Review Article

Pulmonary Circulation

Deregulated angiogenesis in chronic lung diseases: a possible role for lung mesenchymal progenitor cells (2017 Grover Conference Series)

Jonathan A. Kropski[1], Bradley W. Richmond[1], Christa F. Gaskill[1], Robert F. Foronjy[2] and Susan M. Majka[1,3]

[1]Department of Medicine, Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University Medical Center, Nashville, TN, USA; [2]Department of Medicine, Division of Pulmonary and Critical Care Medicine, SUNY Downstate Medical Center, Brooklyn, NY, USA; [3]Department of Medicine, Vanderbilt University, Nashville, TN, USA

Abstract

Chronic lung disease (CLD), including pulmonary fibrosis (PF) and chronic obstructive pulmonary disease (COPD), is the fourth leading cause of mortality worldwide. Both are debilitating pathologies that impede overall tissue function. A common co-morbidity in CLD is vasculopathy, characterized by deregulated angiogenesis, remodeling, and loss of microvessels. This substantially worsens prognosis and limits survival, with most current therapeutic strategies being largely palliative. The relevance of angiogenesis, both capillary and lymph, to the pathophysiology of CLD has not been resolved as conflicting evidence depicts angiogenesis as both reparative or pathologic. Therefore, we must begin to understand and model the underlying pathobiology of pulmonary vascular deregulation, alone and in response to injury induced disease, to define cell interactions necessary to maintain normal function and promote repair. Capillary and lymphangiogenesis are deregulated in both PF and COPD, although the mechanisms by which they co-regulate and underlie early pathogenesis of disease are unknown. The cell-specific mechanisms that regulate lung vascular homeostasis, repair, and remodeling represent a significant gap in knowledge, which presents an opportunity to develop targeted therapies. We have shown that that ABCG2^{pos} multipotent adult mesenchymal stem or progenitor cells (MPC) influence the function of the capillary microvasculature as well as lymphangiogenesis. A balance of both is required for normal tissue homeostasis and repair. Our current models suggest that when lymph and capillary angiogenesis are out of balance, the non-equivalence appears to support the progression of disease and tissue remodeling. The angiogenic regulatory mechanisms underlying CLD likely impact other interstitial lung diseases, tuberous sclerosis, and lymphangioleiomyomatosis.

Keywords

chronic lung disease, angiogenesis, lymphatics, microvasculature, idiopathic pulmonary function, IPF, COPD, emphysema, ABCG2 MPC

Date received: 12 September 2017; accepted: 10 October 2017

Pulmonary Circulation 2017; 8(1) 1–18
DOI: 10.1177/2045893217739807

Vascular dysfunction in chronic lung disease

Microvascular dysfunction and/or structural remodeling (vasculopathy) is frequently, if not ubiquitously, observed in most chronic lung diseases (CLD), including pulmonary fibrosis (PF), chronic obstructive pulmonary disease (COPD)/emphysema and interstitial lung disease associated with systemic sclerosis (SSc). This vasculopathy may be characterized by deregulated angiogenesis, pathologic remodeling, and/or loss of microvessels.

The complex, orchestrated formation of blood vessels that occurs during lung development provides insights into the origins of vasculopathy later in life. The growth of new vessels in the adult can occur through angiogenesis, which is

Corresponding author:
Susan M. Majka, Vanderbilt University, Division of Allergy, Pulmonary and Critical Care Medicine, 1161 21st Avenue S, T-1218 MCN, Nashville, TN 37232, USA.
Email: susan.m.majka@vanderbilt.edu

new vessel growth from existing vessels and vasculogenesis, which is new vessel growth independent of existing vessels.[1–6] The coordination of both processes is important in lung development and it also plays a key role in adult-onset lung disease.

During the fourth week of lung development in humans, hematopoietic progenitor cells initiate vasculogenesis to form vascular lakes.[1,7,8] These structures will ultimately become capillaries and connect with the pulmonary circulation, which begins to develop separately during the seventh and eighth week of gestation. These connections enable the lung to carry out its primary function, which is to deliver blood flow to alveolar capillaries where oxygen absorption and carbon dioxide elimination occurs. The bronchial circulation is a distinct system from the pulmonary circulation. It functions to supports the airway structure of the lung and the bronchial blood supply develops via angiogenesis from the aorta during the ninth and 12th week of gestation.[7–9] When there is obstruction of pulmonary blood flow, ischemic stimuli induce rapid and extensive growth of the bronchial circulation. Indeed, it is the bronchial circulation that supplies the blood vessels that permit tumor growth in lung cancer.[10] In addition to tissue hypoxia, the bronchial vasculature is also sensitive to local inflammatory responses.[11] Inflammation promotes the release of mediators like IL-1 and IL-6 that stimulate the release of vascular endothelial growth factor (VEGF).[12] This increases bronchial vascular density and leads to airway remodeling in asthma.[9,11–15] Though its role in asthma is well-established, the impact of these factors on other inflammatory lung diseases like emphysema and PF is less clear.[16] Some studies have reported increased bronchial vascular density in COPD[17,18] and this may contribute to the mucus production and thickened airways that occur in this disease. However, other studies have shown decreased or no changes in the bronchial vasculature.[19–21] Thus, the role of the bronchial blood vessels in COPD is not as clearly defined as it is in asthma.[22,23] Increases in the bronchial vasculature are noted in bleomycin-induced PF in mice.[24] Despite these findings, most studies have concentrated on alveolar capillaries because the alveolar parenchyma is the region most affected by fibrotic remodeling in this disease.[25–27] This review will largely focus on factors affecting the alveolar microvasculature[28] since alterations in this region have a well-established role in the pathogenesis of both PF and emphysema.

In most forms of CLD, development of secondary pulmonary hypertension (PH) is associated with poor prognosis and worsened survival,[29] underscoring the importance of better understanding the role of the pulmonary vasculature in disease pathogenesis. The etiology of microvascular remodeling and mechanisms through which it contributes to the development and severity of various CLD remain unknown, due in part to a lack of models recapitulating early stage vasculopathy before environmental injuries, disease, or aging. The relevance of vasculopathy to the pathophysiology of CLDs has not been resolved as conflicting evidence depicts angiogenesis as both reparative or pathologic;[30–41] it is possible that both of these are true in specific contexts.

Most work to date investigating the pulmonary vasculature has focused on arterial and capillary networks in the lung; the role of lymphangiogenesis has been largely overlooked. As a balance of existing and de novo capillary and lymphatics is necessary for tissue homeostasis, one might argue that their collective deregulation is paramount to the pathophysiology of disease.

Capillary and lymph angiogenesis

The pulmonary lobule, which measures 2–3 cm in size, is the basic functional unit of the lung. It comprises several terminal bronchioles which run with pulmonary arteries and deep lymphatics in the center. This structural unit is enveloped by interlobular septa containing pulmonary veins and independent superficial lymphatics that are in communication with the pleural lymphatic system. The deep lymphatics travel in the bronchovascular interstitium and drain the airways while the superficial lymphatics are present in the interlobular septa and drain alveolar regions. The structure and function of lymphatic vessels is very different from the circulatory system and they have been shown to be extremely sensitive to interstitial stresses.[42]

In contrast to the circulatory system, lymphatic flow is regulated by the movements of the body and muscle contraction.[42] These structures are interlaced among arterioles and venules of most soft connective tissues of the body to aid in the regulation of fluid balance.[42–47] In the adult lung, lymphatics are localized to the bronchiolar wall and bronchiole associated arteries.[48] Intra-alveolar lymphatics are more difficult to distinguish but are also present, interwoven within the alveolar-capillary interstitium.[45,49] Of note, disorders of these lymphatic systems could promote airway or alveolar injury by impairing the clearance of antigens and lung biomediators.

Angiogenesis is the sprouting and growth of new vasculature from existing vascular structures. Lymphangiogenesis is similar to capillary angiogenesis in that during development and in the maintenance of adult tissue homeostasis, the formation of de novo lymphatics may occur from sprouting of lymphatic endothelial cells (LEC) from existing vessels as well as single LEC or mesenchymal progenitors migrating to a zone where they later connect and form vascular structures.[4,43,44,50–54] Currently, distinguishing between LEC and angiogenic mesenchymal progenitors in vivo is technically challenging due to the promiscuity of lymphatic markers (prox1, lyve1, flt4). In vitro, definition between the cell types relies on functional characteristics and expression of multiple lineage specific markers.[55,56] The regulation of both angiogenic processes involves VEGF signaling.[43,44,50,57] A comparison of the distinct processes of capillary and lymphangiogenesis is reviewed by Adams and Alitalo.[43]

During development, lymphangiogenesis is closely linked with capillary angiogenesis.[43] Their interaction and balance is necessary for maintenance of tissue homeostasis.[43,44,49,51,58] During disease, angiogenesis of one may exceed the other.[4,31,32,41,42,45–47,58–71] Both angiogenesis and lymphangiogenesis are required for wound healing and functional tissue repair, processes deregulated in CLD.[43,50,72] Typically, lymphatic capillaries do not contain mesenchymal cells/pericytes. However during disease, the lymphatic capillaries may attract these cells.[73] In both developmental and pathological instances, mesenchymal cells may also form new lymphatic structures, devoid of endothelium, either connected or separate from existing lymphatic circulation.[52,53,74] These abnormal mesenchymal structures have been described in tumor angiogenesis and termed vascular mimicry.[75–78]

Pulmonary fibrosis

PF (familial, idiopathic [IPF], and associated with SSc) is a debilitating disease characterized by excessive matrix deposition, angiogenesis, and epithelial cell hyperplasia that impedes overall tissue function. Abnormal neovascularization in fibrosis was first characterized in 1963 by Turner-Warwick.[79] The underlying cause of fibrotic remodeling and microvascular dysfunction in the early stages of fibrosis is unknown.[30–32,35,45,49,58,62,70,80–84] Research suggests that impaired cross-talk between endothelial cells and mesenchymal progenitors during disease drives a functional switch of the mesenchyme to a pro-remodeling phenotype, modulating both vascular regression as well as fibrosis.[85] Additionally, the relevance of angiogenesis, microvascular remodeling, and lymphangiogenesis to the pathophysiology of disease are an area of intense investigation.[31]

A heterogenous pattern of microvascular remodeling has been reported in PF. Microvascular density (as defined by CD34+ cells) was reported to be greater during fibrosis in areas of mild remodeling compared to normal lungs, whereas fibroblast foci are described as devoid of vessels.[24,30,31,84,86,87] Similarly, fibrosis in SSc is associated with an initial increase in microvessels, followed by a progressive decrease.[62,65,80–82] Heterogeneity of the microvasculature is largely dependent on the localization and severity of fibrosis.[30–32,84] It is not entirely clear whether these discrepancies in microvascular density represent reactive/compensatory changes or reflect the temporal heterogeneity of the lesions that occur in PF. For example, alveolar capillaries are increased and dilated in non-fibrotic regions but absent in fibrotic interlobular septa.[88–90] These analyses are complicated by the promiscuity of lineage marker expression; for example, CD34 may be expressed on some epithelial cell populations including bronchoalveolar stem cells (BASCs);[91] as such there is a need to more comprehensively define patterns of vascular remodeling in PF using multimarker strategies.

Abnormal lymphangiogenesis is also present in PF and typically associated with the degree of disease severity.[45,49,58,92] El-Chemaly correlated the diameter of lymphatics to disease severity in IPF patients,[45] while the diameter has also been correlated to survival.[93] They further demonstrated that angiogenesis in IPF patients was regulated by short fragment hyaluronan, the extracellular matrix protein, present in the bronchioalveolar lavage fluid and macrophages, not found in healthy controls.[45] Lymphatic microvasculature is localized to areas of remodeling where capillaries are typically absent. Fibrosis disrupts lymphatics in the interlobular septa[94] and this impairs alveolar clearance in the lung.[95] These changes could perpetuate lung fibrosis by impairing the elimination of inflammatory cells that express TGF-β, a profibrotic cytokine that inhibits lymphangiogenesis.[96,97] Abnormal lymphatics have been proposed as a unifying mechanism for fibrogenesis.[58] Intriguingly, in a murine model of PF induced by intratracheal or intraperitoneal bleomycin, the development of abnormal lymphatic structures was driven by platelet-derived growth-factor-beta (PDGF-β), and pharmacologic inhibition of PDGF-β attenuated lymphatic remodeling and improved fibrosis.[98] This is of particular interest as the recently approved antifibrotic treatment nintedanib, among its actions, inhibits PDGF-β/PDGFR-β signaling,[99] raising the possibility that its efficacy may be due in part to effects on lymphatic organization.

