Pericytes in Chronic Lung Disease

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Key Words

Allergic asthma · Chronic obstructive pulmonary disorder · Fibrosis · Inflammation · Lung · Stem cells

Abstract

Pericytes are mesenchymal cells embedded within the abluminal surface of the endothelium of microvessels such as capillaries, pre-capillary arterioles, post-capillary and collecting venules, where they maintain microvascular homeostasis and participate in angiogenesis. In addition to their roles in supporting the vasculature and facilitating leukocyte extravasation, pericytes have been recently investigated as a subpopulation of mesenchymal stem cells (MSCs) due to their capacity to differentiate into numerous cell types including the classic MSC triad, i.e. osteocytes, chondrocytes and adipocytes. Other studies in models of fibrotic inflammatory disease of the lung have demonstrated a vital role of pericytes in myofibroblast activation, collagen deposition and microvascular remodelling, which are hallmark features of chronic lung diseases such as asthma, chronic obstructive pulmonary disorder, pulmonary fibrosis and pulmonary hypertension. Further studies into the mechanisms of the pericyte-to-myofibroblast transition and migration to fibrotic foci will hopefully clarify the role of these cells in chronic lung disease and confirm the importance of pericytes in human fibrotic pulmonary disease.

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Pericytes: An Overview

Pericytes are mesenchymal cells embedded within the abluminal surface of the endothelium of microvessels such as capillaries, pre-capillary arterioles and post-capillary collecting venules, where they maintain microvascular homeostasis and participate in angiogenesis (fig. 1a) [1–3]. The expression of desmin and α -smooth muscle actin (α -SMA) points to the contractile functions of pericytes, by which they are able to control the constriction and dilation of blood vessels, thereby regulating blood flow [4]. Indeed, pericytes occupy a phenotypic continuum with vascular smooth muscle cells (VSMCs), i.e. the mural cells located on larger blood vessels, and therefore share many characteristics [1, 5]. The basement membrane of pericytes is continuous with endothelial cells (ECs), and the two cell types form tight associations by way of adhesion plaques composed of fibronectin and peg-and-socket formations containing tight, gap and adherens junctions [1]. The secretion of platelet-derived growth factor (PDGF)-BB by ECs recruits pericytes to the microvasculature in a paracrine fashion via PDGF receptor (PDGFR)- β [6]. Conversely, pericytes signal to the endothelium via the angiopoietin (Ang)/Tie receptor axis. The Tie2 receptor is expressed on ECs and recognizes the agonist Ang1, secreted by pericytes, as well as the autocrine antagonist Ang2 [6]. The Ang1 signalling loop maintains vascular stability by maintaining pericyte cov-

Fig. 1. **a** Pericytes are mural cells embedded within the abluminal surface of the endothelium of microvessels such as capillaries, pre-capillary arterioles, post-capillary and collecting venules, where they maintain microvascular homeostasis and participate in angiogenesis. The secretion of PDGF-BB by ECs recruits pericytes to the microvasculature in a paracrine fashion via PDGFR- β . **b** Upon

isolation from healthy tissue, pericytes are capable of undergoing trilineage (osteo-, adipo- and chondrogenic) differentiation similar to bone marrow-derived MSCs. **c** However, under inflammatory/fibrotic conditions, pericytes can migrate from the vasculature towards fibrotic foci, where they have been shown to differentiate into a myofibroblast-like phenotype.

erage, whilst Ang2 overexpression is associated with pericyte loss [1]. The vessel coverage of pericytes varies according to species, organ and vessel type and ranges from 1:1 to 10:1 (ECs:pericytes) with the highest abundance in the central nervous system and retina. Sparse coverage is seen in tissues involved in gas and nutrient exchange [2, 3, 7].

