis and chemotaxis as suggested by McGowan and coworkers.

Furthermore, the spatiotemporal abundance of the ligand PDGF-A regulates proliferation, apoptosis, and αSMA expression in lung fibroblasts during alveolarization (22, 32). The Ras-related C3 botulinum toxin substrate 1 (Rac1)/protein kinase B (Akt) pathway has been demonstrated to be involved...
in stretch-mediated actin cytoskeletal remodeling in alveolar epithelial cell monolayers (14). The Rac1 agonist PDGF-A led to Akt phosphorylation in unstretched cells, suggesting a role for PDGF-A in cytoskeletal remodeling (14).

The present study demonstrated that PDGFRα+ cells of the lung express collagen I and thus can be attributed to the fibroblast cell type. To further characterize PDGFRα+ cells, which appear to have progenitor cell features, it was questioned whether PDGFRα+ cells differentially express the phenotypic MSC markers CD44, CD105, CD73, CD146, and CD90 (48).

The present study revealed that MSC markers were indeed differentially expressed in PDGFRα+ cells over the course of late lung development assessed. During the pseudoglandular and canalicular stages, coexpression with PDGFRα was not detected. In the saccular stage, CD44, CD105, and CD90 were expressed in some PDGFRα+ cells. During the alveolar stage at P5, all markers for MSCs were expressed in PDGFRα+ cells of the primary septa. At the site of secondary crest formation, CD73 and CD90 were expressed in PDGFRα+ cells at the base of the secondary septa.

Tip cells of the secondary crests demonstrated coexpression of CD105, CD73, CD146, and PDGF at P5. The differential expression of MSC markers in PDGFRα+ cells might reflect the high differentiation capacity and progenitor cell characteristics of PDGFRα+ cells. A further study by McGowan and McCoy demonstrated that lung fibroblasts with different levels of pdgfra gene expression differentially expressed markers of mesenchymal progenitors and differentiation (34). However, further studies are needed to elucidate the potential MSC nature of PDGFRα+ cells.

Furthermore, the nuclear expression of the reporter gene GFP in the employed PDGFRα/GFP knock-in mice combined with the mostly extracellular expression of markers for MSCs might hinder the recognition of a coexpression within the same cell. Future FACS analyses might validate the findings of the present study obtained by immunohistochemistry. However, the impact of CD90 on lipofibroblasts is well established. The lipogenic phenotype is promoted by CD90 involving PPAR-γ (42, 51).

This is reflected by the data demonstrated in the present study on CD90 expression during the analyzed developmental stages, since CD90 expression mostly coincided with the expression of ADRP. In particular, at P5, CD90 and ADRP were coexpressed in cells at the base of the secondary septa like it has been described for lipofibroblasts (11). Loss of CD90 in vivo impaired alveolarization and increased fibroblast proliferation and increased TGF-β signaling and collagen and elastin production (36).
Furthermore, it has been demonstrated that hyperoxia-induced alveolar epithelial-to-mesenchymal transition is dependent on TGF-β signaling (52). A further study demonstrated that CD90-negative fibroblasts exhibit higher levels of PDGFRα expression and showed increased proliferation in response to PDGF-A compared with CD90+ fibroblasts (17). These findings do not support the observations presented here that PDGFRα tip cells of the secondary crests do express CD90, and αSMA, and thus hold the myogenic character. However, the opposing features of the lipogenic and myogenic...
fibroblast phenotype that seem to be relevant for secondary septation are supported by the data presented here, since ADRP expression was expressed at the base and not at the tip of the secondary crests. With the use of a model of compensatory lung growth after pneumonectomy, this phenomenon has also been studied in realveolarization (12). That study revealed that the number of PDGFRα+ cells transiently increased after pneumonectomy (12).

Furthermore, blockade of fibroblast growth factor (FGF) signaling and PPAR-γ activation (by rosiglitazone) inhibited the differentiation of PDGFRα+ cells into myofibroblasts, a process that is required for alveolarization, and thus resulted in alveolar simplification after pneumonectomy (12). This finding is consistent with the quantitative data of the present study on the abundance of the different fibroblast subtypes. The data demonstrate an increase of αSMA+ PDGFRα+ cells during secondary septation at P5 possibly reflecting the differentiation of PDGFRα+ cells into myofibroblasts upon alveolarization. These findings indicate that PDGFRα+ cells appear in areas of intensive developmentally associated tissue remodeling.