COPD/Emphysema

Emphysema is a form of COPD, characterized by abnormal enlargement of the distal airspaces/alveoli, and is an important contributor to reduced lung function in patients with COPD.[100] Emphysema is a progressive disease that destroys alveolar septa over time and causes a decrease in functional alveolar surface area that impairs the absorption of oxygen. Along with small airways disease and chronic bronchitis, emphysema contributes to persistent airflow obstruction in patients with COPD, resulting in persistent obstruction to expiratory airflow, chronic dyspnea, and death in approximately one-third of affected patients.[101] The narrowing of the airway and the elaboration of thick mucus causes breathlessness by impeding flow in the airways. COPD was ranked sixth among the causes of death globally in 1990 but is projected to be the third most common cause of death by 2020.[102] Current treatment is limited and focuses on halting further lung destruction and preserving lung function and includes smoking cessation, bronchodilators, steroids, and supplemental oxygen.[103] A limited understanding of the cellular pathogenesis of COPD has impeded the development of effective treatments. Recent evidence has identified alterations to the lung microvasculature during the early pathogenesis and heterogeneity of COPD,[104–107] although the underlying mechanisms are not defined.

Emphysematous loss of the alveolar capillary network was first described by Liebow in 1959.[108] However, the

relevance of the microvasculature during the early stages of COPD has not been resolved. The basis for a vascular component contributing to emphysema was suggested by Wiebe and Laursen in 1998, via quantitation of significantly decreased capillary length and density in COPD patients (68%), relative to controls.[109] A functional basis for vasculopathy as a contributor to disease pathogenesis has been demonstrated in clinical studies comparing FEV1 to severity of tissue remodeling and vascular perfusion. Barr et al. defined a relationship between endothelial function, FEV1, and percentage of emphysema using computed tomography (CT) in ex-smokers and demonstrated that endothelial dysfunction was associated with a significant decrease in FEV1.[110] McAllister et al. defined a relationship between emphysema and systemic vascular dysfunction[105] and Sabit et al. showed that evidence of increased arterial stiffness and endothelial dysfunction was related to the severity of airflow obstruction increasing the risk for COPD.[104] Alford et al. documented increased areas of decreased perfusion in individuals with early visual CT evidence of emphysema, relative to emphysema-free smokers and persons who had never smoked.[106] Most recently, imaging of the pulmonary vasculature in COPD patients demonstrated that the extent of loss of vascular function correlates to the degree and heterogeneity of emphysema/COPD.[107] Many studies exploring the mechanism(s) of vasculopathy in COPD have focused on endothelial apoptosis.[111] Alpha-1-antitrypsin has been shown to inhibit caspase-3 activation and apoptosis in endothelial cells, providing a potential mechanism through which vasculopathy contributes to emphysema in patients with alpha-1-antitrypsin deficiency.[112] In addition, *SERPINF1* has been shown to be elevated in patients with COPD and may contribute to increased endothelial cell apoptosis.[113] In addition to the associative studies in humans, animal studies have suggested a causal link between endothelial dysfunction and emphysema. Antagonizing vascular endothelial growth factor receptor (VEGFR) in rodents induced endothelial apoptosis and the subsequent destruction of alveolar lung tissue.[114]

Clinical studies have also linked enhanced lymphangiogenesis to the pathogenesis of COPD. Hardavella et al. correlated the lymphatic microvessel density, as determined by Lyve1 stain, to the degree of airway obstruction, measured by FEV1, in COPD patients vs. non-COPD smokers.[46] Mori et al. followed these proof-of-principle studies with a detailed histochemical quantification of lymphatic distribution and morphological characteristics in healthy control vs. COPD lung tissue.[48] Pathologic, de novo lymphatics were localized in the alveolar parenchyma, not associated with smooth muscle actin positive vessels. They were also found at a higher density in areas of alveolar parenchymal fibrosis. Taken together, these studies suggest that while de novo capillary angiogenesis is halted in COPD, lymphangiogenesis progresses, representing an imbalance of angiogenesis. How these lymphatic vessels contribute to the pathogenesis of COPD remains to be determined. However, it has been speculated that they may channel inflammatory signals to regional lymph nodes where T cell activation occurs. The coordination of T cell activation by this lymphatic system could have a major impact on COPD. T cells promote emphysema by directly injuring the lung epithelium[115] and they promote airway disease by elaborating cytokines like IL-4, -5, and -13, which induce airway obstruction and mucus secretion.

Combined pulmonary fibrosis and COPD (CPFE)

The presence of both emphysema and PF in the same patient is a disorder known as combined pulmonary fibrosis and emphysema (CPFE). Whether this reflects a distinct disease or the chance co-occurrence of two processes is a matter of some debate. This syndrome is characterized by upper-lobe emphysema, lower-lobe fibrosis, and abnormalities of gas exchange that result in dyspnea.[116] Pulmonary function tests (PFTs) differ from the obstructive pattern with increased lung volumes in COPD and the restrictive pattern with reduced lung volumes in lung fibrosis. CPFE patients typically present with near normal (pseudonormalized) lung volumes and with a significantly reduced diffusing capacity for carbon monoxide (DLCO). This reduction reflects the severity of the gas exchange abnormalities that occur in this disorder. Cigarette smoke exposure is a well-recognized risk factor for the development of PF and COPD/emphysema.[117–119] In fact, smoking exhibits deleterious effects on the systemic circulation[118] and exacerbates systemic sclerosis.[117,120] PH is prevalent in CPFE and is the main co-morbidity affecting survival of CPFE patients.[121] In contrast to patients with COPD, CPFE is frequently associated with profound system hypoxemia, suggesting that in these patients, shunt rather than ventilation/perfusion mismatching is a primary driver of hypoxemia. This suggests that there are some distinctions in the patterns of vascular remodeling in CPFE and COPD.

Extracellular matrix remodeling plays an essential role in COPD and PF but the character of the remodeling in these syndromes differs significantly. Alveolar elastin and type III collagen are destroyed in emphysema and replaced by fibrils that are thickened and disorganized.[122] This degrades alveolar tissue and redistributes mechanical forces to disrupt alveolar interdependence and perpetuates lung tissue destruction.[123,124] In contrast, PF is characterized by an excessive deposition of lung collagen that obliterates distal lung tissue structures. Though the pathogenesis is distinct, the loss of alveolar capillary units induces similar clinical symptoms including cough and shortness of breath.

Inflammation, deregulated vascular remodeling, pruning of microvascular structures, and excessive lymphangiogenesis are present in both disorders. These shared mechanisms likely account for the coexistence of both diseases in CPFE. Subtle differences in these processes are probably responsible for the development of fibrosis in some regions and

emphysema in others. For example, studies suggest that inflammation plays a lesser role in the pathogenesis of IPF and this disease does not respond to steroid therapy.[125–127] The inflammatory cells present in IPF appear to contribute to disease pathology by coordinating fibroblast activation and epithelial mesenchymal transition.[128,129] In addition, auto-antibodies produced by B cells attack the endothelium to induce microvascular injury that contributes to PH and lung fibrosis.[130–133] In contrast to IPF, COPD responds clinically to anti-inflammatory therapy with improved lung function and decreased exacerbations.[134] The inflammatory cells in COPD, such as neutrophils, macrophages, lymphocytes, and eosinophils, release mediators that increase mucus production, airway hyper reactivity, and lung tissue destruction.[135] Thus, they play a more direct role in the disease onset and progression. Like PF, auto-immunity triggers the vascular injury that occurs in COPD.[136,137] Since both diseases involve immune-mediated damage to the microvasculature, it is not surprising that PH is so prevalent in CPFE.

How these distinct biological processes are induced in different regions in CPFE is unknown but likely involves changes in inter- and intra-cellular signaling. Kusko et al. recently published a comprehensive study comparing the transcriptome networks of lung tissue between COPD and IPF patients, relative to normal controls. They identified activation of the p53/hypoxia pathway as well as alternative splicing of PDGFA as a common factor in both diseases.[138] This is intriguing since regional variations in alveolar oxygen tension are present in the lung. The apex has comparatively high ventilation to perfusion ratio resulting in increased alveolar oxygen tension. On the other hand, the lung base has the highest blood flow and lowest ventilation-to-perfusion ratio.[139] Thus, oxygen tension at the base is lower than the apex. These regional differences could have differing effects on the p53/hypoxia pathway, potentially explaining why fibrosis occurs at the bases and emphysema at the apex.

Balance of developmental signaling pathways may also play a role. In PF, it has been suggested that there is increased canonical Wnt signaling, while in COPD this is suppressed.[140] We have recently shown that in the bleomycin model of PF, persistent Wnt/B-catenin signaling leads to enhanced proliferation of mesenchymal progenitor cells but impairs differentiation. Since canonical Wnt signaling is required for pulmonary angiogenesis, it may be that in COPD, suppressed Wnt signaling leads to failure of progenitor cell function, while in PF, failure to suppress Wnt signaling culminates in a hyperproliferative but dysfunctional microvascular network. Further work will be required to better elucidate the shared and divergent mechanisms of microvascular remodeling in COPD and PF; however, taken together, the literature supports that there is an inability to sustain functional tissue repair in epithelial, vascular, and mesenchymal compartments in both diseases.

VEGF: friend or foe?

The basis for a vascular component contributing to fibrosis and emphysema has also been demonstrated in rodents via manipulation of VEGF. VEGF is a survival factor for lung endothelial and mesenchymal cells and decreased signaling through its receptors results in loss of distal lung tissue structure.[141–143] Levels of VEGF are increased in early COPD patients,[144–146] and decreased in late COPD.[67] VEGF is overexpressed in the skin of SSc patients[80] despite impaired angiogenesis. It is also abundant in the type 2 pneumocytes and myofibroblasts in IPF lungs.[31,70]

The use of the VEGF receptor tyrosine kinase inhibitor, SU5416, as a VEGF antagonist induces vascular injury, remodeling, subsequent PH, and loss of alveolar tissue structure.[114,147–151] Targeted knockdown of VEGF gene expression in the lung drives septal wall destruction.[152] To date, studies have focused on endothelial cells and VEGF deregulation as the basis for microvascular dysfunction during COPD, while additional contributing cell types have been largely overlooked.

Additionally, VEGF-A splice variant b has been described as anti-angiogenic. Increased levels of VEGF165b have been correlated with PH, fibrosis, peripheral artery disease, and SSc,[65,80,82,146,153–155] while its significance in lymphangiogenesis has not been described. VEGF-A and its receptors are significantly upregulated in asthmatic airways and this expression correlates positively with submucosal vascularity and negatively with FEV1 and airway hyper-responsiveness.[156] Thus, it is conceivable that alternative splicing of VEGF-A could dictate whether fibrotic remodeling or airway obstruction occurs in the lung. Indeed, alternative splicing has been implicated in a number of respiratory diseases including COPD, IPF, and lung cancer.[138,157]

Adult lung mesenchymal progenitors, angiogenesis, and tissue remodeling

There is a precedent for multipotent adult mesenchymal stem or progenitor cells (MPC) to stimulate capillary angiogenesis, as well as lymphangiogenesis. Both bone marrow derived and adipose MPC promote lymphangiogenesis in models of tumor metastases as well as regulate the proliferation of LEC.[158,159] MPC have been hypothesized to be the precursor to pericytes.[160,161] Interestingly, pericytes have also been hypothesized to be mesenchymal stem cells (MSC) in adult tissue.[162] However, this hypothesis was recently challenged by elegant lineage tracing analyses.[163,164] It is likely that pericytes and MSC express similar cell surface determinants and co-localize in the microvasculature, yet are functionally distinct.