The identification of pericytes can be problematic as markers are unspecific and dynamically and differentially expressed depending on the species, type/origin of vessel and pathology [2]. Markers commonly expressed by pericytes include the tyrosine kinase receptor PDGFR- β , the chondroitin sulphate proteoglycan neural glial antigen 2 (NG2), the adhesion molecule CD146, the contrac-

tile proteins desmin and α -SMA, the endosialin CD248, the aminopeptidase CD13, the growth factors Ang1 and Ang2, alkaline phosphatase, the GTPase signalling molecule regulator of G-protein signalling 5 and the ganglioside antigen 3G5 [2, 7, 8]. α -SMA expression is found in the more stellate pericytes of the pre- and post-capillary vessels but is lacking in spindle-shaped capillary pericytes [9]. In humans, pericytes are easily isolated from various tissues, including the lung, using fluorescence-activated cell sorting with the gating strategy CD45-/CD31-/CD56-, CD146^{high} [10, 11]. In mice, pericyte isolation and/or characterization has generally relied on culture methods using cells isolated from the heart [12], ear [13] and brain [14], or through the use of transgenic mice in

which pericytes are labelled in the kidney [15], skeletal tissue [16], liver [17], lung [18–20] and brain [21]. Fluorescence-activated cell sorting is a rapid method and yields pure populations, which is not possible using culture methods alone. Following sorting, pericytes retain the expression of their characteristic markers NG2, CD146, PDGFR- β , alkaline phosphatase and α -SMA over extended periods of culture [11].

Pericytes are thought to represent a subpopulation of mesenchymal stem cells (MSCs) due to their capacity to differentiate into numerous cell types including the classic MSC triad, i.e. osteocytes, chondrocytes and adipocytes, in addition to other cells such as fibroblasts/myofibroblasts, myointimal cells and VSMCs [2]. Initially isolated from murine bone marrow in 1966 [22], MSCs are now believed to reside in all vascularized adult tissue. Their pluripotency [23] and potential for applications in regenerative medicine and tissue engineering renders the isolation, culture and characterization of these cells desirable. In accordance with the minimal criteria proposed by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, an MSC must be plastic adherent, uniformly express the MSC markers CD73, CD90 and CD105 and possess trilineage (osteogenic, adipogenic and chondrogenic) differentiation capabilities [3, 24]. In fulfilment of these criteria, Crisan et al. [11] showed that cultured pericytes from a range of human tissue are plastic adherent, are capable of trilineage differentiation and express CD10, CD13 and CD44. The expression of these markers persisted over extended time periods [11]. Moreover, pericytes from skeletal muscle and non-muscle tissue have been shown to be myogenic both in culture and in SCID-NOD mice injured with cardiotoxin [11] and to differentiate into myofibroblasts in models of fibrosis [15, 17, 18, 20]. The landmark study by Crisan et al. [11] was the first to isolate, culture and differentiate pure populations of pericytes and provided strong evidence that they comprise an MSC-like population possessing the ability to differentiate into multiple tissue types distinct from their origin. The mesenchymal progenitor cell characteristics of pericytes have been observed in cells derived from the retina [25], endometrium [26] and skeletal muscle (fig. 1b) [27].

Although little is known about the MSC capacity of pulmonary pericytes, murine and human resident lung MSCs have been identified using the gating strategy Hoechst 33342^{dim}, CD45⁻, and were found to express the MSC markers ABCG2, CD90, CD105, CD106, CD73, CD44 and Sca1 and to lack haematopoietic markers such as CD45, CD34 and c-kit [28–32]. Although pericyte

marker expression on these cells was not analysed in the majority of these studies, it is likely that some of these resident lung MSCs were of a perivascular nature, as 8.7% of CD45⁻/CD34⁻/Sca1⁺ cells expressed PDGFR- β [29].

Pericytes have been implicated in pathological processes such as fibrosis, tumour formation, hypertension and microangiopathy [2, 7]; their role in chronic lung disease is the subject of this review. Although the presence of pulmonary pericytes was first confirmed in 1974 by electron microscopy [33], there have been a limited number of studies on pericytes in the lung, and their presence has been a somewhat controversial topic. Recent murine immunohistochemical analyses have shown desmin⁺/NG2⁺/PDGFR- β ⁺/ α -SMA⁻ pericyte coverage of both the peribronchiolar/vascular and the alveolar regions of the lung [18, 20]. These cells were shown to be derived from Foxd1-expressing embryonic progenitors, hinting at a mesenchymal lineage [18]. NG2DsRedBAC-transgenic reporter mice have revealed an elongated, branched pulmonary pericyte structure which suggested that the expression of NG2 and 3G5 is irregular [19], again stressing the difficulties in obtaining suitable markers for pericyte investigations. This elongated structure was also shown in human lung and cultured human pulmonary pericytes isolated immunomagnetically using an anti-3G5 antibody coupled to anti-IgM microbeads [19]. In this study, 96% of the pericytes were double positive for 3G5 and NG2 [19]. The above studies confirm the presence of pericytes in both mouse and human lung, paving the way for determining their role in pulmonary pathology.

