Chapter 17
Vascular Repair and Regeneration by Endothelial Progenitor Cells

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Abbreviations

ALI	Acute lung injury
BMPR2	Bone morphogenetic protein receptor 2
BPD	Bronchopulmonary dysplasia
Cdc42	Cell division control protein 42 homolog
COPD	Chronic obstructive pulmonary disease
CXCR4	C-X-C Chemokine receptor type 4
ECFCs	Endothelial colony-forming cells
eNOS	Endothelial nitric oxide synthase
EPC	Endothelial progenitor cell
FoxM1	Forkhead box protein M1
HGF	Hepatocyte growth factor
IGF1	Insulin-like growth factor 1
IL10	Interleukin 10
IPAH	Idiopathic pulmonary arterial hypertension
LPS	Lipopolysaccharide

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MCP1	Monocyte chemoattractant protein-1
MSC	Mesenchymal stem cell
NYHA	New York Heart Association
PAEC	Pulmonary artery endothelial cell
PAH	Pulmonary arterial hypertension
PDE5	Phosphodiesterase 5
Rac1	Ras-related C3 botulinum toxin substrate 1
SDF1	Stromal cell-derived factor 1
VEGF	Vascular endothelial growth factor
VEGFR-2	Vascular endothelial growth factor receptor 2

17.1 EPCs

17.1.1 Discovery of EPCs

Research on vascular grafts paved the way for the description and culture of both early and late outgrowth EPCs. Already in 1963, it was shown that blood contains circulating endothelial cells. In an elegant experiment, young pigs received a special a-cellular Dacron graft consisting of a suspended piece of graft material in the lumen that was kept in place by sutures so that it made no contact with the vascular wall. After 14 days, patches of endothelial cells could be observed on the suspended graft while the sutures holding the suspended graft were not covered with endothelial cells (Stump et al. 1963). Subsequently, in 1998 it was shown that the endothelial cells found on the inside of such grafts are bone marrow derived (Shi et al. 1998). This finding was further supported by the culture of endothelial progenitor cells (EPCs) derived from human peripheral blood (Asahara et al. 1997).

Subsequent experiments demonstrated that there are actually two kinds of EPCs that can be cultured from blood (See Fig. 17.1). The so-called early outgrowth EPCs are present after 7 days of culture express markers of endothelial cells, yet also have monocytic markers and limited proliferation capacity (Rehman et al. 2003). Early outgrowth EPCs can home to ischemic areas, but cannot independently form a vascular network (Sieveking et al. 2008). Their therapeutic potential is believed to depend on the secretion of high levels of growth factors, such as VEGF, SDF1, HGF, and IGF1, that allow them to enhance the network formation of mature endothelial cells (Urbich et al. 2005).

In contrast, late outgrowth EPCs are typically observed after 2–3 weeks of culture and also express endothelial markers. They are more rare than early outgrowth EPCs but are highly proliferative. They can form a vascular network on their own, but do not secrete factors that enhance the network formation of mature endothelial cells (Sieveking et al. 2008). These cells are sometimes also called endothelial colony-forming cells (ECFCs) to highlight their proliferative potential.

	Early outgrowth EPCs	Late outgrowth EPCs (ECFCs)
• Culture time	7-10 days	>7 days
• Endothelial markers	+++	+++
• Monocytic markers	+++	-
• Proliferation	-/+	+++
• Incorporation into vasculature network	-	+++
• Paracrine effects	+++	-/+

Fig. 17.1 Significant differences between early and late outgrowth EPCs. Most importantly, late outgrowth EPCs are highly proliferative and are able to independently form a vascular network. Early outgrowth EPCs lack these characteristics, but secrete a number of growth factors that can help the survival or proliferation

17.1.2 Source of EPCs

While EPCs were originally cultured from peripheral blood, they have subsequently been identified in the bone marrow and even in the endothelium of the intact vessel wall.

In case of the lung, several reports have identified resident EPCs in the lung microcirculation. For example, single cell clonogenic assays of endothelial cells isolated from the pulmonary artery or the pulmonary microvasculature show a hierarchy of proliferative potential. While some cells do not proliferate, others can form large colonies consisting of more than 2,000 cells after 2 weeks of culture, and these colonies were more prevalent in the microvascular endothelial cells. Rare microvascular endothelial cells were able to form very large colonies (>100,000 cells) that show clonogenic potential when reseeded. This highly proliferative population was absent in the main pulmonary artery (Alvarez et al. 2008; Abbas et al. 2003).

