

The role of bone marrow-derived endothelial progenitor cells and angiogenic responses in chronic obstructive pulmonary disease

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Abstract: Increased vascularity of the bronchial sub-mucosa is a cardinal feature of chronic obstructive pulmonary disease (COPD) and is associated with disease severity. Capillary engorgement, leakage, and vasodilatation can directly increase airway wall thickness resulting in airway luminal narrowing and facilitate inflammatory cell trafficking, thereby contributing to irreversible airflow obstruction, a characteristic of COPD. Airway wall neovascularisation, seen as increases in both the size and number of bronchial blood vessels is a prominent feature of COPD that correlates with reticular basement membrane thickening and airway obstruction. Sub-epithelial vascularization may be an important remodelling event for airway narrowing and airflow obstruction in COPD. Post-natal angiogenesis is a complex process, whereby new blood vessels sprouting from extant microvasculature, can arise from the proliferation of resident mature vascular endothelial cells (ECs). In addition, this may arise from increased turnover and lung-homing of circulating endothelial progenitor cells (EPCs) from the bone marrow (BM). Following lung-homing, EPCs can differentiate locally within the tissue into ECs, further contributing to vascular repair, maintenance, and expansion under pathological conditions, governed by a locally elaborated milieu of growth factors (GFs). In this article, we will review evidence for the role of BM-derived EPCs in the development of angiogenesis in the lung and discuss how this may relate to the pathogenesis of COPD.

Keywords: Endothelial progenitor cells; angiogenesis; chronic obstructive pulmonary disease (COPD)

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by fixed airflow obstruction and progressive decline of lung function, in parallel with frequent exacerbations. An emerging hypothesis suggests that pulmonary vascular endothelial dysfunction, as shown by alterations in vessel structure, abnormal endothelial cellular growth, and resistance to apoptosis, is a key component of COPD. It is thought that endothelial cell (EC) damage and abnormal function of pulmonary vessels triggers downstream pulmonary vascular remodelling, leading to

pulmonary hypertension (PH). The abnormal pulmonary vessel function that promotes vascular remodelling in COPD can be attributed to up-regulated vascular permeability, vasodilation, and angiogenesis (1-3).

It was initially proposed that angiogenesis in COPD was caused by proliferation, migration, and remodelling of fully differentiated ECs derived from pre-existing blood vessels. However, accumulating evidence suggests that COPD is not merely a local airway disease, but has a systemic component, involving the activation of hemopoietic events within the bone marrow (BM), leading to the mobilization and homing of endothelial progenitor cells (EPCs) to the lungs where

these cells are involved in disease pathology. Investigating the biological processes through which airway remodelling occurs in COPD may identify novel therapeutic targets, and provide an understanding of its contribution to progressive decline in lung function.

We propose two mechanisms by which airway EPCs may contribute to COPD pathogenesis. Firstly, upon homing to the lungs, EPCs may have dysfunctional activity, thereby preventing normal repair and maintenance of pulmonary vasculature in turn promoting disease pathology (4,5). Alternatively, recruited EPCs to the airways may have increased activity that drives inflammation and enhances EC proliferation in the vascular wall resulting in pulmonary vessel remodelling and PH (6). Conversely, other groups have theorized inadequate or impaired mobilization of EPCs from the BM, thereby preventing repair processes within the pulmonary vasculature (5,7,8).

It is clear, that despite an increasing interest, the contribution of the EPCs to airway remodelling in human COPD has not been fully understood. A significant impediment to investigating the role of EPCs in disease remains the lack of standardized phenotypic definition of these cells and consensus on correct methods for enumeration. The phenotypic and functional overlap between EPCs, hemopoietic progenitor cells (HPC), and mature ECs further complicate this matter. These issues will be addressed in the current article.

EPCs

The initial observation by Asahara *et al.* that human peripheral blood (PB) contains a population of circulating cells capable of forming mature ECs *in vitro* and contributing to the formation of new blood vessels *in vivo* indicated the presence of a population of circulating EPCs (9). Shi *et al.* then showed that genetically tagged donor BM cells covered implanted grafts in the recipients (10), further implicating a contribution of BM-derived cells to vascular changes. To date, two different types of EPCs from adult PB have been identified: early EPCs, which are highly adhesive but have slow proliferative capacity and late EPCs, which are weakly adhesive yet have high proliferative properties (11).

