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Pro-angiogenic Hematopoietic Progenitor Cells and Endothelial Colony Forming Cells in Pathological Angiogenesis of Bronchial and Pulmonary Circulation

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Abstract

Dysregulation of angiogenesis is a common feature of many disease processes. Vascular remodeling is believed to depend on the participation of endothelial progenitor cells, but the identification of endothelial progenitors in postnatal neovascularization remains elusive. Current understanding posits a role for circulating pro-angiogenic hematopoietic cells, which interact with local endothelial cells to establish an environment that favors angiogenesis in physiologic and pathophysiologic responses. In the lung, increased and dysregulated angiogenesis is a hallmark of diseases of the bronchial and pulmonary circulations, manifested by asthma and pulmonary arterial hypertension (PAH), respectively. In asthma T_{Helper}-2 immune cells produce angiogenic factors that mobilize and recruit pro-inflammatory and pro-angiogenic precursors from the bone marrow into the airway wall where they induce angiogenesis and fuel inflammation. In contrast, in PAH, upregulation of hypoxia-inducible factor (HIF) in vascular cells leads to the production of bone marrow-mobilizing factors that recruit pro-angiogenic progenitor cells to the pulmonary circulation where they contribute to angiogenic remodeling of the vessel wall. This review focuses on current knowledge of pro-angiogenic progenitor cells in the pathogenesis of asthma and PAH.

Keywords

angiogenesis; progenitors; endothelium; lung; asthma; pulmonary arterial hypertension

Introduction

In recent years, neovascularization and dysregulation of angiogenesis have been shown to be important contributors to many diseases, including cancer, cardiovascular disease, HIV, and diseases of the pulmonary and bronchial circulations [1–3]. In most areas, the vasculature is primarily quiescent, with very low rates of replication [1]. However, under the influence of factors such as inflammation, vascular injury, hypoxia, and oxidative stress, vessels respond by mobilizing pro-angiogenic cells to sites of remodeling as well as activating local endothelial cells to proliferate [4,5]. These cells, which originally were termed in the literature as “endothelial progenitor cells” (EPC), are actually myeloid cells and interact with the local endothelial cells in a synergistic way to promote vessel formation. EPC are important in vascular homeostasis in both health and disease, including contributing to repairing endothelial injury and neovascularization[4,5]. Because of these critical roles,

identifying these progenitor cells and elucidating the mechanisms by which they exert their putative effects has broad implications for understanding and treating human disease.

Vascular degenerative disorders such as ischemic cardiovascular disease[6–12], cerebrovascular disease[13,14] and diabetes[15–17] are associated with reduced function of circulating pro-angiogenic hematopoietic cells, which are believed to have protective effects on the vasculature in these diseases. In conditions of pathological vascularization, such as, tumor proliferation[1,18] and macular degeneration[19–22], increased numbers of pro-angiogenic progenitors in the blood contribute to the angiogenic remodeling. Whether circulating angiogenic cells are protective or harmful to the lung vascular bed depends on the pathological condition. In conditions characterized by endothelial degeneration such as chronic obstructive pulmonary disease (COPD) [23–25] acute lung injury (ALI) [26–29] and emphysema[30,31], low levels of circulating pro-angiogenic progenitors correlates with poor disease outcome and pro-angiogenic progenitors have been suggested to protect endothelial cell integrity and repair endothelial injury. Asthma and pulmonary arterial hypertension (PAH), typified by angiogenic remodeling of the bronchial and pulmonary vascular bed, respectively, are associated with increased levels of circulating proangiogenic progenitors [2,32–35]. In these diseases, pro-angiogenic cells (and fibrocytes)[28] are believed to exert over-repairing and pro-inflammatory functions.