When it comes to choosing the ideal source of EPCs for treatment, it has been described that cord blood-derived ECFCs proliferate faster than peripheral blood-derived EPCs (Schwarz et al. 2012) and the implantation of cord blood-derived ECFCs leads to more stable and long lasting blood vessels in a collagen/fibronectin gel (Au et al. 2008). Furthermore, combining different cell types, such as MSCs and EPCs might lead to a greater therapeutic benefit as has been shown in the murine hind limb ischemia model (Schwarz et al. 2012). Moreover, dysfunction of early outgrowth EPCs has been described in mice exposed to chronic hypoxia (Marsboom et al. 2008). In patients with a mutation in BMPR2, the most common mutation in families with hereditary pulmonary arterial hypertension (PAH), late outgrowth

EPCs are dysfunctional (Toshner et al. 2009). Therefore, autologous EPCs might not be very effective in some patients and in vitro manipulations such as treatment with statins might be necessary to improve EPC functionality (Alvarez et al. 2008).

17.1.3 Endothelial Regeneration and EPC Contribution

Natural turnover of endothelial cells is very low (Foteinos et al. 2008), and small lesions can actually be repaired by adjoining cells that can sense the loss of contact and migrate within hours to cover the place occupied by the dying cell. This process is not dependent on endothelial proliferation as proliferation of endothelial cells is usually only detected 1–2 days after a major injury (Reidy and Schwartz 1983, 1984). Nevertheless, the microvascular endothelium can proliferate rapidly, for example, in the endometrium during the luteal phase of the menstrual cycle or in response to exercise. Besides proliferation of local endothelial cells, bone marrow-derived EPCs could potentially contribute to vascular repair. Under baseline conditions, only a small fraction of endothelial cells is derived from the bone marrow. For example, 3 months after a bone marrow transplantation, only 1 % of endothelial cells in the aorta or blood vessels of the skin and brain are from a hematopoietic origin (Crosby et al. 2000). However, insights about bone marrow-derived EPCs contributing to endothelial regeneration in pathological situations with more extensive endothelial damage can be obtained from bone marrow and solid organ transplantation studies. For example, in a subgroup of patients receiving a kidney transplant, endothelial cells derived from the host can be observed in the kidney. The variability between patients does not appear to correlate with time after transplantation, but rather the presence of graft rejection. Most patients without signs of rejection had no recipient-derived endothelial cells in the kidney, while in grafts with vascular rejection, almost all kidneys had at least one third of endothelial cells derived from the recipient (Lagaaij et al. 2001). Furthermore, female patients receiving a hematopoietic stem cell transplant from male donors had significant numbers of pulmonary endothelial cells derived from the donor (approximately 40 %) (Suratt et al. 2003). Interestingly, this occurred exclusively in the alveolar capillaries, while no donor-derived endothelial cells were observed in arterioles or major arteries. Of the three patients studied in this report, one patient did not show endothelial chimerism. While the small sample size precludes a definitive conclusion, possible explanations for the lack of chimerism are the short time between transplant and biopsy in this patient (only 50 days compared to more than 6 months in the two other patients) or the different conditioning treatment prior to transplantation (the patients with high chimerism received total body irradiation compared to Busulfan treatment). These studies highlight that in case of significant endothelial damage, EPCs can contribute to endothelial repair.

Obviously, there is great heterogeneity amongst the endothelial cells of different vascular beds. For example, endothelial cells in the brain have tight junctions to

maintain the blood–brain barrier, while endothelial cells in the kidney are fenestrated to allow efficient secretion. Even within a vascular bed, endothelial cells in conduit vessels and microvascular endothelial cells have different characteristics. At this point, it is not clear whether late outgrowth EPCs are able to differentiate in all different phenotypes, but at least one report has demonstrated that EPCs upregulate arterial markers such as ephrin B2 in response to shear stress, suggesting that they can adapt to local conditions (Obi et al. 2009).