However, the quantitative data demonstrated here on the abundance of single PDGFRα cells and single ADRP+ cells are contradictory to previous findings indicating that the number of lipofibroblasts and PDGFRα cells increases during alveolarization (29, 30). This might be due to the different methods that were applied. However, the decline in lipofibroblast abundance over the course of lung development demonstrated by the quantitative data of the present study might be partly due to apoptosis (2).

Further, the present data demonstrated PDGFRα+ cells in the adult lung. With the use of the PDGFRαCreERT2 transgenic mice, a prolonged half-lifetime of the GFP compared with PDGFRα has to be considered. Thus GFP+ cells might already have lost the endogenous PDGFRα expression, representing the progeny of PDGFRα+ cells. The present study aimed to further analyze the differentiation of PDGFRα+ cells during alveolarization using true lineage tracing. The data of the present study demonstrate that PDGFRα+ cells give rise to both myofibroblast and lipofibroblast subtypes, while the timing of the determination of these two lineages is different.

With the use of inducible PDGFRαCreERT2 transgenic mice, it was determined that myofibroblasts, which stem from PDGFRα+ cells, are defined before P2, since induction at P2 or P5 did not give rise to double-positive cells for GFP and αSMA. However, induction at P1 and the usage of the constitutively recombining model (which drove induction from E0) gave rise to myofibroblasts (GFP+ and αSMA+ cells) that were derived from the PDGFRα+ lineage. Because not all αSMA+ cells were also GFP+ at P7, P9, and P14, it has to be suggested that αSMA+ but PDGFRα- cells at this stage may rise from a different progenitor cell type, and contribute largely to the emergence of peribronchial and perivascular smooth muscle cells. In this line, FGF10+ cells have been demonstrated to give rise to parabronchial and vascular smooth muscle cells (15).

Furthermore, FGF signaling is known to be required for the induction of αSMA in PDGFRα+ myofibroblasts (38). Concerning the lipofibroblast lineage, the data presented here demonstrate that PDGFRα+ cells give rise to lipofibroblasts before P5, since induction at P1 and P2 labeled ADRP+ cells, whereas induction at P5 did not (Fig. 9A). Again, double-positive cells were located in the alveolar regions in the primary septa and at the base of the secondary crests, suggesting their participation in secondary septation.

Indeed lipofibroblasts have been reported to play a crucial role in lung development in rodents (20, 29), but lipofibroblasts also have been detected in human lung biopsies (41). A further study questioned the presence of lipofibroblasts in the human lung analyzing different mammalian species, including humans using light and electron microscopic stereology (45).

Different reasons may have caused these contradictory results (1). A recent report revealed that AEC II form alveolospheres when placed in 3D culture (3). Growth and differentiation of these alveolospheres is accelerated when cocultured with PDGFRα+ fibroblasts (3). When hypoxia is used to model the structural changes that occur in the developing lung in BPD, a transition from lipofibroblast to myofibroblast has been described, which was prevented by rosiglitazone treatment (42).

The origin of this lipofibroblast lineage has not been described. However, defective PDGFR signaling has been demonstrated to participate in the development of human BPD (39). The present study demonstrates for the first time using true lineage tracing that the PDGFRα+ fibroblast lineage contributes to the lipofibroblast pool of the lung, and that these lipofibroblasts originating from PDGFRα+ cells are determined before P5.

Because not all lipofibroblasts were labeled in the present model, further sources of the lipofibroblast lineage might exist. Incomplete recombination might in addition have caused labeling of fewer cells and thus fewer lipofibroblasts.

Some important limitations of the approach taken in the present study must be kept in mind. With the use of reporter gene expression, the reporter protein might reveal a different half-lifetime than the endogenous protein. Thus the spatiotemporal appearance of the reporter gene might not have been observed.

The origin of this lipofibroblast lineage has not been described. However, defective PDGFR signaling has been demonstrated to participate in the development of human BPD (39). The present study demonstrates for the first time using true lineage tracing that the PDGFRα+ fibroblast lineage contributes to the lipofibroblast pool of the lung, and that these lipofibroblasts originating from PDGFRα+ cells are determined before P5.

Because not all lipofibroblasts were labeled in the present model, further sources of the lipofibroblast lineage might exist. Incomplete recombination might in addition have caused labeling of fewer cells and thus fewer lipofibroblasts.