Nomenclature

Endothelial progenitor cells were initially described by Asahara and colleagues as a circulating cell with the ability to differentiate into endothelial cells and participate in post-natal vasculogenesis, a process defined by de novo formation of blood vessels through the differentiation of stem cells into endothelial cells [36]. The term EPC has since been used to broadly describe a heterogeneous group of circulating cells that putatively have the capacity to give rise to endothelial cells in vitro or in vivo [5] (Table I). However, conclusive evidence a circulating progenitor cell *differentiates* into endothelial cells during post-natal life is lacking. Instead, the use of the term EPC in the current literature encompasses mainly two categories of cell types: a heterogeneous population of hematopoietic cells that display crucial paracrine angiogenic activity as well as a population of endothelial cells that proliferate to form new blood vessels[4,5,37,38]. Because both of these cell types have been demonstrated to be involved in angiogenesis, i.e. the formation of new blood vessels by sprouting of pre-existing blood vessels, it has been proposed that the former cell type be denoted “pro-angiogenic hematopoietic cell” and the latter cell type as “endothelial colony forming cells” (ECFC) [4]. Pro-angiogenic hematopoietic cells include mature blood cells, such as monocytes, as well as subsets of hematopoietic progenitors with potent angiogenic activities.

Origin and function of pro-angiogenic hematopoietic cells and endothelial colony-forming cells

Common origin of pro-angiogenic hematopoietic cells and ECFC: the hemangioblast

A common origin for hematopoietic and endothelial cells was proposed by Florence Sabin as early as 1917 [39]. Years later, the term “hemangioblast” was subsequently coined to describe a cell which could give rise to hematopoietic and endothelial precursors. Hemangioblasts are well-documented in embryologic development as a bipotent mesodermal stem cell that differentiates into both hematopoietic and endothelial cells. Growing evidence indicate that the hemangioblast persist during adult life as a subpopulation of cells expressing CD34 and CD133 in the bone marrow and uterus [40,41]. As will be subsequently discussed, pro-angiogenic hematopoietic cells and ECFC interact during physiological and pathological vascular remodeling. This post-natal interdependence

is most probably rooted in the common developmental origin of the vascular and hematopoietic systems.

Identification of pro-angiogenic hematopoietic cells

Current evidence shows that Asahara's original work identified a circulating pro-angiogenic hematopoietic progenitor cell [36,42]. These cells were demonstrated to temporarily engraft into injured vessels and form endothelial-like cells in culture [36,43]. The presence of different numbers or impaired function of pro-angiogenic hematopoietic cells in different disease states supports their role as mediators of vascular health. They have, for instance, been demonstrated to correlate with degree of cardiovascular disease, severity of pulmonary hypertension, cancer, and diabetes [44,45] [6–12,15,32]. They have been demonstrated to be derived from the marrow and home in to sites of vascular remodeling [6–12]. Furthermore, preliminary trials have demonstrated a possible therapeutic role for these cells in cardiovascular disease [6–12].

Since Asahara's work, circulating pro-angiogenic hematopoietic cells have been defined using a variety of identification methods, including cell surface marker combinations, functional assays, and colony-forming assays [46]. Different combinations of CD34, CD133, and VEGFR2 (KDR in humans, Flk-1 in mice) have comprised three of the most commonly used markers used to identify circulating pro-angiogenic hematopoietic progenitor cells by flow cytometry [5]. These cells have also been shown to express endothelial markers (CD31, Tie2, E-selectin) as well as pan-hematopoietic surface antigen CD45. Functionally, pro-angiogenic hematopoietic cells possess properties classically expected of endothelial cells, including adherence to fibronectin plates, uptake of acetylated low-density lipoprotein (AcLDL) and binding of the lectin *Ulex Europaeus* agglutinin-1 (UEA-1), but no permanent incorporation in the endothelium. Pro-angiogenic hematopoietic progenitors are identified by their additional ability to form colonies in culture using in vitro colony-forming assays. In these assays CD34⁺CD133⁺ mononuclear cells derived from peripheral blood are plated on fibronectin coated plates and assessed for the formation of colonies following a purification step to remove contaminating monocytes, endothelial cells, and platelets. Pro-angiogenic hematopoietic progenitor cells that form colonies after 7–10 days are dubbed “colony-forming unit, Hill (CFU-Hill),” also described as early outgrowth cells or Colony Forming Unit, Endothelial Cell (CFU-EC) [6]. These colonies are heterogeneous and contain also angiogenic T-cells in addition to CD34⁺CD133⁺ cell derived myeloid progenitor cells [47,48]. The assay is now a commercial kit used to identify circulating pro-angiogenic hematopoietic cells. In contrast, mature pro-angiogenic hematopoietic cells don't form colonies but instead form a layer of non-proliferative cells on fibronectin coated plates [46]. CFU-Hill cells thus represent a subpopulation of CD34⁺CD133⁺ circulating cells which form colonies in vitro and share several features with endothelial cells.

