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Vascular repair and regeneration as a therapeutic target for pulmonary arterial hypertension

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Abstract

The last decade has seen substantial changes in our understanding of the pathobiology of pulmonary arterial hypertension (PAH), a severe and devastating disease without curative treatment. It is now accepted that injury to the endothelial cells of the pulmonary arteries is central for the subsequent development of lumen-obliterative lung vascular lesions. A variety of circulating and lung-resident progenitor and stem cells likely contribute to vascular integrity and evidence for the presence of cells expressing stem and progenitor cell markers is found inside of and in the immediate vicinity of the pulmonary vascular lesions in PAH. The currently available vasodilator therapies mainly target enhanced vasoconstriction in the lung circulation and help to maintain or improve right ventricular function, but do not treat pulmonary vascular remodeling, the underlying cause of the disease. Vascular gene therapy and cell therapy with progenitor and stem cells is a progressing field in the context of the development of novel treatment options for PAH. But the majority of the studies are currently performed on the level of preclinical studies in animal models. The current review provides an overview of the current knowledge on cell- and gene therapy-based approaches for vascular repair and regeneration in PAH.

Keywords

Pulmonary arterial Hypertension; stem cell; progenitor cell; vascular regeneration

INTRODUCTION

Pulmonary arterial Hypertension (PAH) is a severe progressive disease of the pulmonary vasculature characterized by the obliteration of pre-capillary pulmonary arterioles, which leads to right heart failure and death [1]. The classification and subtypes of pulmonary hypertension (PH) have been summarized in the results of the last world symposium on PH in Dana Point [2]. Idiopathic PAH (IPAH) is a subcategory of severe PAH without known cause of the disease [2]. Less than 20% of IPAH patients and a large number of patients with hereditary PAH are heterogeneous for a mutation in bone morphogenic protein (BMP) receptor 2 (BMP2), a receptor of the transforming growth factor- β (TGF- β) family of receptors [3, 4]. Despite decades of research, there is still no curative treatment for this

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crippling disease. Although several vasodilator treatments are currently available and are used regularly in clinical practice, these drugs do not seem to reduce the vascular occlusion, which is the main determinant of increased pulmonary vascular resistance [1, 5]. Whereas previous pathobiological concepts have focused on the role of hypoxia and increased vasoconstriction, it is now accepted that post-apoptotic overgrowth of phenotypically altered, hyper-proliferative cells leads to the vascular obstruction [1, 6]. For a detailed recent review on the pathobiology of PAH please see reference [4]. Advanced severe PAH is characterized by so-called plexiform lesions, which are complex, multicellular vascular lesions [7, 8]. Due to the lack of current curative treatment options for PAH, it is instrumental that novel therapeutic approaches are developed which aim at restoring the vascular architecture of the lung.

In the recent years, our understanding of vascular development and vascular remodeling have increased significantly, in particular due to the advances made in the field of stem and progenitor cell research [9]. Stem cells are generally characterized by their undifferentiated state and their ability to produce progeny which are undergoing differentiation into specialized, terminally differentiated cells in order to replace other terminally differentiated cells which are lost, e.g., due to apoptosis [10]. These differentiated progeny are called progenitor cells, which often exhibit a high proliferation potential that is subsequently lost when progenitor cells undergo terminal differentiation [10]. A fraction of the stem cells maintains their undifferentiated, primitive state in so-called stem cell niches [10]. In the following paragraphs, we provide an overview over stem and progenitor cell populations with relevance for regenerative therapy in the context of pulmonary vascular disease (summary in Figure 1) and we extend our review on the published work that relates to the use of cells and factors for cell-based and gene therapeutic approaches in the context pulmonary vascular diseases (summary in Figure 2).