17.1.4 Long-Term Engraftment Versus Paracrine Effects of EPCs

Currently, there is a discussion in the field about the importance of EPC long-term engraftment in the vasculature versus transient paracrine effects. Proof that long-term engraftment is necessary for functional improvement comes from a murine hind limb ischemia model where ECFCs were transduced with the ganciclovir-inducible Herpes Simplex 1 thymidine kinase suicide gene. When human ECFCs are injected 24 h after hind limb ischemia induction, they improve perfusion after 14 days. Yet subsequent treatment with ganciclovir completely reverses the functional improvement to what is seen in untreated control animals, suggesting that long-term engraftment and vascular incorporation of injected ECFCs is necessary for improving neovascularization (Schwarz et al. 2012). A similar suicide gene approach showed that the functional improvement of early outgrowth EPCs in a murine myocardial infarction also depends on long-term engraftment. EPC injection 1 day after myocardial infection improves the ejection fraction and capillary density after 2 weeks. Yet these improvements disappear if mice are subsequently treated with ganciclovir (which was not observed in mice receiving EPCs that do not express the suicide gene) (Ziebart et al. 2008). Using high resolution confocal microscopy in a murine hind limb ischemia model, it was shown that bone marrow-derived cells do not actually become endothelial cells, but rather accumulate around nascent blood vessels (Ziegelhoeffer et al. 2004). Similar observations were made in a melanoma model of angiogenesis, where the bone marrow-derived cells were identified as pericytes or monocytes (Rajantie et al. 2004). Other possibilities for EPCs to modify the vasculature are cell fusion and the release of microparticles or microvesicles. For example, in vitro studies have demonstrated the potential of bone marrow-derived cells to fuse with and obtain phenotypic markers of other cell types including embryonic stem cells, although this event appears to be rare (Terada et al. 2002). Endothelial cell-derived microparticles can induce the proliferation of mature endothelial cells (Dignat-George and Boulanger 2011), and microRNAs appear to mediate many of the effects of microparticles (Zhang et al. 2014).

17.1.5 EPCs Can Be Used as a Biomarker or as a Therapeutic Agent

Indirect evidence for the importance of EPCs for vascular health comes from studies demonstrating that patients with coronary artery disease have less and functionally impaired early outgrowth EPCs (Vasa et al. 2001) and even in healthy patients, lower EPC numbers are associated with worse endothelium-dependent vasodilation and a higher cardiovascular risk as assessed with the Framingham risk score (Hill et al. 2003). Therefore, levels of circulating EPCs might be a valuable biomarker for the severity of disease and disease progression.

Moreover, EPC transfer has been tested as a therapy for myocardial infarction, hind limb ischemia, and wound healing. In the next section, we will focus on the work that has been performed using EPCs either as a biomarker or as a therapy specifically for pulmonary diseases.

17.2 Role of EPCs in Pulmonary Vascular Regeneration During Disease

EPCs have been studied in several pulmonary diseases including bronchopulmonary dysplasia (BPD), PAH, acute lung injury (ALI), and chronic obstructive pulmonary disease (COPD). We will summarize the current knowledge for each of these conditions (see Fig. 17.2).

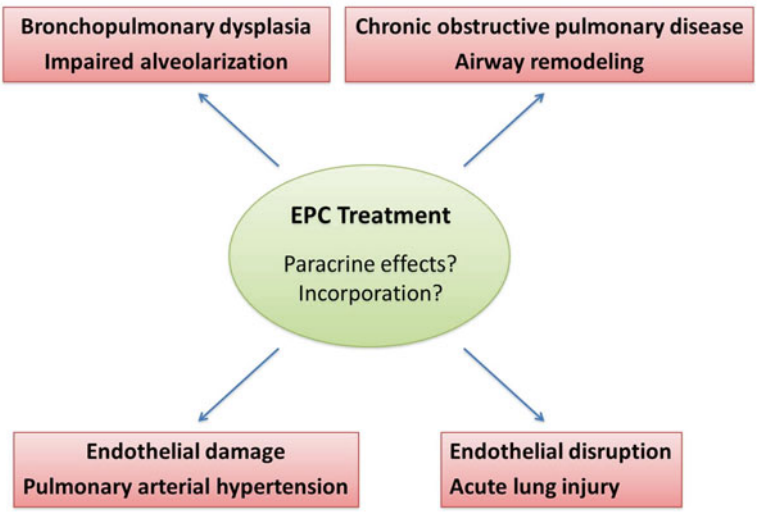

Fig. 17.2 Different pulmonary diseases for which EPC therapies are being considered

17.2.1 Bronchopulmonary Dysplasia

BPD is associated with premature birth and neonatal respiratory distress syndrome. Disruption of the normal lung development results in alveolar simplification and decreased vascular density. Although novel therapies such as prenatal steroids, postnatal surfactant, and improved ventilator strategies have led to a better survival, the prevalence of BPD has not declined.

Pulmonary vascular and alveolar development occur concurrently and recent evidence indicates that disrupted pulmonary blood vessel development can directly lead to impaired alveolarization and BPD (Stenmark and Abman 2005; Thebaud and Abman 2007; Jakkula et al. 2000). Therefore, a current line of thinking is that EPCs could be useful to improve vascularity, which in turn will lead to a normalized alveolar development.