Pro-angiogenic hematopoietic cells are not endothelial but plastic myeloid cells

Because they exhibited many of the same functional properties as endothelial cells, expressed classic endothelial cell markers, and possessed an endothelial phenotype in vitro, many groups originally believed that pro-angiogenic hematopoietic cells were endothelial progenitor cells. However, subsequent analysis of these cells revealed that they were in fact hematopoietic cells, primarily myeloid cells, that were derived from the bone marrow [4,42,47,48]. Furthermore, despite exhibiting some properties of endothelial cells, pro-angiogenic hematopoietic cells are unable to pass more rigorous tests of endothelial cell function. CFU-Hill cells lack the capacity to independently form capillary-like structures in vitro (via tube formation assay) or in vivo as assessed by vessel formation in a collagen gel implanted in immunodeficient mice [49].

ECFC are proliferative endothelial cells

In contrast to pro-angiogenic hematopoietic cells, ECFC, also known as late outgrowth endothelial cells, or blood outgrowth endothelial cells, give rise to true endothelial cells. ECFC were identified by Ingram and colleagues using a single cell in vitro colony-forming assay [37,50]. It is currently unclear if this single cell is a unipotent or multipotent stem cell or just a fully differentiated endothelial cell with high proliferative potential. Unlike pro-angiogenic hematopoietic cells, ECFC cannot yet be identified by a specific set of cell surface markers and cannot be enumerated by flow cytometry. However, they have been shown to stain positively for endothelial cell markers, such as CD34, CD146, CD31, Flk-1 and CD105 [37]. Notably, they do not express CD133 nor CD45 as pro-angiogenic hematopoietic cells do, and thus are not hematopoietic cells [37]. Unlike pro-angiogenic hematopoietic cells, ECFC have been demonstrated to meet more rigorous tests of endothelial cell characteristics and function. They are able to form functional vessels that inosculate with the murine circulation when implanted in immunodeficient mice, thus providing evidence that they become structural cells of the endothelium in mature blood vessels [49].

ECFC can be found in the endothelium, but are rare in the circulation, and have significant proliferative potential [4]. They have been isolated from endothelial cells from several tissues such as the umbilical vein, human aorta, and pulmonary artery. However it has been documented that in the circulation only 1 in 10^8 plated human mononuclear cells is an ECFC. Whether circulating ECFC are actively mobilized or passively sloughed off due to sheared stress remain to be determined. ECFC also possess significant proliferative potential, and have been shown to achieve over 100 population doublings [37,50]. ECFC function is altered in some disease processes, for example impaired angiogenic tube formation by ECFC has been documented in diabetes [51] and PAH [52], but there is much work ahead to understand the mechanisms via which ECFC affect health and disease.

Pro-angiogenic hematopoietic cells and ECFC participate synergistically in angiogenesis

Given that both ECFC and pro-angiogenic cells have independently demonstrated potential to participate in angiogenesis, the question arose as to whether and how these cells interact to repair or remodel blood vessels. Yoon et al demonstrated that CFU-Hill and ECFC in fact participate synergistically in neovascularization [43]. In a mouse hind limb ischemia model as well as in Matrigel plug model, both these types of cells enhanced angiogenesis, but injecting of a mixture of both populations of cells yielded the most robust angiogenic response [43]. Pro-angiogenic hematopoietic cells express greater quantities of pro-angiogenic cytokines, such as hepatocyte growth factor (HGF), VEGF, IL-8, and G-CSF [43,48,53]. These cytokines are potent mobilizers of hematopoietic cells, and VEGF and HGF have positive effects on endothelial cell growth and survival, and induce paracrine release of growth factors in tissues. Ex vivo expanded CFU-Hill cells also incorporate temporarily into newly formed vessels in vivo, and studies in animal cancer models suggest that they form a temporary endothelium in the early stages of angiogenesis[54], most probably by vascular mimicry [55]. CFU-Hill cells likely thus exert their pro-angiogenic effects through paracrine interactions with ECFC which most likely respond to pro-angiogenic chemokines secreted by mobilized pro-angiogenic hematopoietic cells [56].