STEM AND PROGENITOR CELLS AND THE PULMONARY CIRCULATION CIRCULATING AND LUNG-RESIDENT ENDOTHELIAL PROGENITOR AND STEM CELLS

Endothelial progenitor cells (EPCs) have been originally identified as circulating cells with pro-angiogenic properties which express markers of both progenitor cells and endothelial cells (ECs) and can be differentiated into ECs *in vitro* [11]. Various isolation techniques have yielded important subsets of cells: the endothelial cell colony-forming units (CFU-ECs, also “early-outgrowth EPCs”) and the endothelial colony-forming cells (ECFCs, also “late-outgrowth EPCs”). Whereas CFU-ECs are more likely hematopoietic progenitors, can differentiate into macrophages, but lack the ability to establish functional vasculature, ECFCs have a higher proliferative potential and can form perfused vascular structures *in vivo* [12]. Circulating EPCs have been shown to be increased in IPAH, and ECFCs from PAH patients with BMPR2 mutations are hyperproliferative, but impaired in their ability to form vascular tubes [13, 14].

EPCs resident to the vascular wall have been first shown in the arterial wall of the systemic circulation and may reside within the endothelium or at the border region between tunica media and tunica adventitia [15, 16]. It is interesting that the arterial wall contains in fact a complete hierarchy of these EPCs [16]. These resident EPCs are characterized by a high proliferation potential and the ability for clonal expansion [16]. In the lung vasculature, the microvascular pulmonary endothelium contains a high fraction of lung resident EPCs which are responsible for the significant proliferation potential [17].

Recently, cells residing in human and murine lungs have been shown to be able to replace cells of the vascular wall: Clonogenic human lung stem cells can regenerate all cells of the airways, vascular tree and alveolar wall when injected into the lungs of cryoinjured mice

[18]. Endothelial cells isolated from the lungs of mice contain a population of vascular endothelial stem cells (VESC) with a high proliferative capacity, clonal expansion potential and the ability to generate functional blood vessels [19]. The common denominator of both of these studies is the presence of stem cells with high proliferative and regenerative potential, which may serve as replacement pool for various cell types, including ECs, in the lung circulation.

LUNG-RESIDENT AND BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

Mesenchymal Stem Cells (MSCs) have been originally identified in the bone marrow (BM) as plastic adherent, non-hematopoietic cells, which form fibroblast-like colonies, have a high proliferation potential and the ability to undergo differentiation into multiple mesenchymal and vascular lineages [20–22]. MSCs are also interesting for cellular therapeutic approaches because of their low immunogenic profile combined with anti-inflammatory properties [21, 23, 24]. In the BM, MSCs are important to maintain the integrity of the hematopoietic stem cell (HSC) niche and MSCs also regulate the trafficking of HSCs between BM, circulation and other organs [21]. MSCs themselves can also be mobilized from the BM and circulate to other organs in response to tissue injury [21]. The lung contains tissue-resident MSCs, and multipotent MSCs have been isolated from the lung vascular lesions of patients with chronic thromboembolic PAH [25, 26]. It is currently unclear, whether lung resident or BM-derived MSCs are part of the pathobiological process of lung vascular obliteration in PAH, or are present in remodeled vessel walls in an attempt to repair the injured vessel wall.

HEMATOPOIETIC STEM AND PROGENITOR CELLS

Hematopoietic stem cells (HSCs) and HPCs share a common developmental source with the ECs and are the common source for cells of the hematopoietic lineages [27, 28]. HSCs reside in the BM in the so-called hematopoietic stem cell niches, which are maintained by BM-resident MSCs [29]. In PAH patients, alterations of the BM composition and reticulin fibrosis were identified, suggesting a subclinical myeloproliferative process [30]. Putative HPCs have been shown in the arterial wall in the systemic circulation of mice and in pulmonary artery walls of chronic hypoxic mice [31, 32]. Cells with a similar marker profile have also been identified in the plexiform lesion of patients with IPAH [33, 34]. Transplantation of myeloid progenitors derived from human PAH patients, but not from healthy controls, to immunodeficient mice resulted in pulmonary vascular remodeling, in particular intravascular thrombosis [35]. These data indicate that BM-derived myeloid/hematopoietic progenitor/stem cells may contribute to the development of lung vascular lesions and PAH.