In Vitro Experiments

To determine the paracrine effects of late outgrowth EPCs on pulmonary artery endothelial cells (PAECs), EPCs were isolated from the cord blood of both term and preterm infants and then used to condition medium. Cells were exposed to room air or hyperoxia (50 % oxygen for 24 h). Regardless of whether PAECs were exposed to hyperoxia, conditioned medium from EPCs increased their proliferation and angiogenic network formation. However, if preterm EPCs were exposed to hyperoxia, the conditioned medium became far less effective, while term EPCs were not influenced by hyperoxia. This suggests that EPCs from preterm infants are particularly sensitive to hyperoxia and that autologous EPC transfer might not be an appropriate treatment option for infants with BPD (Baker et al. 2013).

Animal Experiments

Exposure of neonatal mice to hyperoxia (80 % oxygen) for 10 days leads to airway simplification and a 72 % reduction in vascular density (Balasubramaniam et al. 2007). The same treatment also decreased EPC numbers in the lungs, blood, and bone marrow while adult mice actually had increased EPC numbers in the lungs and bone marrow. Interestingly, in vitro exposure of bone marrow-derived EPCs to hyperoxia leads to apoptosis (Balasubramaniam et al. 2007).

Bleomycin administration leads to pulmonary fibrosis in adult rats, yet if neonatal rats are exposed for 14 days to bleomycin, they develop BPD and pulmonary hypertension (Baker et al. 2013). Intravenous injection of either late-outgrowth EPCs or of their conditioned medium reduces right ventricular hypertrophy. EPCs from term infants were equally effective when exposed to hypoxia, yet preterm EPCs exposed to hyperoxia do not significantly decrease

right ventricular hypertrophy. In contrast, no improvement of alveolar simplification, vessel density, or pulmonary artery muscularization was observed in response to any of the conditioned media (Baker et al. 2013).

Clinical Studies

In a first study that looked at preterm infants (gestational age <32 weeks and birth weight <1,500 g), there was a reduction of the numbers of late-outgrowth EPCs in the cord blood of infants that would later on develop BPD (Borghesi et al. 2009). Remarkably, levels of late-outgrowth EPCs do not correlate with gestational age or birth weight, suggesting that levels of EPCs would be an independent predictor of the risk to develop BPD (Baker et al. 2012). Not only levels of cord blood EPCs, but also of circulating EPCs in the infant can be used to predict risk: using a similar study population, investigators found that those infants that would later on develop BPD, had similar levels of circulating EPCs at birth, yet had significant lower levels at 7 or 21 days (Qi et al. 2013; Paviotti et al. 2011). The reduction in circulating EPC numbers could be reversed by administration of inhaled nitric oxide (Qi et al. 2013).

17.2.2 Pulmonary Arterial Hypertension

PAH is a disorder characterized by an increase in the pulmonary vascular resistance due to a narrowing of the pulmonary blood vessels. This ultimately leads to right ventricular failure and prognosis remains poor despite the introduction of new therapies including prostacyclin, Phosphodiesterase 5 (PDE 5) inhibitors, and endothelin-1 receptor antagonists. The current 5-year survival rate of patients is only 61 % (Thenappan et al. 2010).

Endothelial damage is considered to be an early event in the onset of PAH (Tuder et al. 2001b) and vascular abnormalities in PAH patients include increased muscularization of the pulmonary arteries and formation of plexiform lesions consisting of disordered proliferating endothelial cells (Tuder et al. 2001a). These lesions are observed in both patients with idiopathic or heritable PAH and in patients that develop PAH in association with other diseases such as scleroderma. Interestingly, in patients with idiopathic PAH (IPAH), these endothelial cells have a monoclonal origin, suggesting that a single endothelial cell gave rise to the plexiform lesion. This is reminiscent of Kaposi's sarcoma and suggests that in the presence of endothelial damage, some endothelial cells can acquire a hyperproliferative phenotype, possibly due to a somatic mutation (Lee et al. 1998).

Further proof for the importance of endothelial damage in the development of PAH comes from the observation that levels of soluble E-selectin, a marker of endothelial inflammation and injury, are increased in PAH patients (Cella et al. 2001). Moreover, novel therapies for PAH are based on supplying endothelial-derived compounds such as prostacyclin and nitric oxide that are reduced in PAH patients.