The current understanding of the role MPC/pericytes play in adult lung disease, de novo angiogenesis, and vascular remodeling is controversial.[30–32] Pericytes are the "smooth muscle cell" of the microvessels/capillary beds[73] and provide stability to the vasculature by direct contact

with endothelium and regulation of vascular tone.[73] Mice lacking pericytes die before birth due to hemorrhage.[165] They participate in wound healing, tissue repair, vascular remodeling/vasculopathy, and fibrosis.[33,35,37–40,73,87,166–183]

While these hypotheses are intriguing, the studies of tissue-resident stromal progenitor cells as well as the origin of pericyte lineages in the adult have been complicated by a lack of unique markers to define specific cell types within heterogeneous mesenchymal and lineage specified pericyte populations. The lack of suitable markers to trace mesenchymal progenitor subpopulations and their differentiation in the adult lung has limited our understanding of their role in homeostasis and disease. However, the importance of mesenchymal precursors during disease has been underscored by current limitations. Agha et al. recently published a summary of MSC and their roles in fibrotic diseases of multiple organs.[184]

Currently, there is no exclusive vascular cell marker to distinguish microvascular endothelium or pericytes from multipotent mesenchyme and their derived lineages in the adult lung. For example, PDGFR-β, Gli-1, and Tbx4 have been used to trace mesenchymal cells during fibrosis and development, but these markers label mixed mesenchymal and derived lineage populations as well as epithelial lineages in the adult, respectively (Table 1).[40,185–187] Other current models rely on SM22 or SM-MHC driven Cre systems to manipulate genes in lung smooth muscle[179,188,189] but SM22 and SM-MHC demonstrate systemic expression. Endothelial cell lineage tracing has been reported using Tie-2Cre,[190] which labels mesenchymal cells as well as circulating bone marrow derived angioblasts.[191] Similarly, lymphatic vascular endothelial hyaluronan receptor-1 (lyve-1) labels both endothelium and mesenchymal progenitors.[52,53,74,192,193] Our recent studies validated the ATP binding cassette G2 (ABCG2) as a reasonable label for perivascular adult mesenchymal progenitors, with the caveat that low dose tamoxifen is required for specificity.[194]

A second limitation to understanding the function of multipotent mesenchyme in the adult lung is the ability to translate the findings from populations identified in rodent tissues to a comparable population of primary patient cells. Because ABCG2 is present at the cell surface, we have been able to isolate populations of adult lung MPC from lung tissue explants from normal and disease lungs and characterize them in parallel to the murine models and cells.[36,55,194,195] We have been able to define tissue-specific signatures of MPC as well as pathways related to signaling, matrix, inflammation, and angiogenesis disrupted in disease.[194–196]

Table 1. Labeling pulmonary mesenchymal subpopulations.

Lineage trace marker	Protein	Putative cell population tracing	Specificity	Refs.
ABCG2	MDR transporter	Lung MPC	Perivascular adult mesenchymal progenitors	36,166,194,196,204,208,209
ADRP	Perilipin2, adipose differentiation related protein	Lipofibroblasts/ myofibroblasts	Alveolar typeII cells Lipofibroblasts	251,252
Foxd1	Forkhead family Transcription factor	Foxd1 pericytes	Developing vascular/ mesenchymal lineages, pericytes, endothelium	35,253
Gli1	Transcription factor, associated with sonic hedgehog signaling	Lung MSC, lung mesenchyme, fibroblasts	Mesenchyme, fibroblasts/ pericytes	33,186,254–257
NG2 (cspg4)	Neural/glial antigen 2, membrane proteoglycan	Differentiated pericytes	Differentiated pericytes Neural precursors	38,258
PDGFRβ	Tyrosine kinase receptor for PDGFB	SMC precursors	Fibroblasts, mesenchyme, differentiated pericytes, progenitors	183,259,260
SMA (acta2)	Conserved protein involved in cytoskeletal structure and integrity	Vascular SMC	Differentiated pericytes, smooth muscle,	179,251
Tbx4	T-box family Transcription factor	Developing lung mesenchyme (vascular precursors)	Smooth muscle, endothelium, fibroblasts, pericytes, vascular progenitors	40,173,261,262
Tbx18	T-box family Transcription factor	Differentiated pericytes	Differentiated pericytes, smooth muscle, glomerular mesangial cells	164,263

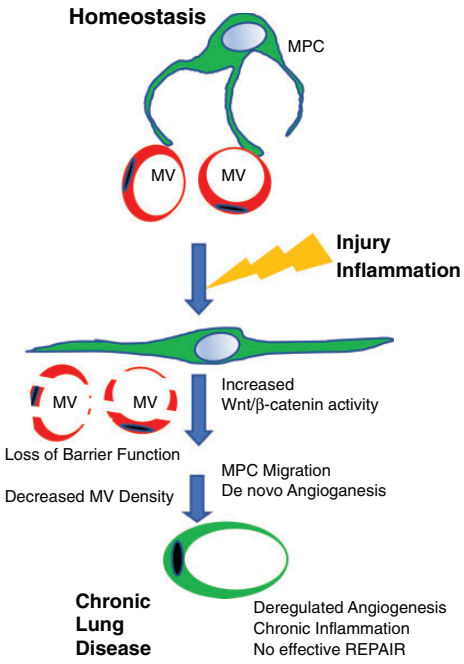

Fig. 1. Hypothetical model showing a role for MPC during the development of vasculopathy in disease. During tissue homeostasis, MPC participates in the regulation of capillary microvessels. Injury results in increased MPC β-catenin activity, and loss of MPC–MVEC interactions. MVEC respond with decreased barrier function. MV density decrease while MPC increase migration and abnormal angiogenesis. We speculate that prolonged vascular remodeling at the expense of repair results in CLD.

ABCG2 mesenchymal progenitor cells and angiogenesis

The validation of ABCG2 as a marker of adult mesenchymal progenitors enabled us to determine their relationship to pericytes as well as putative function using an inducible murine lineage tracing model system.[36,166,194] The selection of ABCG2 as a marker to define this lung mesenchymal progenitor population was based on historical evidence that the expression of this multi-drug resistance transporter selects for a "side population" of cells that demonstrate stem cell-like properties in adult tissues.[191,197–203] Side population selection was used to identify multipotent mesenchymal and vascular precursors in the lung.[194,204–211]

Transitioning from the so-called "side population" (SP) of cells to lineage tracing and expression of ABCG2 in adult lung facilitated lineage-tracing studies to elucidate MPC location and function.[36,194,195] ABCG2 MPC are a perivascular population of cells that share properties of differentiated NG2 pericytes and are more closely related to pericytes than fibroblasts.[36] However, MPC significantly differ from NG2 pericytes in functional properties including contraction.[36,194] We chose to evaluate naïve vs. canonical Wnt activated MPC[194] because Wnt/β-catenin signaling is biologically relevant, due to its association with tissue homeostasis and many adult pulmonary and vascular diseases.[36,212–218] While Wnt signaling in adult CLD has been well studied in terms of the epithelium, little is understood about its role in MPC regulation of the microvasculature and pathological angiogenesis.

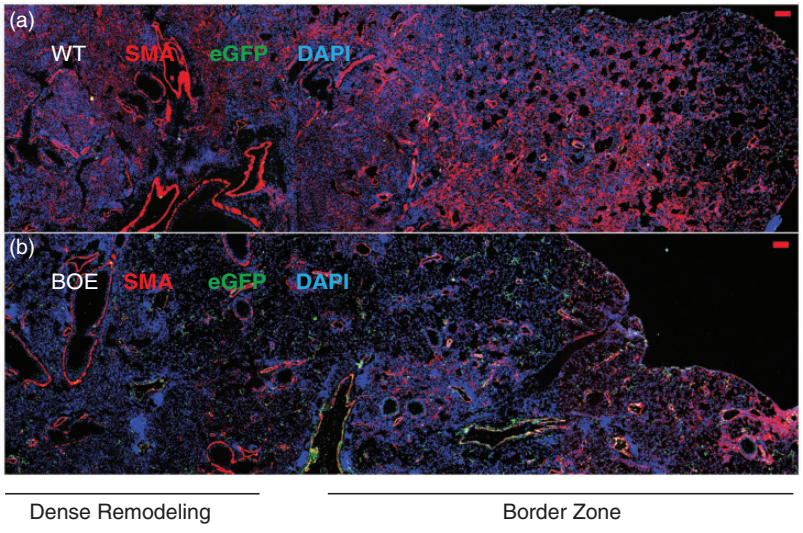

Fig. 2. Overview of remodeling and angiogenesis in bleomycin injured mouse lung tissue. β-catenin stabilization in MPC was achieved by engineering a conditional activator [β-catenin lacking degradation sites; Catnb$^{lox/p}$(ex3)], targeted to lung MPC using ABCG2CreERT2 with reporters:*Rosa26* $^{mtomato/mGFPlox\text{-}stop}$.[36,166,200,249,250] Mice were induced with intraperitoneal low dose tamoxifen (0.5 mg total).[194] Groups: All room air exposure; Control, or Wnt activated/β-catenin over-expressors (βOE). Two weeks following induction, 0.15 U of bleomycin or PBS vehicle was administered intratracheally and mouse lung tissue harvested on day 14 peak fibrosis and analyzed. Immunostaining was performed on lung tissue sections to localize smooth muscle alpha actin (SMA) and eGFP-labeled MPC lineage cells. SMA-labeled myofibroblasts in areas of remodeling as well as muscularized microvessels, airways and vasculature. (a) Representative WT or (b) β–catenin over-expressor (βOE) mouse lung tissue sections. DAPI was used to stain nuclei (blue). Scale bars = 100 μM.

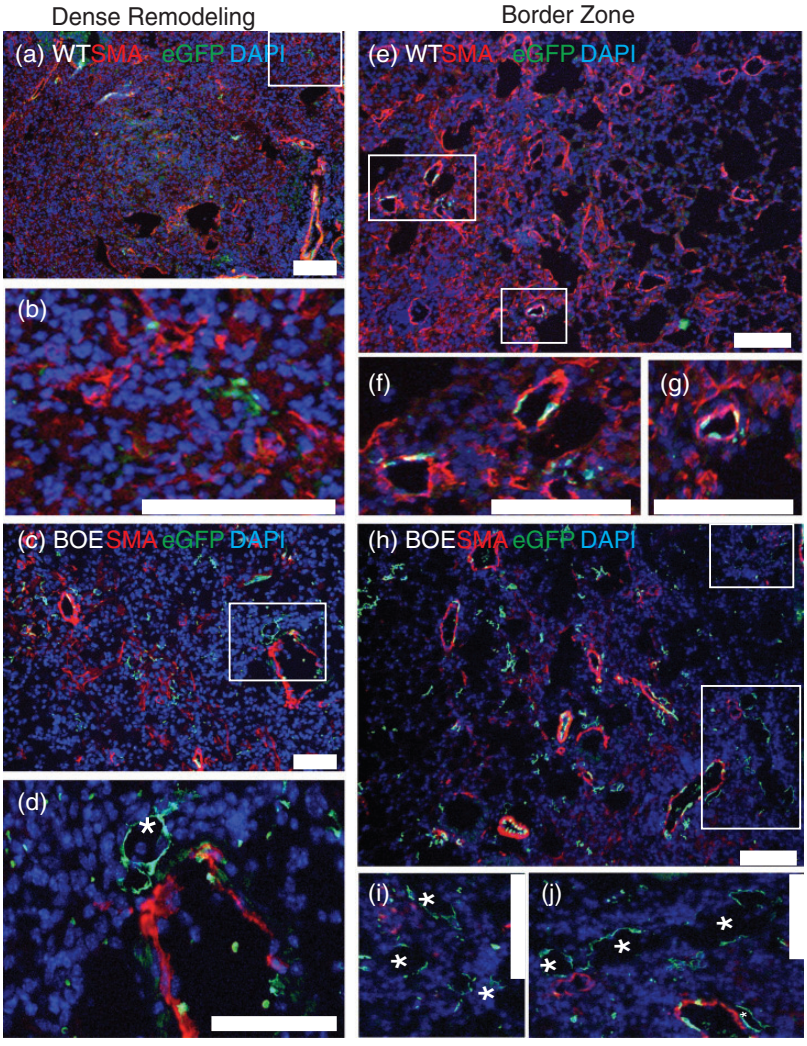

Fig. 3. MPC contribution to abnormal angiogenesis in bleomycin injured mouse lung tissue. Mice were induced with intraperitoneal low dose tamoxifen (0.5 mg total).[194] Two weeks following induction, 0.15 U of bleomycin or PBS vehicle was administered intratracheally and mouse lung tissue harvested on day 14 peak fibrosis and analyzed. Immunostaining was performed on lung tissue sections to localize smooth muscle alpha actin (SMA) and eGFP-labeled MPC lineage cells. Representative images were taken in the areas of (a–d) dense remodeling and (e–j) border zones outlined in Fig. 2. (a, b, e, g) WT or (c, d, h, j) β–catenin over-expressor (βOE) mouse lung tissue sections. DAPI was used to stain nuclei (blue). Scale bars = 100 μM.