Lung Circulations

The majority of work on pro-angiogenic progenitors is related to the systemic vasculature. However, the pulmonary circulation is distinct from the systemic circulation. The human lung possesses a dual blood supply consisting of oxygenated blood supplied by the systemic circulation forming the bronchial circulation and deoxygenated blood pumped to the lungs

by the right heart comprising the pulmonary circulation. The pulmonary circulation develops as individual segments by vasculogenesis on the leading tips of the dichotomously branching airways, driven by VEGF secreted by the airway epithelial cells[57]. As the airways grow, these blood vessels subsequently fuse to expand the pulmonary vascular network. Alveolar capillaries, where the gas exchange occurs, sprout from this network by angiogenesis. The bronchial circulation is formed mainly by angiogenesis from the dorsal aorta [57] [58]. Under normal conditions, about 5% of the total blood volume to the lungs is supplied by the bronchial circulation, which vascularizes the airway wall and the large pulmonary vessels [2]. While the bronchial and pulmonary circulations are often thought of as separate, functional connections exist between the two systems. The bronchial circulation gives rise to the vasa vasorum of the pulmonary arteries and thus provides the blood supply that nourishes the vessels of the pulmonary circulation[59,60]. Anastomoses can arise between both circulations, and this is particularly pronounced in several cardiac and pulmonary disease states. Enlargement of the bronchial circulation is a hallmark of some congenital heart diseases, as well as in thromboembolic pulmonary vascular disease and bronchopulmonary dysplasia [59]. Thus pulmonary vascular stenosis and elevated pulmonary artery pressures are related to proliferation and dilation of the bronchial vessels, establishing a link between pulmonary vascular disease and bronchial angiogenesis.

Remodeling of the Bronchial Circulation

Recent work has demonstrated that disturbances of bronchial angiogenesis are characteristic for diseases of the airway such as asthma and COPD. Asthma is characterized by T_{Helper}-2 type inflammation and hyperreactivity of the small airways, resulting in episodic attacks that are triggered by stimuli including allergens, exercise, and emotion. COPD is associated with chronic airway inflammation and alveolar loss, including chronic bronchitis, a clinically by cough and sputum production, and emphysema, characterized by alveolar loss and enlargement. This section will focus on the role of pro-angiogenic hematopoietic progenitor cells and ECFC in diseases of the bronchial circulation, and in particular on asthma, where this has been the most studied.

Increased angiogenesis and angiogenic factors in asthma

Numerous reports have noted that there is increased angiogenesis of the bronchial circulation in asthma. Dunnill demonstrated hypervascularity of the small airways in asthma more half a decade ago. Since then increased angiogenesis in asthma has been conclusively confirmed by several other groups and related to decreased lung function and asthma severity [61–64]. The combination of increased airway vascularity and vessel permeability contributes to inner airway wall thickening resulting in airway edema and narrowing of the lumen, diminishing air flow and thus leading to the obstructive symptoms characteristic of asthma. An increase in angiogenic factors, particularly VEGF, has been associated with greater airway vascularity in asthma [65]. Bronchoalveolar lavage fluids from patients with asthma express greater quantities of potent angiogenic factors, including VEGF, angiogenin, monocyte chemotactic protein, basic fibroblast growth factor (bFGF), nerve growth factor, IL-8 and GM-CSF [2,10,33,66]. Other studies have demonstrated VEGF concentration in bronchial biopsies correlate with airway vessel density, basement membrane thickness, and mast cell quantity [67]. VEGF is also elevated in bronchoalveolar fluid of asthmatic children, and levels were inversely related to airflow[10]. COPD patients also have increased airway vascularity albeit to a lesser extent than asthma patients, and more often characterized largely by vessel dilation[68]. In another model of chronic airway inflammation, the bacterium *Mycoplasma pulmonis* mouse model, TNF- α [69,70] and the angiopoietin/Tie2 pathway were shown to be involved in key aspects of bronchial vascular remodeling, including vascular permeability and capillary-to-venule transformation[71,72]. Importantly, this model also suggests that bronchial vascular remodeling exacerbates airway

inflammation by increasing influx of inflammatory cells into the airways and thickening of the airway wall due to leaky vessels.