The Role of Pericytes in Coordinating the Inflammatory Response

Many chronic diseases of the lung are associated with a robust inflammatory response, in particular asthma and chronic obstructive pulmonary disorder (COPD) [34] as well as, more controversially, pulmonary fibrosis [35]. It is well known that the vascular endothelium plays a vital role in the extravasation and tissue infiltration of inflammatory cells. However, recent studies have demonstrated an equally important role of pericytes in facilitating leukocyte extravasation. Following the well-described processes of tethering and rolling on activated ECs, adhesion, crawling and transendothelial migration, leukocytes breach the vascular basement membrane and encounter pericytes that surround the blood vessel and effectively

form an additional barrier to be crossed [36]. Gaps between pericytes are associated with regions of low expression of the basement proteins laminin and collagen type IV and high expression of ICAM-1, which essentially form portals that have been shown to be preferential sites for cell migration [37]. Recent evidence has suggested that migrating neutrophils and monocytes actively search for these regions during extravasation [38].

In vitro investigations into this process using human EC/pericyte cocultures have revealed that pericytes alone are less efficient at facilitating neutrophil transmigration than ECs, while EC/pericyte bilayers support intermediate levels of transmigration. This deficiency in pericyte-mediated neutrophil transmigration was attributed to the altered expression pattern of ICAM-1 on pericytes in the EC/pericyte bilayers, in which pericytes formed a confluent layer rather than providing gaps for transmigrating cells, as has been described in vivo [39]. Subsequent studies from the same research group improved on this model by incorporating a pericyte-deposited basement membrane, with ECs and pericytes seeded on opposing sides of a Transwell membrane. Interestingly, in the presence of a basement membrane, EC/pericyte composites performed comparably to ECs alone following activation with TNF- α [40]. Other studies have shown that pericytes can further contribute to coordinating the immune response by responding to damage-associated molecular patterns such as necrotic cell lysates or TNF- α . The pro-inflammatory pericyte phenotype was characterized by upregulated ICAM-1 expression and a release of the chemoattractant MIF; using two-photon microscopy, these changes were found to greatly enhance leucocyte extravasation. Following extravasation, pericytes instructed leucocytes by increasing TLR expression and enhanced the ability of innate immune cells to scan the local microenvironment [41].

This interplay between leucocytes and pericytes during acute inflammatory responses was elegantly described by Proebstl et al. [42] using real-time confocal intravital imaging. To achieve this, chimeric mice were generated to express *RFPcherry* in pericytes and *GFP* in neutrophils. These authors showed that pericytes facilitate transmigration in vivo in an ICAM-1-, Mac-1- and LFA-1-dependent manner. The permissive extravasation sites between pericytes previously described by this research group were found to be enlarged in inflamed tissues in a process mediated by a change in the shape of pericytes that was driven by inflammatory mediators such as IL-1 β and TNF- α [42]. Interestingly, only a small proportion of these gaps (9%) were found to support the extravasation

of the majority (70%) of neutrophils; these gaps were characterized by a high expression of KC and ICAM-1 and were of a size preferred by transmigrating neutrophils, i.e. 8–50 μm^2 [42]. Mechanistically, the opening of these gaps between pericytes may depend on direct contact with neutrophils, which induces relaxation of the pericyte cytoskeleton via RhoA/ROCK inhibition [43]. Other clues to the mechanisms by which pericytes mediate leucocyte extravasation can be taken from studies on the blood-brain barrier, of which pericytes are an important component [44]. Pericytes have been shown to directly modulate inflammatory responses at the blood-brain barrier by responding to and producing inflammatory cytokines and NO [45] as well as by altering their expression of integrins with a switch from $\alpha 1$ to $\alpha 2$ integrin [46].

