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The Emerging Role of Stem/Progenitor Cells in Pulmonary Vascular Disease

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Abstract

Pulmonary hypertension is a severe disease characterized by small pulmonary artery obstruction from vascular proliferation and remodeling leading to elevated mean pulmonary arterial pressure, increased pulmonary vascular resistance, right ventricular failure and death. Current treatments include prostacyclin analogs, endothelin receptor antagonists and phosphodiesterase type 5 inhibitors, which largely address mechanisms of endothelial dysfunction that were identified over 2 decades ago. Despite advances in understanding the disease mechanisms and the development of new pharmacological therapies, the prognosis of pulmonary hypertension remains poor. Recent advances in stem cell biology have unraveled the potential of stem/progenitor cells to repair damaged organs and offer the possibility for cell-based treatment for intractable diseases. This review summarizes the emerging role of stem/progenitor cells in the pathophysiology and the treatment of pulmonary hypertension.

Introduction

Pulmonary hypertension [1] is defined by an increase in pulmonary arterial pressure exceeding 25 mmHg at rest [2]. The five categories of PH have recently been revised in the Dana Point classification [3]. Pulmonary arterial hypertension (PAH) represents a subset of PH and is characterized by pulmonary arterial obstruction, increased pulmonary vascular resistance, leading to right ventricular failure and death.

Despite advances in therapeutic interventions targeting the vascular endothelium including prostacyclins, endothelin receptor blockers and phosphodiesterase type 5 inhibitors; the mortality and morbidity of PAH remain high. Animal models, and increasingly human studies, have advanced our understanding about the pathogenesis of PAH and enabled the development of novel pharmacological therapies. While at present there is no perfect preclinical model that completely recapitulates human PAH [4], all models have provided invaluable insight into the pathophysiology of PH, including the emerging role of stem/progenitor cells.

Pathophysiology of PAH

PAH is considered a highly specific vasculopathy that is limited to the lung, in particular the pre-capillary arteriolar bed. Histological features of PAH include intimal hyperplasia, medial hypertrophy, adventitial proliferation/fibrosis, occlusion of small arteries, thrombosis in situ, and infiltration of inflammatory/progenitor cells. The hallmark plexiform lesions are often found in advanced PAH and represent complex "glomeruloid" structures of poorly organized vessels surrounded by abnormal and proliferative endothelial-like cells. In addition to the abnormalities of the resident cells, increasing evidence points to the contribution of migrating cells to the pathogenesis of PAH [5].

Endothelium

Endothelial dysfunction is considered to play a major role in the pathogenesis of PAH. Disturbed proliferation of endothelial cells and altered production of endothelial-derived vasoactive mediators lead to structural remodeling of the pulmonary vasculature [6]. Angioproliferative "plexiform" lesions, which contribute to lumen obliteration, are found in PAH. The current understanding is that there is initial apoptosis of endothelial cells followed by disorganized proliferation of phenotypically abnormal vascular cells with endothelial and myofibroblast characteristics. It has been suggested that endothelial progenitor cells or other bone marrow–derived cells migrate, to the injured endothelium, [7] although it is unclear whether these serve to repair or promote this pathological process as discussed below.

Pulmonary artery smooth muscle cells (PASMCs)

In PAH, there is an increased proliferation of PASMCs. Pericytes differentiate into SMCs as a result of muscularization of distal pulmonary arteries, further thickening the SMC layer. Neointima is formed by recruitment of myofibroblasts with deposition of extracellular matrix between endothelium and internal elastic lamina, causing obliteration. Furthermore, increased proliferation and migration of SMCs, along with the progressive thickening of the proximal intra-acinar and pre-acinar muscular artery walls, also results in obliteration [8]. Recent evidence suggests there are similarities between cancer cells in cancer and PASMCs in PAH. PASMCs in experimental and human PAH exhibit a cancer-like glycolytic phenotype that drives cells to be resistant to apoptosis and amenable to specific therapeutic targeting [9].

Recently described circulating smooth muscle progenitor cells are characterized by the expression of markers of mesenchymal/smooth muscle lineage markers, such as, endoglin (CD105), α-SM-actin, calponin, SM myosin heavy chain, SM22, or platelet-derived growth factor receptor-β [10,11]. These cells may contribute to atherosclerotic plaque formation by producing extracellular matrix proteins [12]. However, their role in PH remains unexplored.

