Arterial endothelial cells: still the craftsmen of regenerated endothelium

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Abstract

For more than a decade, a prevailing hypothesis in research related to arterial disease has been that circulating endothelial progenitor cells (EPCs) provide protection by their innate ability to replace dysfunctional or damaged endothelium. This paradigm has led to extensive investigation of EPCs in the hope of finding therapeutic targets to control their homing and differentiation. However, from the very beginning, the nomenclature and the phenotype of EPCs have been subject to controversy and there are currently no specific markers that can unambiguously identify these cells. Moreover, many of the initial observations that EPCs differentiate to endothelial cells in the course of arterial disease have been criticized for methodological problems. The present review discusses the contrasting experimental evidence as to the role of EPCs in contributing to relining of the endothelium and highlights some of the methodological pitfalls and terminological ambiguities that confuse the field.

Keywords

Endothelial progenitor cells • Endothelial cell • Atherosclerosis • Allograft vasculopathy • Mechanical injury

1. Introduction

The healthy endothelium forms a continuous lining of the vascular system that controls the passage of nutrients and oxygen from the blood into tissues, serves the removal of cellular and metabolic waste products, and regulates arterial reactivity through synthesis and release of vasoactive molecules.1 The basal rate of endothelial cell (EC) proliferation in healthy blood vessels is low but certain regions display a higher rate.2 Moreover, EC proliferation increases with age and the presence of cardiovascular risk factors.3

Common for cardiovascular diseases is the loss of normal endothelial function. Prolonged or exaggerated endothelial activation leads to dysfunction and loss of integrity with apoptosis and necrosis.1,4 At the atherosclerosis-prone regions of the apolipoprotein E-deficient (apoE−/−) mouse, for example, the endothelial turnover rate and proliferation are increased early before the development of atherosclerotic plaques.5

The means whereby the uninterrupted endothelial lining is maintained and regenerated have been debated for a long time (Figure 1). In the mid-1900s, several experiments showed that regeneration of damaged endothelium involves EC mitosis.6,7 However, other investigators also reported that the endothelium may be derived from other types of cells, including circulating cells in the blood and undifferentiated cells from the subendothelial space.8 A number of careful studies in the 1970s further supported the dominant role of local EC mitosis and migration in endothelial regeneration after vascular damage,9,10 although they provided little information concerning the actual mechanisms that control the movement and the proliferation of ECs at injury sites.11 Additionally, it was shown that small areas of endothelial injury can be repaired solely through EC migration, whereas larger areas of injury are repaired by both proliferation and migration.9,12 These findings are in line with indirect evidence obtained in wound healing assays, which supports the role of EC proliferation and migration in endothelial repair.13 Moreover, pathological processes that cause damage to the endothelium can also cause detachment of ECs resulting in a very low number of circulating mature ECs in the bloodstream that might participate in endothelial repair.14 None of these studies, however, was able to determine whether local proliferation is the only mechanism underlying EC renewal and regeneration.

In a landmark study, Asahara et al.13 isolated putative endothelial progenitor cells (EPCs) from the peripheral blood. During short-term culturing, some of these CD34+ mononuclear blood cells acquired endothelial-like characteristics, and they homed to sites of angiogenesis when injected intravenously into animals with hind limb ischaemia. The foundation for research in EPC-mediated repair of the endothelium was laid (Figure 1). During the last decade, numerous studies have undertaken to define the role of EPCs in vascular disease as well as their potential use as a biomarker of cardiovascular disorders. However, attempts to identify and describe EPCs and their biological
properties have yielded conflicting results. As discussed in the present review, much of the controversy may be fostered by inconsistent terminology and common methodological pitfalls.