β-catenin signaling regulates MSC/MPC cell specification, cell differentiation, renewal, proliferation, and angiogenesis.[4,166,194,213,219–235] We found that conditional genetic stabilization of β-catenin in ABCG2^{pos} MPC resulted in expansion of this progenitor pool in the lung. However, the MPC did not assume contractile function or expression of smooth muscle alpha actin, while microvessel expression of smooth muscle alpha actin decreased. Alteration of proper MPC function in the lung, via dysregulated Wnt/β-catenin signaling, facilitated the finding that MPC regulate lung microvascular homeostasis and function (Fig. 1).[194] Loss of MPC function resulted in a decreased microvascular density, contractility, and smooth muscle cell homoeostasis. In a murine model of bleomycin-induced fibrosis, wild-type ABCG2^{pos} MPC associated with both smooth muscle alpha actin or Factor VIII positive microvessels in alveolar tissue peripheral to the actively remodeling regions (Figs. 2 and 3).[194] However, in the lung tissue of Wnt activated MPC (termed βOE), we detected MPC contribution to atypical vascular structures in the fibrotic and peripheral areas of remodeling. These microvessels were atypical because they were devoid of both Factor VIII expressing endothelium and smooth muscle alpha actin (Fig. 3).[194] Of note, in response to bleomycin injury we detected migration of MPC, or derived cells, along the existing alveolar vasculature (arterioles, capillaries, and veins; Figs. 3 and 4).

Because lymphangiogenesis is deregulated in CLD, it is reasonable to hypothesize that MPC may also influence this process during injury and disease. We therefore analyzed the MPC lineage labeled cells and abnormal vascular structures for the expression of lyve-1.[4,43,45,49,51–53,58,158] Lyve-1 is a

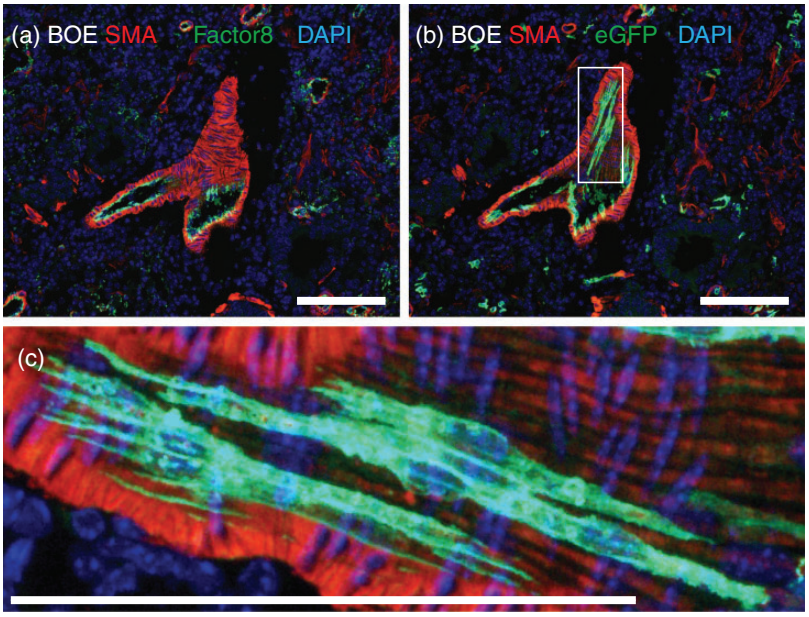

Fig. 4. Enhanced intravascular migration of βOE MPC following bleomycin injury. Immunostaining was performed on day 14 bleomycin injured β–catenin over-expressor (βOE) mouse lung tissue sections to localize smooth muscle alpha actin (SMA) expressing vasculature (a–c), Factor VIII positive endothelium (a) and eGFP-labeled MPC lineage cells (a–c). DAPI was used to stain nuclei (blue). Scale bars = 100 μM.

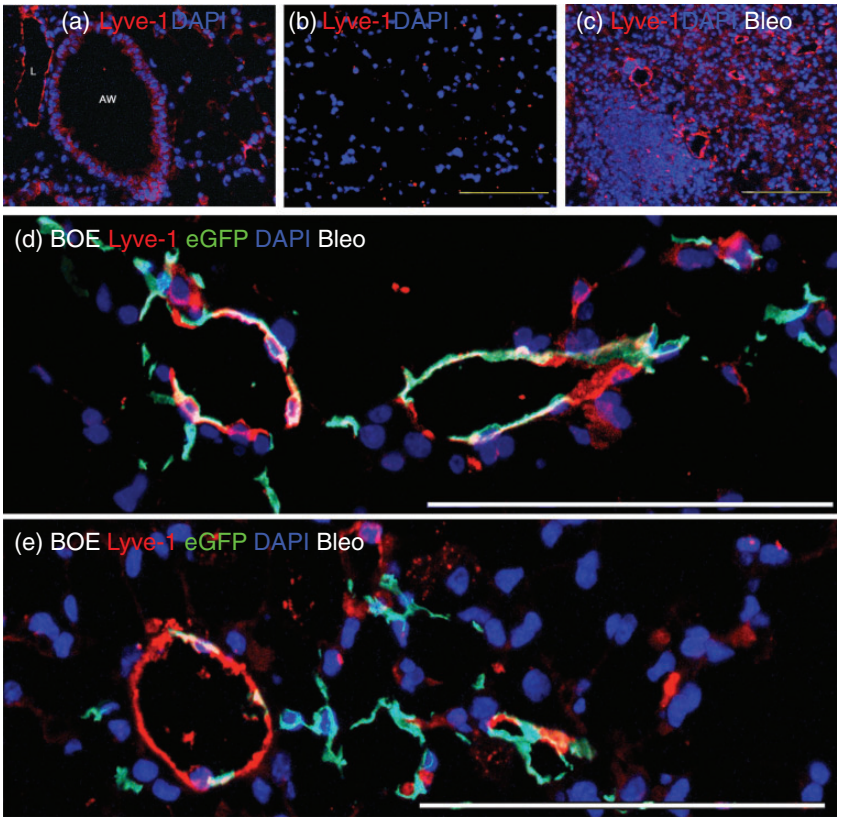

Fig. 5. MPC contribute to de novo lymphangiogenesis. Immunostaining was performed on day 14 bleomycin vehicle control (a, b) or injured (c–e) β–catenin over-expressor (βOE) mouse lung tissue sections to localize smooth muscle alpha actin (SMA) expressing vasculature, lyve-1 expressing cells/lymphatics and eGFP-labeled MPC lineage cells. DAPI was used to stain nuclei (blue). Scale bars = 100 μM.

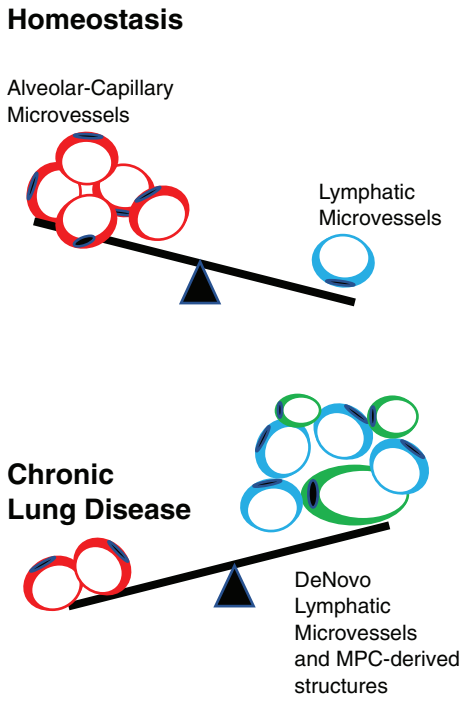

Fig. 6. Balance of capillary and lymphangiogenesis: hypothetical model of vasculopathy in CLD. Abnormal MPC vascular structures = green.

receptor for hyaluronan, necessary for cell migration and metastases as well as a typical marker for lymphangiogenesis and existing lymphatic vasculature (Fig. 5a–c).[44,52,53,74,192,236] However, lyve-1 expression is not limited to lymphatic endothelium.[56] We found that the MPC lineage and the de novo vascular structures exhibited heterogeneous expression of lyve-1 (Fig. 5d and e). Heterogeneous expression of lyve-1 may be due to stage of vessel formation and cell (MPC or endothelial) migration.[43,49–53] We therefore speculate that during disease MPC contribute to abnormal angiogenesis that contributes to the imbalance of capillary and lymphatic microvasculature (Fig. 6).

Summary

Conclusions

Capillary and lymphangiogenesis are deregulated in both IPF and COPD, although the mechanisms by which they co-regulate and underlie early pathogenesis of disease are unknown. We have shown that that ABCG2^{pos} MPC influence both the capillary microvasculature and lymphatic angiogenesis. A balance of both is required for normal tissue homeostasis and repair. Our current models suggest that when lymph and capillary angiogenesis are out of balance, the non-equivalence appears to support the progression of disease and tissue remodeling. The angiogenic regulatory mechanisms underlying both COPD/emphysema, IPF, and SSc likely impact additional CLD including other interstitial lung diseases, tuberous sclerosis (TSC), and lymphangioleiomyomatosis.

Future directions

Ongoing studies are designed to elucidate the mechanisms by which MPC influence capillary and lymphangiogenesis as well as understanding the balance between them in normal lung tissue, following injury, and during repair. The MPC niche and environment regulates their function which in turn impacts the vascular microenvironment (Fig. 1). This is a seemingly complex interaction because the niche involves endothelium, epithelium, vascular smooth muscle, extracellular matrix, and environmental influences.

Cigarette smoke is a major risk factor for CLD including COPD/emphysema and IPF, and is therefore a reasonable candidate factor to deregulate the MPC niche. Both cigarette smoke and hypoxia upregulate the expression of ABCG2.[237–239] Additionally, the MPC express the hyaluronan receptors CD44 and lyve-1 which function to regulate cell migration, Wnt, mTOR, and VEGF signaling as well as phenotype and function.[68,192,240–243] Hyaluronan is cleaved into high and low molecular weight fragments differentially during disease and has been characterized as altered in COPD and IPF.[46,56,68,240,241,244–246] These receptors cluster with ABCG2 at the cell surface[247] and likely regulate cell-specific niches and their responses to the microenvironments, including proliferation, apoptosis, response to oxidant stress, and metabolism.[247,248]

A fundamental question that remains is how MPC reprogrammaing disrupts capillary and lymphatic networks to hinder the physiologic function of the lung. Establishing these mechanisms would provide key new insights into the pathogeneis of lung disease. Therefore, it is essential to examine these aspects of the MPC niche, including ABCG2 regulation of cell signaling, proliferation, and migration, using murine models of emphysema and fibrosis as well as isolated human MPC. The ultimate goal of these studies is to identify novel therapeutic targets to restore MPC and vascular function and promote pulmonary tissue repair.

Acknowledgments

The authors thank Dr. M.M. Taketo for providing the $^{fl}\Delta$Ex3 *catnb* mice.

Conflict of interest

The author(s) declare that there is no conflict of interest.

Funding

This work was funded by grants to S.M. Majka from the NIH/NHLBI R01HL116597. Additional funding was provided by NIH/NHLBI K08HL130595 and the Francis Family Foundation to J.A. Kropski.

2017 Grover Conference Series

This review article is part of the 2017 Grover Conference Series. The American Thoracic Society and the conference organizing committee gratefully acknowledge the educational grants provided for the support of this conference by Actelion Pharmaceuticals US, Inc., Gilead Sciences, Inc., and United Therapeutics Corporation. Additionally, the American Thoracic Society is grateful for the support of the Grover Conference by the American Heart Association, the Cardiovascular Medical Research and Education Fund, and the National Institutes of Health.