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Adventitia

There is increased production of extracellular matrix in the adventitia, including collagen, elastin, fibronectin and tenascin. Adventitial fibroblasts also become hyperproliferative and display increased sensitivity to serotonin [13]. A population of Mesenchymal progenitor cells – cells that are able to differentiate into at least one of the mesenchymal lineages – are present in adventitia and can give rise to fibroblasts, myofibroblasts and smooth muscle cells in response to vascular injury and play a role in vascular remodeling [14,15].

Similar to what has been described in pulmonary fibrosis, circulating *fibrocytes* seem to contribute to the pulmonary vascular remodeling in PAH [5,16]. *Fibrocytes* are currently defined as bone marrow–derived mesenchymal progenitors that co-express hematopoietic stem cell antigens, markers of the monocyte lineage, and fibroblast products [17]. These cells contribute to extracellular matrix remodeling and can further differentiate into myofibroblasts both *in vitro* and *in vivo,* under permissive microenvironmental conditions. The combination of collagen production and expression of CD45 (or one of the hematopoietic or myeloid antigens, such as CD11b, CD13 or CD34) is considered as sufficient criteria to discriminate *fibrocytes* from leukocytes, dendritic cells, endothelial cells, and tissue resident fibroblasts *in vivo* and *in vitro*. The resulting cell population produces more collagen and fibronectin than the relatively immature fibrocyte. Given their contribution to the pathogenesis of PAH, fibrocytes may hold promise as biomarkers or therapeutic targets.

These insights into the cellular and molecular basis of PAH have led to the development of new agents tackling more than endothelial dysfunction and aiming at stopping/reversing the abnormal cell and extracellular matrix accumulation. Promising pharmacological options include: (i) Rho kinase inhibitors [18]; [14] tyrosine kinase inhibitors (platelet-derived growth factor and epidermal growth factor receptor inhibitors); (iii) multikinase inhibitors (for tyrosine kinase and serine/threonine kinase), (iiv) elastase inhibitors [19]; (v) metabolic modulators [20], and (vi) peroxisome proliferator–activated receptors activators [21], all of which reverse PH in rodent models and early success in human PAH appear in case reports [22].

These observations also highlight the potential role of stem/progenitor cells in the pathophysiology of PAH. Recent insight into stem cell biology has enabled the isolation and characterization of a variety of stem/progenitor cells from various organs, including the lung. While this field of investigation is still relatively young, exciting findings about the role of stem/progenitor cells in respiratory health and disease have opened new therapeutic avenues for cell-based strategies for PH.

Definition and Types of Stem Cells

Stem cells are cells that have the capacity of self renewal and the ability to undergo differentiation into multiple phenotypes [23,24], therefore are known to play an important role in organogenesis, regeneration and tissue repair and maintenance. Stem cells have the capacity to produce one or more lineages. Depending on this differentiation potency of stem cells, they can be classified as totipotent (differentiate into all cell types e.g. zygote), pluripotent (differentiate into cells from all three germ layers), multipotent (capable of producing more than one cell lineage) or unipotent (differentiate into one cell type).

Stem cells can also be classified according to their tissue of origin [25]:

Embryonic Stem Cell [26]: They are derived from the blastocyst of an embryo from *in vitro* fertilization. Under appropriate conditions, ESCs are pluripotent and can differentiate into specialized somatic cells. ESCs have garnered a lot of controversy because of the ethical issues regarding the destruction of a human embryo. Some of the ethical and technical limitations of ESCs may be overcome by the recent advent of induced pluripotent stem cells (see below).

