

Chapter 15

Lung Vascular Regeneration and Repair

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Overview of Proposed Mechanisms for Replacing or Regenerating Vascular Endothelium

Given the astounding number of publications retrieved with the search term “angiogenesis” (new vessel growth from preexisting vessels) in the Pubmed database (74,557 papers identified on November 1, 2014), it is shocking how little is known about certain fundamental properties of vascular endothelium; we do not know the baseline level of endothelial cell turnover required for blood vessel homeostasis or during vessel repair in most vertebrate organisms. A great deal is known about somatic stem cell contributions to tissue maintenance for some cell lineages such as blood, gut, and skin [1]. For example, in the adult human body, billions of circulating blood cells are replaced on an hourly basis in such an exquisite fashion that the circulating blood cell counts do not change. All of the circulating blood cells are derived from hematopoietic stem cells and downstream hematopoietic progenitor cells residing in the bone marrow compartment [2]. The adult mouse small intestinal mucosal lining is completely replaced every 3–5 days via a hierarchy comprised of intestinal stem cells and transient amplifying cells [3]. This cycle of intestinal cell turnover occurs several hundred times during the 2-year lifespan of an average laboratory mouse. Similarly, in the adult human, an estimated 100 billion intestinal epithelial cells are lost and replaced daily [4]. Finally, the entire epidermal layer of human skin is estimated to be replaced

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every 28 days [5]. These few examples, highlight the dynamic nature of cellular turnover that constitutes normal tissue homeostasis. One would naturally anticipate that the endothelial lining of the vast circulatory system feeding all of these dynamic tissues and organs must also harbor some replacement mechanism, especially since it is well recognized that circulating apoptotic and necrotic endothelial cells are present in human subjects. It is apparent that no one can yet answer some of the simple questions: (1) Do all endothelial cells possess proliferative potential or is this a heterogeneous property only displayed by some cells (as is the case for many epithelial tissues and organs)? (2) How is proliferative potential maintained in endothelial cells when mature endothelial cells comprising the vascular intimal lining must be constrained by tight inter-endothelial junctions to control barrier properties and homogeneously participate in other tissue specific functions? (3) Are there certain blood vessels that require more endothelial repair and regeneration than other vessels in the body and do they, therefore, contain endothelial cells with greater proliferative potential than those vessels requiring less repair? Compare these questions to those raised by Dr. Rudolph Altschul six decades ago; “As already mentioned, cell division of the endothelium is a difficult problem. There are several important questions which are still not definitively answered and which are ignored by many investigators. It is difficult for me to understand how the pathology and pathogenesis of arteriosclerosis can be successfully investigated as long as we do not know: (a) when the mitotic division of endothelium occurring in fetal life comes to an end, if at all; (b) how much desquamation or molting of endothelial cells occurs during life; and (c) whether or not it is correct to assume that desquamated endothelial cells are being replaced by means of direct division (of adjacent cells) [6].”

Whether endothelial cells in vessels proliferated at all was a matter of controversy prior to 1950, with some authors concluding that endothelial cells were highly proliferative in the fetal period, but then ceased to divide soon after birth and were maintained within the vessel lifelong [6]. By the 1960s, it had become well accepted that vascular endothelial cells multiply by mitosis from adjacent endothelial cells as a mechanism of repair [7–11]. In the 1970s, it became clear that a variety of physiologic stressors could influence the rate of endothelial proliferation within the aorta (age of the host, various metabolic disorders, and infection or exposure to endotoxin) [12–15]. Some evidence also emerged that not all endothelial cells lining the aorta possessed the same proliferative potential. Particularly in young rodents, some aortic endothelial cells displayed little proliferation while other areas displayed clustered foci of high replication rates approaching 60 % [16]. The question that remains elusive is to understand what factors induce the endothelial cells to begin replicating within the vessels?

One stimulus for inducing local endothelial cell proliferation was to experimentally inflict an endothelial denudation injury to a vessel. For example, careful insertion of a stainless steel wire into the femoral or carotid artery of a rodent can literally scrape and remove a narrow strip of endothelial cells and to allow assessment of the cellular events of the reparative process [13, 17, 18]. It is apparent that small injuries can be repaired simply by surviving neighboring endothelial cell migration and spreading to cover the defect, but larger injuries require proliferation of nearby

endothelial cells [19]. Use of tritiated thymidine infusion studies into rodents suggested endothelial turnover in normal tissues to be highly variable and range from a few months [20] to 47–10,000 days [21]. Several groups note that <1 % of endothelial cells are labeled with a brief pulse of tritiated thymidine [5, 22]. However, tools to assess the origin of the proliferating cells were limited in the late 1970s and 1980s and some questions remained about whether some of the repair of the damaged vessel was contributed by circulating blood-borne cells rather than the resident endothelial cells [23, 24]. Indeed, formation of endothelial cells from circulating blood lymphocytes at sites of thrombosis had been postulated as early as 1950 [25].

In 1997, Asahara and colleagues [26] reported that a subset of human circulating blood cells, some expressing CD34 (15.7 % of peripheral blood cells) and/or vascular endothelial growth factor receptor 2 (VEGFR2) (also called kinase insert domain receptor or KDR) (20 % of peripheral blood cells) displayed the ability to form clusters of round cells on fibronectin-coated dishes within 12 h of plating. Spindle-shaped adherent cells emerged from the base of the clusters and these cells possessed the capacity form cellular networks and tube-like structures. These spindle-shaped cells displayed limited expression of hematopoietic antigens CD45 (27.2 %), but increasing amounts of “endothelial” antigens such as CD34 (32.8 %), CD31 (71.5 %), Tie2 (54.6 %), and general uptake of the lectin *Ulex Europaeus* Agglutinin 1 (UEA1) upon in vitro culture in the presence of vascular endothelial growth factor (VEGF) and serum. Injection of a fraction of human blood cells (isolated by magnetic bead isolation of CD34⁺ cells) into athymic nude mice that had undergone unilateral hind limb femoral artery excision revealed localization of the human cells into the capillary bed of the muscle of the ischemic limb in areas of neovascularization. The authors termed these blood cells progenitor endothelial cells and proposed a number of potential uses for these cells as vehicles for treating human disease states [26]. With great speed, the field of “endothelial progenitor cell” biology grew from a few hundred papers in 1997 to a recent total of 15,568 cited works (keyword: endothelial progenitor cell, Pubmed, November 8, 2014). A new paradigm arose that bone-marrow-derived circulating endothelial progenitor cells were recruited to sites of vascular injury or repair and these cells differentiated into replacement endothelial cells that formed a portion of the neovasculature via a process of postnatal vasculogenesis (reviewed in [27–29]).

A critical reevaluation of the methods to identify and classify the circulating endothelial progenitor cells has revealed that the vast majority of these EPCs represent various subsets of proangiogenic hematopoietic stem and progenitor cells (HSPC) that are not direct precursors of any endothelial cells [30–32]. The HSPC are recruited to the site of vessel injury and repair to provide paracrine support to initiate or augment local resident endothelial cell migration and proliferation to provide for the vascular repair (Fig. 15.1). While HSPC may adhere to the exposed vascular basement membrane (or to adherent platelets), the HSPC do not reprogram into endothelial cells (change their fate from blood to endothelium) that provide long-term repair of the endothelial intima [31]. Proof of the role of the local resident endothelial cells in providing the endothelial reparative function has been provided by the use of a number of transgenic murine model systems

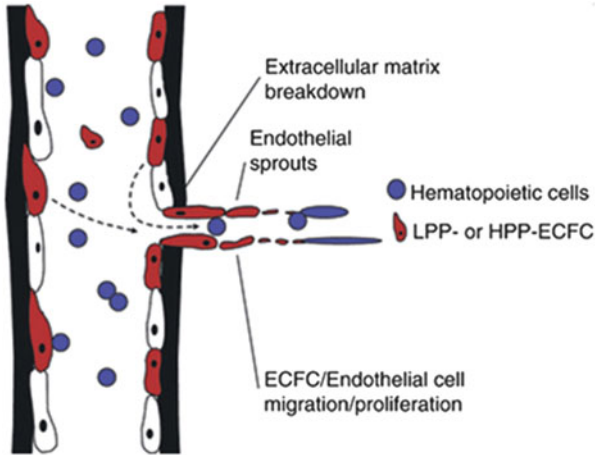


Fig. 15.1 Circulating and resident cells involved in neovascularization. This figure depicts the rare circulating low proliferative potential (LPP) and high proliferative potential endothelial colony forming cells (HPP-ECFC) that may become the new vessels at neovascularization sites. More abundant circulating hematopoietic cell subsets modulate the initiation, recruitment, and formation of the new vessels via stimulation of the circulating and resident ECFC