References

1. deMello DE and Reid LM. Embryonic and early fetal development of human lung vasculature and its functional implications. *Pediatr Dev Pathol* 2000; 3(5): 439–449.
2. Almeida FM, Saraiva-Romanholo BM, Vieira RP, et al. Compensatory lung growth after bilobectomy in emphysematous rats. *PLoS ONE* 2017; 12(7): e0181819.
3. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2000; 6(4): 389–395.
4. Carmeliet P and Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature* 2011; 473(7347): 298–307.
5. Shalaby F, Rossant J, Yamaguchi TP, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 1995; 376(6535): 62–66.
6. Zeng X, Wert SE, Federici R, et al. VEGF enhances pulmonary vasculogenesis and disrupts lung morphogenesis in vivo. *Dev Dyn* 1998; 211(3): 215–227.
7. Hislop A. Developmental biology of the pulmonary circulation. *Paediatr Respir Rev* 2005; 6(1): 35–43.
8. Hislop AA. Airway and blood vessel interaction during lung development. *J Anat* 2002; 201(4): 325–334.
9. Hamer J. The human bronchial circulation in health and disease. *Proc R Soc Med* 1970; 63(1): 105.
10. Nguyen-Kim TDL, Frauenfelder T, Strobel K, et al. Assessment of bronchial and pulmonary blood supply in non-small cell lung cancer subtypes using computed tomography perfusion. *Invest Radiol* 2015; 50(3): 179–186.
11. Horvath G and Wanner A. Bronchial arterial circulation in the human. In: Yuan JXJ, Garcia JGN, West JB, et al. (eds) *Textbook of Pulmonary Vascular Disease*. Boston, MA: Springer US, 2011, pp.439–450.
12. Lee CG, Link H, Baluk P, et al. Vascular endothelial growth factor (VEGF) induces remodeling and enhances T(H)2-mediated sensitization and inflammation in the lung. *Nat Med* 2004; 10(10): 1095–1103.
13. Doherty T and Broide D. Cytokines and growth factors in airway remodeling in asthma. *Curr Opin Immunol* 2007; 19(6): 676–680.
14. Hoshino M, Takahashi M and Aoike N. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol* 2001; 107(2): 295–301.
15. Salvato G. Quantitative and morphological analysis of the vascular bed in bronchial biopsy specimens from asthmatic and non-asthmatic subjects. *Thorax* 2001; 56: 902–906.
16. Kotoulas C, Panagiotou I, Tsipas P, et al. Experimental studies in the bronchial circulation Which is the ideal animal model? *J Thoracic Dis* 2014; 6(10): 1506–1512.
17. Calabrese C, Bocchino V, Vatrella A, et al. Evidence of angiogenesis in bronchial biopsies of smokers with and without airway obstruction. *Respir Med* 2006; 100(8): 1415–1422.
18. Hiroshima K, Iyoda A, Shibuya K, et al. Evidence of neoangiogenesis and an increase in the number of proliferating cells within the bronchial epithelium of smokers. *Cancer* 2002; 95(7): 1539–1545.
19. Hashimoto M, Tanaka H and Abe S. Quantitative analysis of bronchial wall vascularity in the medium and small airways of patients with asthma and COPD. *Chest* 2005; 127(3): 965–972.
20. Zanini A, Chetta A, Imperatori AS, et al. The role of the bronchial microvasculature in the airway remodelling in asthma and COPD. *Respir Res* 2010; 11: 132.
21. Paredi P and Barnes PJ. The airway vasculature: recent advances and clinical implications. *Thorax* 2009; 64(5): 444.
22. Chetta A, Zanini A, Torre O, et al. Vascular remodelling and angiogenesis in asthma: morphological aspects and pharmacological modulation. *Inflamm Allergy Drug Targets* 2007; 6: 41–45.
23. Zanini A, Chetta A, Saetta M, et al. Chymase-positive mast cells play a role in the vascular component of airway remodelling in asthma. *J Allergy Clin Immunol* 2007; 120: 329–333.
24. Peão MND, Águas AP, de Sá CM, et al. Neoformation of blood vessels in association with rat lung fibrosis induced by bleomycin. *Anat Rec* 1994; 238(1): 57–67.
25. Mlika M, Bacha S, Braham E, et al. The inter-connection between fibrosis and microvascular remodeling in idiopathic pulmonary fibrosis: Reality or just a phenomenon. *Respir Med Case Rep* 2016; 17: 30–33.
26. Cudkowicz L and Armstrong JB. The bronchial arteries in pulmonary emphysema. *Thorax* 1953; 8(1): 46–58.
27. Reid JA and Heard BE. The capillary network of normal and emphysematous human lungs studied by injections of Indian ink. *Thorax* 1963; 18(3): 201–212.
28. Townsley MI. Structure and composition of pulmonary arteries, capillaries and veins. *Compr Physiol* 2012; 2: 675–709.
29. Austin ED, Kawut SM, Gladwin MT, et al. Pulmonary hypertension: NHLBI Workshop on the Primary Prevention of Chronic Lung Diseases. *Ann Am Thorac Soc* 2014; 11(Suppl. 3): S178–S85.
30. Barratt S and Millar A. Vascular remodelling in the pathogenesis of idiopathic pulmonary fibrosis. *QJM* 2014; 107(7): 515–519.
31. Hanumegowda C, Farkas L and Kolb M. Angiogenesis in pulmonary fibrosis: Too much or not enough? *Chest* 2012; 142(1): 200–207.
32. Keane MP. Angiogenesis and pulmonary fibrosis. *Am J Respir Crit Care Med* 2004; 170(3): 207–209.
33. Birbrair A, Zhang T, Files D, et al. Type-1 pericytes accumulate after tissue injury and produce collagen in an organ-dependent manner. *Stem Cell Res Ther* 2014; 5(6): 122.
34. Duffield JS. The elusive source of myofibroblasts: problem solved? *Nat Med* 2012; 18(8): 1178–1180.
35. Hung C, Linn G, Chow Y-H, et al. Role of lung pericytes and resident fibroblasts in the pathogenesis of pulmonary fibrosis. *Am J Respir Crit Care Med* 2013; 188(7): 820–830.
36. Marriott S, Baskir RS, Gaskill C, et al. ABCG2(pos) lung mesenchymal stem cells are a novel pericyte subpopulation

that contributes to fibrotic remodeling. *Am J Physiol* 2014; 307(8): C684–C698.
37. Noble PW, Barkauskas CE and Jiang D. Pulmonary fibrosis: patterns and perpetrators. *J Clin Invest* 2012; 122(8): 2756–2762.
38. Rock JR, Barkauskas CE, Cronce MJ, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci* 2011; 108(52): 1475–1483.
39. Steinhauser ML and Lee RT. Pericyte progenitors at the crossroads between fibrosis and regeneration. *Circ Res* 2013; 112(2): 230–232.
40. Xie T, Liang J, Liu N, et al. Transcription factor TBX4 regulates myofibroblast accumulation and lung fibrosis. *J Clin Invest* 2016; 126(8): 3063–3079.
41. Voelkel NF, Douglas IS and Nicolls M. Angiogenesis in chronic lung disease. *Chest* 2007; 131(3): 874–879.
42. Scavelli C, Weber E, Agliano M, et al. Lymphatics at the crossroads of angiogenesis and lymphangiogenesis. *J Anat* 2004; 204(6): 433–449.
43. Adams RH and Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. *Nat Rev Mol Cell Biol* 2007; 8(6): 464–478.
44. Avraamides CJ, Garmy-Susini B and Varner JA. Integrins in angiogenesis and lymphangiogenesis. *Nat Rev Cancer* 2008; 8(8): 604–617.
45. El-Chemaly S, Malide D, Zudaire E, et al. Abnormal lymphangiogenesis in idiopathic pulmonary fibrosis with insights into cellular and molecular mechanisms. *Proc Natl Acad Sci* 2009; 106(10): 3958–3963.
46. Hardavella G, Tzortzaki EG, Siozopoulou V, et al. Lymphangiogenesis in COPD: Another link in the pathogenesis of the disease. *Respir Med* 2012; 106(5): 687–693.
47. Pepper MS, Tille JC, Nisato R, et al. Lymphangiogenesis and tumor metastasis. *Cell Tissue Res* 2003; 314: 167–177.
48. Mori M, Andersson CK, Svedberg KA, et al. Appearance of remodelled and dendritic cell-rich alveolar-lymphoid interfaces provides a structural basis for increased alveolar antigen uptake in chronic obstructive pulmonary disease. *Thorax* 2013; 68: 521–531.
49. Yamashita M, Iwama N, Date F, et al. Characterization of lymphangiogenesis in various stages of idiopathic diffuse alveolar damage. *Hum Pathol* 2009; 40: 542–551.
50. Bianchi A, Painter KJ and Sherratt JA. Spatio-temporal models of lymphangiogenesis in wound healing. *Bull Math Biol* 2016; 78(9): 1904–1941.
51. Baluk P and McDonald DM. Markers for microscopic imaging of lymphangiogenesis and angiogenesis. *Ann N Y Acad Sci* 2008; 1131: 1–12.
52. Buttler K, Kreysing A, von Kaisenberg CS, et al. Mesenchymal cells with leukocyte and lymphendothelial characteristics in murine embryos. *Dev Dyn* 2006; 235(6): 1554–1562.
53. Conrad C, Niess H, Huss R, et al. Multipotent mesenchymal stem cells acquire a lymphendothelial phenotype and enhance lymphatic regeneration in vivo. *Circulation* 2009; 119(2): 281–289.
54. Akeson AL, Wetzel B, Thompson FY, et al. Embryonic vasculogenesis by endothelial precursor cells derived from lung mesenchyme. *Dev Dyn* 2000; 217(1): 11–23.
55. Gaskill C and Majka SM. A high-yield isolation and enrichment strategy for human lung microvascular endothelial cells. *Pulm Circ* 2017; 7(1): 108–116.
56. Gordon EJ, Gale NW and Harvey NL. Expression of the hyaluronan receptor LYVE-1 is not restricted to the lymphatic vasculature; LYVE-1 is also expressed on embryonic blood vessels. *Dev Dyn* 2008; 237(7): 1901–1909.
57. Lohela M, Bry M, Tammela T, et al. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. *Curr Opin Cell Biol* 2009; 21(2): 154–165.
58. Yamashita M. Lymphangiogenesis and lesion heterogeneity in interstitial lung diseases. *Clin Med Insights Circ Respir Pulm Med* 2015; 9(Suppl. 1): 111–121.
59. Cursiefen C, Chen L, Borges LP, et al. VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. *J Clin Invest* 2004; 113: 1040–1050.
60. Kumasaka T, Seyama K, Mitani K, et al. Lymphangiogenesis in lymphangioleiomyomatosis: its implication in the progression of lymphangioleiomyomatosis. *Am J Surg Pathol* 2004; 28: 1007–1016.
61. Condliffe R and Howard LS. Connective tissue disease-associated pulmonary arterial hypertension. *F1000Prime Rep* 2015; 7: 06.
62. Hirigoyen D, Burgos PI, Mezzano V, et al. Inhibition of angiogenesis by platelets in systemic sclerosis patients. *Arthritis Res Ther* 2015; 17: 332.
63. Hopkins N and McLoughlin P. The structural basis of pulmonary hypertension in chronic lung disease: remodelling, rarefaction or angiogenesis? *J Anat* 2002; 201(4): 335–348.
64. Howell K, Preston RJ and McLoughlin P. Chronic hypoxia causes angiogenesis in addition to remodelling in the adult rat pulmonary circulation. *J Physiol* 2003; 547(Pt 1): 133–145.
65. Manetti M, Guiducci S, Ibba-Manneschi L, et al. Impaired angiogenesis in systemic sclerosis: the emerging role of the antiangiogenic VEGF165b splice variant. *Trends Cardiovasc Med* 2011; 21(7): 204–210.
66. Matarese A and Santulli G. Angiogenesis in chronic obstructive pulmonary disease: a translational appraisal. *Transl Med UniSa* 2012; 3: 49–56.
67. Siafakas NM, Antoniou KM and Tzortzaki EG. Role of angiogenesis and vascular remodeling in chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2007; 2(4): 453–462.
68. Slevin M, Krupinski J, Gaffney J, et al. Hyaluronan-mediated angiogenesis in vascular disease: Uncovering RHAMM and CD44 receptor signaling pathways. *Matrix Biol* 2007; 26(1): 58–68.
69. Tuder RM, Chacon M, Alger L, et al. Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. *J Pathol* 2001; 195(3): 367–374.
70. Tzouvelekis A, Anevlavis S and Bouros D. Angiogenesis in interstitial lung diseases: a pathogenetic hallmark or a bystander? *Respir Res* 2006; 7(1): 82.
71. Tahergorabi Z and Khazaei M. Imbalance of angiogenesis in diabetic complications: the mechanisms. *Int J Prev Med* 2012; 3(12): 827–838.
72. El-Chemaly S, Levine SJ and Moss J. Lymphatics in lung disease. *Ann N Y Acad Sci* 2008; 1131: 195–202.