	CFU-Hill	**CAC**	**ECFC**
Replating ability	-	-	+
In vitro tube formation	+/-	+/-	+
In vivo de novo vessel formation	-	-	+
Homing to ischemic sites in vivo	+	+	+
Paracrine augmentation of angiogenesis	+	+	+/-
Phagocytosis of bacteria	+	+	-
Clonal proliferative status	-	-	+
Non-specific esterase expression	+	+	-
Phenotypic appearance	$CD34^{+/-}$	$CD34^{+/-}$	$CD34^{+}$
	$CD133^{+}$	$CD133^{+}$	$CD133^{-}$
	$VEGFR2^{+}$	$VEGFR2^{+}$	$VEGFR2^{+}$
	$CD45^{+/-}$	$CD45^{+/-}$	$CD45^{-}$
	$CD14^{+/-}$	$CD14^{+/-}$	$CD14^{-}$
	$CD115^{+}$	$CD115^{+}$	$CD115^{-}$
	$CD31^{+}$	$CD31^{+}$	$CD31^{+}$
	$ALDH^{bright}$	$ALDH^{bright}$	$ALDH^{bright/lo}$
	acLDL uptake	acLDL uptake	acLDL uptake
	UEA-1 lectin binding	UEA-1 lectin binding	UEA-1 lectin binding
	$eNos^{+}$	$eNos^{+}$	$eNos^{+}$
	von $Willebrand^{+}$	von $Willebrand^{+}$	von $Willebrand^{+}$

Properties in bold distinguish cells in ECFC assay from cells in CFU-Hill and CAC assays. The properties credited to an EPC are only fully displayed by ECFC and not CFU-Hill and CAC. CFU-Hill indicates colony forming unit-Hill; CAC, circulating angiogenic cells; ECFC, endothelial colony forming cells; VEGFR2, vascular endothelial growth factor receptor 2; ALDH, aldehyde dehydrogenase; AcLDL, acetylated low density lipoprotein; UEA-1, Ulex europeaus agglutinin-1; eNOS, endothelial nitric oxide synthase

Table 1: Characteristics and cell surface markers of cells comprising the adherent population in the commonly used assays of "EPC" identification (From Hirschi et al. 2008).

Somatic stem and progenitor cells: These cells can be isolated from adult human tissues as well as from cord blood. They are not as potent as ESCs and they have increasing degrees of fate restriction. These cells include amongst others, mesenchymal stem/stromal cells (MSCs) and endothelial progenitor cells (EPCs). The therapeutic potential of these cells has already been explored in animal models of PH as well as in clinical pilot studies.

Induced pluripotent stem cell (iPS): One of the most transformative contributions to the field of stem cell biology in the last decade is the engineering of pluripotency into somatic cells by the ectopic expression of transcription factors linked to pluripotency. Dr. Yamanaka's group was the first to reprogram mouse [27] and then human [28] fibroblasts through retroviral transduction by screening a panel of 24 transcription factors that are highly expressed in ES cells. This cadre of genes was progressively reduced to four that encode the transcription factors octamer 3/4 (Oct4), SRY box–containing gene 2 (Sox2), Kruppel-like factor 4 (Klf4), and c-Myc. iPS cell pluripotency is highly similar to ESCs [29,30]. The ability of mouse iPS cells to generate an entire mouse, and of human iPS cells to form teratomas *in vivo* [31] indicates in the most stringent tests that they are pluripotent cells and suggests that the defined factor reprogramming approach produces cells with a developmental potential similar to that of ES cells.

One of the numerous advantages of iPS cells over ESCs is the ability to engineer patient-specific iPS cells. This should soon be harnessed in the field of PH to provide (1) unprecedented insights into disease mechanisms and (2) a useful platforms for drug discovery. The generation of iPS from patients with known bone morphogenetic protein receptor 2 (BMPR2), ALK-1 or endoglin mutations may be the first step/good example for iPS technology becoming relevant for PH research.

The therapeutic effects of stem/progenitor cells in pulmonary hypertension and lung injury *in vivo* are summarized in table 2.

Endothelial progenitor cells (EPCs): Circulating EPCs in adult human peripheral blood were originally identified in 1997 by Asahara et al. [32], which challenged the paradigm that vasculogenesis is a process restricted to embryonic development. The ability to isolate a circulating cell that displays potential to give rise to cells appearing endothelial-like *in vitro* and with the potential to incorporate at sites of neoangiogenesis *in vivo*, spawned a new field of investigation. The isolation and further characterization defined the basic and translational properties of these presumed bone marrow-derived circulating EPCs [33]. Numerous preclinical studies in animal models suggest a high probability for successful clinical translation of EPCs as biomarkers, or cell therapies to treat ischemic disorders via new vessel formation. Hence, there is considerable interest in the potential of these cells to promote vasculogenesis and overcome endothelial dysfunction in PH.