[33–37]. For example, Purhonen et al. [38] used chimeric mice reconstituted with fluorescently-labeled bone marrow cells to demonstrate that no bone-marrow-derived endothelial or endothelial progenitor cell contributes to repair or incorporation into the site of tumor neovascularization upon implantation of cancer cells. However, evidence was presented for robust recruitment of bone marrow labeled cells to the perivascular site of endothelial repair and some cells differentiate into periendothelial macrophages (an expected hematopoietic derivative). One recent review [39] highlights the many approaches used to present evidence for and against the direct conversion of marrow-derived endothelial progenitor cells into endothelial cells at sites of vascular injury or disease. The preponderance of evidence does not support the contribution of circulating bone marrow-derived endothelial progenitor cells into the endothelium regenerated in injured arterial vessels, rather, arterial endothelial injuries appear to be repaired by migration and proliferation of local resident endothelial cells. For example, transgenic mice prone to develop atherosclerosis were transplanted with bone marrow cells expressing a fluorescence reporter and no bone-marrow-derived circulating cells were shown to contribute to the endothelium covering the site of plaque development in the aorta of affected mice [40] (Fig. 15.2). Similarly, re-endothelialization of a denuded segment of the carotid artery (induced by wire insertion) did not occur through recruitment of circulating bone-marrow-derived cells expressing a fluorescence reporter [37], but rather through migration and possible proliferation of resident endothelial cells from adjacent non-injured endothelial intima [33–35]. This recent review concludes that the paradigm of the 1970s stating that injury to arterial vascular endothelium is repaired by migrating and proliferating local resident endothelium remains valid and without contribution of circulating cells [39].

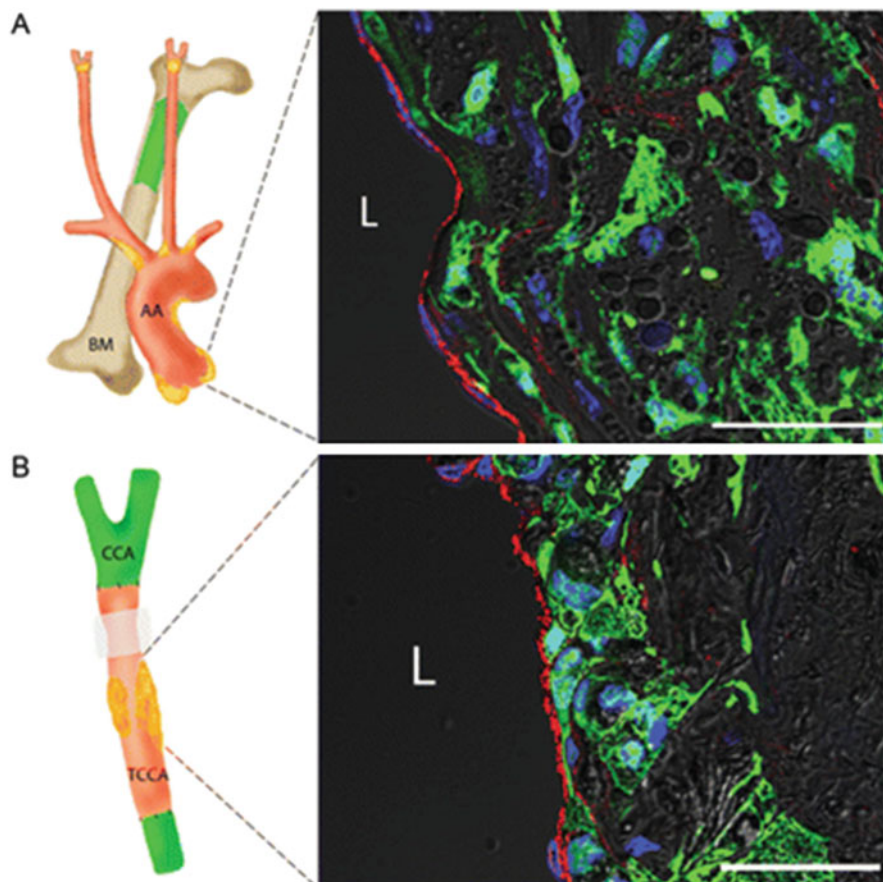


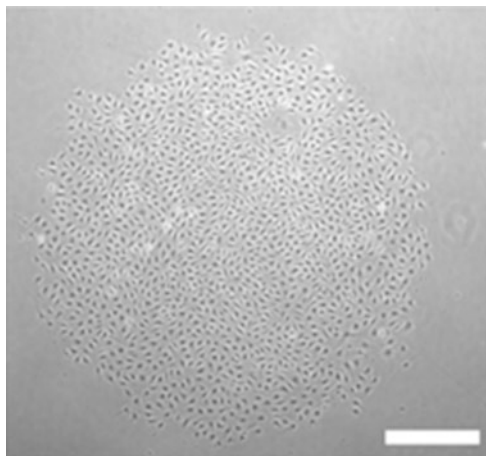
Fig. 15.2 Plaque ECs were derived from the local arterial wall. (a) Experiment to investigate whether or not bone marrow (BM)-derived endothelial progenitor cells (EPCs) contribute to plaque ECs during atherogenesis. An aortic root plaque from an $\text{apoE}^{-/-}$ mouse transplanted with BM from an $\text{eGFP}^+\text{apoE}^{-/-}$ mouse. No $\text{eGFP}^+\text{vWF}^+$ double-positive cells are present. (b) An experiment to investigate whether or not any types of blood-borne EPCs contribute to plaque ECs during atherogenesis. A common carotid artery (CCA) segment from an $\text{apoE}^{-/-}$ mouse was orthotopically transplanted into $\text{eGFP}^+\text{apoE}^{-/-}$ mice (isotransplantation except for the eGFP transgene). None of the vWF^+ cells is eGFP^+ . Green indicates eGFP ; red vWF ; blue nuclei; gray DIC. L lumen; BM bone marrow; AA aortic arch; CCA common carotid artery; TCCA transplanted common carotid artery. Scale bars = 50 μm . Reproduced with permission from Hagensen et al. [39]

In contrast, it has been known for more than 50 years that rare viable circulating endothelial cells (CECs) are present in the blood stream of goats, baboons, dogs, pigs, and human subjects and that these CECs can function to form an intraluminal endothelial lining covering implanted Dacron materials or intravascular devices [41]. Of interest, the circulating blood cells in young pigs also contain fibroblast and

smooth muscle cells that form an adventitial subendothelial network on Dacron materials suspended in the flowing blood in the thoracic aorta. A variety of factors appear to play important roles in determining whether a large implanted device will develop an intact coating of endothelial cells and these include, (1) host species, (2) age of host, (3) device surface composition, and (4) dimensions of the device (reviewed in [42]).

The CEC present in adult peripheral and umbilical cord blood display heterogeneity in clonal proliferative potential [43, 44]. Given the ability of the CEC to generate clonal colonies of endothelial cells, these cells have been called endothelial colony forming cells (ECFC) (Fig. 15.3). Cord blood and adult blood ECFC display similar cell surface antigen expression of proteins common to all endothelial cells, fail to express blood markers such as CD45 or CD14, and form human blood vessels that integrate with the host circulation upon transplantation into immunodeficient mice [30, 45–49]. Differences in expression of telomerase and maintenance of telomeres exists between cord blood and adult peripheral blood ECFC, consistent with significantly greater population doublings achieved by cultured cord blood ECFC before reaching replicative senescence. No unique identifying marker has yet been found to identify the ECFC within the human blood cells to permit prospective isolation, though all of the viable ECFC present in cord blood are present within a subset of cells with the phenotype $CD34^+CD31^+CD146^+CD45^-CD133^-$ [50]. It is unclear what role the circulating ECFC play within the human vasculature, however, recent studies implicate a role for the *in vivo* selectin-mediated adhesion of circulating human ECFC at the site of thrombus stabilization via recruited neutrophils. This neutrophil and ECFC interaction activates the proangiogenic functions of the ECFC and may be involved in vessel repair [51]. The number of circulating ECFC has been shown to highly correlate with the severity of coronary artery stenosis in symptomatic patients after adjusting for age, gender, cardiac risk factors, left ventricular ejection fraction, angiotensin-converting enzyme inhibitor or statin use [52]. The presence of higher

Fig. 15.3 Representative photomicrograph of endothelial colony. Colony formed 7 days after adult and cord blood-derived endothelial cells were plated at low cell density ($\times 50$ magnification). Scale bar represents 100 μm



concentrations of circulating ECFC is associated with reduced microvascular obstruction after acute myocardial infarction, leading to reduced infarct size and less left ventricular remodeling in patients compared to those patients in whom circulating ECFC could not be isolated [53]. The circulating concentration of ECFC has also been documented to increase tenfold following an acute myocardial infarction in human subjects [54], but a comparison of patient outcomes based upon the extent of the acute mobilization of these cells during myocardial infarction has not yet been conducted. These data all suggest a relationship between the presence of myocardial ischemic protection and circulating ECFC concentrations in human subjects. Of interest, the source of the circulating ECFC remains unknown.