73. Armulik A, Genov G and Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell* 2011; 21(2): 193–215.
74. Karunamuni G, Yang K, Doughman YQ, et al. Expression of lymphatic markers during avian and mouse cardiogenesis. *Anat Rec (Hoboken)* 2010; 293(2): 259–270.
75. Ozerdem U, Monosov E and Stallcup WB. NG2 proteoglycan expression by pericytes in pathological microvasculature. *Microvasc Res* 2002; 63(1): 129–134.
76. Ozerdem U and Stallcup WB. Early contribution of pericytes to angiogenic sprouting and tube formation. *Angiogenesis* 2003; 6(3): 241–249.
77. Folberg R, Hendrix MJC and Maniotis AJ. Vasculogenic mimicry and tumor angiogenesis. *Am J Pathol* 2000; 156(2): 361–381.
78. Chang YS, di Tomaso E, McDonald DM, et al. Mosaic blood vessels in tumors: Frequency of cancer cells in contact with flowing blood. *Proc Natl Acad Sci* 2000; 97(26): 14608–14613.
79. Turner-Warwick M. Precapillary systemic-pulmonary anastomoses. *Thorax* 1963; 18(3): 225–237.
80. Distler JHW, Gay S and Distler O. Angiogenesis and vasculogenesis in systemic sclerosis. *Rheumatology* 2006; 45(Suppl. 3): iii26–iii27.
81. Konttinen YT, Mackiewicz Z, Ruuttila P, et al. Vascular damage and lack of angiogenesis in systemic sclerosis skin. *Clin Rheumatol* 2003; 22(3): 196–202.
82. Manetti M, Guiducci S and Matucci-Cerinic M. The crowded crossroad to angiogenesis in systemic sclerosis: where is the key to the problem? *Arthritis Res Ther* 2016; 18: 36.
83. Strieter RM, Belperio JA and Keane MP. CXC chemokines in angiogenesis related to pulmonary fibrosis. *Chest* 2002; 122(Suppl. 6): 298S–301S.
84. Farkas L and Kolb M. Pulmonary microcirculation in interstitial lung disease. *Proc Am Thorac Soc* 2011; 8(6): 516–521.
85. Cipriani P, Di Benedetto P, Ruscitti P, et al. Impaired endothelium-mesenchymal stem cells cross-talk in systemic sclerosis: a link between vascular and fibrotic features. *Arthritis Res Ther* 2014; 16(5): 442.
86. Farkas L, Farkas D, Ask K, et al. VEGF ameliorates pulmonary hypertension through inhibition of endothelial apoptosis in experimental lung fibrosis in rats. *J Clin Invest* 2009; 119(5): 1298–1311.
87. Fernandez IE and Eickelberg O. New cellular and molecular mechanisms of lung injury and fibrosis in idiopathic pulmonary fibrosis. *Lancet* 2012; 380(9842): 680–688.
88. Ebina M, Shimizukawa M, Shibata N, et al. Heterogeneous increase in CD34-positive alveolar capillaries in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2004; 169(11): 1203–1208.
89. Gracey DR, Divertie MB and Brown AL. Alveolar-capillary membrane in idiopathic interstitial pulmonary fibrosis. *Am Rev Respir Dis* 1968; 98(1): 16–21.
90. Renzoni EA. Neovascularization in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2004; 169(11): 1179–1180.
91. Kim CFB, Jackson EL, Woolfenden AE, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005; 121(6): 823–835.
92. Lara AR, Cosgrove GP, Janssen WJ, et al. Increased lymphatic vessel length is associated with the fibroblast reticulum and disease severity in usual interstitial pneumonia and nonspecific interstitial pneumonia. *Chest* 2012; 142(6): 1569–1576.
93. Mandal RV, Mark EJ and Kradin RL. Organizing pneumonia and pulmonary lymphatic architecture in diffuse alveolar damage. *Hum Pathol* 2008; 39(8): 1234–1238.
94. Ebina M, Shibata N, Ohta H, et al. The disappearance of subpleural and interlobular lymphatics in idiopathic pulmonary fibrosis. *Lymphat Res Biol* 2010; 10(4): 199–207.
95. Ebina M. Remodeling of airway walls in fatal asthmatics decreases lymphatic distribution; beyond thickening of airway smooth muscle layers. *Allergol Int* 2008; 57: 165–174.
96. Oka M, Iwata C, Suzuki HI, et al. Inhibition of endogenous TGF-β signaling enhances lymphangiogenesis. *Blood* 2008; 111(9): 4571–4579.
97. Tammela T and Alitalo K. Lymphangiogenesis: molecular mechanisms and future promise. *Cell* 2010; 140(4): 460–476.
98. Meinecke A-K, Nagy N, Lago GD, et al. Aberrant mural cell recruitment to lymphatic vessels and impaired lymphatic drainage in a murine model of pulmonary fibrosis. *Blood* 2012; 119(24): 5931–5942.
99. Wollin L, Wex E, Pautsch A, et al. Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. *Eur Respir J* 2015; 45: 1434–1445.
100. Snider GL, Kleinerman J, Thurlbeck WM, et al. The definition of emphysema. *Am Rev Respir Dis* 1985; 132(1): 182–185.
101. Fletcher CH. Terminology in chronic obstructive lung diseases. *J Epidemiol Community Health* 1978; 32(4): 282–288.
102. Murray CJL and Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* 1997; 349(9061): 1269–1276.
103. Global Initiative for Chronic Obstructive Lung Disease (GOLD). *Global Strategy for the Diagnosis, Management and Prevention of COPD*. 2017. Available at: http://gold-copd.org/.
104. Sabit R, Bolton CE, Edwards PH, et al. Arterial stiffness and osteoporosis in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007; 175(12): 1259–1265.
105. McAllister DA, Maclay JD, Mills NL, et al. Arterial stiffness is independently associated with emphysema severity in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007; 176(12): 1208–1214.
106. Alford SK, van Beek EJR, McLennan G, et al. Heterogeneity of pulmonary perfusion as a mechanistic image-based phenotype in emphysema susceptible smokers. *Proc Natl Acad Sci* 2010; 107(16): 7485–7490.
107. Yu N, Wei X, Li Y, et al. Computed tomography quantification of pulmonary vessels in chronic obstructive pulmonary disease as identified by 3D automated approach. *Medicine (Baltimore)* 2016; 95(40): e5095.
108. Liebow AA. Pulmonary emphysema with special reference to vascular changes. *Am Rev Respir Dis* 1959; 80(1P2): 67–93.
109. Wiebe BM and Laursen H. Lung morphometry by unbiased methods in emphysema: bronchial and blood vessel volume, alveolar surface area and capillary length. *APMIS* 1998; 106(1–6): 651–656.
110. Barr RG, Mesia-Vela S, Austin JHM, et al. Impaired flow-mediated dilation is associated with low pulmonary function and emphysema in ex-smokers: the Emphysema and Cancer Action Project (EMCAP) Study. *Am J Respir Crit Care Med* 2007; 176(12): 1200–1207.
111. Serban KA and Petrache I. Alpha-1 antitrypsin and lung cell apoptosis. *Ann Am Thorac Soc* 2016; 13(Suppl. 2): S146–S149.

112. Petrache I, Fijalkowska I, Medler TR, et al. a-1 antitrypsin inhibits caspase-3 activity, preventing lung endothelial cell apoptosis. *Am J Pathol* 2006; 169(4): 1155–1166.
113. Shaw JG, Vaughan A, Dent AG, et al. Biomarkers of progression of chronic obstructive pulmonary disease (COPD). *J Thorac Dis* 2014; 6(11): 1532–1547.
114. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, et al. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest* 2000; 106(11): 1311–1319.
115. Fairclough L, Urbanowicz RA, Corne J, et al. Killer cells in chronic obstructive pulmonary disease. *Clin Sci (Lond)* 2008; 114(8): 533–541.
116. Lin H and Jiang S. Combined pulmonary fibrosis and emphysema (CPFE): an entity different from emphysema or pulmonary fibrosis alone. *J Thorac Dis* 2015; 7(4): 767–779.
117. Leask A. When there is smoke there is scleroderma: evidence that patients with scleroderma should stop smoking. *J Cell Commun Signal* 2011; 5(1): 67–68.
118. Yanbaeva DG, Dentener MA, Creutzberg EC, et al. Systemic effects of smoking. *Chest* 2007; 131(5): 1557–1566.
119. Oh CK, Murray LA and Molfino NA. Smoking and idiopathic pulmonary fibrosis. *Pulm Med* 2012; 2012: 808260.
120. Hudson M, Lo E, Lu Y, et al. Cigarette smoking in patients with systemic sclerosis. *Arthritis Rheum* 2011; 63(1): 230–238.
121. Cottin V, Nunes H, Mouthon L, et al. Combined pulmonary fibrosis and emphysema syndrome in connective tissue disease. *Arthritis Rheum* 2011; 63(1): 295–304.
122. Finlay GA, O'Donnell MD, O'Connor CM, et al. Elastin and collagen remodeling in emphysema. A scanning electron microscopy study. *Am J Pathol* 1996; 149(4): 1405–1415.
123. Paré PD and Mitzner W. Airway-parenchymal interdependence. *Compr Physiol* 2012; 2(3): 1921–1935.
124. Suki B and Parameswaran H. Computational modeling helps uncover mechanisms related to the progression of emphysema. *Drug Discov Today Dis Models* 2014; 70(27–28): 4245–4249.
125. Bringardner BD, Baran CP, Eubank TD, et al. The role of inflammation in the pathogenesis of idiopathic pulmonary fibrosis. *Antioxid Redox Signal* 2008; 10(2): 287–301.
126. Gauldie J. Inflammatory mechanisms are a minor component of the pathogenesis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2002; 165(9): 1205–1206.
127. Juarez MM, Chan AL, Norris AG, et al. Acute exacerbation of idiopathic pulmonary fibrosis—a review of current and novel pharmacotherapies. *J Thorac Dis* 2015; 7(3): 499–519.
128. Baran CP, Opalek JM, McMaken S, et al. Important roles for macrophage colony-stimulating factor, CC chemokine ligand 2, and mononuclear phagocytes in the pathogenesis of pulmonary fibrosis. *Am J Respir Crit Care Med* 2007; 176(1): 78–89.
129. Antunes RdS, Madge L, Soroosh P, et al. The TNF family molecules LIGHT and lymphotoxin αβ induce a distinct steroid-resistant inflammatory phenotype in human lung epithelial cells. *J Immunol* 2015; 195(5): 2429–2441.
130. François A, Gombault A, Villeret B, et al. B cell activating factor is central to bleomycin- and IL-17-mediated experimental pulmonary fibrosis. *J Autoimmun* 2015; 56: 1–11.
131. Feghali-Bostwick CA and Wilkes DS. Autoimmunity in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2011; 183(6): 692–693.
132. Lee JS, Kim EJ, Lynch KL, et al. Prevalence and clinical significance of circulating autoantibodies in idiopathic pulmonary fibrosis. *Respir Med* 2013; 107(2): 249–255.
133. Magro CM, Waldman WJ, Knight DA, et al. Idiopathic pulmonary fibrosis related to endothelial injury and antiendothelial cell antibodies. *Hum Immunol* 2006; 67(4): 284–297.
134. Santos S, Marin A, Serra-Batlles J, et al. Treatment of patients with COPD and recurrent exacerbations: the role of infection and inflammation. *Int J Chron Obstruct Pulmon Dis* 2016; 11: 515–525.
135. Caramori G, Casolari P, Barczyk A, et al. COPD immunopathology. *Semin Immunopathol* 2016; 38(4): 497–515.
136. Karayama M, Inui N, Suda T, et al. Antiendothelial cell antibodies in patients with COPD. *Chest* 2010; 138(6): 1303–1308.
137. Taraseviciene-Stewart L, Douglas IS, Nana-Sinkam PS, et al. Is alveolar destruction and emphysema in chronic obstructive pulmonary disease an immune disease? *Proc Am Thorac Soc* 2006; 3(8): 687–690.
138. Kusko RL, Brothers JF, Tedrow J, et al. Integrated genomics reveals convergent transcriptomic networks underlying chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2016; 194(8): 948–960.
139. Galvin I, Drummond GB and Nirmalan M. Distribution of blood flow and ventilation in the lung: gravity is not the only factor. *Br J Anaesth* 2007; 98(4): 420–428.
140. Lehmann M, Baarsma HA and Königshoff M. WNT signaling in lung aging and disease. *Ann Am Thorac Soc* 2016; 13(Suppl. 5): S411–S416.
141. Kotch LE, Iyer NV, Laughner E, et al. Defective vascularization of HIF-1α-null embryos is not associated with VEGF deficiency but with mesenchymal cell death. *Dev Biol* 1999; 209(2): 254–267.
142. Ylikorkala A, Rossi DJ, Korsisaari N, et al. Vascular abnormalities and deregulation of VEGF in Lkb1-deficient mice. *Science* 2001; 293(5533): 1323–1326.
143. Majka S, Fox K, McGuire B, et al. Pleiotropic role of VEGF-A in regulating fetal pulmonary mesenchymal cell turnover. *Am J Physiol Lung Cell Mol Physiol* 2006; 290(6): L1183–L1192.
144. Suzuki M, Betsuyaku T, Nagai K, et al. Decreased airway expression of vascular endothelial growth factor in cigarette smoke-induced emphysema in mice and COPD patients. *Inhal Toxicol* 2008; 20(3): 349–359.
145. Papaioannou AI, Kostikas K, Kollia P, et al. Clinical implications for vascular endothelial growth factor in the lung: friend or foe? *Respir Res* 2006; 7(1): 128.
146. Marwick JA, Stevenson CS, Giddings J, et al. Cigarette smoke disrupts VEGF165-VEGFR-2 receptor signaling complex in rat lungs and patients with COPD: morphological impact of VEGFR-2 inhibition. *Am J Physiol Lung Cell Mol Physiol* 2006; 290(5): L897.
147. Alder JK, Guo N, Kembou F, et al. Telomere length is a determinant of emphysema susceptibility. *Am J Respir Crit Care Med* 2011; 184(8): 904–912.
148. Demura Y, Taraseviciene-Stewart L, Scerbavicius R, et al. N-acetylcysteine treatment protects against VEGF-receptor blockade-related emphysema. *COPD* 2004; 1(1): 25–32.