In the respiratory tract, the role of pericytes in mediating vascular stability during inflammation has been investigated using a mouse model of *Mycoplasma pulmonis* infection. This study demonstrated that PDGF- β signalling in pericytes, which is important in maintaining EC-pericyte contacts, plays a vital role in maintaining vascular stability following *M. pulmonis* infection, which is characterized by an extensive vascular remodelling and increased pericyte coverage of the microvasculature in the large airways. Specifically, the inhibition of PDGF- β signalling by the DNA aptamer AX102 in the context of acute *M. pulmonis* infection led to a greatly reduced pericyte coverage of the microvasculature of the trachea, vascular swelling and increased vascular leakiness; this was associated with an increased infectious burden in the airways determined by *M. pulmonis* 16S RNA expression [47]. It is tempting to speculate that this loss of pericytes on the respiratory microvasculature may have compromised leucocyte extravasation, thereby reducing bacterial clearance and exacerbating the disease, but further studies are needed to test this hypothesis.

The Pathology and Mechanisms of Organ Fibrosis

Fibrotic disease is a major cause of mortality throughout the world with no currently available treatment [48]. Fibrosis is the permanent scarring, thickening and hardening of tissue due to dysregulated repair mechanisms and the deposition of extracellular matrix (ECM) proteins. Typically, it occurs following chronic sustained inflammation and usually in a person with a genetic predisposition [49–51]. In the lung, fibrotic diseases, known collectively as interstitial lung disorders, manifest in

many ways and are associated with environmental exposure (asbestos, antigens and viruses) or chronic inflammatory disease (rheumatoid arthritis and sarcoidosis) or, more commonly, are idiopathic [52]. Lung fibrosis, especially idiopathic lung fibrosis (IPF), has a poor prognosis and leads to a high mortality rate [53].

Following tissue insult, epithelial cells and ECs orchestrate the development of a temporary ECM, and cytokine release from an influx of leucocytes propels the inflammatory response and results in fibroblast recruitment [49, 53]. Lymphocytes secrete profibrotic cytokines and growth factors such as transforming growth factor (TGF)- β and PDGF [49]. Fibroblasts become activated α -SMA+ myofibroblasts and secrete ECM proteins, particularly collagen [53]. Due to its potent effects on collagen and other ECM protein secretion, TGF- β is thought to be a key molecule involved in fibrogenesis [50]. In the healthy lung, the temporary ECM is degraded by matrix metalloproteases, which are in turn regulated by tissue inhibitors of metalloproteases. Under conditions of chronic inflammation, however, these mechanisms are dysregulated in such a way that collagen and ECM proteins are deposited at a greater rate than at which they are degraded, resulting in fibrotic scar formation [49].

The origin of myofibroblasts, the cells considered to be the major contributors to fibrosis, remains under debate; these cells may be derived from tissue-resident cells, bone marrow-derived MSCs or cells that have undergone epithelial-to-mesenchymal or endothelial-to-mesenchymal transition [54]. In vogue, the pericyte, with its MSC-like capabilities, has been shown to significantly contribute to the myofibroblast population via lineage tracing in skin [55], muscle [55], kidney [56], liver [17, 57] and lung [18, 20] models of fibrosis. Moreover, these studies provide in vivo evidence that epithelial-to-mesenchymal transition, which until recently had been the favoured candidate, is not a major mechanism of fibrosis, whilst MSCs and resident fibroblasts play an important role.