Whereas circulating apoptotic and necrotic endothelial cells have long been thought to be sloughed vascular endothelial cells that increase in most cardiovascular diseases and cancer [55, 56], the specific site and mechanisms for generating viable ECFC remain obscure [42]. Duong et al. [57] have reported that directly plating of pulmonary artery endothelial cells (PAEC) isolated from vessels from patients with pulmonary hypertension revealed significantly greater number of clonogenic ECFC from the patient samples than from control subject lung vessels. They concluded that patient-derived PAEC contain significantly more ECFC that display greater proliferative potential than control subject PAEC. We have also identified ECFC resident in the endothelium isolated from umbilical cord and aortic endothelium of human subjects [58] that display high proliferative clonogenic potential. In human subjects with peripheral arterial disease, circulating ECFC display diminished proliferative potential that is matched by diminished ECFC numbers and proliferative potential by resident arterial samples [59]. Lin et al. [44] identified the most proliferative circulating ECFC to arise from the donor bone marrow tissue in patients that had undergone a sex-mismatched bone marrow transplant. Whether the ECFC were derived from vessels contained in the marrow tissue or from the hematopoietic cells that reconstituted the host subjects has not been clarified.

In sum, the strength of evidence supports an important role for resident local vascular endothelial cells to participate in repair of experimentally and genetic-induced endothelial injury or disruption in rodent models and that process is facilitated via paracrine molecules provided by circulating proangiogenic hematopoietic cells (previously called endothelial progenitor cells) recruited from the bone marrow. There is limited evidence for circulating endothelial cell contribution to vascular injury models in mice or rats, however, in larger mammalian species, substantial evidence implicates the presence of viable circulating endothelial cells that can cover intravascular implanted devices and circulating ECFC play a role in protection of the cardiac microcirculation following an acute infarction that promotes better patient outcomes.

Vascular Regeneration in Post-pneumonectomy Lung Growth

It has been recognized for more than a century that surgical removal of lung tissue in numerous animal species is associated with compensatory lung growth by the remaining lung tissue (reviewed in [60]). While compensatory growth was successfully demonstrated in dogs and rabbits in 1881, attempts to successfully remove lung tissue in human subjects failed to lead to successful outcomes until the 1930s (reviewed in [61]). Whether or not the human lung responds to lung resection with compensatory growth appears to be related to many factors including age. Several reports have documented long-term increases in lung volume (without an increase in residual volume) indicative of new alveolar growth following pneumonectomy [62–64]. The debate over whether the human adult lung can undergo compensatory growth following pneumonectomy remains controversial (reviewed in [60]). Continued advances in studying the mechanisms leading to compensatory growth in animal model systems have provided important understanding for how the host responds to loss of lung tissue with changes in cardiac and respiratory physiology, while advances in respiratory support and numerous surgical tools and approaches have steadily improved outcomes in this challenging operation.

Many physiologic factors are known to regulate the compensatory growth of the lung to pneumonectomy in animal model systems. These variables include the species, sex, and age of the animal tested [65, 66]. The impact of stretch on the remaining lung is known to be a major factor in the extent of compensatory lung growth observed post-pneumonectomy [67, 68]. Since the entire cardiac output is diverted into the remaining lung post-pneumonectomy, significant changes in pulmonary blood flow also impact the compensatory growth response [69, 70]. The specific roles played by the numerous cell types that comprise the lung in the realveolarization process that exemplifies compensatory lung growth have only recently begun to be elucidated.

Since sprouting angiogenesis is a prominent feature of the pulmonary capillary bed during normal lung development [71–73] and there is a parallel increase in the number of endothelial cells and the nearly 30 % increase in alveoli post-pneumonectomy [74], numerous authors have considered that angiogenesis may play a prominent role in post-pneumonectomy lung growth [75–77]. It is well known that a host of angiogenic growth factors are elevated in the lungs of animals post-pneumonectomy [78–82]. In fact, there are several waves of changes in gene expression that occur in the capillary bed post-pneumonectomy that may drive the angiogenic response [83].

Ding et al. [84] have provided recent analysis of some of the cellular and molecular mechanisms involved in compensatory lung growth following pneumonectomy in mice. Within 2 weeks of removal of the left lung, a 1.5-fold increase in right lung weight and 1.8-fold increase in right lung volume was observed. A significant increase in proliferation was documented in Clara cell secreted protein (CCSP) expressing bronchoalveolar stem cells (BASC) as early as day 3 post-

pneumonectomy prior to any change in proliferation of type II alveolar epithelial cells (AECIIs) or pulmonary capillary endothelial cells (PCECs). However, proliferation in the AECIIs and PCECs is significantly increased by day 7, such that the proliferating PCECs constitute 7 % of the total lung mononuclear cells. By day 15 post-pneumonectomy, both AECIIs, which are known to be precursors of the type 1 AEC, and PCECs are increased threefold over sham control populations. Since growth activated endothelial cells are known to secrete a variety of angiocrine factors that can promote tissue repair and regeneration [85, 86], Ding et al. examined whether PCEC displayed evidence of VEGFR2 or fibroblast growth factor receptor 1 (FGFR1) activation. Though the overall level of VEGFR2 was not increased in PCEC, VEGFR2 phosphorylation was significantly increased on days 1–9 post-pneumonectomy, while FGFR1 was increased on days 3–11 post-pneumonectomy. These results implicated activation of these growth factor receptors in the PCEC as a potential key element in the PCEC expansion that occurred during lung regrowth. When VEGFR2 and/or FGFR1 were subsequently deleted specifically in endothelial cells in transgenic mice, left lung pneumonectomy failed to increase proliferation in the BASC population of the right lung at day 3 post-pneumonectomy, completely abrogated proliferation in the AECII and PCEC in the remaining lung at day 7 and beyond, and severely diminished restoration of lung weight and total lung volume in the right lung post-pneumonectomy. These results suggested that the growth factor-stimulated PCEC may be playing a significant role in the realveolarization of the lung post-pneumonectomy. In a series of additional studies the authors provided evidence that the PCEC of the regenerating right lung secreted high concentrations of matrix metalloproteinase 14 (MMP14), that PCEC MMP14 enhanced proliferation of AECII cells and BASC in a cell–cell contact interaction, and that inhibition of PCEC MMP14 with a blocking antibody *in vivo* blocked alveolarization in the remaining lung post-pneumonectomy. The specific mechanism through which MMP14 stimulated alveolarization appeared to be via unmasking of cryptic epidermal growth factor (EGF)-like ligands (from cleaved laminin matrix molecules) that stimulated the epithelial regrowth via the EGF receptor. Indeed, intravenous infusion of recombinant EGF restored alveogenesis post-pneumonectomy even in transgenic mice lacking both VEGFR2 alleles and one FGFR1 allele in PCEC. Of interest, intravenous infusion of lung PCEC, but not liver sinusoidal endothelial cells, from wild-type littermate controls also rescued alveogenesis and lung regrowth post-pneumonectomy in transgenic mice lacking PCEC expression of VEGFR2 and FGFR1. Ding et al. concluded that pneumonectomy induces a pulmonary specific activation of PCEC in the remaining lung that results in release of specific angiocrine factors that promote alveogenesis from BASC and AECII precursors and this collective endothelial–epithelial interaction leads to compensatory lung growth in adult mice (Fig. 15.4).