149. Golpon HA, Coldren CD, Zamora MR, et al. Emphysema lung tissue gene expression profiling. *Am J Respir Cell Mol Biol* 2004; 31(6): 595–600.
150. Tuder RM, Yoshida T, Fijalkowka I, et al. Role of lung maintenance program in the heterogeneity of lung destruction in emphysema. *Proc Am Thorac Soc* 2006; 3(8): 673–679.
151. Tuder RM, Zhen L, Cho CY, et al. Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol* 2003; 29(1): 88–97.
152. Tang K, Rossiter HB, Wagner PD, et al. Lung-targeted VEGF inactivation leads to an emphysema phenotype in mice. *J Appl Physiol* 2004; 97(4): 1559–1566.
153. Suzuki S, Yoshihisa A, Yokokawa T, et al. Association between levels of anti-angiogenic isoform of vascular endothelial growth factor A and pulmonary hypertension. *Int J Cardiol* 2016; 222: 416–420.
154. Manetti M, Guiducci S, Romano E, et al. Overexpression of VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, leads to insufficient angiogenesis in patients with systemic sclerosis. *Circ Res* 2011; 109(3): e14.
155. Kikuchi R, Nakamura K, MacLauchlan S, et al. An antiangiogenic isoform of VEGF-A contributes to impaired vascularization in peripheral artery disease. *Nat Med* 2014; 20(12): 1464–1471.
156. Hoshino M, Nakamura Y and Hamid QA. Gene expression of vascular endothelial growth factor and its receptors and angiogenesis in bronchial asthma. *J Allergy Clin Immunol* 2001; 107(6): 1034–1038.
157. Pio R and Montuenga LM. Alternative splicing in lung cancer. *J Thorac Oncol* 2009; 4(6): 674–678.
158. Maertens L, Erpicum C, Detry B, et al. Bone marrow-derived mesenchymal stem cells drive lymphangiogenesis. *PLoS ONE* 2014; 9(9): e106976.
159. Yan A, Avraham T, Zampell JC, et al. Adipose-derived stem cells promote lymphangiogenesis in response to VEGF-C stimulation or TGF-β1 inhibition. *Future Oncol* 2011; 7(12): 1457–1473.
160. Crocker DJ, Murad TM and Geer JC. Role of the pericyte in wound healing: An ultrastructural study. *Exp Mol Pathol* 1970; 13(1): 51–65.
161. Hirschi KK and D'Amore PA. Pericytes in the microvasculature. *Cardiovasc Res* 1996; 32(4): 687–698.
162. Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 2008; 3(3): 301–313.
163. Guimaraes-Camboa N, Cattaneo P, Sun Y, et al. Pericytes of multiple organs do not behave as mesenchymal stem cells in vivo. *Cell Stem Cell* 2017; 20(3): 345–359.
164. Blocki A, Wang Y, Koch M, et al. Not all MSCs can act as pericytes: functional in vitro assays to distinguish pericytes from other mesenchymal stem cells in angiogenesis. *Stem Cells Dev* 2013; 22(17): 2347–2355.
165. Hellstrom M, Gerhardt H, Kalen M, et al. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *J Cell Biol* 2001; 153(3): 543–554.
166. Chow K, Fessel JP, KaoriIhida S, et al. Dysfunctional resident lung mesenchymal stem cells contribute to pulmonary microvascular remodeling. *Pulm Circ* 2013; 3(1): 31–49.
167. Dulmovits BM and Herman IM. Microvascular remodeling and wound healing: A role for pericytes. *Int J Biochem Cell Biol* 2012; 44(11): 1800–1812.
168. Humphreys BD. Targeting pericyte differentiation as a strategy to modulate kidney fibrosis in diabetic nephropathy. *Semin Nephrol* 2012; 32(5): 463–470.
169. Kida Y and Duffield JS. Pivotal role of pericytes in kidney fibrosis. *Clin Exp Pharmacol Physiol* 2011; 38(7): 467–473.
170. Lin S-L, Kisseleva T, Brenner DA, et al. Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. *Am J Pathol* 2008; 173(6): 1617–1627.
171. Okada H, Strutz F, Danoff TM, et al. Possible pathogenesis of renal fibrosis. *Kidney Int Suppl* 1996; 54: S37–38.
172. Patel MS, Taylor GP, Bharya S, et al. Abnormal pericyte recruitment as a cause for pulmonary hypertension in Adams–Oliver syndrome. *Am J Med Genet A* 2004; 129A(3): 294–299.
173. Kumar ME, Bogard PE, Espinoza FH, et al. Defining a mesenchymal progenitor niche at single cell resolution. *Science* 2014; 346(6211): 1258810.
174. Morikawa S, Baluk P, Kaidoh T, et al. Abnormalities in pericytes on blood vessels and endothelial sprouts in tumors. *Am J Pathol* 2002; 160(3): 985–1000.
175. Nehls V and Drenckhahn D. Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin. *J Cell Biol* 1991; 113(1): 147–154.
176. Paquet-Fifield S, Schluter H, Li A, et al. A role for pericytes as microenvironmental regulators of human skin tissue regeneration. *J Clin Invest* 2009; 119(9): 2795–2806.
177. Barham W, Frump AL, Sherrill TP, et al. Targeting the Wnt pathway in synovial sarcoma models. *Cancer Discov* 2013; 3(11): 1286–1301.
178. Ricard N, Tu L, Le Hiress M, et al. Increased pericyte coverage mediated by endothelial derived FGF-2 and IL-6 is a source of smooth muscle-like cells in pulmonary hypertension. *Circulation* 2014; 129(15): 1586–1597.
179. Sheikh AQ, Lighthouse JK and Greif DM. Recapitulation of developing artery muscularization in pulmonary hypertension. *Cell Rep* 2014; 6(5): 809–817.
180. Simonavicius N, Ashenden M, van Weverwijk A, et al. Pericytes promote selective vessel regression to regulate vascular patterning. *Blood* 2012; 120(7): 1516–1527.
181. Siroky BJ, Yin H, Dixon BP, et al. Evidence for pericyte origin of TSC-associated renal angiomyolipomas and implications for angiotensin receptor inhibition therapy. *Am J Physiol Renal Physiol* 2014; 307(5): F560–F570.
182. Yuan K, Orcholski ME, Panaroni C, et al. Activation of the Wnt/planar cell polarity pathway is required for pericyte recruitment during pulmonary angiogenesis. *Am J Pathol* 2015; 185(1): 69–84.
183. Armulik A, Abramsson A and Betsholtz C. Endothelial/pericyte interactions. *Circ Res* 2005; 97(6): 512–523.
184. El Agha E, Kramann R, Schneider RK, et al. Mesenchymal stem cells in fibrotic disease. *Cell Stem Cell* 2017; 21(2): 166–177.
185. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. *Cytokine Growth Factor Rev* 2004; 15(4): 255–273.
186. Kramann R, Schneider Rebekka K, DiRocco Derek P, et al. Perivascular Gli1+ progenitors are key contributors to