Pericytes in Organ Fibrosis

With the advent of genetic fate-tracking techniques, it has become possible to observe changes in the localization and behaviour of pericytes in organ fibrosis. In 2008, Lin et al. [15] observed that collagen type I $\alpha 1$ (collagen 1 $\alpha 1$)-expressing cells (genetically labelled with eGFP) in a mouse model of renal fibrosis express markers for pericytes, including NG2 and PDGFR- β . Further analysis indicated that the number of pericytes expressing collagen

1 $\alpha 1$ increased over time following renal injury, and that these cells acquired α -SMA expression and migrated into interstitial fibrotic areas [15]. A follow-up study from the same research group [56] used a Cre-mediated labelling strategy to demonstrate that Foxd1-expressing renal stromal cells (VSMCs, pericytes and mesangial cells) respond to renal injury by proliferating and increasing their expression of α -SMA. In fact, the authors stated that this cell population, also positive for PDGFR- β expression, represented nearly the entire myofibroblast population in their model of renal fibrosis [56]. Taken together with their previous studies on collagen 1 $\alpha 1$ -expressing cells, these authors concluded that the vast majority of myofibroblasts in renal fibrosis are derived from pericytes, not epithelial cells, and that efforts to develop novel treatments for renal fibrosis would be best served by focusing on pericyte biology rather than epithelial-to-mesenchymal transition.

Similar outcomes have been observed in mouse models of hepatic fibrosis. Liver-resident pericytes are also known as hepatic stellate cells (HSCs); recent studies have shown that HSCs are the primary source of collagen in the liver following injury and the initiation of fibrosis. Using a combination of a reporter mouse line (eGFP-expressing collagen 1 $\alpha 1$ -producing cells) and flow cytometry to detect vitamin A-expressing HSCs in alcohol- or carbon tetrachloride-driven mouse models of liver fibrosis, it was found that the majority of myofibroblasts were derived from HSCs [57]. Further evidence to support a role for pericytes in liver fibrosis has been recently provided by lineage tracing of HSCs in three discrete models of liver disease, including models of toxic, cholestatic and fatty liver damage [17]. This lineage tracing strategy, based on Cre expression driven by lecithin-retinol acyltransferase, was found to reliably label 99% of HSCs in the healthy liver; these cells were also positive for the pericyte markers desmin and PDGFR- β . Following injury to the liver, HSCs were found to be the dominant source of myofibroblasts [17], in line with investigations into the role of pericytes in kidney fibrosis.

Pericytes in IPF

There have been only two in vivo studies investigating the role of pericytes in IPF [18, 20]; both studies revealed heterogeneous populations of lung-resident MSCs contributing to the fibrotic phenotype and provided evidence that pericytes are major, but not sole, contributors to myofibroblast progenitor populations and collagen deposition.

Foxd1 is a forkhead transcription factor activated in MSC progenitors of the VSMC-pericyte lineage during embryonic development and is silenced once progenitor cells enter lung buds and differentiate into the pulmonary mesenchyme [18]. Using Foxd1-Cre and collagen 1 α 1-GFP transgenic mice, Hung et al. [18] identified two populations of lung-resident pericytes derived from the Foxd1 lineage and expressing the pericyte markers PDGFR- β , NG2 and CD146. One population expressed collagen 1 α 1 and was PDGFR- α -, whilst the other was negative for both. A third lung-resident cell type was identified: collagen 1 α 1+, which did not express pericyte markers and was considered to be a classic fibroblast. Despite the pericyte-like morphology and close approximation to the microvasculature of both populations of Foxd1 progenitor-derived cells, transcriptomics revealed that collagen 1 α 1- cells expressed genes relating to vascular development, whilst collagen 1 α 1+ cells possessed transcripts associated with matrix remodelling and wound repair-related pathways [18]. These findings may hint at an intermediate phenotype between a pericyte and a collagen 1 α 1+ non-Foxd1-derived fibroblast, in line with an MSC-like nature. In this same study, using a bleomycin-driven murine model of IPF, 45–68% of the myofibroblasts (defined by α -SMA expression) were shown to be derived from cells of the Foxd1 lineage, and 30% of these cells were found to produce collagen within fibrotic foci. Using a similar model of bleomycin-driven lung injury in mice, Rock et al. [20] showed an increased expression of the pericyte markers PDGFR- β , NG2 and desmin within fibrotic foci. Using NG2CreER BAC-transgenic mice crossed with ROSA-farnesylated GFP reporter mice, it was shown that pericytes proliferate under fibrotic conditions, although, in contrast to the results of Hung et al. [18], few took on the α -SMA+ phenotype of a myofibroblast. However, the number of pericytes contributing to the myofibroblast population in this study may have been underestimated due to the inefficiency of the recombination process used.