a. **Role of EPCs in respiratory health and disease:** In lipopolysaccharide-induced murine lung injury EPCs (Sca-1+, Flk-1+) are rapidly released into the circulation and contribute together with other bone marrow-derived progenitor cells to lung repair [34]. In elastase-induced emphysema, bone marrow-derived cells develop characteristics of endothelial cells and contribute to the repair of alveolar capillary wall [35,36]. Patients with acute lung injury have 2-fold higher

Cell Type	Source/Route	Animal Model	Findings	Reference
ELPC and eNOS transduced ELPCs	Bone marrow / IV	MCT	When administered 3 days after MCT induced PAH - complete prevention of PAH 3 weeks after injury – prevented further increase in RVSP ENOS transduced ELPCs - normalized pulmonary hemodynamics and improved survival	Zhao, 2005 – PMID 15692087
Adrenomedullin gene-transduced EPCs	Umbilical cord blood/IV	MCT	Decrease in PVR and mean PAP, inhibition of increase in medial wall thickness	Nagaya, 2003 – PMID 12835224
EPC	Peripheral blood, culture media/ injection directly into lung parenchyma	MCT	EPC transplantation in lungs improved mean PAH, arterial pressure, CO, PVR and improved neovascularization	Takahashi, 2004 – PMID15265294
CGRP transfected EPCs	Peripheral blood/IV	Shunt operation – abdominal aorta to IVC	Decreased PAH and vascular remodeling	Zhao Q, 2007 – PMID17643632
EPCs	Peripheral blood/IV	Clinical study	Increase in mean distance walked in 6 mins and improvement in mean PAP, PVR and CO	Wang XX, 2007 – PMID17418297
Prostacyclin synthase-MSCs	Bone marrow/IV	MCT	Improved PH and pulmonary arteriolar remodeling and decreased RVH	Takemiya, 2009 – PMID 19838762
MSC	Bone marrow/IV	MCT	Improved right ventricular function, decreased right ventricular peak systolic pressure, pulmonary artery narrowing, alveolar septum thickening and RVH	Umar, 2009 – PMID19783775
MSC	Bone marrow /Intratracheal	MCT	Improved endothelium- dependent responses and decreased pulmonary arterial resistance and PVR	Baber, 2006 – PMID 16980338
MSC	Bone Marrow	Hyperoxia (95% O_2)	Attenuated alveolar and vascular lung injury and pulmonary hypertension in neonatal rats	Van Haaften, 2009 – PMID 19713449
MSC	Bone Marrow	Hyperoxia (75% O_2)	Reduced alveolar loss and prevented pulmonary hypertension in neonatal mice	Aslam M, 2009 – PMID 19713447
eNOS expressing MSCs	Bone marrow/IV	MCT	Improvement in right ventricular impairment, decrease in RVSP, RV/body weight ratio decreased	Kanki-Horimoto, 2006 – PMID 16820570
MSC expressing HO-1 transgene	Bone marrow/IV	Chronic hypoxia	Prevented and reversed PAH, reversed RV hypertrophy and vascular remodeling. HO-1 prevented against oxidative damage	Liang, 2011 – PMID20957739

MCT =Monocrotaline, RV= Right Ventricle, RVSP= Right Ventricular Systolic Pressure, CO= Cardiac Output, PVR= Pulmonary Vascular Resistance, RVH= Right Ventricular Hypertrophy, PAP=Pulmonary Arterial Pressure, IVC=Inferior Vena Cava, IV=Intravenous

Table 2: Stem/Progenitor cells in Pulmonary Hypertension.

number of circulating EPCs than healthy control subjects [37], suggesting the mobilization of EPCs may play some biological role in lung disease. Similar to the prognostic role of EPCs described in ischemic diseases, illness severity [37] and improved survival [38] appear to correlate with increased circulating EPCs in acute lung injury as well. Circulating EPCs are significantly increased in patients with pneumonia while patients with low EPC counts have persistent fibrotic changes even after recovery from pneumonia. In patients with chronic lung disease, the EPC (CD34+, CD133+, KDR+, kinase-domain region, also known as VEGF receptor 2 or fetal liver kinase-1/Flk-1) count is decreased and correlates with disease-severity [39]. In the developing lung, arrested alveolar growth in hyperoxia-induced BPD in neonatal mice is associated with decreased circulating, lung and bone marrow EPC (CD45-, Sca-1+, CD133+, VEGFR-2+) [40].