Other investigators have focused on understanding the different patterns of neovascularization observed in the remaining lung following pneumonectomy. New blood vessel growth can occur via sprouting angiogenesis from preexisting vessels or by non-sprouting intussusceptive angiogenesis [87]. During intussusceptive vessel remodeling, the opposing walls of the vessel are bridged together and

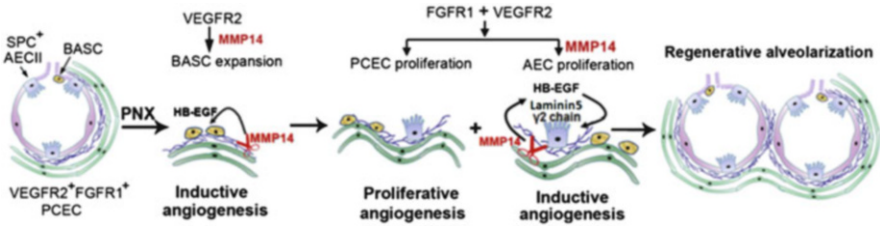


Fig. 15.4 Proposed model illustrating the inductive role of VEGFR2 and FGFR1 primed PCECs in lung regenerative alveolarization. Upon left pneumonectomy, activation of VEGFR2 in PCECs leads to MMP14 production and HB-EGF release to stimulate the expansion of epithelial progenitor cells (BASCs and AECIIs). Subsequent activation of FGFR1 along with VEGFR2 stimulates proliferation of PCECs maintaining MMP14 expression. MMP14 unmasks cryptic EGFR ligands through shedding of HB-EGF and cleaving laminin5 γ 2 chain, which by activating EGFR induce proliferation of SPC + E-cadherin + AECs. After pneumonectomy, sequential propagation of epithelial cells induced by PCEC-derived MMP14 and increase in bioavailability of EGFR ligands, culminates in full reconstitution of physiologically functional alveolar-capillary sacs. Proliferation of the PCECs mediated through VEGFR2 and FGFR1, vascularizes the regenerating lung tissue to restore the blood supply and gas exchange function. *Abbreviations:* VEGFR2 vascular endothelial growth factor receptor 2; FGFR1 fibroblast growth factor receptor 1; PCEC pulmonary capillary endothelial cells; MMP14 matrix metalloproteinase 14; HB-EGF heparin binding epidermal growth factor; BASCs basal alveolar stem cells; AECIIs alveolar epithelial type II cells; EGFR epidermal growth factor receptor; SPC surfactant protein C; E-cadherin epithelial-cadherin. Reproduced with permission from Ding et al. [84]

become sealed as a pillar that now separates the vessel into two vessels and as the new vessels mature, small holes 1–5 μ m in diameter appear in between the vessels where the pillars originally formed [88–90] (Fig. 15.5). A detailed analysis of post-pneumonectomy lung growth by scanning electron microscopy, microCT scans, synchrotron radiation tomographic microscopy, and light and transmission electron microscopy has revealed that there is asymmetric growth of the right lung when the left lung is removed in rodents with the right cardiac lobe displaying the greatest expansion into the left pleural space [75, 91]. Surprisingly, many of the features of normal lung development are observed in the regenerating lung with upfolding of new alveolar septa and a duplicated capillary bed. This duplication is caused by massive intussusceptive angiogenesis and appears to be critical for the septal alveolarization in the regenerating lung just as it is in normal lung development [91] (Fig. 15.6). These data strongly implicate the resident capillary endothelial cells as the primary agents driving the vascular reparative response. However, the capillary endothelial cells do not function in a vacuum, as there are robust increases in recruited CD11b⁺ myeloid cells, alveolar macrophages, and AECII in close apposition to the pulmonary capillary endothelial cells and these recruited cells display an increase in angiogenic growth factor secretion during this phase of angiogenesis; disruption of the recruitment of these accessory cells diminishes the angiogenic response and the extent of lung regeneration indicating an important role for these diverse cell types [91–93].

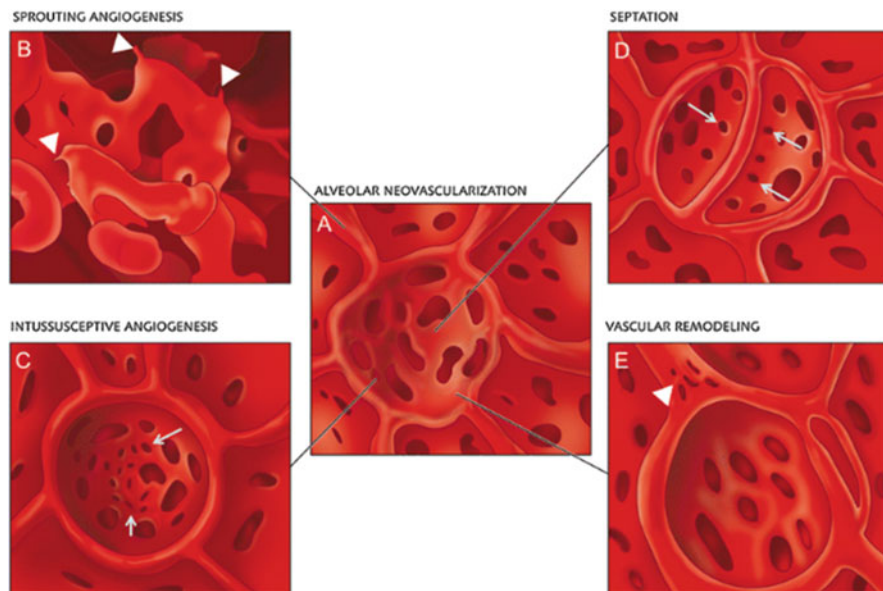


Fig. 15.5 Schematic illustration of alveolar neovascularization (a) as seen in microvascular corrosion cast replicas with sprouting angiogenesis (b), which is frequently seen subpleurally and in compact growth zones. Sprouts are evident as blind ends or protrusions (*arrowheads*). (c) Alveolar intussusceptive angiogenesis, recognizable by the presence of numerous small caliber holes with diameters between 1 and 5 μm as hallmarks of pillar formation (*arrows*). (d) The formation of new alveolar septa is accompanied by the occurrence of parallelly orientated intussusceptive pillars (*arrows*)—visible in the cast as holes—ensuring a rapid expansion of the alveolar microvascular network. Vascular remodeling also occurs on the AER vessels (*arrowheads*). Intussusceptive pillars may merge and split up the primary vessel. Reproduced with permission from Ackermann et al. [91]

Chamoto et al. [92, 94] have recently proposed that blood-borne CD34^+ endothelial progenitor cells displaying enhanced proliferative status are recruited into the pulmonary circulation post-pneumonectomy and become resident endothelial cells during the period of compensatory lung growth. Whereas only 1 % of lung endothelial cells expressed CD34 at homeostasis, a dramatic 12-fold increase in CD34^+ lung endothelial cells was detected at day 7 post-pneumonectomy and the frequency of CD34^+ endothelial cells returned to nearly normal levels by day 21 following lung resection. At day 7 post-pneumonectomy, cell division was fourfold greater in the CD34^+ lung endothelial cells than in the CD34^- lung endothelial cells. In an attempt to interrogate the contribution of blood-borne CD34^+ cells to the CD34^+ lung endothelial pool post-pneumonectomy, the authors surgically connected a wild-type mouse with a transgenic constitutive green fluorescence protein (GFP) expressing mouse via parabiosis [92, 95]. Following a 28-day recovery period, the wild-type parabiont underwent left pneumonectomy and 7 days later the regenerating right lung was analyzed for evidence of GFP^+

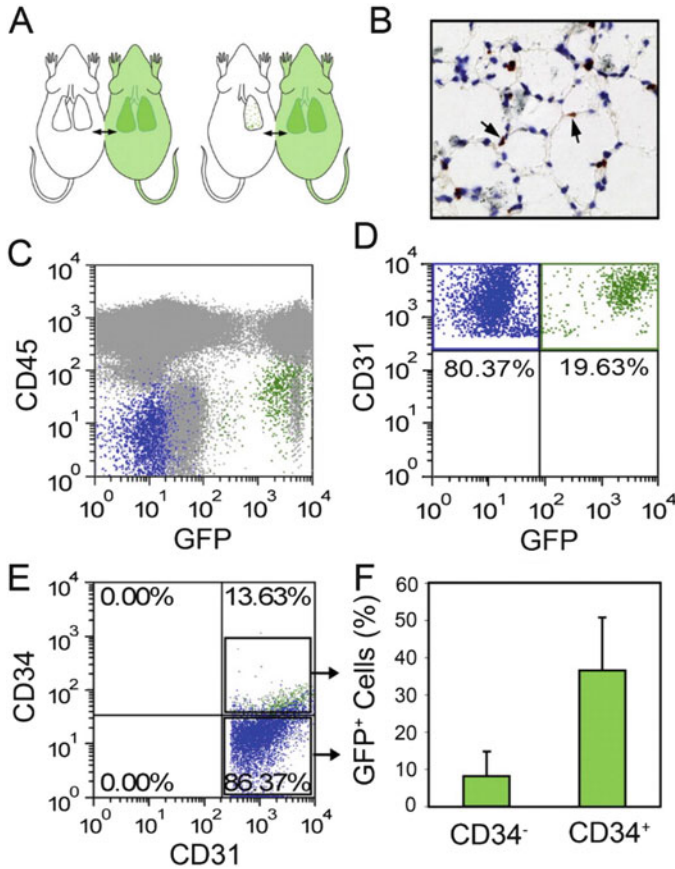


Fig. 15.6 Parabolic demonstration of blood-borne CD34⁺ endothelial cells on day 7 after pneumonectomy. (a) Schematic of experimental design involving a left pneumonectomy in the wild-type parabolic of wild-type/GFP⁺ parabolic twins (C57/B6). Parabiosis was established for 28 days prior to left pneumonectomy. The remaining lung was studied 7 days after pneumonectomy. (b) Anti-GFP avidin–biotin complex (ABC) immunohistochemical staining demonstrated GFP⁺ cells within the alveolar septae. Flow cytometry of the lung digests demonstrated GFP⁺ cells that were CD45⁻ (c) and CD31⁺ (d). (e, f) Analysis of CD31⁺ lung endothelial cells demonstrated that most of the GFP expression occurred in the CD34⁺ endothelial cell population. Reproduced with permission from Chamoto et al. [92]