These studies provide evidence that, as in other tissues, pericytes are key cells involved in pulmonary fibrosis and contribute to a significant proportion of the myofibroblast population. However, it is clear that other cells, such as the collagen 1 α 1+ fibroblasts, which comprise 53% of the collagen-producing cells [18], are also responsible for collagen deposition. Further studies are certainly warranted to investigate whether targeting the pericyte-to-myofibroblast transition is a viable treatment strategy for IPF.

Pericytes in COPD

COPD is a progressive, poorly reversible disease in which airflow is restricted as a result of peribronchial fibrosis, emphysematous changes and reduced elastic recoil. Currently, there are no studies directly investigating the role of pericytes in COPD; however, fewer α -SMA+ myofibroblasts have been shown in the alveoli and bronchioles of COPD patients compared with non-smokers, with greater numbers of α -SMA+ myofibroblasts in the bronchi [58]. Fibroblasts also exhibit deficient contractility and migration in COPD patients [59]. These findings might suggest dysregulated pulmonary repair mechanisms involving myofibroblasts, which, as previously discussed, have been shown to be, at least in part, derived from pericytes.

Despite the fact that there has been no research into pericytes in COPD, clues to their potential role can be garnered from studies investigating the vascular response in this disorder, as pericytes participate in vascular homeostasis and remodelling, and respond to and secrete vascular growth factors. Although little is known about microvasculature remodelling in COPD, a slightly increased vascularity has been observed [60, 61]. An increased expression and gene polymorphisms of TGF- β ₁ are observed in COPD [62], and this growth factor is considered a major mediator of COPD due to its proliferative effect on fibroblasts [63]. However, the TGF- β ₁ signalling pathway is also involved in the control of the contractility of retinal pericytes [64], and therefore could mediate the vessel dilation observed in COPD. Furthermore, a smoking-induced impairment of vasodilator responses persists in COPD patients despite smoking cessation [65]. These studies suggest that dysregulated pericyte contractile activity may play a part in COPD pathogenesis.

An increased expression of vascular endothelial growth factor (VEGF), which has vasculo- and angiogenic properties, has been observed in chronic bronchitis, an aspect of COPD pathology in some patients. Moreover, VEGF levels were found to negatively correlate with forced expiratory volume in 1 s (FEV₁) [66]. Of note, VEGF was increased in the VSMCs of the bronchioles and alveoli [66]. In patients with emphysema, however, VEGF levels were decreased and positively correlated with FEV₁, suggesting different mechanisms with regard to the microvasculature between different phenotypes of COPD [66, 67]. VEGF may contribute to emphysema by inducing EC apoptosis [68], which could contribute to the microvessel loss observed in emphysematous COPD subtypes. Pericytes secrete VEGF [2, 69] and play a strong role in an-

giogenesis and vascular homeostasis, so it is reasonable to postulate that pericytes might be involved in microvascular changes associated with COPD.

Ang2 functions as part of the Ang-Tie2 receptor axis that mediates blood vessel maturation and is mainly expressed during vascular remodelling by ECs and reportedly by perivascular cells as well [6]. Ang2 has been shown to be increased in the serum of COPD patients during exacerbations [70]. Ang2 facilitates the loosening of pericyte-endothelial interactions, leading to vessel destabilization, and, in conjunction with VEGF, promotes angiogenesis. Finally, the cell surface expression of CD146, a marker found on most pericytes, has been shown to be decreased in rat lung in an experimental model of emphysema and patients with COPD, leading to increased microvascular permeability, whilst soluble CD146 was increased in bronchoalveolar lavage fluid [71]. Although this study was performed to investigate the role of the endothelium in COPD, it cannot be ruled out that pericytes did not contribute to CD146 expression [71]. Taken together, although these studies do not provide direct evidence for a role of pericytes in COPD, they highlight areas that require further investigation.