FIBROCYTES

Fibrocytes are a subpopulation of circulating progenitor cells, which co-express leukocyte and hematopoietic progenitor cell markers and mesenchymal lineage markers, and have been mainly implicated in the course and prognosis of fibrotic disease, such as idiopathic pulmonary fibrosis (IPF) [36–38]. It has been demonstrated that fibrocytes accumulate around the pulmonary arteries in chronic hypoxic mice, and that a prostacyclin-analogue inhibits the recruitment of circulating fibrocytes to the lung vasculature of chronic hypoxic mice, which was associated with a slight reduction in lung vascular remodeling [39, 40]. The biology of fibrocytes in the context of PAH has to be better understood before these cells may be considered for applications such as vehicle for gene therapy or drug delivery.

VASCULAR REGENERATION AND REPAIR FOR PULMONARY HYPERTENSION

Because of the concept of post-apoptotic cell overgrowth as the underlying pathogenic problem in the pathobiology of PAH, the administration of vaso-protective, pro-angiogenic factors would be expected to induce vascular regeneration and repair in the context of PAH. However, due to the nature of the complex vascular lesions, which are characterized by the presence of abnormal, hyperproliferative cells expressing EC markers, it is questionable whether simply boosting the angiogenic potential would in fact be beneficial or would actually promote disease progression. The choice of an appropriate model mimicking the human disease as closely as possible will be important to make valid pre-clinical decisions on the potential benefits of vascular regenerative therapy [41–44]. The most promising preclinical and clinical data are available for cell-based therapeutic approaches, where genetic manipulation, mostly of progenitor or stem cells, to overexpress a particular gene is combined with the transplantation of these modified cells. Such a cell transfer strategy may be useful for the treatment of a variety of lung diseases [45, 46]. The concept behind this approach is that these cells will migrate to the remodeled vessels and will therefore deliver the gene product at the site of vascular injury. Whereas this concept is intriguing, it needs to be considered that our current knowledge regarding the true “intentions” of these progenitor or stem cells accumulating in the lung vascular lesions is limited. Another important problem is the generation of cell cultures suitable for clinical application. A summary of potential strategies can be found in Figure 2.

THERAPEUTIC APPROACHES USING PROGENITOR AND STEM CELLS WITHOUT GENETIC MANIPULATION

Granulocyte-colony stimulating factor (G-CSF) is an important colony stimulating factor that enhances neutrophil counts and neutrophil activity [47, 48]. G-CSF has also been used to mobilize hematopoietic cells from the bone marrow [49]. In a rat model, G-CSF reduced myocardial infarct size and the frequency of ventricular arrhythmias [50, 51]. Whereas G-CSF administration in the MCT rat model of PAH resulted in improved survival and reduced PAH [52], G-CSF treatment worsened α -Naphthylthiourea-induced PH [53], indicating a highly context-dependent effect of G-CSF treatment and BM cell mobilization.

The administration of more or less defined progenitor cell populations has been investigated, mainly in pre-clinical animal models, in the context of PAH. The concept underlying such an approach is based on the assumption that progenitor cells have may help to repair pulmonary vasculature. One frequently investigated cell population is constituted of BM-derived MSCs [23]. Several groups have shown that transplantation of BM-derived MSCs can reduce vascular remodeling and PAH in the monocrotaline (MCT) model [54–56], in a high flow-induced model of PAH [57, 58] and a model of bronchopulmonary dysplasia and associated PH [59]. It is interesting that even intratracheal administration of MSCs results in reduced PAH in the MCT model [60]. In contrast, MSCs are not integrated in the pulmonary arteries of chronic hypoxic rats despite repeated infusions [61]. MSCs derived from human embryonic stem cells also reduce PAH in the MCT model of PAH according to a study by Zhang *et al.*[62].