blood-borne cells (Fig. 15.6). Though poorly visualized by fluorescence microscopy, GFP⁺ cells were detectable by immunohistochemistry in the alveolar septa. Approximately 11% (range 4–20%) of the CD31⁺ lung endothelial cells were GFP⁺ and more than 1/3 of the CD34⁺ endothelial cells in the lung were GFP⁺ (Fig. 15.6). Intravenous infusion of several different fluorochrome labeled lectin molecules prior to sacrifice of the animals and digestion of the lung tissue, demonstrated uniform labeling of the CD34⁺ and the CD34⁻ lung endothelial cells, suggesting integration of the CD34⁺ cells into the endothelial intima. These and

other data led the authors to conclude that blood-borne bone marrow-derived CD34⁺ EPCs were recruited and integrated into the lung circulation during the post-pneumonectomy lung growth. While the use of flow cytometry enhanced the ability of the authors to detect the presence of the dim GFP⁺ cells within the lung endothelium, this tool lacks the ability to provide anatomic localization of the cells within the vasculature. Ohle et al. [96] have previously reported that confocal microscopy analysis of putative bone marrow-derived GFP⁺ EPC contributions to injured lung has revealed that in essentially all events examined, the flat GFP⁺ cells near a vessel lumen were located in a periendothelial location and were not *bona fide* GFP⁺ endothelial cells. Since Chamoto et al. [92] showed that CD34⁺ expression increases in the lung post-pneumonectomy independent of recruited GFP⁺ cells (Fig. 15.6e), it is possible that some of the recruited CD34⁺GFP⁺ cells from the GFP⁺ parabiont may have been derived as mobilized lung endothelial cells. A direct analysis of the cells circulating in the blood of the parabionts at rest and following pneumonectomy would be required to test this hypothesis. At present the majority of published work (above) suggests that the primary endothelial cells that participate in the angiogenic response that drives neoalveolarization and lung regeneration post-pneumonectomy arise from lung resident endothelial cells.

Vascular Repair and Regeneration in Lung Injury

Abounding evidence suggest the supportive role of circulating proangiogenic hematopoietic cells in the repair of acute and chronic lung injury. In the classic model of murine acute inflammatory lung injury induced by lipopolysaccharide (LPS), there was a rapid release of putative EPCs into the circulation that contribute in concert with other bone marrow-derived progenitor cells to lung repair [97]. Transplantation of autologous putative EPCs as a therapeutic strategy attenuated endotoxin-induced lung injury in rabbits [98]. Huang and Zhao [99] elegantly demonstrated the importance of the resident local vascular endothelial cells and their interaction with circulating bone-marrow-derived progenitor cells (BMPC) to insure lung repair by using a mouse model with endothelial cell-restricted disruption of FoxM1 (FoxM1 CKO) [100]. In wild-type mice, BMPC treatment improved LPS-induced lung inflammation and survival and lead to a rapid induction of FoxM1 expression. Conversely, these effects were abrogated in FoxM1 CKO mice and BMPC treatment failed to induce lung EC proliferation suggesting that endothelial expression of the reparative transcriptional factor FoxM1 is required for the protective effects of BMPCs. Human studies also suggest a supportive reparative role of circulating putative EPCs in acute and chronic lung diseases. Patients with acute lung injury have twofold higher numbers of circulating putative EPCs than healthy control subjects, suggesting some biological role for the mobilization of these cells during lung disease [101]. Interestingly and similar to the prognostic role of EPCs in ischemic vascular diseases, improved patient survival in acute lung injury correlates with increased circulating putative EPCs [102] and severity of

illness [101]. Likewise, the number of circulating putative EPCs is significantly increased in patients with pneumonia and patients with low EPC counts tend to have persistent fibrotic changes in their lungs even after recovery from pneumonia. Likewise, the number of circulating putative EPCs is significantly increased in patients with pneumonia and patients with low EPC counts tend to have persistent fibrotic changes in their lungs even after recovery from pneumonia [103]. Decreased numbers and impaired mobilization of circulating putative EPCs have also been reported in patients with pulmonary fibrosis and chronic obstructive pulmonary disease (COPD), but remain controversial [103–105]. More interesting is the study of the function of these cells. Blood outgrowth endothelial cells (BOECs; also called ECFC) from smokers and COPD patients showed increased DNA damage and senescence as well as impaired angiogenic ability compared to nonsmokers [106]. BOECs senescence could be reversed by activating the protein deacetylase SIRT1. In summary, these data largely confirm the supportive role of circulating proangiogenic hematopoietic cells in the pathophysiology and repair of acute and chronic lung injury (though in many cases it is unclear exactly which circulating blood cell subset was comprising the EPC population evaluated).

Additional evidence for the important role of lung resident endothelial cells in lung repair comes from the literature in the developing lung. Bronchopulmonary dysplasia (BPD), the chronic lung disease that follows ventilator and oxygen therapy for acute respiratory failure after premature birth, now predominantly occurs in infants born <28 weeks gestation, is characterized by an arrest in alveolar development and decreased lung vascular growth. Borghesi et al. [107] studied ECFC in the cord blood of 98 preterm infants (gestational age <32 weeks). ECFCs in cord blood were lower in infants who later developed BPD and even though ECFCs decreased with decreasing gestational age, extremely low gestational age infants (gestational age <28 weeks) with higher numbers of cord blood ECFCs were protected from BPD. The endothelial and hematopoietic cell subsets studied by flow cytometry were comparable in infants with and without BPD and rapidly decreased after birth. Conversely, the same group showed that the percentages of circulating angiogenic cells ($CD34^+VEGFR-2^+$; $CD34^+CD133^+VEGFR-2^+$; and $CD45^-CD34^+CD133^+VEGFR-2^+$ cells) in the peripheral blood at birth, day 7 and day 28 of life in 142 preterm neonates (gestational age <32 weeks) were not able to predict the development of BPD [107]. Baker et al. quantified ECFCs and the angiogenic circulating progenitor cells (CPC)/nonangiogenic-CPC ratio (CPC/non-CPC) in cord blood samples from 62 preterm infants (24–36 weeks gestational age) [108]. ECFC number and CPC/non-CPC ratio were significantly decreased in cord blood of preterm infants who subsequently developed moderate or severe BPD. These data suggest that decreased circulating ECFCs in the fetal circulation impairs postnatal vasculogenesis thereby contributing to the severity of chronic lung disease in preterm infants.

Histological abnormalities of BPD are recapitulated in neonatal rodents exposed to chronic hyperoxia [109]. This model exhibits decreased circulating, lung and bone-marrow-derived putative EPCs [110]. Interestingly, hyperoxic adult mice did not display alveolar damage and had increased circulating putative EPCs, implying

that decreased EPCs may contribute to the arrested lung growth seen in the neonatal animals. Infusion of these bone marrow-derived angiogenic cells restored alveolar and lung vascular growth in neonatal mice exposed to hyperoxia [111]. The recent discovery in the adult rat lung of resident microvascular endothelial progenitor cells that share features of human cord blood and lung ECFCs provided an opportunity to explore the role of resident ECFCs in experimental BPD models. Alphonse et al. [112] were able to show that the developing human fetal and neonatal rat lungs contained ECFCs with robust proliferative potential, secondary colony formation, and de novo blood vessel formation in vivo when transplanted into a matrigel plug under the skin of immunodeficient mice [112]. In contrast, human fetal lung ECFCs exposed to hyperoxia in vitro and neonatal rat ECFCs isolated from hyperoxic alveolar growth-arrested rat lungs mimicking BPD proliferated less, showed decreased clonogenic capacity, and formed fewer capillary-like networks. This suggested that impaired ECFC function may contribute to explain the incapacity of lung repair and the persistent alveolar growth in BPD. It also provided the rationale to test the therapeutic potential of exogenous supplementation of human cord blood-derived ECFCs. Because of the increased toxicity of hyperoxia on ECFCs [113, 114], intrajugular administration of human cord blood-derived ECFCs was performed after established oxygen-induced arrested alveolar growth. ECFCs restored lung function, alveolar and lung vascular growth, and attenuated pulmonary hypertension in immune-deficient rats and mice. Lung ECFC colony- and capillary-like network-forming capabilities were also restored. The therapeutic benefit persisted at 10 months of age with no adverse effects and persistent improvement in lung structure, exercise capacity, and pulmonary hypertension. However, lung engraftment, similar to what has been observed with other types of cell therapies (mesenchymal stromal cells for example) was low, suggesting that the effect of these exogenous ECFCs was through a paracrine effect. Accordingly, administration of cell-free ECFC-derived conditioned media exerted similar therapeutic benefits in the hyperoxia- and bleomycin-induced models of BPD in rodents [112, 115]. Overall, these findings further support the crucial role of lung resident endothelial cells in lung repair and offers promising new therapeutic strategies to prevent/restore lung damage.