Subsequent studies in pathological conditions such as myocardial infarction, limb ischemia, wound healing, atherosclerosis, and tumour vascularisation have, to varying degrees, supported the view that EPCs promote angiogenic sprouts and formation of new blood vessels in

adult tissue (12). These studies suggest that while BM-derived EPCs contribute to early angiogenic switch, once neovascularisation is established, the traffic of these cells is limited and extra-medullary processes perpetuate the local tissue neovascularisation process (13,14). It is possible, that targeting of the lung-homing and local proliferative potential of EPCs in pulmonary tissue could modulate airway remodelling in COPD, however additional research is needed to clarify.

Phenotypic identification of EPCs

EPCs have been phenotypically difficult to isolate from HPCs given that they express similar markers including, CD34, CD31, and vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2) (7). Initially, EPCs were defined as cells positive for CD34 and VEGFR2, however these markers are also expressed on mature ECs and were therefore deemed insufficient to identify the population of interest (13,14). Since then, additional immature hematopoietic stem cell markers have been included. CD133 is a 5-transmembrane glycoprotein expressed by pluripotent hematopoietic stem cells, but not mature ECs (15-17). In particular, Peichev *et al.* reported that 2% of mobilized human PB CD34⁺ cells co-expressed VEGFR2 and CD133, whereas CD34⁺VEGFR2⁺ EC from human umbilical vein failed to express CD133, thus circulating CD34⁺VEGFR2⁺CD133⁺ cells were thought to represent an EPC phenotype (16). Since then, subsequent papers have identified EPCs using the markers CD34, VEGFR2, and CD133.

There is a paucity of studies that formally compare the functional properties of PB-derived CD34⁺VEGFR2⁺CD133⁺ cells in hematopoietic and endothelial assays (reviewed in Alaiti *et al.* 2010) (18). Interestingly, more than 99% of CD34⁺VEGFR2⁺CD133⁺ cells also express CD45⁺, which is a leukocyte marker. Given that the endothelial lineage is negative for CD45, even at the mRNA level (19), it is not surprising that in some reports where purified PB EPCs were identified as CD34⁺VEGFR2⁺CD133⁺CD45⁺ cells, these cells did not give rise to endothelial colonies *in vitro* (20). As such, some research groups have used CD45^{dim}, in addition to other standard EPC markers to accurately enumerate this cell population (21-24). Other studies have utilized co-expression of CD34 and fibroblast GF receptor-1 (FGFR-1), or CD34 and VEGFR3 to identify EPCs (25,26).

With respect to animal models, mice do not express

CD133, thus other markers have been employed to identify EPCs in the murine model. These markers include stem-cell antigen-1 (Sca-1), which has consistently been used as a primitive marker for stem cells (27), c-kit, which is a cytokine receptor for stem cell factor, and VEGF2R. Thus, in murine models, cells expressing Sca-1⁺c-kit⁺VEGF2R⁺ have been consistently characterized as EPCs (27).

The majority of peer-reviewed studies in humans have employed the CD34⁺VEGFR2⁺CD133⁺ or CD34⁺VEGFR2⁺CD45^{dim} phenotype to enumerate EPCs, however there still remains a lack of clear consensus in the literature as to the exact phenotypic profile that should be employed for identifying EPCs. An effective enumeration strategy may be utilized the CD34⁺VEGFR2⁺CD133⁺CD45^{dim} phenotype as this removes a major population contaminating leukocytes from the EPC regions.

Role of EPCs in angiogenesis and airway remodelling

Angiogenesis is a complex, multi-phase process that generates new vessels via a balanced equilibrium between pro-angiogenic and anti-angiogenic factors (28-30). During chronic inflammation, vascular remodelling processes are initiated by the predominance of pro-angiogenic growth factors (GFs). Airway angiogenesis has been correlated with airway hyper-responsiveness and is thought to contribute to disease pathology by either supporting growth of other hyperplastic cells, such as airway smooth muscle, or contributing to airway thickness by providing additional permeable vasculature, leading to oedema and airflow obstruction (28-32). Asahara was the first to propose that activated EPCs migrate to sites of angiogenesis, terminally differentiate into mature ECs, and incorporate into new vessels, further propagating disease pathogenesis (6).