Pericytes in Asthma

Allergic asthma is a respiratory disease driven by aero-allergen exposure and the subsequent allergen-driven immune response. The pathology of allergic asthma is characterized by airway remodelling, encompassing changes to the airway epithelium (excess mucus production and epithelial shedding) and subepithelial regions (angiogenesis, fibrosis and airway smooth muscle thickening) [72]. These structural changes to the airway wall and the pulmonary microvasculature ultimately lead to impaired lung function [73]. As with COPD, there are no currently published studies that have directly investigated the role of pericytes in allergic asthma. However, unpublished data from our laboratory suggest that, in a mouse model of house dust mite-induced allergic airway disease, pericytes are one of several cell types in the lung that contribute to airway smooth muscle thickening.

The vascular component in asthma has been more extensively studied compared with COPD. An increased number of vessels, increased vessel activity (vasodilation, leakage and leucocyte extravasation) and increased growth factor secretion have been observed in asthmatics compared with healthy control subjects [74]. Moreover, increased vascularity correlated with disease severity and

likely contributed to the robust inflammatory response seen in severe asthma [74]. Interestingly, the capillaries of the lower airways are not usually fenestrated in the healthy lung [75], but this phenomenon has been observed in asthmatics [76]. Moreover, vascular remodelling, characterized by a thickening of the VSMC layer, EC proliferation and perivascular fibrosis, has been observed in the bronchi of mice subjected to respiratory ovalbumin (OVA) [77] and chronic house dust mite [78] exposure.

In addition to angiogenesis and vessel stability, VEGF mediates capillary fenestration and, as in certain subtypes of COPD, has been shown to be elevated in asthmatics and in IL-13-driven murine models of asthma [79]. Similarly, elevated levels of Ang2 in induced sputum correlate with asthma severity [80]. In a mouse model of allergic airway disease driven by OVA sensitization and challenge, Tie2 expression has been observed in macrophages and epithelial cells, which did not express this Ang ligand under control conditions [80]. VEGF and Ang2 levels were also elevated in bronchoalveolar lavage fluid as well as whole lung tissue and expressed by VSMCs and MSCs [80]. Conversely, also in an OVA-driven model of allergic airway disease, Ang1 expression was decreased with respect to control mice and was thought to confer protection against airway inflammation [81]. Further research in this field will certainly clarify the role of Ang and vascular stability in asthma.

PDGF-BB/PDGFR- β signalling is another important aspect of pericyte-endothelial interactions. Unpublished data from our group using a murine model of allergic asthma have shown that a disruption of this signalling pathway leads to pericyte mobilization away from the airway microvasculature and accumulation in the airway smooth muscle bundles; this process was found to significantly contribute to airway hyperreactivity in this model. Interestingly, the impact of PDGF-BB/PDGFR- β signalling in mediating airway remodelling in allergic airway disease was shown in a study by Hirota et al. [82], in which the ectopic overexpression of PDGF-BB in the airway lumen of OVA-sensitized mice led to airway smooth muscle cell proliferation and increased airway hyperresponsiveness compared with mice exposed to the surrogate allergen OVA alone. Although the contribution of microvascular pericytes to these changes was not investigated, it has been shown that pericytes undergo chemotaxis towards a strong source of PDGF-BB [1]. Conversely, the inhibition of PDGF-BB/PDGFR- β signalling by tyrosine kinase inhibitors (imatinib and masitinib) is currently being investigated as a means of reversing airway remodelling in animal models of

asthma, and phase II studies are currently underway in human asthmatics (ClinicalTrials.gov identifier NCT01097694). This drug class has been quite extensively studied in cancer models; interestingly, the effect of imatinib intervention depends on the PDGF-BB expression level in tumour cells, since a PDGFR- β blockade in high PDGF-BB-expressing tumours significantly inhibited tumour growth and metastases by increasing pericyte coverage and stabilizing the microvasculature, whereas in low PDGF-BB-expressing tumours, imatinib treatment reduced pericyte coverage and promoted tumour cell dissemination [83]. Tyrosine kinase inhibitors have shown therapeutic promise in cat [84] and mouse [85] models of allergic asthma, with reductions observed in airway inflammation and remodelling, leading to improved lung function. However, it must be emphasized that the compounds used in these studies on allergic asthma (masitinib and imatinib) have profound effects on the inflammatory response as well, greatly complicating the interpretation of results on structural changes and lung function. In any case, these studies highlight the importance of understanding the role of vascular mediators in asthma. As pericytes are involved in these signalling pathways and in vascular homeostasis, it seems probable that they contribute to asthma pathobiology, and their contribution to these mechanisms requires further investigation.