Also animal models demonstrate that endothelial damage contributes to disease onset, as inhibition of VEGFR-2, an important receptor involved in endothelial survival, leads to an exacerbated disease state (Taraseviciene-Stewart et al. 2001).

A number of factors including increased shear stress, viral infection, or hypoxia sometimes in combination with genetic mutations can lead to PAEC injury. To repair the damaged endothelial cells, either local endothelial cells can proliferate or circulating EPCs can be recruited to repair the damage. In the absence of adequate repair, smooth muscle cells will proliferate and lead to vascular narrowing.

Animal Models

A widely used model to study pulmonary hypertension is the use of the plant pyrrolizidine alkaloid monocrotaline. This compound becomes activated in the liver to monocrotaline pyrrole, a reactive alkylating compound that subsequently causes endothelial damage (Roth and Reindel 1991). Mainly the lungs are affected because this is the first vascular bed encountered by the activated compound after the liver. A single injection of monocrotaline causes a significant increase in the pulmonary artery pressure of rats together with increased muscularization of the pulmonary arteries (Marsboom et al. 2012).

Endothelial damage is an important aspect of monocrotaline-induced pulmonary hypertension and injections of EPCs show beneficial effects in at least two different species. In rats, injection in the jugular vein of bone marrow-derived early outgrowth EPCs 3 days after monocrotaline almost completely prevented the rise in right ventricular systolic pressures and right ventricular hypertrophy. Reversal experiments, where EPCs are administered 3 weeks after monocrotaline injection, also had a positive effect on pressures, hypertrophy, and survival. These effects were modestly improved by transducing EPCs with eNOS (Zhao et al. 2005). Of note, labeled EPCs were present for at least 3 weeks in the distal arterioles of the lungs. In another manuscript, nude rats received monocrotaline followed 7 days later by early outgrowth EPCs derived from human umbilical cord mononuclear cells. EPC transfer lowers the mean pulmonary artery pressure with 14 %, and an even greater effect (−29 %) was observed when EPCs were transduced with the vasodilator adrenomedullin (Nagaya et al. 2003). Similar improvements were observed for muscularization and survival. The beneficial effect of EPC transfer has also been shown in dogs. Dogs receiving an intravenous injection of monocrotaline pyrrole develop severe pulmonary hypertension after 6 weeks (Takahashi et al. 2004). In this model, injection of early outgrowth EPCs derived from peripheral blood (10–14 days of culture) into the lung parenchyma of the lower lobes can improve pulmonary artery pressure and cardiac output and lowers pulmonary vascular resistance (Takahashi et al. 2004).

These positive findings are in contrast with results in the chronic hypoxia model of pulmonary hypertension, where transplantation of early outgrowth EPCs was not able to lower right ventricular pressures or hypertrophy (Marsboom et al. 2008). A possible explanation is that chronic hypoxia leads to EPC dysfunction (reduced adhesion, migration to SDF1α, and incorporation into a vascular network), suggesting

that EPC treatment for PAH patients that also experience hypoxemia might have less therapeutic benefit (Marsboom et al. 2008).

Clinical Studies

Based on promising results in the monocrotaline model of pulmonary hypertension, preliminary small-scale clinical studies have been performed to assess the safety and therapeutic efficacy of autologous EPC transfer in IPAH patients (see Table 17.1). The first study used IPAH patients with New York Heart Association (NYHA) functional class II and III (mild to significant symptoms during activity, but comfortable at rest) (Wang et al. 2007). Patients received standard therapy (but no patients on prostacyclin were included) and were randomized to either also receive autologous EPCs ($n = 15$) or only continue standard treatment ($n = 16$). EPCs were isolated from peripheral blood, cultured for 5 days, and on average 11 million EPCs were subsequently administered intravenously. After 12 weeks, patients receiving EPCs walked on average an additional 50 m during the 6-min walk test, which was the primary end point for this trial. In contrast, the control group's walking distance only improved by less than 6 m on average. The improvement in the EPC group was associated with lower pulmonary artery pressures and vascular resistance and improved cardiac output. No complications related to EPC therapy were observed (Wang et al. 2007). The same group also studied the safety and feasibility of EPC transfer in 13 children with IPAH. Similar improvements in exercise capacity and pulmonary artery pressures were observed, yet this study did not contain a control group (Zhu et al. 2008). Interestingly, at least two other trials with EPCs have been registered on the website of ClinicalTrials.gov. The first study by Canadian researchers is an open-label trial looking at the safety and functional improvement in response to intravenous injection of eNOS transduced EPCs (NCT00469027). The trial was projected to start in 2006, but to the best of our knowledge no results have been published. The other study is based in China and would be the first double-blind clinical trial involving EPCs for the treatment of PAH (NCT00372346). It was also projected to start in 2006, but until now no results have been published. At this point, it is not clear whether recruitment issues, a lack of funding, or possible side-effects are responsible for the delay in publication, but results of these trials will be crucial to assess the therapeutic efficacy of EPCs in PAH.