b. **Role of EPCs in PAH:** In PAH, vascular remodeling involves hyperproliferative and apoptosis-resistant pulmonary artery endothelial cells, which are thought to arise as a consequence of an initial endothelial injury and apoptosis. Therefore, it is unclear whether circulating EPCs will reduce vascular injury and promote repair, or whether they might contribute to an angioproliferative response and thus potentially worsen the disease. Levels of circulating CD34+ CD133+ bone marrow-derived proangiogenic precursors were higher in peripheral blood from patients with PAH than in healthy controls and correlated with pulmonary artery pressure, whereas levels of resident endothelial progenitors in PAH pulmonary arteries were comparable to those of healthy controls [41]. Colony-forming units of endothelial-like cells (CFU-ECs) derived from CD34+ CD133+ bone marrow precursors of PAH patients secreted high levels of matrix metalloproteinase-2, had greater affinity for angiogenic tubes, and spontaneously formed disorganized cell clusters that were hyper-responsive to transforming growth factor-β or BMP-2. In NOD SCID mice with PAH, subcutaneous injection with CFU-ECs within Matrigel plugs, but not with control CFU-ECs, produced cell clusters in the Matrigel and proliferative lesions in surrounding murine tissues. Thus, mobilization of high levels of proliferative bone marrow-derived proangiogenic precursors is a characteristic of PAH and may participate in the pulmonary vascular remodeling process [41]. Conversely, Junhui et al. [42] found reduced number and activity of circulating, AC133+(CD133), KDR+ EPCs in patients with idiopathic PAH. Likewise, Diller et al. [43] found decreased number of circulating CD34+, CD34+/AC133+, CD34+/KDR+, and CD34+/AC133+/KDR+ progenitor cells in Eisenmenger patients compared with healthy control subjects. Reductions in EPC numbers correlated with New York Heart Association functional class, 6-minute walk distance, and plasma brain-type natriuretic peptide levels. The capacity of cultured peripheral blood mononuclear cells to form colonies and incorporate into tube-like structures was impaired in Eisenmenger patients. Idiopathic PAH patients had reduced numbers of EPCs, and the number of circulating EPCs correlated with invasive hemodynamic parameters in this cohort. Interestingly, treatment with the phosphodiesterase inhibitor sildenafil was associated with a dose-dependent rise in EPC numbers within the idiopathic PAH population, resulting in levels consistently above those found with other therapies. Accordingly, Hansmann et al. demonstrated that in patients with IPAH, PAH associated with congenital heart or connective tissue disease, the number of CD34+/KDR+ and CD34+/KDR+/CD31+/CD45- (so-called "late" EPCs, i.e. EPCs in advanced differentiation) were about half the numbers of matched controls [44]. The authors used a novel microfluidic device for easy capture and rapid enumeration of EPCs by surface markers (CD34+/KDR+/CD31+/CD45-) that may prove useful for evaluation of disease severity and guidance of therapeutic interventions [44].

Conversely, there have been studies that show a detrimental role of EPCs in vascular remodeling [45]. Toshner et al. found that EPC markers, especially in the plexiform lesions, were upregulated in all patients with PAH [46]. In addition, the number of circulating angiogenic progenitors (CD133+, CD34+, VEGFR2+) were increased. The late outgrowth progenitor cells showed a hyperproliferative phenotype and impaired vascular network formation in patients with PAH with BMPRII mutation. Proangiogenic progenitor cells (CD34+, CD133+) also contribute to the pathogenesis of asthma and PAH [47]. These reports suggest that while EPCs may show therapeutic benefit, they may also potentially contribute to disease progression. Further studies are therefore required to elucidate which EPC phenotype has a beneficial role and which may have a deleterious effect.