References

1. Fuchs E (2009) The tortoise and the hair: slow-cycling cells in the stem cell race. *Cell* 137(5): 811–819
2. Orkin SH, Zon LI (2008) Hematopoiesis: an evolving paradigm for stem cell biology. *Cell* 132(4):631–644
3. Barker N, van de Wetering M, Clevers H (2008) The intestinal stem cell. *Genes Dev* 22(14): 1856–1864
4. Barker N (2014) Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. *Nat Rev Mol Cell Biol* 15(1):19–33

5. Hoath SB, Leahy DG (2003) The organization of human epidermis: functional epidermal units and phi proportionality. *J Invest Dermatol* 121(6):1440–1446
6. Altschul R (1954) *Endothelium: its development, morphology, function, and pathology*. Macmillan, New York
7. Florentin RA, Nam SC, Lee KT, Thomas WA (1969) Increased 3H-thymidine incorporation into endothelial cells of swine fed cholesterol for 3 days. *Exp Mol Pathol* 10(3):250–255
8. Poole JC, Sanders AG, Florey HW (1958) The regeneration of aortic endothelium. *J Pathol Bacteriol* 75(1):133–143
9. Robertson HR, Moore JR, Mersereau WA (1959) Observations on thrombosis and endothelial repair following application of external pressure to a vein. *Can J Surg* 3:5–16
10. Stehbens WE (1965) Endothelial cell mitosis and permeability. *Q J Exp Physiol Cogn Med Sci* 50:90–92
11. Wright HP (1968) Endothelial mitosis around aortic branches in normal guinea pigs. *Nature* 220(5162):78–79
12. Caplan BA, Schwartz CJ (1973) Increased endothelial cell turnover in areas of in vivo Evans blue uptake in the pig aorta. *Atherosclerosis* 17(3):401–417
13. Prescott MF, Muller KR (1983) Endothelial regeneration in hypertensive and genetically hypercholesterolemic rats. *Arteriosclerosis* 3(3):206–214
14. Schwartz SM, Benditt EP (1977) Aortic endothelial cell replication. I. Effects of age and hypertension in the rat. *Circ Res* 41(2):248–255
15. Schwartz SM, Gajdusek CM, Reidy MA, Selden SC 3rd, Haudenschild CC (1980) Maintenance of integrity in aortic endothelium. *Fed Proc* 39(9):2618–2625
16. Schwartz SM, Benditt EP (1976) Clustering of replicating cells in aortic endothelium. *Proc Natl Acad Sci U S A* 73(2):651–653
17. Li F, Downing BD, Smiley LC, Mund JA, Distasi MR, Bessler WK et al (2014) Neurofibromin-deficient myeloid cells are critical mediators of aneurysm formation in vivo. *Circulation* 129(11):1213–1224
18. Wara AK, Manica A, Marchini JF, Sun X, Icli B, Tesmenitsky Y et al (2013) Bone marrow-derived kruppel-like factor 10 controls reendothelialization in response to arterial injury. *Arterioscler Thromb Vasc Biol* 33(7):1552–1560
19. Schwartz SM, Gajdusek CM, Selden SC 3rd (1981) Vascular wall growth control: the role of the endothelium. *Arteriosclerosis* 1(2):107–126
20. Tannock IF, Hayashi S (1972) The proliferation of capillary endothelial cells. *Cancer Res* 32(1):77–82
21. Hobson B, Denekamp J (1984) Endothelial proliferation in tumours and normal tissues: continuous labelling studies. *Br J Cancer* 49(4):405–413
22. Sholley MM, Cavallo T, Cotran RS (1977) Endothelial proliferation in inflammation. I. Autoradiographic studies following thermal injury to the skin of normal rats. *Am J Pathol* 89(2):277–296
23. Haudenschild C, Studer A (1971) Early interactions between blood cells and severely damaged rabbit aorta. *Eur J Clin Invest* 2(1):1–7
24. Schwartz SM, Stemerman MB, Benditt EP (1975) The aortic intima: II. Repair of the aortic lining after mechanical denudation. *Am J Pathol* 81(1):15–42
25. Moschowitz E (1950) Hyperplastic arteriosclerosis versus atherosclerosis. *J Am Med Assoc* 143(10):861–865
26. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T et al (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275(5302):964–967
27. Asahara T, Kawamoto A, Masuda H (2011) Concise review: circulating endothelial progenitor cells for vascular medicine. *Stem Cells* 29(11):1650–1655
28. Fadini GP, Losordo D, Dimmeler S (2012) Critical reevaluation of endothelial progenitor cell phenotypes for therapeutic and diagnostic use. *Circ Res* 110(4):624–637
29. Yoder MC (2012) Human endothelial progenitor cells. *Cold Spring Harb Perspect Med* 2(7):a006692

30. Medina RJ, O'Neill CL, Sweeney M, Guduric-Fuchs J, Gardiner TA, Simpson DA, Stitt AW (2010) Molecular analysis of endothelial progenitor cell (EPC) subtypes reveals two distinct cell populations with different identities. *BMC Med Genomics* 3:18
31. Ohtani K, Vlachojannis GJ, Koyanagi M, Boeckel JN, Urbich C, Farcas R, Bonig H, Marquez VE, Zeiher AM, Dimmeler S (2011) Epigenetic regulation of endothelial lineage committed genes in pro-angiogenic hematopoietic and endothelial progenitor cells. *Circ Res* 109(11):1219–1229
32. Rose JA, Erzurum S, Asosingh K (2015) Biology and flow cytometry of proangiogenic hematopoietic progenitors cells. *Cytometry A* 87(1):5–19
33. Hagensen MK, Raarup MK, Mortensen MB, Thim T, Nyengaard JR, Falk E, Bentzon JF (2012) Circulating endothelial progenitor cells do not contribute to regeneration of endothelium after murine arterial injury. *Cardiovasc Res* 93(2):223–231
34. Itoh Y, Toriumi H, Yamada S, Hoshino H, Suzuki N (2010) Resident endothelial cells surrounding damaged arterial endothelium reendothelialize the lesion. *Arterioscler Thromb Vasc Biol* 30(9):1725–1732
35. Nishimura R, Kawasaki T, Sekine A, Suda R, Urushibara T, Suzuki T et al (2015) Hypoxia-induced proliferation of tissue-resident endothelial progenitor cells in the lung. *Am J Physiol Lung Cell Mol Physiol* 308(8):L746–L758. doi:10.1152/ajplung.00243.2014
36. Rinkevich Y, Lindau P, Ueno H, Longaker MT, Weissman IL (2011) Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip. *Nature* 476(7361):409–413
37. Tsuzuki M (2009) Bone marrow-derived cells are not involved in reendothelialized endothelium as endothelial cells after simple endothelial denudation in mice. *Basic Res Cardiol* 104(5):601–611
38. Purhonen S, Palm J, Rossi D, Kaskenpaa N, Rajantie I, Yla-Herttuala S et al (2008) Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. *Proc Natl Acad Sci U S A* 105(18):6620–6625
39. Hagensen MK, Vanhoutte PM, Bentzon JF (2012) Arterial endothelial cells: still the craftsmen of regenerated endothelium. *Cardiovasc Res* 95(3):281–289
40. Hagensen MK, Shim J, Thim T, Falk E, Bentzon JF (2010) Circulating endothelial progenitor cells do not contribute to plaque endothelium in murine atherosclerosis. *Circulation* 121(7):898–905
41. Stump MM, Jordan GL Jr, Debakey ME, Halpert B (1963) Endothelium grown from circulating blood on isolated intravascular dacron hub. *Am J Pathol* 43:361–367
42. Yoder MC (2010) Is endothelium the origin of endothelial progenitor cells? *Arterioscler Thromb Vasc Biol* 30(6):1094–1103
43. Ingram DA, Mead LE, Tanaka H, Meade V, Fenoglio A, Mortell K et al (2000) Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 104(9):2752–2760
44. Lin Y, Weisdorf DJ, Solovey A, Hebbel RP (2000) Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest* 105(1):71–77
45. Au P, Daheron LM, Duda DG, Cohen KS, Tyrrell JA, Lanning RM et al (2008) Differential in vivo potential of endothelial progenitor cells from human umbilical cord blood and adult peripheral blood to form functional long-lasting vessels. *Blood* 111(3):1302–1305
46. Medina RJ, O'Neill CL, Humphreys MW, Gardiner TA, Stitt AW (2010) Outgrowth endothelial cells: characterization and their potential for reversing ischemic retinopathy. *Invest Ophthalmol Vis Sci* 51(11):5906–5913
47. Melero-Martin JM, De Obaldia ME, Kang SY, Khan ZA, Yuan L, Oettgen P, Bischoff J (2008) Engineering robust and functional vascular networks in vivo with human adult and cord blood-derived progenitor cells. *Circ Res* 103(2):194–202
48. Melero-Martin JM, Khan ZA, Picard A, Wu X, Paruchuri S, Bischoff J (2007) In vivo vasculogenic potential of human blood-derived endothelial progenitor cells. *Blood* 109(11):4761–4768

49. Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F et al (2007) Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 109(5):1801–1809
50. Mund JA, Estes ML, Yoder MC, Ingram DA Jr, Case J (2012) Flow cytometric identification and functional characterization of immature and mature circulating endothelial cells. *Arterioscler Thromb Vasc Biol* 32(4):1045–1053
51. Hubert L, Darbousset R, Panicot-Dubois L, Robert S, Sabatier F, Fallague K et al (2014) Neutrophils recruit and activate human endothelial colony-forming cells at the site of vessel injury via P-selectin glycoprotein ligand-1 and L-selectin. *J Thromb Haemost* 12(7):1170–1181
52. Guven H, Shepherd RM, Bach RG, Capoccia BJ, Link DC (2006) The number of endothelial progenitor cell colonies in the blood is increased in patients with angiographically significant coronary artery disease. *J Am Coll Cardiol* 48(8):1579–1587
53. Meneveau N, Deschaseaux F, Seronde MF, Chopard R, Schiele F, Jehl J et al (2011) Presence of endothelial colony-forming cells is associated with reduced microvascular obstruction limiting infarct size and left ventricular remodelling in patients with acute myocardial infarction. *Basic Res Cardiol* 106(6):1397–1410
54. Massa M, Campanelli R, Bonetti E, Ferrario M, Marinoni B, Rosti V (2009) Rapid and large increase of the frequency of circulating endothelial colony-forming cells (ECFCs) generating late outgrowth endothelial cells in patients with acute myocardial infarction. *Exp Hematol* 37(1):8–9
55. Blann AD, Woywodt A, Bertolini F, Bull TM, Buyon JP, Clancy RM et al (2005) Circulating endothelial cells. Biomarker of vascular disease. *Thromb Haemost* 93(2):228–235
56. Woywodt A, Blann AD, Kirsch T, Erdbruegger U, Banzet N, Haubitz M, Dignat-George F (2006) Isolation and enumeration of circulating endothelial cells by immunomagnetic isolation: proposal of a definition and a consensus protocol. *J Thromb Haemost* 4(3):671–677
57. Duong HT, Comhair SA, Aldred MA, Mavrakis L, Savasky BM, Erzurum SC, Asosingh K (2011) Pulmonary artery endothelium resident endothelial colony-forming cells in pulmonary arterial hypertension. *Pulm Circ* 1(4):475–486
58. Ingram DA, Mead LE, Moore DB, Woodard W, Fenoglio A, Yoder MC (2005) Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. *Blood* 105(7):2783–2786
59. Prasain N, Lee MR, Vemula S, Meador JL, Yoshimoto M, Ferkowicz MJ et al (2014) Differentiation of human pluripotent stem cells to cells similar to cord-blood endothelial colony-forming cells. *Nat Biotechnol* 32(11):1151–1157
60. Paisley D, Bevan L, Choy KJ, Gross C (2014) The pneumonectomy model of compensatory lung growth: insights into lung regeneration. *Pharmacol Ther* 142(2):196–205
61. Fuentes PA (2003) Pneumonectomy: historical perspective and prospective insight. *Eur J Cardiothorac Surg* 23(4):439–445
62. Laros CD, Westermann CJ (1987) Dilatation, compensatory growth, or both after pneumonectomy during childhood and adolescence. A thirty-year follow-up study. *J Thorac Cardiovasc Surg* 93(4):570–576
63. Stiles QR, Meyer BW, Lindesmith GG, Jones JC (1969) The effects of pneumonectomy in children. *J Thorac Cardiovasc Surg* 58(3):394–400
64. Werner HA, Pirie GE, Nadel HR, Fleisher AG, LeBlanc JG (1993) Lung volumes, mechanics, and perfusion after pulmonary resection in infancy. *J Thorac Cardiovasc Surg* 105(4):737–742
65. Khadempour MH, Ofulue AF, Sekhon HS, Cherukupalli KM, Thurlbeck WM (1992) Changes of growth hormone, somatomedin c, and bombesin following pneumonectomy. *Exp Lung Res* 18(3):421–432
66. Sekhon HS, Thurlbeck WM (1992) A comparative study of postpneumonectomy compensatory lung response in growing male and female rats. *J Appl Physiol* 73(2):446–451

67. Brown LM, Rannels SR, Rannels DE (2001) Implications of post-pneumonectomy compensatory lung growth in pulmonary physiology and disease. *Respir Res* 2(6):340–347
68. Fisher JM, Simnett JD (1973) Morphogenetic and proliferative changes in the regenerating lung of the rat. *Anat Rec* 176(4):389–395
69. Dane DM, Yilmaz C, Estrera AS, Hsia CC (2013) Separating in vivo mechanical stimuli for postpneumonectomy compensation: physiological assessment. *J Appl Physiol* 114(1):99–106
70. McBride JT, Kirchner KK, Russ G, Finkelstein J (1992) Role of pulmonary blood flow in postpneumonectomy lung growth. *J Appl Physiol* 73(6):2448–2451
71. Metzger RJ, Klein OD, Martin GR, Krasnow MA (2008) The branching programme of mouse lung development. *Nature* 453(7196):745–750
72. White AC, Lavine KJ, Ornitz DM (2007) FGF9 and SHH regulate mesenchymal Vegfa expression and development of the pulmonary capillary network. *Development* 134(20):3743–3752
73. Yamamoto H, Yun EJ, Gerber HP, Ferrara N, Whitsett JA, Vu TH (2007) Epithelial-vascular cross talk mediated by VEGF-A and HGF signaling directs primary septae formation during distal lung morphogenesis. *Dev Biol* 308(1):44–53
74. Fehrenbach H, Voswinckel R, Michl V, Mehling T, Fehrenbach A, Seeger W, Nyengaard JR (2008) Neoalveolarisation contributes to compensatory lung growth following pneumonectomy in mice. *Eur Respir J* 31(3):515–522
75. Konerding MA, Gibney BC, Houdek JP, Chamoto K, Ackermann M, Lee GS et al (2012) Spatial dependence of alveolar angiogenesis in post-pneumonectomy lung growth. *Angiogenesis* 15(1):23–32
76. Takeda SI, Ramanathan M, Estrera AS, Hsia CC (1999) Postpneumonectomy alveolar growth does not normalize hemodynamic and mechanical function. *J Appl Physiol* 87(2):491–497
77. Yan X, Bellotto DJ, Foster DJ, Johnson RL Jr, Hagler HK, Estrera AS, Hsia CC (2004) Retinoic acid induces nonuniform alveolar septal growth after right pneumonectomy. *J Appl Physiol* 96(3):1080–1089
78. Jancelewicz T, Grethel EJ, Chapin CJ, Clifton MS, Nobuhara KK (2010) Vascular endothelial growth factor isoform and receptor expression during compensatory lung growth. *J Surg Res* 160(1):107–113
79. Landesberg LJ, Ramalingam R, Lee K, Rosengart TK, Crystal RG (2001) Upregulation of transcription factors in lung in the early phase of postpneumonectomy lung growth. *Am J Physiol Lung Cell Mol Physiol* 281(5):L1138–L1149
80. Sakamaki Y, Matsumoto K, Mizuno S, Miyoshi S, Matsuda H, Nakamura T (2002) Hepatocyte growth factor stimulates proliferation of respiratory epithelial cells during post-pneumonectomy compensatory lung growth in mice. *Am J Respir Cell Mol Biol* 26(5):525–533
81. Yuan S, Hannam V, Belcastro R, Cartel N, Cabacungan J, Wang J et al (2002) A role for platelet-derived growth factor-bb in rat postpneumonectomy compensatory lung growth. *Pediatr Res* 52(1):25–33
82. Zhang Q, Bellotto DJ, Ravikumar P, Moe OW, Hogg RT, Hogg DC et al (2007) Post-pneumonectomy lung expansion elicits hypoxia-inducible factor-1 α signaling. *Am J Physiol Lung Cell Mol Physiol* 293(2):L497–L504
83. Lin M, Chamoto K, Gibney BC, Lee GS, Collings-Simpson D, Houdek J et al (2011) Angiogenesis gene expression in murine endothelial cells during post-pneumonectomy lung growth. *Respir Res* 12:98
84. Ding BS, Nolan DJ, Guo P, Babazadeh AO, Cao Z, Rosenwaks Z et al (2011) Endothelial-derived angiocrine signals induce and sustain regenerative lung alveolarization. *Cell* 147(3):539–553
85. Ding BS, Nolan DJ, Butler JM, James D, Babazadeh AO, Rosenwaks Z et al (2010) Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. *Nature* 468(7321):310–315