BM-derived mononuclear cells reduce canine MCT-induced PAH and these cells were detected in the endothelial layer of the lung vessels [63]. In another study by Spees *et al.* using bone marrow-chimeric animals, BM-derived progenitor cells were detected in the lung of MCT rats as interstitial fibroblasts, myofibroblasts, hematopoietic cells, Clara cells, vascular endothelial cells and smooth muscle cells, indicating that transplanted bone marrow-derived cells may present with a rather heterogeneous phenotype in the lung [64].

EPCs have also been used in preclinical and early clinical studies for vascular regeneration in the context of PAH: Canine and rodent MCT-induced PAH was reduced after the transplantation of blood- or BM-derived EPCs [65, 66]. In contrast, human CFU-ECs derived from the peripheral blood did not prevent or reduce mortality or vascular remodeling in MCT-induced PAH [67]. Wang *et al.* have investigated the transplantation of autologous EPCs in a randomized controlled trial in IPAH patients and found an increase in the 6 minute walk distance after 12 weeks [68].

CXC chemokine ligand 12 (CXCL12) belongs to the CXC family of chemokines, which contains four highly conserved cysteine residues and the first two cysteine residues, separated by a variable amino acid, determine the name of this chemokine family [69]. CXCL12 (also known as stromal cell derived factor 1 α) signals through CXC chemokine receptor 4 (CXCR4). CXCR4 signaling is not only important for chemotaxis and migration of circulating and resident cells towards a CXCL12 gradient, but also for cell survival and proliferation [70]. Whereas activation of the CXCL12/CXCR4 axis has been shown to aid regeneration in the heart [71], elevated expression of CXCR4 and its ligand CXCL12 are found in plexiform lesions in PAH [14]. Indeed, CXCR4 inhibition resulted in reduced accumulation of putative HPCs and reduced vascular remodeling and PAH [31, 72].

Overall, the results of studies interfering with BM-derived cell mobilization and migration shows conflicting results, whereas a transfer of more selected cell populations such as MSCs or EPCs appears to be more beneficial for the treatment of PAH. Carefully designed clinical evaluations need to provide substantial proof for the safety and efficacy of these treatment strategies before cell therapies should be considered as an established treatment option for PAH.

THERAPEUTIC APPROACHES USING ANGIOGENIC THERAPIES AND GENETICALLY MODIFIED CELLS

Vascular Endothelial Growth Factor (VEGF) is a central angiogenic and growth factor that is highly expressed in the ECs of the lung vasculature. Inhibition of VEGF receptors induces EC apoptosis and severe obliterative PAH when combined with exposure to chronic hypoxia [73, 74]. Although VEGF expression was found to be increased in human PAH lungs [75] and in lungs of chronic hypoxic rats [76, 77], it was reduced in the lungs of MCT rats [78] and in the lungs of chronic pulmonary diseases which are frequently associated with pulmonary hypertension, such as IPF [79, 80] and emphysema [81]. Gene therapeutic approaches with VEGF overexpression have been successful to reduce pulmonary vascular remodeling in the chronic hypoxia model of PAH [82], the MCT model of PAH [83] and our animal model of experimental lung fibrosis and associated PH [84]. One may suggest caution because VEGF overexpression could also promote the development of angioobliterative angiogenic vasculopathy, as could be suggested by the findings of high levels of VEGF expression in plexiform lesions of PAH patients [75].