These studies in animal models of kidney, liver and lung fibrosis point to a vital role of pericytes in myofibroblast activation, collagen deposition and microvascular remodelling observed in chronic lung disease (fig. 1c). However, there may be considerable differences in the mechanisms driving organ fibrosis in humans and those active in animals such as the mouse, most importantly in terms of the duration of the disease. Further studies into the mechanisms of the pericyte-to-myofibroblast transition and migration to fibrotic foci are certainly warranted, in addition to a confirmation of the importance of pericytes in human fibrotic disease.

Pericytes in Pulmonary Hypertension

Pulmonary hypertension is characterized by structural changes to the walls of pulmonary vessels, with prominent fibrosis of the vessel wall, an accumulation of α -SMA+ cells and inflammatory cell infiltration [86], ultimately leading to increased blood pressure in the pulmonary vasculature and heart failure. Currently, the molecular mechanisms responsible for these processes are

incompletely understood. In recent years, pericyte proliferation has been investigated as a causative factor of pulmonary hypertension.

Clinically, abnormal pericyte coverage of the pulmonary vasculature has been implicated in the pathogenesis of several forms of pulmonary hypertension, including congenital Adams-Oliver syndrome [87] and pulmonary capillary haemangiomatosis [88]. In particular, in the latter study, *in situ* hybridization and immunohistochemistry revealed a higher expression of PDGF-BB by type II pneumocytes and ECs as well as increased PDGFR- β expression by pericytes and VSMCs associated with proliferating capillaries. This hypothesis was investigated in greater depth by Ricard et al. [19], using human material obtained during lung transplantations in pulmonary hypertension patients as well as from a chronic hypoxia-driven rodent model of pulmonary hypertension. An overabundance of pericytes was found to be associated with remodelled distal vessels in the lung in both the clinical samples and the rodent model; this was associated with an increased expression of FGF-2 and IL-6 by ECs, although no difference in PDGF-BB expression was observed [19]. The authors of this study concluded that pulmonary hypertension is driven by EC abnormalities that ultimately promote excess pericyte coverage, pulmonary smooth muscle hyperplasia and vascular remodelling. An increased coverage of the microvasculature with pericytes and VSMCs has also been observed in other animal models of pulmonary hypertension. Using a rat model of pulmonary hypertension driven by hyperoxia, Jones et al. [89] employed immunogold staining and high-resolution imaging to assess the PDGFR- β /PDGF-BB pathway. PDGFR- β was found to be highly overexpressed in perivascular cells in hypertensive lungs compared with control lungs; PDGF-BB was similarly overexpressed in the early stages of the disease [89]. It has been postulated by a number of authors that receptor tyrosine kinase inhibitors may be effective treatments for pulmonary hypertension as they have been shown to reduce pericyte density in solid tumour models [90]. However, the efficacy and safety of this intervention will need to be assessed in clinical trials on pulmonary hypertension patients.

Future Directions

It is clear that the vascular component of airway disease is of significance, and, despite being a key regulator of vascular mechanisms such as angiogenesis and vessel contractility, pericytes have been studied only recently in

the context of IPF and pulmonary arterial hypertension. Moreover, the roles of this cell type in chronic lung diseases such as asthma and COPD are not completely understood at the present time. In addition to vascular homeostasis, pericytes participate in inflammatory reac-

tions and possess MSC-like progenitor capabilities, and are therefore likely to play multiple roles in lung pathobiology. A comprehensive understanding of the involvement of this cell type in chronic lung disorders may lead to the development of new therapeutic strategies.

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