Some important limitations of the approach taken in the present study must be kept in mind. With the use of reporter gene expression, the reporter protein might reveal a different half-lifetime than the endogenous protein. Thus the spatiotemporal appearance of the reporter gene might not have been observed.
fully reflect the features of the endogenous protein (endogenous PDGFRA expression compared with GFP expression in PDGFRA<sup>CreERT2</sup>;mT/mG knock-in mice).

Furthermore, use of the CreERT2 stop <i>loxP</i> system recombination might not be completed. The tamoxifen availability and half-lifetime might have caused a prolonged recombination period compared with the chosen time points of induction. Finally, the promoter system of the stop <i>loxP</i> reporter mice might have a different strength driving expression as the endogenous promotor and thus might change the abundance of the labeled cells.

There is no doubt that PDGFRA<sup>+</sup> mesenchymal cells and PDGF-A signaling play a crucial role in septum formation and the generation of αSMA<sup>+</sup> myofibroblasts and elastin fibers (8, 26). However, it remains unresolved how the three fibroblast
subtypes, namely lipofibroblasts, myofibroblasts, and PDGFR\alpha\textsuperscript{-} cells, interact to ensure proper septum formation and growth. The present study demonstrated that PDGFR\alpha\textsuperscript{+} cells can give rise to a part of myofibroblasts and lipofibroblasts during alveolarization (Fig. 10B). Furthermore the data of the present study narrow the time period for the commitment of the PDGFR\alpha\textsuperscript{-} lineage to myofibroblasts and lipofibroblasts (Fig. 10A). Further studies are needed to elucidate differentiation of lipofibroblasts and myofibroblasts back to PDGFR\alpha progenitor cells during regeneration (Fig. 10B). A distinct and balanced regulation of the differentiation of PDGFR\alpha cells toward the lipogenic or myogenic phenotype seems to be essential for alveolarization. The present study demonstrated both ways of differentiation and characterized the presence of PDGFR\alpha cells in a spatiotemporal manner during lung development. Further studies are needed to discover the factors regulating the differentiation of PDGFR\alpha cells in alveolarization and regeneration to be exploited for the development of new therapeutic strategies for structural lung diseases of the neonatal and adult lung.

**ACKNOWLEDGMENTS**

We thank Botond Roska for providing the PDGFR\alpha\textsuperscript{Cre} mice.

**GRANTS**

This study was financially supported by the Max Planck Society, the LOEWE initiative, the Excellence Cluster Cardio Pulmonary System of the German Research Foundation, and Rhön Klinikum AG.
DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


Fig. 10. Schematic summary of the key lineage tracing results of the present study and integration of the core findings into a model demonstrating open questions regarding the differentiation of fibroblast subtypes during lung development and regeneration. A: with the use of a constitutive Cre expression model, labeling of PDGFRα<sup>+</sup> cells throughout lung development demonstrated that PDGFRα<sup>+</sup> cells give rise to a part of the lipofibroblast subpopulation and a part of the myofibroblast population at P5 and P7, respectively. With the use of an inducible Cre<sup>ERT2</sup> activation model, PDGFRα<sup>+</sup> cells gave rise to a part of fibroblasts and a part of myofibroblasts at P7 and P9, respectively, when labeled at P1. In contrast, labeling of PDGFRα<sup>+</sup> cells at P2 resulted in labeling of a part of lipofibroblasts at P14 without labeling myofibroblasts. Labeling of PDGFRα<sup>+</sup> cells at P5 demonstrated that lipofibroblasts and myofibroblasts at P14 were not generated from these PDGFRα<sup>+</sup> cells. B: schematic model of cellular transition during alveolar development demonstrated by the present study (dark arrows) and hypothetical transition during disease and regeneration still waiting to be elucidated (bright arrows). PDGFRα<sup>+</sup> progenitor cells (PG) reside in the alveolar septum and generate parts of the lipofibroblast (LF) pool and the myofibroblast (MF) pool, including the tip cells of the secondary crest during postnatal lung development (dark arrows, capillary (C)). The lineage for the lipofibroblast pool is defined before P5. The lineage generating the myofibroblast pool is defined before P2. Both lineages differentially and transiently express mesenchymal stem cell markers such as CD44, CD105, CD73, CD146, and CD90 upon the differentiation processes into lipofibroblasts and myofibroblasts. Future studies are needed to demonstrate if there is a direct transition of lipofibroblasts into myofibroblasts or reprogramming of differentiated fibroblasts back to precursor cells during disease and regeneration, respectively (bright arrows).