Prognostic Value of Circulating EPC Numbers in PAH

The development of an EPC capture chip coated with an anti-CD34 antibody, allows measurements on small blood samples (200 μL) without the delays of associated with the isolation and culturing of EPCs from large whole blood samples. Immunostaining for CD31, VEGFR2, and CD45 allows for a rapid (less than 1 h) determination of circulating EPC numbers. This chip therefore could be useful in

Table 17.1 Clinical trials using EPCs for the treatment of PAH

Clinical trial #	Description of trial	Location of trial	Primary goals	Patient population	Number of patients	Trial design
NCT00257413 (2007)	Safety and efficacy study of transplantation of EPCs to treat idiopathic pulmonary arterial hypertension[a]	Zhejiang University	Feasibility, safety, and initial clinical outcome of intravenous infusion of autologous EPCs	Idiopathic PAH	15 NYHA class II–III receiving EPCs vs. 16 controls	Randomized, single-blind
NCT00372346	Safety and efficacy study of transplantation of EPCs to treat idiopathic pulmonary arterial hypertension	Zhejiang University	Feasibility, safety, and initial clinical outcome of intravenous infusion of autologous EPCs	Idiopathic PAH	40 patients, NYHA class II–III	Randomized, double-blind 2006–2007
NCT00641836	Safety and feasibility of autologous endothelial progenitor cells transplantation in patients with idiopathic pulmonary arterial hypertension	Zhejiang University	Ideal quantity of EPCs for therapy, the duration of the therapeutic effect, and moreover, the potential toxicity of such therapy.	Idiopathic PAH	98 patients	Follow-up study, non-randomized and open label. 2005–2007
NCT00469027	Pulmonary hypertension: assessment of cell therapy (PHACeT)	St. Michael's Hospital, Toronto and Sir Mortimer B. Davis–Jewish General Hospital, Montreal, Canada	Safety of autologous progenitor cell-based gene therapy of heNOS	Idiopathic, familial, or anorexigen-induced PAH	18 patients	Non-randomized and open label. 2006–2012. Two centre, phase 1 trial

[a]Wang XX, Zhang FR, Shang YP, Zhu JH, Xie XD, Tao QM, Chen JZ. Transplantation of autologous endothelial progenitor cells may be beneficial in patients with idiopathic pulmonary arterial hypertension: A pilot randomized controlled trial. *J Am Coll Cardiol*. 2007;49:1566–1571

following EPC numbers over time to monitor therapeutic efficacy (Hansmann et al. 2011). However, at this point it is not clear that these are markers truly specific for EPCs, how well they can discriminate between minimally proliferative and highly proliferative EPCs, and whether there is any prognostic value of EPC number determination in PAH. Some researchers have found decreased while other have found increased numbers of circulating EPCs in PAH patients. For example, CD34+/AC133+/VEGFR2+ and AC133+/VEGFR2+ circulating cells are reduced in IPAH patients (Diller et al. 2008; Junhui et al. 2008), while others have shown an increase in CD34+/AC133+/VEGFR2+ and CD34+/AC133+ cells in IPAH patients (Toshner et al. 2009; Asosingh et al. 2008). At this point, it is unclear how this discrepancy can be reconciled, but one possibility is that differences in patient selection in combination with relatively small patient samples lead to a large variability (e.g., the studies showing a decrease in circulating EPC numbers had 20 and 55 patients, while the studies showing an increase had 16 and 17 IPAH patients). When we look at the biggest study so far, using 55 IPAH patients, the reduction in circulating EPCs did not correlate with exercise capacity of NYHA functional class assessment (Diller et al. 2008). Also, incorporation of early outgrowth EPCs into a vascular network was not different between IPAH and control patients (Diller et al. 2008). Highlighting the heterogeneity of findings, another publication has actually found reduced adhesion to the extracellular matrix component fibronectin and decreased migration towards VEGF in EPCs from IPAH (Junhui et al. 2008). Therefore, it is currently not clear whether circulating EPC numbers and their functionality can be used to predict disease outcome and response to therapy.