Pro-angiogenic progenitor cells contribute to increased angiogenesis and airway inflammation

Increased angiogenic factors in asthma were long believed to be secondary to the inflammatory process and thus incidental to the airway inflammation. The identification of pro-angiogenic hematopoietic progenitor cells as mediators of angiogenesis in several disease models offered a new perspective to study angiogenesis in asthma and is shedding new light on the roles of vascular remodeling in airway inflammation. We reported increased mobilization of CD34⁺CD133⁺ pro-angiogenic progenitor cells in the circulation of patients with asthma compared to controls, as shown by flow cytometry and CFU-Hill assays [33]. These progenitors exhibited increased proliferation and angiogenic activity as assessed by in vitro tube formation assays. At the time of clinical diagnosis, asthma is already established and thus it is difficult to analyze the temporal relation between angiogenesis and inflammation. The commonly used ovalbumin model of allergic airway inflammation recapitulates the human situation of increased progenitor cell mobilization and angiogenesis, allowing us to use this model to dissect pro-angiogenic progenitor cell biology, angiogenesis and inflammation in vivo [33]. In this model pro-angiogenic progenitor cell (VEGFR2⁺c-Kit⁺Sca1⁺) mobilization and lung specific recruitment started as early as 6 hours post-allergen exposure and increased steadily over time, followed by angiogenesis in the lung within 24 – 48 hours of allergen exposure. Of note, significant recruitment of inflammatory cells, mainly eosinophils, was observed only after initiation of angiogenesis. This report provided evidence that pro-angiogenic progenitors are early responders to allergen exposure and that angiogenesis precede inflammation in the genesis of asthma. Further evidence for this was offered by Doyle and colleagues, who showed that inhibiting pro-angiogenic cell recruitment via the SDF-1 pathway inhibitor AMD3100 reduced angiogenesis in the OVA mouse model and decreased airway hyperresponsiveness [73]. In addition, a prior study by our group showed that CFU-Hill cells from patients with asthma express a higher level of eotaxin, a main eosinophil chemoattractant, and that this was also true in the OVA mouse model [74]. Taken together, these findings suggest a role for pro-angiogenic progenitor cell recruitment during angiogenesis to induce or enhance the eosinophilic response in asthma. Thus increased airway angiogenesis may be a critical part of asthma pathogenesis, perhaps by increasing migration of eosinophils into the airway, and may not be merely secondary to airway inflammation (Fig 1).

Despite these findings, questions remain. For example, the role of ECFC itself in bronchial angiogenesis is unexplored. Further work addressing the mechanisms of progenitor cell mobilization, initiation of asthmatic angiogenesis and their overall contribution to the airway remodeling and inflammation will aid in the development of novel therapeutic strategies.

Remodeling of the Pulmonary Circulation

Dysregulation of angiogenesis has also been implicated in diseases of the pulmonary circulation. This is particularly true in pulmonary arterial hypertension (PAH), a disease characterized by both increased pulmonary vascular tone and increased and aberrant pulmonary vascular remodeling. PAH is the most common disorder of the pulmonary circulation. It is defined pathologically as a panvasculopathy of the pulmonary arteries, affecting elastic, muscular, and nonmuscular arteries [75,76]. It is characterized by intimal lesions with smooth muscle hypertrophy, muscularization of normally non-muscular arterioles, and “plexiform lesions” consisting of disorganized clonal proliferation of endothelial cells [77–79]. Clinically, it is defined by resting pulmonary artery pressures greater than 25 mm Hg and includes idiopathic, familial, and acquired forms [80]. PAH has

a devastating prognosis, with eventual progression to right heart failure and death. While medical treatments for PAH have primarily aimed for reduction in pulmonary vascular tone, efforts to develop treatments that target the dysregulation in vascular remodeling remain hampered by controversies over our understanding of the mechanisms that give rise to the vascular lesions.