Various studies have supported the aforementioned theory of EPCs playing a contributing role to angiogenesis. When first investigated, cultured cells isolated from human PB not only demonstrated morphological characteristics of mature ECs after one week, but also expressed von Willebrand factor (vWf) and decreased expression of CD133, suggesting differentiation of EPCs into mature ECs (33). When plated on fibronectin, these cells began to form tube-like structures, which resembled vasculature (33). This differentiation and contribution to angiogenesis was further demonstrated *in vivo*, by injecting a bolus of human CD133⁺CD34⁺VEGF2R⁺ cells into a mouse model of hind-limb ischemia (33). Here it was found that a significant

fraction of capillaries in the re-perfused limb were of human origin, suggesting an essential role of EPCs in post-natal vasculogenesis (33). Kalka *et al.* demonstrated that infusion of *ex vivo* expanded human EPCs, could be detected in newly formed vessels in mice with ischemic limbs, which lead to subsequent increased rates of blood flow recovery and capillary density, with reduction in limb loss. Furthermore, in a rat model of myocardial infarction, infusion of human CD34⁺ cells could be detected in newly formed capillaries. An *in vitro* study, further supported these findings, by demonstrating green fluorescent protein (GFP) labelled EPCs cultured on fibronectin with human umbilical vein endothelial cells (HUVEC) appeared to be completely incorporated into newly formed tube-like structures (28). Similarly, work by Peinado *et al.* using CD133⁺ cells, showed cells with positive immunoreactivity to this antigen were in present in denuded areas of the endothelium and within the intimal layer of pulmonary arteries from COPD patients (34). CD133⁺ EPC numbers within vessel walls were associated with contractile response to hypoxic stimulus, with an additional association between wall thickness and EPC number in the intima, VEGF and VEGFR mRNA expression (34).

Although, these studies demonstrate a role for EPCs in angiogenesis, it is not well understood whether this function can contribute to repair or pathogenesis in airway disease. Studies demonstrate that transplantation of autologous EPCs or unfractionated BM cells facilitates vascular repair, whereas others have suggested that circulating EPCs participate in pathogenesis of various diseases by inducing smooth muscle cell (SMC) proliferation and neo-intimal formation at sites of vascular injury (34,35). Thus, EPCs may exert an opposite effect on injured tissue by either promoting endothelial repair when differentiated into mature ECs, or vascular remodelling when lineage-committed to SMCs.

Recent literature also proposes that EPCs have the potential to produce GFs that stimulate local angiogenic responses in a paracrine fashion. Evidence from mouse tumour models suggest minimal incorporation of EPCs into the vascular wall and instead support the view that EPCs promote angiogenesis by secreting factors such as VEGF, stromal-derived factor-1 (SDF-1), and granulocyte-macrophage colony stimulating factor (GM-CSF), which promote recruitment of mature ECs and tissue vascularisation (36,37).

It is clear that the activity of EPCs can contribute to pulmonary vasculature change, including angiogenesis.

However, more research is needed to determine the extent to which this activity contributes to the pathogenesis of COPD. Specifically, the underlying mechanism that promotes the role of EPC in angiogenesis, and whether the targeting of these cells will serve as a novel therapeutic avenue.

Enumeration of circulating and local EPCs in COPD

To date, three methods have been described to enumerate EPCs in COPD. The first method quantified EPCs by the culturing of blood mononuclear cells as early growth EPC-colony forming units (CFUs). Their therapeutic potential is believed to depend on high levels of GF including VEGF, SDF-1, and GFG that enhance network formation of ECs (37). The second method involves culturing of late outgrowth EPCs, typically observed 2–3 weeks post-culture and express endothelial markers. These cells are highly proliferative and can form a vascular network of their own, thus they are often referred to as EC-CFUs. The third method utilizes flow cytometry to identify and enumerate EPCs using expression of specific immunofluorescent endothelial markers. While most studies indicate lower levels of circulating EPCs, with impaired function, in COPD patients compared to healthy controls, others do not find a significant difference. These discrepancies in the literature may be due to differences in the methods and phenotype used to enumerate EPCs and a failure to accommodate for co-morbidities, gender, and age between control groups. The latter point is particularly important, given that smoking history, age, and sex all have significant influences on EPC number and function (8,38-40).