17.2.3 Acute Lung Injury and Acute Respiratory Distress Syndrome

Damage to the pulmonary endothelium leads to a disruption of the alveolar-capillary membrane in patients with ALI and its most severe form acute respiratory distress syndrome. The endothelial disruption leads to pulmonary edema formation, hypoxemia, and respiratory failure. Despite improved ventilatory strategies, there is a mortality rate of 30–50 %. ALI occurs frequently in association with sepsis.

At the cellular level, endotoxins (lipopolysaccharide or LPS) result in the loss of endothelial barrier function and edema formation, which in turn can lead to hypoxemia. Endothelial repair with EPCs might be a novel mechanism to restore endothelial barrier function and accelerate the recovery.

Animal Experiments

Work from our group has previously shown that LPS-induced injury triggers a small population of lung endothelial cells to proliferate, which is dependent on the FoxM1 transcription factor and is necessary for restoring the endothelial barrier

(Zhao et al. 2006). In addition, we have also shown that bone marrow-derived mononuclear cells can decrease LPS-induced lung microvascular permeability and edema formation by improving the endothelial barrier function (Zhao et al. 2009). Mechanistically, bone marrow-derived mononuclear cells secrete the phospholipid sphingosine-1-phosphate, which upon binding to its receptor on endothelial cells activates the Rho GTPases Cdc42 and Rac1 and leads to the assembly of adherens junctions as well as the restoration of the endothelial barrier (Zhao et al. 2009).

Moreover, 1 week after intranasal delivery of LPS to mice, multiple bone marrow-derived cells can be observed in the lungs (Yamada et al. 2004) of which a subset expresses CD34, a marker observed on hematopoietic and endothelial cells. The number of circulating early-outgrowth EPCs was also increased in response to LPS (Yamada et al. 2004). Indirect evidence for the importance of these circulating EPCs in lung repair was obtained by giving mice a sublethal irradiation followed by LPS administration which would suppress or eliminate bone marrow stem and progenitor cells. This leads to the formation of emphysema-like lesions in the lungs, which were absent when the bone marrow was reconstituted (Yamada et al. 2004).

Rats receiving bone marrow-derived early outgrowth EPCs 30 min after an intravenous injection of LPS have improved survival (Mao et al. 2010). Histological analysis revealed incorporation of EPCs in the vascular wall up to 14 days, and the incorporation is not observed in control rats that did not receive LPS injection. EPCs decreased the typical thickening of the alveolar wall seen after LPS treatment and reduced the number of inflammatory cells present in the lung. The wet-to-dry ratio of the lung also was significantly reduced after EPC treatment, thus indicating improved barrier function. Finally, plasma levels of endothelin-1 were decreased, while the anti-inflammatory cytokine IL10 was increased after EPC injection (Mao et al. 2010).

Clinical Studies

ALI leads to an increase in circulating late-outgrowth EPC numbers and their numbers within 72 h after the onset of ALI is an independent predictor of survival. Patients with high levels of EPCs have a twofold higher survival rate even when other factors such as age, gender, and severity of disease are included in the multivariate analysis (Burnham et al. 2005). While the exact mechanism of the survival benefit is not known, it can be hypothesized that mobilization of EPCs could potentially repair damaged pulmonary endothelium. Also, in humans suffering from pneumonia, an increased number of circulating early-outgrowth EPCs has been observed (Yamada et al. 2005).

17.2.4 Chronic Obstructive Pulmonary Disease

COPD patients have airflow obstruction due to chronic inflammation of the airways. Endothelium-dependent relaxation is impaired in pulmonary arteries of patients with mild COPD, suggesting that endothelial dysfunction or injury is present at the

initial stages of COPD (Peinado et al. 1998). Smoking appears to be a major culprit since smokers with a normal lung function have structural abnormalities in the pulmonary arteries and also express less endothelial nitric oxide synthase (Barbera et al. 2001). Endothelium-derived nitric oxide is a potent vasodilator with antiproliferative effects on smooth muscle cells and its downregulation is a hallmark of endothelial dysfunction.

The occurrence of endothelial damage likely explains why COPD patients are at increased risk for developing pulmonary hypertension. Mild pulmonary hypertension defined as mean pulmonary artery pressure higher than 25 mmHg is present in approximately half of COPD patients, with around 4 % of patients having severe pulmonary hypertension (pulmonary artery pressure of more than 35 mmHg). The development of pulmonary hypertension is associated with a significant shorter survival (37 % 5-year survival rate versus 63 % in COPD patients without pulmonary hypertension) (Andersen et al. 2012). Therefore, EPC therapy might be useful to extend the life expectancy of COPD patients.