injury-induced organ fibrosis. *Cell Stem Cell* 2015; 16(1): 51–66.
187. Li C, Li M, Li S, et al. Progenitors of secondary crest myofibroblasts are developmentally committed in early lung mesoderm. *Stem Cells* 2015; 33(3): 999–1012.
188. West J, Harral J, Lane K, et al. Mice expressing BMPR2R899X transgene in smooth muscle develop pulmonary vascular lesions. *Am J Physiol Lung Cell Mol Physiol* 2008; 295: L744–755.
189. Tada Y, Majka S, Carr M, et al. Molecular effects of loss of BMPR2 signaling in smooth muscle in a transgenic mouse model of PAH. *Am J Physiol Lung Cell Mol Physiol* 2007; 292: L1556–1563.
190. Qiao L, Nishimura T, Shi L, et al. Endothelial fate mapping in mice with pulmonary hypertension. *Circulation* 2014; 129(6): 692–703.
191. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 2001; 107(11): 1395–1402.
192. Banerji S, Ni J, Wang S-X, et al. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. *J Cell Biol* 1999; 144(4): 789–801.
193. Gu B, Alexander JS, Gu Y, et al. Expression of lymphatic vascular endothelial hyaluronan receptor-1 (LYVE-1) in the human placenta. *Lymphatic Res Biol* 2006; 4(1): 11–17.
194. Gaskill CF, Carrier EJ, Kropski JA, et al. Disruption of lineage specification in adult pulmonary mesenchymal progenitor cells promotes microvascular dysfunction. *J Clin Invest* 2017; 127(6): 2262–2276.
195. Gaskill C, Marriott S, Pratap S, et al. Shared gene expression patterns in mesenchymal progenitors derived from lung and epidermis in pulmonary arterial hypertension: identifying key pathways in pulmonary vascular disease. *Pulm Circ* 2016; 6: 483–497.
196. West JD, Austin ED, Gaskill C, et al. Identification of a common Wnt-associated genetic signature across multiple cell types in pulmonary arterial hypertension. *Am J Physiol Cell Physiol* 2014; 307(5): C415–C430.
197. Majka SM, Jackson KA, Kienstra KA, et al. Distinct progenitor populations in skeletal muscle are bone marrow derived and exhibit different cell fates during vascular regeneration. *J Clin Invest* 2003; 111(1): 71–79.
198. McKinney-Freeman SL, Majka SM, Jackson KA, et al. Altered phenotype and reduced function of muscle-derived hematopoietic stem cells. *Exp Hematol* 2003; 31(9): 806–814.
199. Tanaka KK, Hall JK, Troy AA, et al. Syndecan-4-expressing muscle progenitor cells in the SP engraft as satellite cells during muscle regeneration. *Cell Stem Cell* 2009; 4(3): 217–225.
200. Fatima S, Zhou S and Sorrentino BP. Abcg2 expression marks tissue-specific stem cells in multiple organs in a mouse progeny tracking model. *Stem Cells* 2012; 30(2): 210–221.
201. Tadjali M, Zhou S, Rehg J, et al. Prospective isolation of murine hematopoietic stem cells by expression of an Abcg2/GFP allele. *Stem Cells* 2006; 24(6): 1556–1563.
202. Zhou S, Schuetz JD, Bunting KD, et al. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med* 2001; 7: 1028–1034.
203. Asakura A and Rudnicki MA. Side population cells from diverse adult tissues are capable of in vitro hematopoietic differentiation. *Exp Hematol* 2002; 30(11): 1339–1345.
204. Summer R, Fitzsimmons K, Dwyer D, et al. Isolation of an adult mouse lung mesenchymal progenitor cell population. *Am J Respir Cell Mol Biol* 2007; 37(2): 152–159.
205. Summer R, Kotton DN, Liang S, et al. Embryonic lung side population cells are hematopoietic and vascular precursors. *Am J Respir Cell Mol Biol* 2005; 33(1): 32–40.
206. Summer R, Kotton DN, Sun X, et al. Side population cells and Bcrp1 expression in lung. *Am J Physiol Lung Cell Mol Physiol* 2003; 285(1): L97–104.
207. Irwin D, Helm K, Campbell N, et al. Neonatal lung side population cells demonstrate endothelial potential and are altered in response to hyperoxia-induced lung simplification. *Am J Physiol Lung Cell Mol Physiol* 2007; 293: L941–951.
208. Martin J, Helm K, Ruegg P, et al. Adult lung side population cells have mesenchymal stem cell potential. *Cytotherapy* 2008; 10(2): 140–151.
209. Jun D, Garat C, West J, et al. The pathology of bleomycin-induced fibrosis is associated with loss of resident lung mesenchymal stem cells that regulate effector T-cell proliferation. *Stem Cells* 2011; 29(4): 725–735.
210. Majka SM, Beutz MA, Hagen M, et al. Identification of novel resident pulmonary stem cells: form and function of the lung side population. *Stem Cells* 2005; 23(8): 1073–1081.
211. Chow KS, Jun D, Helm KM, et al. Isolation & characterization of Hoechst(low) CD45(negative) mouse lung mesenchymal stem cells. *J Vis Exp* 2011; (56): e3159.
212. Baksh D, Boland GM and Tuan RS. Cross-talk between Wnt signaling pathways in human mesenchymal stem cells leads to functional antagonism during osteogenic differentiation. *J Cell Biochem* 2007; 101(5): 1109–1124.
213. Etheridge SL, Spencer GJ, Heath DJ, et al. Expression profiling and functional analysis of Wnt signaling mechanisms in mesenchymal stem cells. *Stem Cells* 2004; 22(5): 849–860.
214. Kalani MYS, Cheshier SH, Cord BJ, et al. Wnt-mediated self-renewal of neural stem/progenitor cells. *Proc Natl Acad Sci* 2008; 105(44): 16970–16975.
215. Kirton JP, Crofts NJ, George SJ, et al. Wnt/β-catenin signaling stimulates chondrogenic and inhibits adipogenic differentiation of pericytes. *Circ Res* 2007; 101(6): 581–589.
216. Huang S-MA, Mishina YM, Liu S, et al. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* 2009; 461(7264): 614–620.
217. Reya T, Duncan AW, Ailles L, et al. A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* 2003; 423(6938): 409–414.
218. Laumanns IP, Fink L, Wilhelm J, et al. The noncanonical WNT pathway is operative in idiopathic pulmonary arterial hypertension. *Am J Respir Cell Mol Biol* 2009; 40(6): 683–691.
219. Bukowska J, Ziecik AJ, Laguna J, et al. The importance of the canonical Wnt signaling pathway in the porcine endometrial stromal stem/progenitor cells: implications for regeneration. *Stem Cells Dev* 2015; 24(24): 2873–2885.
220. Cha S-W, Tadjuidje E, Tao Q, et al. Wnt5a and Wnt11 interact in a maternal Dkk1-regulated fashion to activate both canonical and non-canonical signaling in Xenopus axis formation. *Development* 2008; 135(22): 3719.

221. Choi H-J, Park H, Lee H-W, et al. The Wnt pathway and the roles for its antagonists, DKKS, in angiogenesis. *IUBMB Life* 2012; 64(9): 724–731.
222. Dravid G, Ye Z, Hammond H, et al. Defining the role of Wnt/beta-catenin signaling in the survival, proliferation, and self-renewal of human embryonic stem cells. *Stem Cells* 2005; 23(10): 1489–1501.
223. Gore AV, Swift MR, Cha YR, et al. Rspo1/Wnt signaling promotes angiogenesis via Vegfc/Vegfr3. *Development* 2011; 138(22): 4875.
224. Kahn M. Can we safely target the WNT pathway? *Nat Rev Drug Discov* 2014; 13(7): 513–532.
225. Katoh M and Katoh M. WNT signaling pathway and stem cell signaling network. *Clin Cancer Res* 2007; 13(14): 4042–4045.
226. Ling L, Nurcombe V and Cool SM. Wnt signaling controls the fate of mesenchymal stem cells. *Gene* 2009; 433(1–2): 1–7.
227. Morrisey EE and Hogan BLM. Preparing for the first breath: genetic and cellular mechanisms in lung development. *Dev Cell* 2010; 18(1): 8–23.
228. Park JS, Valerius MT and McMahon AP. Wnt/beta-catenin signaling regulates nephron induction during mouse kidney development. *Development* 2007; 134(13): 2533–2539.
229. Reynolds SD, Zemke AC, Giangreco A, et al. Conditional stabilization of β-catenin expands the pool of lung stem cells. *Stem Cells* 2008; 26(5): 1337–1346.
230. Ring A, Kim Y-M and Kahn M. Wnt/catenin signaling in adult stem cell physiology and disease. *Stem Cell Rev* 2014; 10(4): 512–525.
231. Sun Z, Gong X, Zhu H, et al. Inhibition of Wnt/β-catenin signaling promotes engraftment of mesenchymal stem cells to repair lung injury. *J Cell Physiol* 2014; 229(2): 213–224.
232. Zhao C, Blum J, Chen A, et al. Loss of β-catenin impairs the renewal of normal and CML stem cells in vivo. *Cancer Cell* 2007; 12(6): 528–541.
233. de Jesus Perez VA, Alastalo TP, Wu JC, et al. Bone morphogenetic protein 2 induces pulmonary angiogenesis via Wnt-beta-catenin and Wnt-RhoA-Rac1 pathways. *J Cell Biol* 2009; 184(1): 83–99.
234. Karar J and Maity A. PI3K/AKT/mTOR pathway in angiogenesis. *Front Mol Neurosci* 2011; 4: 51.
235. Korn C, Scholz B, Hu J, et al. Endothelial cell-derived non-canonical Wnt ligands control vascular pruning in angiogenesis. *Development* 2014; 141(8): 1757–1766.
236. Yang X-M, Han H-X, Sui F, et al. Slit-Robo signaling mediates lymphangiogenesis and promotes tumor lymphatic metastasis. *Biochem Biophys Res Commun* 2010; 396(2): 571–577.
237. An Y, Kiang A, Lopez JP, et al. Cigarette smoke promotes drug resistance and expansion of cancer stem cell-like side population. *PLoS ONE* 2012; 7(11): e47919.
238. Zhao Y, Butler EB and Tan M. Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis* 2013; 4: e532.
239. Krishnamurthy P, Ross DD, Nakanishi T, et al. The stem cell marker Bcrp/ABCG2 enhances hypoxic cell survival through interactions with heme. *J Biol Chem* 2004; 279(23): 24218–24225.
240. Bourguignon LYW, Wong G, Earle CA, et al. Interaction of low molecular weight hyaluronan (LMW-HA) with CD44 and toll-like receptors promotes the actin filament-associated protein (AFAP-110)-actin binding and MyD88-NFκB signaling leading to pro-inflammatory cytokine/chemokine production and breast tumor invasion. *Cytoskeleton (Hoboken)* 2011; 68(12): 671–693.
241. Li Y, Jiang D, Liang J, et al. Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. *J Exp Med* 2011; 208(7): 1459–1471.
242. Schmitt M, Metzger M, Gradl D, et al. CD44 functions in Wnt signaling by regulating LRP6 localization and activation. *Cell Death Differ* 2015; 22(4): 677–689.
243. Solis MA, Chen Y-H, Wong TY, et al. Hyaluronan regulates cell behavior: a potential niche matrix for stem cells. *Biochem Res Int* 2012; 2012: 346972.
244. Dentener M, Vernooy J, Hendriks S, et al. Enhanced levels of hyaluronan in lungs of patients with COPD: relationship with lung function and local inflammation. *Thorax* 2005; 60(2): 114–119.
245. Liang J, Jiang D and Noble PW. Hyaluronan as a therapeutic target in human diseases. *Adv Drug Deliv Rev* 2016; 97: 186–203.
246. Noble PW. Hyaluronan and its catabolic products in tissue injury and repair. *Matrix Biol* 2002; 21(1): 25–29.
247. Qin Z, Dai L, Bratoeva M, et al. Cooperative roles for emmprin and LYVE-1 in the regulation of chemoresistance for primary effusion lymphoma. *Leukemia* 2011; 25(10): 1598–1609.
248. Brechbuhl HM, Gould N, Kachadourian R, et al. Glutathione transport is a unique function of the ATP-binding cassette protein ABCG2. *J Biol Chem* 2010; 285(22): 16582–16587.
249. Harada N, Tamai Y, Ishikawa T, et al. Intestinal polyposis in mice with a dominant stable mutation of the beta-catenin gene. *Embo J* 1999; 18(21): 5931–5942.
250. Muzumdar MD, Tasic B, Miyamichi K, et al. A global double-fluorescent Cre reporter mouse. *Genesis* 2007; 45(9): 593–605.
251. El Agha E, Moiseenko A, Kheirollahi V, et al. Two-way conversion between lipogenic and myogenic fibroblastic phenotypes marks the progression and resolution of lung fibrosis. *Cell Stem Cell* 2017; 20: 571.
252. Schultz CJ, Torres E, Londos C, et al. Role of adipocyte differentiation-related protein in surfactant phospholipid synthesis by type II cells. *Am J Physiol Lung Cell Mol Physiol* 2002; 283(2): L288.
253. Sims-Lucas S, Schaefer C, Bushnell D, et al. Endothelial progenitors exist within the kidney and lung mesenchyme. *PLoS ONE* 2013; 8(6): e65993.
254. Watkins DN, Berman DM, Burkholder SG, et al. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature* 2003; 422(6929): 313–317.
255. Pepicelli CV, Lewis PM and McMahon AP. Sonic hedgehog regulates branching morphogenesis in the mammalian lung. *Curr Biol* 1998; 8(19): 1083–1086.
256. Liu L, Kugler MC, Loomis CA, et al. Hedgehog signaling in neonatal and adult lung. *Am J Respir Cell Mol Biol* 2013; 48(6): 703–710.

257. Peng T, Frank DB, Kadzik RS, et al. Hedgehog actively maintains adult lung quiescence and regulates repair and regeneration. *Nature* 2015; 526(7574): 578–582.
258. Price MA, Wanshura LEC, Yang J, et al. CSPG4, a potential therapeutic target, facilitates malignant progression of melanoma. *Pigment Cell Melanoma Res* 2011; 24(6): 1148–1157.
259. Sheikh AQ, Misra A, Rosas IO, et al. Smooth muscle cell progenitors are primed to muscularize in pulmonary hypertension. *Sci Transl Med* 2015; 7(308): 308ra159.
260. Hellstrom M, Kalon M, Lindahl P, et al. Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* 1999; 126(14): 3047–3055.
261. Naiche LA and Papaioannou VE. Loss of Tbx4 blocks hindlimb development and affects vascularization and fusion of the allantois. *Development* 2003; 130(12): 2681.
262. Zhang W, Menke DB, Jiang M, et al. Spatial-temporal targeting of lung-specific mesenchyme by a Tbx4enhancer. *BMC Biol* 2013; 11(1): 111.
263. Xu J, Nie X, Cai X, et al. Tbx18 is essential for normal development of vasculature network and glomerular mesangium in the mammalian kidney. *Dev Biol* 2014; 391(1): 17–31.