Enumeration of EPCs with early outgrowth EPC-CFUs in COPD

The EPC-CFU assay was originally discovered by Hill *et al.* in 2003, and employed to overcome difficulties associated with quantifying small numbers of circulating cells by flow cytometry (40). Various studies have enumerated PB EPCs by measuring EPC-CFUs in mononuclear cell cultures in COPD patients. Takahashi *et al.* found impaired mobilization and proliferation of EPC-CFUs in COPD patients compared to healthy controls (4). Similarly, Liu *et al.* reported reduced numbers of EPC-CFUs isolated from human PB in COPD patients relative to healthy controls (5). Contrary to these findings, Brittan *et al.* demonstrated a 4-fold increase in the number of EPC-CFUs in COPD

patients compared with healthy controls (41). Variation in these studies findings may be attributed to differences in smoking history and co-morbidities between the subject populations.

It is important to note that although EPC-CFUs express a variety of EC characteristics, they represent a heterogeneous population that includes myeloid cells (42,43). Accordingly, it is not known whether these colonies arise directly from circulating EPCs or the progeny of myeloid cells that indirectly contribute to angiogenesis via paracrine processes (6,44).

Enumeration of EPCs with late outgrowth CFUs in COPD

EC-CFUs have been thought to originate in BM together with EPCs, however EC-CFUs differ with respect to having a robust proliferative potential, express endothelial markers but not hematopoietic or monocytic markers, and form *de novo* blood vessels when transplanted into immune-deficient mice (43,45). It has been suggested that only EC-CFUs are true EPCs with clonogenic and proliferative potential. Utilization of EC-CFUs has been used to not only help enumerate EPCs in COPD patients, but also assess the function of these cells in the context of angiogenic capacity.

Liu *et al.* showed that the number of late outgrowth EC-CFUs and their adherence to HUVEC tubules was reduced in COPD patients, with fewer VEGFR2⁺ staining cells incorporated into injured tissue sites (5). Similarly, Yang *et al.* found fewer adhering EPCs to HUVEC in COPD patients, along with reduced proliferative capacity and nitric oxide (NO) production (46). Paschalaki *et al.* investigated EPC dysfunction in smokers by isolating and expanding EC-CFUs from PB of healthy controls, healthy smokers, and COPD patients (47). EC-CFUs from smokers and COPD patients had increased DNA double-strand breaks and senescence compared to non-smokers (47). They further showed using an *in vivo* mouse model that EC-CFUs from COPD patients had impaired angiogenic activity and increased apoptosis (47). This is consistent with previous reports of increased DNA damage and senescence in peripheral lungs of normal smokers and to a greater degree in COPD patients compared to non-smoking controls (48-50). Michaud *et al.* found that EPCs isolated from PB of smokers had reduced proliferative capacity and participation in tube formation, along with reduction in EC-specific markers (8). Collectively, these findings suggest that the dysfunction of circulating EPC may contribute to

impaired blood vessel repair and growth in both smokers and COPD patients.