Endothelial Nitric Oxide Synthase (eNOS) is important for the endothelial production of nitric oxide as a vasodilator targeting the pulmonary artery smooth muscle cells [85]. The observation of decreased eNOS expression in pulmonary arteries of PAH patients and newborns with persistent PH provides a good rationale to attempt to increase eNOS expression as a treatment option [86, 87]. However, conflicting results have been published and indicate that eNOS may be increased in the lungs of PAH patients and chronic hypoxic rats [88, 89]. A study by Mason *et al.* has provided a possible explanation for this controversy [90]: The authors found that eNOS expression was reduced in small pulmonary arterioles of PAH patients, but eNOS was strongly expressed in the plexiform lesions of PAH patients. eNOS gene therapy has been demonstrated in several publications to be effective to treat PAH in animal models [83, 91, 92]. One mechanism may be the

preservation of microvascular architecture [92]. An early-phase clinical trial is investigating the tolerability of eNOS-enhanced autologous EPCs delivered into the pulmonary circulation of patients with severe PAH (the Pulmonary Hypertension and Cell Therapy trial, PHACeT) (<http://clinicaltrials.gov>, Identifier: NCT00469027). Results from two small clinical trials which examined the transplantation of autologous EPCs in patients with IPAH suggest that this approach may be safe and useful [68, 93]. However, the long-term use of cell-based eNOS gene therapy may require careful evaluation, because enhancing eNOS levels or activation may potentially promote the progression of complex angiogenic lesions [94].

Prostacyclin (PGI₂) is the most important arachidonic acid metabolite of ECs and vascular smooth muscle cells, a powerful vasodilator and inhibitor of cell growth [95]. The expression of PGI₂ synthase, the enzyme responsible for PGI₂ biosynthesis, has been shown to be decreased in the pulmonary vasculature of IPAH patients [95]. Pulmonary overexpression of PGI₂ synthase protects or reduces PAH in animal models [96, 97]. Gene transfer of PGI₂ synthase into skeletal muscles and the liver was also able to reduce PAH in rats [98, 99]. The transplantation of MSCs transduced to overexpress PGI₂ synthase reduced PAH and pulmonary vascular remodeling in MCT-treated rats [100].

Angiopoietins (Angs) represent a family of ligands for the Tie tyrosine kinase receptors Tie-1 and Tie-2, which are mostly found on ECs [101–104]. The well investigated Angs are Ang-1 and Ang-2. Whereas Ang-1 is frequently produced by cells of mesenchymal lineage, including smooth muscle cells, Ang-2 is mostly secreted by ECs [105–108]. Quiescent ECs produce very low levels of Ang-2, but activated ECs drastically increase their Ang-2 production [108–110]. Ang-2 can, depending on the angiogenic context, either promote angiogenesis or induce regression of vessels [109, 111]. Two studies have yielded controversial results regarding the relevance of Ang-1 in PAH: Whereas cell-based gene transfer of Ang-1 reduced PAH in the MCT model [112], Ang-1 overexpression in the lungs of healthy rats resulted in the development of PAH [113]. These data suggest a context-specific effect of Ang-1 on the pulmonary vasculature.

BMPR2 loss of function is associated with the mutations found in hereditary and sporadic severe PAH [4]. One possible strategy is gene transfer of wildtype *BMPR2*, which was successful in preclinical studies in chronic hypoxic rats and MCT-treated rats [114, 115]. A second potential strategy would be restoration of intracellular signaling of mutant *BMPR2* [116].

SUMMARY AND PERSPECTIVE

The pathobiology of PAH is complex and our current therapeutic approaches are not curative. Although there has been considerable progress in the understanding of the pathobiology of stem and progenitor cell biology as well as approaches targeting vascular repair and regeneration in the context of lung vascular remodeling and PAH, there is considerable work to be done to understand the specific role of angiogenic factors and stem or progenitor cell populations in this complex disease. It is very likely that the curative treatment of severe PAH will require a multimodal approach that will utilize angiogenic vascular repair and stem/progenitor cell therapy. Because such therapies may also enhance the process of vascular remodeling, careful preclinical studies in models mimicking as many of the features of severe PAH as possible need to be conducted to rule out unwanted adverse effects.