In a rabbit model of brush injury-induced bronchial denudation, transplantation of tissue-engineered implants containing mature endothelial cells can improve airway regeneration (less airway remodeling and luminal narrowing) (Zani et al. 2008). Although similar experiments have not been performed with EPCs, these findings at least suggest that improving endothelial function by cell transplantation may be beneficial in COPD.

Animal Models

Elastase-induced lung injury mimics the lung pathology seen during COPD. It was shown that an intraperitoneal injection of hepatocyte growth factor leads to a recovery of alveolar structure. While hepatocyte growth factor might have a direct effect on the proliferation of lung epithelial cells (Sakamaki et al. 2002), it is intriguing that an almost sevenfold increase of circulating EPCs was observed and that bone marrow-derived cells were observed in the vascular wall (Ishizawa et al. 2004). Further research is however necessary to confirm the importance of EPCs in improving alveolar structure.

Clinical Studies

Patients with COPD or restrictive lung disease have an approximately twofold reduction of circulating EPC numbers measured as CD34+/CD133+/VEGFR2+ cells/ml blood (Fadini et al. 2006). Interestingly, within a group of COPD patients, those with lower arterial pO_2 levels actually have the highest EPC numbers, suggesting that hypoxemia is inducing mobilization of EPCs. In the patients with restrictive lung disease, patients with lower total lung volumes also have the lowest EPC numbers. As in the COPD patients, those with hypoxemia (in these patients

measured as increased hematocrit levels) have higher levels of EPCs. Given the heterogeneous disease background in patients with restrictive lung disease (idiopathic pulmonary fibrosis, obesity-related ventilatory impairment, sarcoidosis, tuberculosis, amyotrophic lateral sclerosis, and kyphoscoliosis) and that some of the patients were smoking, it is difficult to draw definitive conclusions. Nevertheless, it appears that in all of these settings the number of circulating EPCs is reduced.

17.3 Remaining Challenges and Future Directions

Animal experiments have demonstrated the feasibility and efficacy of EPC-mediated vascular repair in the lungs of animals, mainly rodents. Future research should therefore focus on larger animals to establish the numbers of cells needed in patient trials and which cell type (early or late-outgrowth EPCs) is more beneficial in each of the pulmonary vascular diseases. Small animal research would be useful to identify ways to increase the homing of EPCs to the lungs in case insufficient EPCs can be obtained from patients and to better understand any potential side-effects of EPC therapy.

Although initial clinical trials have shown that EPC transfer is safe (as described above), it should be pointed out that under certain conditions, EPCs could actually have detrimental effects. For example, early outgrowth EPCs release a large amount of tissue factor which could contribute to coagulation, while late outgrowth EPCs release the inflammatory chemokine MCP1 (Zhang et al. 2009). Early outgrowth EPCs migrate towards the chemokine stromal cell-derived factor-1 (SDF1) (Marsboom et al. 2008) and blocking CXCR4 can largely prevent (but not reverse) chronic hypoxia-induced vascular remodeling, increased right ventricular pressures, and right ventricular hypertrophy (Gambaryan et al. 2011). This suggests that the homing of progenitor cells (including EPCs) could even contribute to disease progression. Similarly, elimination of circulating monocytes also prevents the development of pulmonary hypertension (Frid et al. 2006). This highlight the complex role that EPCs can play pulmonary vascular disease, having both beneficial and detrimental effects; and that research is needed to identify the optimal EPC type and timing of therapy to maximize the benefits of cell therapy.

Finally, and most importantly, to establish EPC transplantation as a clinical therapy, well designed and double blind clinical trials are necessary. While safety is a serious concern, the lessons learned from larger animal experiments in combination with dose-escalating trials should allow to minimize the risk for patients. At the same time, it is imperative that the results of ongoing clinical trials in PAH patients are published as soon as possible so that other clinical investigators can learn from these initial trials. For example, the numbers of injected cells needed for clinical improvement would benefit trials for other pulmonary vascular disorders as well.

17.4 Conclusion

While animal experiments have shown that EPC transfer can be used to treat several pulmonary vascular diseases, the translation of these findings into clinical practice is clearly lacking. It still needs to be established whether early or late outgrowth EPCs are more effective in the treatment of BPD, PAH, ALI, and COPD. The authors believe that the use of larger animals might be more appropriate to address safety and efficacy of EPC transfer before moving to clinical trials.

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