Flow cytometry enumeration of EPCs in COPD

Initial reports in COPD, have shown a significant decrease in PB CD34⁺ cells and hemopoietic-CFUs, compared to healthy controls (51). However, given these studies did not use specific EPC markers, they could not infer whether EPCs were selectively reduced in COPD patients. Pizarro *et al.* measured CD34⁺CD45^{dim}CD133⁺ cells at baseline and reported that COPD patients had lower numbers of PB EPCs compared to healthy controls, however COPD patients with PH had greater numbers of PB EPCs compared to those without PH (52). Interestingly, they found no difference in PB EPC number between smoking and non-smoking COPD patients, suggesting that the reduction of EPCs was a consequence of the disease itself and not related to smoking. Conversely, Michaud *et al.* found reduced numbers of PB EPCs in smokers versus non-smokers (8), and determined that low serum antioxidant levels in smokers correlated with reduced availability of NO and EPC functional activity. They further showed that proliferation and migration in response to VEGF was significantly impaired in EPCs isolated from smokers, with reduced expression of VEGFR2 (8). Similarly, Yue *et al.* reported decreased PB EPCs in smokers with cardiac arterial disease, which was associated with elevated pulmonary arteries systolic pressure (53). In addition, Kondo *et al.* reported that PB EPCs (CD45^{dim}CD34⁺VEGFR2⁺CD133⁺) were reduced in chronic smokers compared to non-smokers, with EPCs incapable of forming colonies (38). They further showed that cessation of smoking led to rapid restoration of EPC counts (38). The aforementioned findings suggest that potential mechanisms responsible for the negative effect of smoking on EPCs include, impairment of EPC mobilization from the BM, increased oxidative stress, decreased NO availability and impaired differentiation towards an endothelial phenotype.

Fadini *et al.* expanded on these findings by showing that PB EPCs, defined as CD34⁺VEGFR2⁺CD133⁺ cells, were decreased in COPD patients relative to healthy controls, which correlated with disease severity (54). Similarly, Yang *et al.* found PB CD34⁺VEGFR2⁺CD133⁺ cell numbers in COPD to be reduced compared to healthy controls (46). Sala *et al.* further demonstrated that PB CD34⁺VEGFR2⁺ cells were higher in exacerbated COPD patients compared to those with stable COPD and healthy controls but no

significant difference between EPCs in stable COPD patients and healthy controls (55). A positive association between PB EPC numbers and VEGF levels in stable and exacerbated COPD patients (55). Doyle *et al.* employing PB CD34⁺VEGFR2⁺ and CD34⁺VEGFR2⁺CD133⁺ gating strategies found that both gated populations of EPCs were reduced in COPD patients, which correlated inversely with the extent of emphysema. For example, both populations were related to extent of panlobular emphysema and diffusion capacity. We have recently shown that while HPCs (CD45^{dim}CD34⁺ cells) are comparable between COPD patients and healthy controls, PB EPCs (CD34⁺CD45^{dim}CD133⁺ cells) were markedly decreased (24). Interestingly, Guerin *et al.* found CD45⁻CD133⁺ cells to be significantly higher in hypoxic COPD patients compared to non-hypoxic COPD patients, suggesting that HPCs are mobilized from BM to PB in patients with hypoxia. Lastly, Huertas and colleagues found a greater reduction in PB CD34⁺VEGFR2⁺ cells in COPD patients with low BMI versus normal BMI, suggesting greater systemic impairment (56).

In contrast to the aforementioned studies, there have been several reports of contradictory results. Janssen *et al.* found no difference between PB CD45^{dim}CD34⁺ or CD45^{dim}CD34⁺VEGFR2⁺ cells in COPD patients and healthy controls. However, they found PB CD45⁺CD34⁺VEGFR⁺CD133⁺ cells to correlate with airway obstruction and were lowest in subjects with severe emphysema (57). They also reported that the ability to form EC-CFUs was reduced in COPD subjects (57). Brittan *et al.* demonstrated that while PB CD34⁺VEGFR2⁺CD133⁺ cells were not reduced in COPD patients compared to healthy controls, these cells had greater EPC-CFU forming capacity in COPD patients (41). Lastly, Caramori *et al.* found no relationship between PB CD34⁺VEGFR2⁺CD133⁺ cells and lung function score of patients with emphysema (49).

Despite the variability in the methodology, exclusion criteria, and outcome parameters with respect to enumerating circulating EPCs, it seems that the evidence points to COPD being associated with a reduction in the number BM-derived EPCs in the peripheral circulation. The mechanism and/or purpose for this decrease remains unclear. We have recently found that PB CD34⁺CD45^{dim}CD133⁺ cells are significantly decreased in COPD patients, and for the first time shown an associated increase in sputum CD34⁺CD45^{dim}CD133⁺ cells. This suggests that the reduction in circulating EPC numbers is due to increased recruitment of these cells to sites of pulmonary vasculature injury. Recruitment of EPCs to

the pulmonary vasculature may in turn lead to disruption of normal maintenance and repair of the airways, thereby contributing to COPD pathogenesis. This is in part supported by Peinado *et al.* who found increased CD133⁺ cell adhesion to vascular endothelial surfaces and intimal layers in pulmonary arteries of COPD patients compared to healthy controls (34).