Upregulation of HIF contributes to endothelial cell dysfunction in PAH

Widespread endothelial dysfunction is a hallmark of PAH, including vasodilator-vasoconstrictor imbalance, a metabolic switch favoring glycolysis, and a hyper-proliferative endothelial cell phenotype [81–83]. PAH pulmonary artery endothelial cells (PAEC) display decreased apoptosis, increased proliferative capacity, and impaired ability to form organized tubes in an in vitro assay. This is particularly evident in plexiform lesions, which are comprised of disorganized clonally expanded endothelial cells. The increased proliferation is also dependent on the transcription factor STAT3, as blocking STAT3 in vitro reduced proliferation [81]. A common pathway linking several aspects of endothelial dysfunction in PAH is dysregulation of the hypoxia inducible factor (HIF) axis. HIF regulates the expression of many molecules involved in angiogenesis and mobilization of pro-angiogenic progenitor cells, including VEGF, SDF-1, Epo, HGF, and SCF [84] [82,83]. In turn, STAT3 regulates the expression of HIF, and both HIF and STAT3 are upregulated in the pulmonary endothelium and in plexiform lesions [85–87]. Dysregulation of HIF signaling and its effects on increasing angiogenesis is thus believed play a central role in the pathogenesis of PAH through contributing to increased PAEC proliferation as well as the increased production of pro-angiogenic factors.

Involvement of pro-angiogenic progenitor cells in angiogenesis in PAH

Since PAH is a disease characterized by increased vascular remodeling, elevated numbers of circulating pro-angiogenic cells in PAH patients might be expected. In animal models of hypoxic PAH circulating bone marrow-derived pro-angiogenic cells are elevated [35,88,89]. In humans, however, the data is conflicting. In patients with PAH secondary to idiopathic pulmonary fibrosis, Fadini and colleagues reported a decrease in circulating CD34⁺CD133⁺KDR⁺ cells [90]. Diller and colleagues studied patients with the idiopathic form of PAH (IPAH) as well as PAH secondary to Eisenmenger syndrome and demonstrated a decrease in circulating CD34⁺CD133⁺KDR⁺ cells in both patient populations, as well as fewer and dysfunctional CFU-Hill cells in the Eisenmenger syndrome population [91]. Interestingly, despite fewer CD34⁺CD133⁺KDR⁺ cells in IPAH patients in this study, the group found more CFU-Hill cells relative to controls. Diller et al argued that reduced levels of circulating pro-angiogenic cells in PAH may represent a reduction in the capacity to mobilize these cells to participate in vascular repair. In contrast to the above findings, studies by our group and others have demonstrated an increase in circulating pro-angiogenic cells [32,52,84]. Toshner and colleagues also reported elevated circulating CD34⁺CD133⁺KDR⁺ cells in patients with PAH compared to controls. The group also noted increased staining for c-kit (the receptor for stem cell factor) in the lungs of PAH patients, as well as SDF-1 and its receptor CXCR4, particularly in plexiform lesions, suggesting that these cells were homing to sites of vascular remodeling in the lung [52]. Using flow cytometry our group confirmed increased recruitment of CD34⁺CD133⁺ progenitors in the pulmonary artery wall of PAH patients [84]. Animal studies have demonstrated a similar targeting of the pulmonary vasculature by pro-angiogenic progenitor cells [35,92].

These discrepancies most likely reflect differences in the markers used to define pro-angiogenic cells (CD34⁺KDR⁺ cells vs. CD34⁺CD133⁺ cells) [32,91], stage of patient

disease (the presence of plexiform lesions typically denotes late disease stages), as well as by differences in flow cytometry experimental design or cell preparation techniques [93,94].

Therapeutic potential of pro-angiogenic cells

The discovery of pro-angiogenic progenitor cells as key players in vascular remodeling have made them targets of potential cellular vehicles for gene therapy in PAH. In animal models of PAH, infusion of ex vivo expanded healthy pro-angiogenic cells transfected with eNOS reduced RV systolic pressure and improved survival [95–97]. These works led to human studies of pro-angiogenic cell transplantation [98,99], demonstrating improved 6 minute walk distance and reduced pulmonary vascular resistance. However, the trial design was limited by the fact that it included only a single 12-week follow-up, lack of sham treatment, and lack of blinding [94]. These studies have now spurred a randomized controlled trial investigating eNOS transfected pro-angiogenic cells as a therapy for pulmonary hypertension (ClinicalTrials.gov Identifier: NCT00469027). The above studies demonstrate a possible role for autologous angiogenic cell transplant in the treatment of PAH and suggest that subsets of pro-angiogenic cells may have a protective application in PAH. However, since pro-angiogenic hematopoietic cells do not permanently incorporate into the endothelium, the long-term effects of such treatment are unknown.