c. **Controversy around the definition of EPCs:** A major limitation has been the lack of a clear definition of how to unambiguously identify circulating EPCs, and thus, deciding which cell population to infuse into patients. Various methods exist for the isolation and identification of EPCs [48,49] (Figure 1). Most of the above mentioned studies relied on the expression of cell surface markers such as CD34, CD133, and VEGFR2 (KDR/Flk-1); however, there is yet no clear agreement on what markers define a "true" EPC. Alternatively, EPCs can be derived by culturing MNCs in conditions that promote EC specification. Schematically, there are three major methods to culture of EPCs from circulating mononuclear cells (MNC). In one assay, nonadherent MNCs cultured on fibronectin form so-called colony forming unit-Hill (CFU-Hill) within 5-9 days [50]. CFU-Hills display some phenotypic and functional characteristics of endothelial cells, including expression of cell surface antigens (CD31, CD105, CD144, CD146, vWF, and VEGFR2) and uptake of AcLDL. However, they also express hematopoietic-specific antigens CD45 and CD14, display nonspecific esterase and phagocytic capabilities consistent with monocytes/macrophages, and cannot be propagated long term in culture [49]. A second assay identifies adherent, so called "circulating angiogenic cells" (CACs) following 4 days of culture in endothelial specific conditions [51,52]. Likewise, CACs resemble endothelial cells phenotypically but are also enriched for hematopoietic-derived monocytes/macrophages. Less studied cells are the so-called endothelial colony forming cells (ECFCs) [53-57]. Cells plated on collagen I in endothelial growth media form cobblestone-like adherent colonies within 6 days from umbilical cord blood MNCs, or 14-21 days from adult peripheral MNCs [56]. A very similar population can also arise from the prolonged (~2 week) culture of MNCs on fibronectin and have been termed late outgrowth EPCs. By definition, a true EPCs is a cell that can be clonally and serially replated in culture and will give rise to endothelium by differentiation *in vitro* or *in*

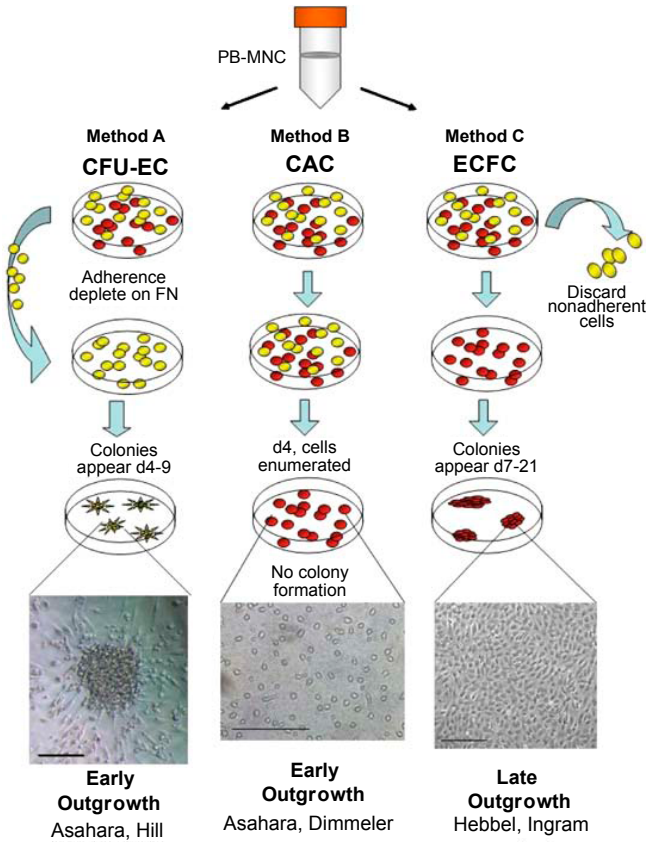

Figure 1: Common Methods of EPC culture (from Prater et al., 2007, with permission).