Another potential mechanism to account for the reduction in EPCs in COPD could be due to increased cellular apoptotic rates. Fadini *et al.* reported that COPD patients currently smoking had increased numbers of apoptotic EPCs, assessed via expression of Annexin V, compared to healthy controls (54). This was in parallel with a reduction in EPCs, suggesting that smoking products may reduce the number of EPCs through enhanced apoptosis. The potential relationship of smoking and apoptosis as a mechanism accounting for reduced EPCs needs to be further elucidated.

It is clear from the above findings that there is discrepancy across various studies with respect to PB EPC numbers when enumerated via flow cytometry. However, there seems to be substantial evidence pointing towards smoking as a key modulator of EPC number and function in COPD, and that cessation of smoking may attenuate these changes. Further delineating the exact relationship between smoking and EPCs will be integral to the understanding of the pathogenesis of COPD.

Mobilization and migration of EPCs in COPD

Depletion of the circulating pool of EPCs due to the homing to sites of injury within the pulmonary vasculature may explain the reduced blood levels of EPC in COPD patients. This has been additionally hypothesized in patients with cardiovascular risk factors, and is supported by findings of vascular impairment being inversely related to circulating levels of EPC (40).

Several studies have shown that the pro-angiogenic factor, VEGF, promotes mobilization of EPCs and their incorporation into sites of neovascularisation (6,58-61). VEGF can mediate angiogenesis by inducing proliferation, differentiation, survival and chemotaxis of HPCs, EPCs, and ECs through VEGF receptors (58,59,62,63). VEGF administration in mice has led to a subsequent increase in circulating EPCs, with enhanced proliferative and migratory activity (6). Furthermore, in humans with lower limb ischemia that received VEGF gene transfer, there was an overall increase by >200% in circulating EPCs (61).

These results suggest that VEGF over-expression can mobilize EPCs in humans. In addition to VEGF, other angiogenic GFs, including hepatocyte growth factor (HGF) and SDF-1, stimulate EPC mobilization, recruitment, and migration of EPCs (64). Not surprisingly, over-expression of these GFs is evident in patients with COPD.

Studies have shown that VEGF is increased in pulmonary arteries and plasma of COPD patients, in parallel with prominent vascular remodelling and decreased PB EPCs (52,65). Huertas *et al.* reported greater levels of VEGF and HGF in COPD patients with low BMI, which correlated with lung function impairment (56). Kanazawa *et al.* observed a positive relationship between sputum VEGF concentration and FEV₁ in stable COPD patients (66). Furthermore, Valipour *et al.* demonstrated that in patients with exacerbated COPD, there was markedly higher VEGF, compared to stable COPD and healthy controls, which correlated with disease severity (67).

SDF-1 is a member of the CXC chemokine family, which is essential for the retention of CD34⁺ progenitor cells in the BM (68). Evidence suggests that interfering with CXCR4/SDF-1 axis can initiate mobilisation and release of progenitors from the BM, thus an increase in SDF-1 within the airways, may result in the homing of these progenitors to lung tissue (69-71).

Despite evidence for up-regulated expression of GFs in COPD, the underlying driving force of this increase is largely unknown. Furthermore, whether there is increased mobilization and lung-homing in response to these GFs remains less clear. We have recently reported a greater migrational responsiveness of HPCs to SDF-1, from COPD subjects compared to healthy controls, which was associated with greater numbers of CXCR4⁺ HPCs and EPCs in sputum (24). Conversely, Kim *et al.* found that in COPD, circulating EPCs have significantly lower migratory capacity at baseline and in response to VEGF, compared to non-smoking healthy controls (7). Similarly, Liu and Xie reported decreased migration of EPCs and expression of CXCR4 in COPD patients, suggesting dysfunctional BM responsiveness to systemic inflammatory signals (5). Michaud *et al.* reported decreased migratory responsiveness of EPCs from smokers compared to non-smokers (8). Conversely, Heiss *et al.* demonstrated that acute secondhand smoke exposure lead to increased plasma VEGF levels over a 24 h period, with immediate increases in PB EPC numbers for up to 2.5 h following smoke exposure, suggesting the increased VEGF promotes EPC mobilization into the PB.