Detrimental Potential of pro-angiogenic progenitor cells fueling pathological remodeling

Despite differences in reported pro-angiogenic hematopoietic cell mobilization, all groups have reported dysfunction of these cells. Our group has reported that CFU-Hill derived from the peripheral blood of PAH patients exhibited increased and disorganized angiogenesis in in vitro and in vivo matrigel assays[32]. Diller and others found that CFU-Hill cells were impaired in their tube-like structure forming ability [52,91]. However, whether these findings are attributable to *deficient* or *excessive* angiogenic potential of the pro-angiogenic progenitor cells remains to be determined.

Since pro-angiogenic progenitor cells are bone marrow-derived, these observations further implied that there may be a fundamental alteration in the bone marrow cells that give rise to mobilized pro-angiogenic cells. In this context, our group recently demonstrated that CD34⁺CD133⁺ progenitor cells were elevated in the marrow of PAH patients compared to healthy controls [84]. The bone marrow in PAH patients also demonstrated increased activation of the JAK-STAT pathway, including STAT3 and STAT5, and stained strongly for reticulin, suggesting a myeloproliferative component in PAH [84,100]. Furthermore, non-affected family members of the PAH patients also displayed strong marrow reticulin staining and increased numbers of CD34⁺CD133⁺ pro-angiogenic progenitor cells, suggesting that the myeloproliferative process may predispose or precede the vasculopathy in PAH [84]. One possible explanation for this is that PAH PAEC may be activating bone marrow. As mentioned above, HIF is well-documented as being elevated in PAH PAEC, and HIF-inducible bone marrow-activating and pro-angiogenic factors such as Epo, VEGF, stem cell factor (SCF), and HGF are elevated in the circulation of patients with PAH. Altogether, these recent findings suggest a role for interactions between the pulmonary vasculature and the bone marrow, rising the possibility that PAH may be a myelo-pulmonary disorder (Fig 2).

Role of Bronchial Circulation in the Remodeling of the Pulmonary Vasculature

Vaso vasorum in the pulmonary artery wall is derived from the bronchial circulation. Accumulating evidence show that this anatomical connection between the two lung circulation play a role in the vascular remodeling in PAH. CD34⁺CD133⁺c-kit⁺ progenitors, which are increased in the circulation of IPAH patients, migrate to the vaso vasorum in the adventitia of the pulmonary arteries in human IPAH[101] and hypoxic calf model [35]. It is

unclear whether adventitial remodeling and vaso vasorum expansion may actually be a contributor to further vascular remodeling or whether it is a by-product. One explanation is that due to anastomoses between the bronchial and pulmonary circulations, it is possible that increased congestion and subsequent vascular wall stretch in the bronchial circulation from elevated pulmonary pressures leads to vaso vasorum expansion. Furthermore, if vaso vasorum is a contributor to pulmonary vascular disease, rather than a consequence, we might expect adventitial remodeling to precede or at least be concurrent with medial and intimal remodeling. Hypoxia-induced pulmonary adventitial remodeling in rats and calves *does* precede medial remodeling and occurs early, but this is not the case pigs, where medial remodeling dominates early [102, 103, 104]. The chronology is unknown in humans. Nevertheless, remodeling of the vaso vasorum is a consistent finding in PAH and may provide a conduit for progenitor and inflammatory cells to participate in further vascular remodeling.

Conclusions

The endothelial progenitor cell concept has been adding new insights into vascular biology. This heterogeneous group of cells is crucial for new blood vessel formation, but none of the currently described cell types appears to be a true endothelial progenitor cell. Guidelines from international vascular biology societies would therefore be helpful to reach consensus within the scientific community about a nomenclature that fits the actual function of the cells.