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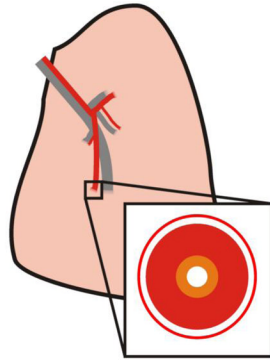
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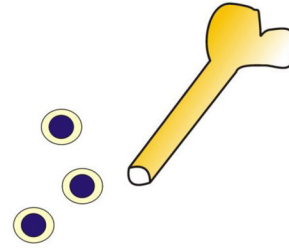
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Pulmonary vasculature and Lung



EPCs/ VESCs
MSCs
HPCs/HSCs

Bone marrow and circulation



EPCs/ VESCs
MSCs
HPCs/HSCs
Fibrocytes

Figure 1. Potential sources of stem and progenitor cells in the context of PAH.
 EPC – endothelial progenitor cell; VESC – vascular endothelial stem cell; MSC – mesenchymal stem cell; HPC – hematopoietic progenitor cell; HSC – hematopoietic stem cell.

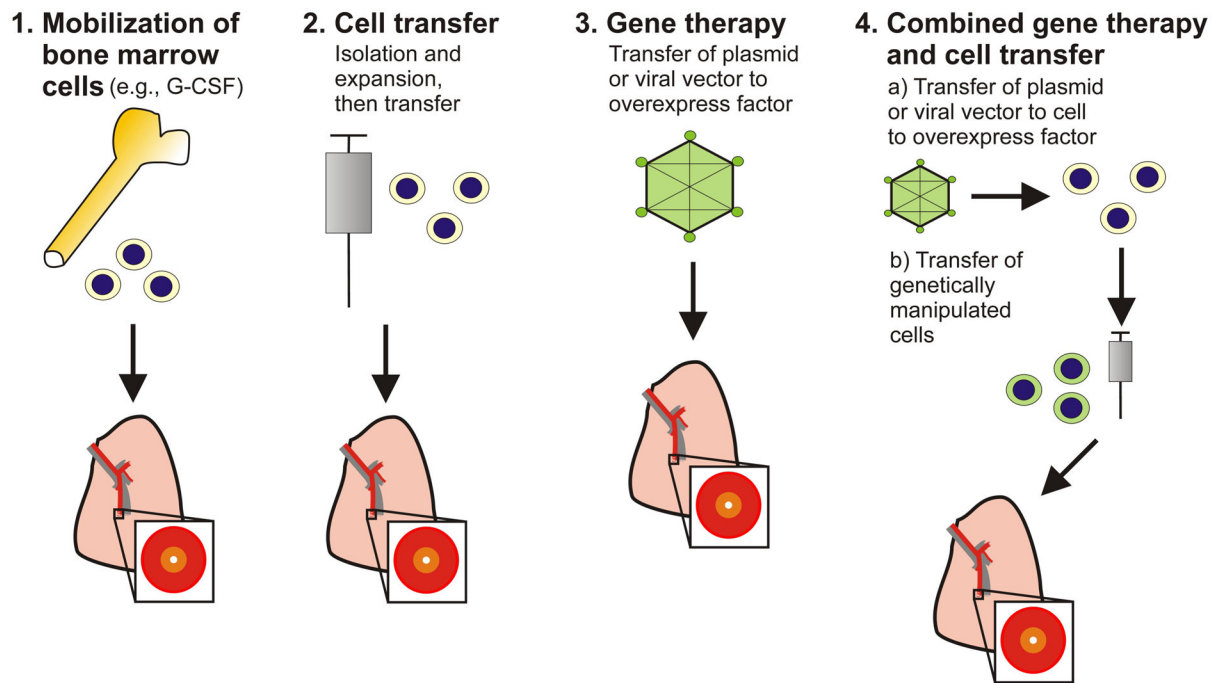


Figure 2. Potential treatment strategies targeting vascular repair and regeneration in the context of PAH

Note that these strategies have been mainly studied in preclinical animal experiments. A detailed overview can be found in the main text. One common way to mobilize bone marrow cells (1.) is the intravenous injection of granulocyte-colony stimulating factor (G-CSF). For cell transfer, cells are usually injected intravenously.