The observed difference between these studies may be attributed to the use of flow cytometry (HPCs) versus culture (EPC-CFU) to enumerate or identify EPCs, as well as different methodologies to assess migrational responsiveness of these cells. More work is required to understand the mobilization and trafficking of EPCs, and whether these cells pose as an effective therapeutic target for COPD. In addition, a clearer understanding is required to determine what produces the underlying increase in GF production in patients with COPD.

Clinical implications of EPCs to treat COPD

Given the potential protective role of EPCs in COPD pathogenesis, it is important to determine whether transplantation of EPCs poses as a viable option to treat this respiratory disease. Shi *et al.* determined that intra-tracheal transplantation of EPCs in murine mice with COPD improved lung function and decreased anti-oxidant activity in BALF, as well as decreased levels of MMP-2, MMP-9, and TUNEL-positive cells in lung tissues, compared to non-EPC treated mice. These findings suggest that EPCs can help to attenuate the pathophysiology of COPD by decreasing inflammatory infiltration, decelerate apoptosis, and inhibiting proteolytic enzyme activity. Furthermore, these findings support one of the current theories that reduced EPC airway infiltration or dysfunctional EPC activity may prevent proper repair and maintenance of pulmonary vasculature homeostasis in COPD. As such, infusion or delivery of EPCs to the airways in COPD patients may, in turn, reduce underlying pathogenesis.

To our knowledge, no have been done to determine the efficacy of EPC transplantation to treat COPD. However, it is noteworthy that several studies have looks at the use of mesenchymal stem cell (MSC) transplantation to treat COPD. MSCs are non-hematopoietic multipotent stromal cells that have the ability to differentiate into tissue-derived from a single germ layer. MSCs have been reported to exhibit immunomodulatory effects by inhibiting T cell proliferation and secreting anti-inflammatory cytokines and GFs. Furthermore, studies have shown that MSCs can infiltrate to sites of lung injury and differentiate into specific cell types to allow for regeneration. Hui *et al.* demonstrated in a rat model that treatment with MSCs after 6-month exposure to cigarette smoke exhibited restored alveolar architecture compared to untreated rats. In addition, Gu *et al.* demonstrated that cigarette smoke-exposed mice had significant attenuation of alveolar structure

destruction following treatment with MSCs. A more recent human clinical trial evaluated the safety and efficacy of administering MSCs to treat moderate to severe COPD, received as 4 monthly infusions. However, no significant changes in pulmonary function were detected during the study. These findings, in part, support the protective theory of stem cells towards COPD pathogenesis, however there is a need to carry out larger clinical trials to determine the most effective dosage, route of administration and treatment schedule to evaluate efficacy more accurately. Treatment with stem cells may be a viable route of therapy for COPD, however more studies are required to determine if EPC transplantation would be an efficacious treatment option.

Conclusions

It is clear that the current consensus implicates circulating EPCs in the process of new blood vessel formation and vascular repair in the pathogenesis of COPD. However, controversy persists as to the exact methodology and phenotypic profiling that should be used to study EPCs. Finding a unique cell surface marker that would allow for accurate enumeration and isolation or enrichment of these cells would clarify the exact role of these cells in COPD pathogenesis. Although we have recently demonstrated that PB CD34⁺CD45^{dim}CD133⁺ cells are significantly decreased in COPD patients, with an associated increase in the airways CD34⁺CD45^{dim}CD133⁺ cells, it is unknown whether the presence of EPCs within the airways promotes COPD progression or repair. As such, there remains a large gap in knowledge pertaining to the regulation and modification of EPC homing, differentiation, and proliferation. Furthermore, a more thorough understanding is required with respect to the underlying mechanisms driving GF production in COPD patient and how this modulates EPC function and number. The aforementioned research will determine whether pharmacologic activation or inhibition of EPC mobilization to the airways poses as a novel therapeutic avenue to treat COPD.

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Footnote

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