vivo. In contrast to CFU-Hills and CACs, which display various monocyte/macrophage phenotypes and function (Table 1) [58], ECFCs are characterized by (i) robust proliferative potential, [14] secondary and tertiary colony formation upon replating, and (iii) de novo blood vessel formation *in vivo* when transplanted into immunodeficient mice [59]. Current efforts focus on improved characterization of circulating EPCs. Using a combined protocol including polychromatic flow cytometry, colony assays, immunomagnetic selection, and electron microscopy, Mund et al. were able to reliably identify ECFCs and mature circulating endothelial cells (CD34+, CD31+, CD146+, CD105+, CD45-) in circulating peripheral blood and cord blood with ECFCs being increased in cord blood and extremely rare in the peripheral blood of healthy adults [60]. In summary, evidence suggests that CFU-Hill and CACs are not true EPCs, but modified myeloid lineage cells that participate in neoangiogenesis. Nonetheless, these cells still display therapeutic benefit, but they do not require direct transdifferentiation into the endothelial monolayer of new vessels. In contrast, ECFC display all the features of a true EPC: clonal proliferative capacity, hierarchy of proliferative potential, and *de novo* vessel forming ability *in vivo*. However, in the only study directly comparing the therapeutic potential of early versus late outgrowth EPCs in the treatment of experimental PAH, only early population of endothelial-like, culture-modified monocytes were able to prevent MCT-induced PAH [61]. In addition, recent studies suggest that highly proliferative ECFCs may contribute to plexiforms lesions [47]. Thus, while the highly proliferative forms of "true" EPCs may be potentially exciting, both their efficacy and safety need to be defined in rigorous preclinical testing before any consideration to use these cell populations in translational clinical studies.

All together, these findings suggest that EPCs leave the bone marrow, enter the circulation and then migrate to the pulmonary vasculature and perivascular tissue where they contribute to repair the injured endothelium and help restoring lung integrity. Since endothelial dysfunction plays a role in PH and EPCs are responsible for endothelial homeostasis and neovascularization, various studies have been conducted to explore the therapeutic potential of EPCs in PAH.

d. Therapeutic potential of EPCs in PH: One of the first studies was performed in dehydromonocrotaline induced PH in dogs. EPC transplantation in the lungs using a bronchoscope, improved mean pulmonary arterial pressure, cardiac output and pulmonary vascular resistance and improved neovascularization of the lung [62]. Subsequent studies seem to suggest that the therapeutic activity of EPCs could be enhanced by genetic engineering. Zhao et al. [63] explored the role of bone marrow derived endothelial like progenitor cells in MCT-induced PH in rats. ELPC were characterized by Dil acetylated LDL uptake, UEA-1 lectin staining, and immunostaining to detect vWF and Flk-1 expression. Endothelial like progenitor cells administered 3 days after MCT completely prevented PH. Endothelial like progenitor cells incorporated in the pulmonary microvasculature. When endothelial like progenitor cells were administered 3 weeks after MCT, they prevented further increase in right ventricular systemic pressure. Importantly, only endothelial like progenitor cells transduced with eNOS were able to significantly reverse established PH. Adrenomedullin gene-transduced EPCs, derived from umbilical cord blood, improved MCT-induced PH in rats to a greater extent than EPCs alone [64]. EPCs transfected with calcitonin gene related peptide significantly decreased PH and vascular remodeling in immunodeficient rats with an abdominal aorta to inferior vena cava shunt operation [65].

Decreased BMPR2 expression and derangement of TGF-ß signaling has been implicated in the pathogenesis of PAH. In a recent study, adenoviral BMPR2 gene delivery to pulmonary vascular endothelium in chronic hypoxia and MCT –induced PAH, showed improved pulmonary and cardiac function and reduced vascular remodeling. In addition, increase in TGF-ß was prevented by BMPR2 treatment and endothelial-mesenchymal transition, brought on by TGF-ß1, was partially improved [66]. The therapeutic potential of BMPR2 upregulation alone or in combination with genetically engineered EPCs in PAH remains to be explored.

In a pilot clinical study to test the feasibility and safety of intravenous infusion of autologous EPCs in patients with IPAH, EPC therapy improved exercise outcomes and hemodynamics in patients with idiopathic PAH [67]. This treatment was also found to be safe in children with idiopathic PAH [68]. An early phase clinical study has also been initiated (http://clinicaltrials.gov/, Identifier: NCT00469027) in Canada, to investigate the safety and potential efficacy of eNOS-enhanced autologous EPCs in patients with severe PAH, refractory to all available therapies. The Pulmonary Hypertension And Cell Therapy (PHACeT) trial is a dose escalation study, ranging from 7 to 50 million cells. Currently 7 out of 12 patients have been enrolled into the trial and received the therapy.