There is a growing role of endothelium and pro-angiogenic hematopoietic progenitor cells in the mechanisms of lung diseases, such as asthma, acute lung injury, chronic obstructive pulmonary disease, emphysema and PAH. Further work is needed to define via which exact mechanisms pro-angiogenic progenitors and endothelial cells interact to maintain endothelial integrity and repair endothelial injury. However, emerging evidence indicate a role for pro-angiogenic progenitors beyond angiogenesis. Angiogenesis in asthma appears to depend on mobilization of pro-angiogenic progenitor cells, which are also pro-inflammatory. This new insight points towards a causal link between increased bronchial angiogenesis and airway inflammation. The role of ECFC in this process needs to be studied. Perhaps targeting angiogenesis in the future could be an avenue for treating human patients with asthma. The increased angiogenesis in pulmonary arterial hypertension appears to depend on endothelial dysfunction, with upregulation of the HIF-axis and the secretion of several HIF-inducible factors that promote angiogenesis. While endothelial injury appears to be an important component of PAH pathogenesis, recent evidence also shows an underlying myeloproliferative process that may affect pro-angiogenic progenitor cell function. Interactions between ECFC and pro-angiogenic progenitors, and how they contribute to remodeling or may promote repair, remains to be studied.

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Figure 1. Hematopoietic pro-angiogenic progenitor cells in remodeling of the bronchial circulation and airway inflammation in asthma

Inflammation and angiogenesis in the airway mucosa are cardinal features of asthma. Within hours after allergen inhalation CD34⁺CD133⁺ pro-angiogenic progenitors are mobilized from the bone marrow into the peripheral blood circulation and recruited into the airways. Interactions with the airway mucosal blood vessels induce release of pro-inflammatory cytokines, such as eotaxin, by the pro-angiogenic progenitors which attracts inflammatory cells. Release of soluble angiogenic factors by the progenitor cells and interactions with local endothelial cells induce sprouting of the bronchial blood vessels. Resultant increased vascular bed facilitates influx of inflammatory cells into the lungs. The combined effects of pro-angiogenic progenitors on angiogenesis and chemoattraction of immune cells fuels asthmatic airway inflammation. Illustration by David Schumick, BS, CMI. Reprinted with the permission of the Cleveland Clinic Center for Medical Art & Photography © 2011. All Rights Reserved.

Figure 2. Hematopoietic pro-angiogenic progenitor cells in the remodeling of the pulmonary arterial in PAH

Initial endothelial cell injury and endothelial HIF activation predispose the pulmonary endothelium to pathological proliferation. Endothelial cell-derived myeloproliferative factors activate bone marrow resident CD34⁺CD133⁺ pro-angiogenic progenitor cells, which are mobilized into the peripheral circulation. Local SDF-1 secretion by the dysregulated pulmonary artery endothelial cells attracts the pro-angiogenic progenitor cells from the circulation into the pulmonary artery wall. Pro-angiogenic progenitors may also arrive in the pulmonary artery wall via the vasa vasorum, which is derived from the bronchial circulation. Interactions between the endothelial cells and the pro-angiogenic progenitors results in pathological angiogenesis that further activates HIF-mediated release of progenitor cell mobilization and proliferation factors. This circular stimulation amplifies the aberrant endothelial proliferation and remodeling for the vascular wall. Illustration by David Schumick, BS, CMI. Reprinted with the permission of the Cleveland Clinic Center for Medical Art & Photography © 2011. All Rights Reserved.

Table I

Overview of endothelial progenitor cells.

Nomenclature	Markers	Detection Assay	Function
Hemangioblast	CD34 ⁺ C-kit ⁻ CD133 ⁺	Blast formation assay	Bipotent stem cells, differentiate into endothelial cells and hematopoietic stem cells Vasculogenesis
Pro-angiogenic hematopoietic (progenitor) cell, formerly called "endothelial progenitor cells" Early outgrowth cells	Progenitor cell antigens: CD34 ⁺ CD133 ⁺ subsets (human); Sca-1 ⁺ C-kit ⁺ subsets (murine), Tie-2 ⁺ UEA-1, AcLDL	Flow cytometry CFU-Hill assay	Temporary endothelium lining, vascular mimicry Potent paracrine angiogenic effects Angiogenesis
Endothelial Colony Forming Cell (ECFC) Late outgrowth cells	(no specific markers yet)	ECFC assays	True endothelial cells, become structural cells of endothelium Angiogenesis

UEA-1: Ulex Europaeus agglutinin-1, AcLDL: acetylated low-density lipoprotein