86. Hooper AT, Butler JM, Nolan DJ, Kranz A, Iida K, Kobayashi M et al (2009) Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. *Cell Stem Cell* 4(3):263–274
87. Carmeliet P, Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. *Nature* 473(7347):298–307
88. Burri PH, Tarek MR (1990) A novel mechanism of capillary growth in the rat pulmonary microcirculation. *Anat Rec* 228(1):35–45
89. Caduff JH, Fischer LC, Burri PH (1986) Scanning electron microscope study of the developing microvasculature in the postnatal rat lung. *Anat Rec* 216(2):154–164
90. Djonov VG, Kurz H, Burri PH (2002) Optimality in the developing vascular system: branching remodeling by means of intussusception as an efficient adaptation mechanism. *Dev Dyn* 224(4):391–402
91. Ackermann M, Houdek JP, Gibney BC, Ysasi A, Wagner W, Belle J (2014) Sprouting and intussusceptive angiogenesis in postpneumonectomy lung growth: mechanisms of alveolar neovascularization. *Angiogenesis* 17(3):541–551
92. Chamoto K, Gibney BC, Ackermann M, Lee GS, Lin M, Konerding MA et al (2012) Alveolar macrophage dynamics in murine lung regeneration. *J Cell Physiol* 227(9):3208–3215
93. Chamoto K, Gibney BC, Lee GS, Ackermann M, Konerding MA, Tsuda A, Mentzer SJ (2013) Migration of CD11b+ accessory cells during murine lung regeneration. *Stem Cell Res* 10(3):267–277
94. Chamoto K, Gibney BC, Lee GS, Lin M, Collings-Simpson D, Voswinckel R et al (2012) CD34+ progenitor to endothelial cell transition in post-pneumonectomy angiogenesis. *Am J Respir Cell Mol Biol* 46(3):283–289
95. Gibney BC, Chamoto K, Lee GS, Simpson DC, Miele LF, Tsuda A et al (2012) Cross-circulation and cell distribution kinetics in parabiotic mice. *J Cell Physiol* 227(2):821–828
96. Ohle SJ, Anandaiah A, Fabian AJ, Fine A, Kotton DN (2012) Maintenance and repair of the lung endothelium does not involve contributions from marrow-derived endothelial precursor cells. *Am J Respir Cell Mol Biol* 47(1):11–19
97. Yamada M, Kubo H, Kobayashi S, Ishizawa K, Numasaki M, Ueda S et al (2004) Bone marrow-derived progenitor cells are important for lung repair after lipopolysaccharide-induced lung injury. *J Immunol* 172(2):1266–1272
98. Lam CF, Liu YC, Hsu JK, Yeh PA, Su TY, Huang CC et al (2008) Autologous transplantation of endothelial progenitor cells attenuates acute lung injury in rabbits. *Anesthesiology* 108(3):392–401
99. Huang X, Zhao YY (2012) Transgenic expression of foxm1 promotes endothelial repair following lung injury induced by polymicrobial sepsis in mice. *PLoS One* 7(11), e50094
100. Zhao YD, Huang X, Yi F, Dai Z, Qian Z, Tirupathi C et al (2014) Endothelial foxm1 mediates bone marrow progenitor cell-induced vascular repair and resolution of inflammation following inflammatory lung injury. *Stem Cells* 32(7):1855–1864
101. Burnham EL, Taylor WR, Quyyumi AA, Rojas M, Brigham KL, Moss M (2005) Increased circulating endothelial progenitor cells are associated with survival in acute lung injury. *Am J Respir Crit Care Med* 172(7):854–860
102. Yamada M, Kubo H, Ishizawa K, Kobayashi S, Shinkawa M, Sasaki H (2005) Increased circulating endothelial progenitor cells in patients with bacterial pneumonia: evidence that bone marrow derived cells contribute to lung repair. *Thorax* 60(5):410–413
103. Malli F, Koutsokera A, Paraskeva E, Zakyntinos E, Papagianni M, Makris D et al (2013) Endothelial progenitor cells in the pathogenesis of idiopathic pulmonary fibrosis: an evolving concept. *PLoS One* 8(1), e53658
104. Smadja DM, Mauge L, Nunes H, d’Audigier C, Juvin K, Borie R et al (2013) Imbalance of circulating endothelial cells and progenitors in idiopathic pulmonary fibrosis. *Angiogenesis* 16(1):147–157

105. Brittan M, Hoogenboom MM, Padfield GJ, Tura O, Fujisawa T, Maclay JD et al (2013) Endothelial progenitor cells in patients with chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol* 305(12):L964–L969
106. Paschalaki KE, Starke RD, Hu Y, Mercado N, Margariti A, Gorgoulis VG et al (2013) Dysfunction of endothelial progenitor cells from smokers and chronic obstructive pulmonary disease patients due to increased DNA damage and senescence. *Stem Cells* 31(12): 2813–2826
107. Borghesi A, Massa M, Campanelli R, Garofoli F, Longo S, Cabano R et al (2013) Different subsets of circulating angiogenic cells do not predict bronchopulmonary dysplasia or other diseases of prematurity in preterm infants. *Int J Immunopathol Pharmacol* 26(3):809–816
108. Baker CD, Balasubramaniam V, Mourani PM, Sontag MK, Black CP, Ryan SL et al (2012) Cord blood angiogenic progenitor cells are decreased in bronchopulmonary dysplasia. *Eur Respir J* 40(6):1516–1522
109. O'Reilly MA (2012) Angiotensin II: tapping the cell cycle machinery to kill endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 303(7):L575–L576
110. Balasubramaniam V, Mervis CF, Maxey AM, Markham NE, Abman SH (2007) Hyperoxia reduces bone marrow, circulating, and lung endothelial progenitor cells in the developing lung: implications for the pathogenesis of bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol* 292(5):L1073–L1084
111. Balasubramaniam V, Ryan SL, Seedorf GJ, Roth EV, Heumann TR, Yoder MC et al (2010) Bone marrow-derived angiogenic cells restore lung alveolar and vascular structure after neonatal hyperoxia in infant mice. *Am J Physiol Lung Cell Mol Physiol* 298(3):L315–L323
112. Alphonse RS, Vadivel A, Fung M, Shelley WC, Critser PJ, Ionescu L et al (2014) Existence, functional impairment, and lung repair potential of endothelial colony-forming cells in oxygen-induced arrested alveolar growth. *Circulation* 129(21):2144–2157
113. Baker CD, Ryan SL, Ingram DA, Seedorf GJ, Abman SH, Balasubramaniam V (2009) Endothelial colony-forming cells from preterm infants are increased and more susceptible to hyperoxia. *Am J Respir Crit Care Med* 180(5):454–461
114. Fujinaga H, Baker CD, Ryan SL, Markham NE, Seedorf GJ, Balasubramaniam V, Abman SH (2009) Hyperoxia disrupts vascular endothelial growth factor-nitric oxide signaling and decreases growth of endothelial colony-forming cells from preterm infants. *Am J Physiol Lung Cell Mol Physiol* 297(6):L1160–L1169
115. Baker CD, Seedorf GJ, Wisniewski BL, Black CP, Ryan SL, Balasubramaniam V, Abman SH (2013) Endothelial colony-forming cell conditioned media promote angiogenesis in vitro and prevent pulmonary hypertension in experimental bronchopulmonary dysplasia. *Am J Physiol Lung Cell Mol Physiol* 305(1):L73–L81