Plastic-adherent in standard culture conditions
≥ 95% of the MSCs must express CD105, CD73 and CD90 and lack expression of CD45, CD34, Cd14 or Cd11b, CD79α or CD19, HLA-DR
Must be able to differentiate *in vitro* into osteoblasts, adipocytes, chondroblasts

Table 3: Minimal criteria for defining MSC [70].

Mesenchymal stem/stromal cells (MSCs)

MSCs are plastic adherent adult stem cells originally described in the bone marrow with the ability to form cells within the osteogenic, chondrogenic, and adipogenic lineages [69]. While the definition of MSCs is still evolving, minimal criteria to define a human MSC have been established by the International Society for Cellular Therapy (Table 3) [70]. MSCs, because of their ease of culture and pleiotropic properties, are the major stem cell-based therapy explored in a great variety of experimental lung diseases [71]. Their therapeutic potential has also been explored in PH.

Intratracheal administration of rat MSCs in MCT-induced PH improved endothelium-dependent vasodilatation of pulmonary arteries and decreased pulmonary vascular resistance. Despite the intratracheal administration, immunolabeled cells were not detected in the pulmonary vessel wall [72].

Bone marrow derived MSC from donor rats with MCT induced PAH when intravenously administered to recipient rats with MCT induced PAH (to mimic autologous transplantation) improved right ventricular function, pulmonary artery narrowing, alveolar septum thickening and right ventricular hypertrophy [73]. In neonatal PH associated with oxygen-induced lung injury, intratracheal MSCs prevented the decrease in pulmonary arterial acceleration time, right ventricular hypertrophy and decreased lung angiogenesis [74].

Similar to EPC therapy, MSCs were used as a vector for delivery of prostacyclin synthase gene. MSCs engineered to overexpress the prostacyclin synthase gene engrafted in the lung and restored prostacyclin synthesis in MCT-induced PH. This was associated with an improvement in PH, pulmonary arteriolar remodeling and decreased right ventricular hypertrophy [75]. Similar results were described with MSCs overexpressing eNOS [76]. More recently, MSCs overexpressing human hemeoxygenase-1 transgene in the lung, prevented and reversed chronic hypoxia induced PAH in mice [77].

This study also explored the paracrine effect of cell-based therapies. Indeed, the limited amount of cell engraftment suggests that alternate mechanisms account for the therapeutic benefit of these cells [78]. *In vitro* experiments showed that conditioned medium of these MSCs attenuated hypoxia-induced lung inflammation and inhibition of smooth muscle cell proliferation [77], suggesting new therapeutic avenues for the treatment of PH. Likewise, Aslam et al showed that a single injection of MSC conditioned media prevented vascular changes and right ventricular hypertrophy in a hyperoxic neonatal mouse model [79].

Compared to EPCs, MSCs are immmunopriviliged and thus provides the advantage of making heterologous MSC transplantation a potential approach for clinical therapies [78]. Given that engraftment seems to play a minor role in the therapeutic benefit of MSCs, the legitimate question remains which cell-based strategy is the most advantageous in terms of feasibility, efficacy and safety: whole cell administration or cell-free conditioned media applications, or delivery of certain conditioned media components.

Conclusion

More than a century has passed since the first recorded description of pulmonary vascular disease by Romberg in 1891 [80]. Since then we have developed a greater understanding of the pathophysiology, diagnosis and treatment of pulmonary vascular disease. Despite these advances, pulmonary vascular disease remains a disease with grim prognosis and early diagnosis remains a challenge. In order to combat the poor outcome of pulmonary vascular disease, a better understanding of the disease mechanism is required to alter the natural history of the disease. In addition, better diagnostic tools need to be developed for early diagnosis. The current treatment focuses on restoring the vasomotor tone of the pulmonary vasculature. New insights into the pathophysiology of pulmonary vascular disease will allow focusing on therapies that alter and/or reverses the disease process. Increased understanding of the biology of stem cells and their involvement in the pathophysiology of pulmonary vascular disease have opened new therapeutic avenues for cell-based therapies and clinical proof of concept and safety trials using EPCs are currently under way. Other therapeutic targets may include the modulation of resident and circulating stem/progenitor cells that contribute to the pathophysiology of pulmonary vascular disease. Finally, the recent discovery of iPS cells will undoubtedly shed new light on some of the genetic causes of pulmonary vascular disease and provide a platform for drug testing.

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