2. Origin and characterization of EPCs

EPCs can reside in the bone marrow (BM), the peripheral blood, the vascular adventitia, and/or the endothelium itself.14–16 Their identification and characterization has been based on a variety of techniques that fall roughly into two categories: flow cytometric assays for cell surface markers and cell culture assays. Asahara et al.13 defined EPCs as cells positive for the haematopoietic stem cell (HSC) marker CD34 and the vascular endothelial growth factor receptor 2 (VEGFR2 or KDR in humans). Since CD34 is not exclusively expressed in HSCs but also in mature ECs, the immature HSC marker AC133 has been used to better discriminate between EPCs and mature ECs.17 Both populations (CD34+ or AC133+) may differentiate into cells that express EC markers in vitro13,18 and enhance neovascularization when injected in animal models after ischaemia.13,19 However, although CD34+VEGFR-2+AC133+ cells are widely accepted to represent ‘true EPCs’ in humans,20 they have never been proved to differentiate into ECs in vivo. Moreover, recent studies show that mobilized adult peripheral blood CD34+VEGFR-2+AC133+ cells in fact represent an enriched population of CD45+ haematopoietic precursors, which do not differentiate to ECs in vitro.21

Based on cell culture assays, two distinct EPC phenotypes derived from human peripheral blood mononuclear cells have been described.22 When seeded in culture dishes, colonies of cells with an elongated and spindle shape were observed similar to that of the EPCs first reported by Asahara et al.13 These cells die within 4 weeks and were called ‘early’ EPCs in contrast to a cell population displaying an endothelial-like cobblestone shape after 2 to 3 weeks, surviving up to 12 weeks and named endothelial colony forming cells (ECFCs) or ‘late’ EPCs.23 Flow cytometry indicates that the few circulating ECFCs are CD146+CD34+CD31+CD45-AC133- cells, similar to mature circulating and resident ECs.24 The origin of ECFCs is not yet clear, but similar cells can be identified in primary cultures of umbilical venous and aortic ECs, suggesting that they may dislodge into the blood from the vessel wall.25

In vivo, both types of EPCs apparently show similar vasculogenic capacity22 but since ECFCs have higher proliferation potency and may incorporate into perfused blood vessels, they are considered by some to represent true EPCs.26 In a model of hind limb ischaemia, ECFCs enhanced revascularization in synergy with early EPCs.27

The use of the formation of ‘EC’ colonies after culture of peripheral blood cells to demonstrate early EPCs should be interpreted with caution. Prokopi et al. demonstrated that conventional methods for isolating mononuclear cells after 7 days of culture lead to contamination with platelet microparticles (MPs).28 These are taken up in mononuclear cells, which thereby acquire artefactual ‘EC’ characteristics such as the presence of CD31 and von Willebrand Factor (vWF), binding of lectins and even angiogenic properties.28

Overall, the use of different marker combinations and culture assays to isolate or measure progenitor cells indicate that the term EPC does not represent a single, well-defined cell type. Rather, the term is used to describe a number of different and heterogeneous cell populations that circulate in the blood or can be derived through short- or long-term culturing of blood cells.

3. EPCs and cardiovascular disease

Consistent with their putative function in endothelial turnover and repair, several reports indicate that the concentration of EPCs, defined in various ways, is associated with cardiovascular disease. Thus, risk factors for atherosclerosis correlate inversely with the number of EPCs in the blood in vivo and with the results of in vitro assays of EPC function.29–35 Furthermore, a low number of EPCs, defined as CD34+VEGFR2+ cells, are associated with the presence of coronary artery disease (CAD), and predicts the occurrence of cardiovascular events and death from cardiovascular causes in patients.30,31 Using flow cytometry and short-term culture assays to
measure EPC numbers, similar correlations have been obtained between reduced EPC numbers and the extent of atherosclerosis in other vascular beds than the coronaries,33 allograft vasculopathy (AV) in transplanted hearts,34 and diffuse in-stent restenosis after percutaneous coronary intervention (PCI).35 In contrast, Güven et al.36 reported that the number of EPCs was actually increased among patients with significant CAD when EPCs were defined by their ability to give rise to endothelial-cell-like colonies during long-term culture (late EPCs).

Stem cells usually exist in a quiescent state but start to differentiate and to be mobilized into the systemic circulation upon specific stimulation. For example, putative EPCs can be mobilized in patients with vascular diseases by granulocyte-colony stimulating factor, statins, or by exercise.37 Whether the associations between EPC number and cardiovascular disease reflect alterations in the consumption rate or regulation of the release of putative EPCs is unknown.

4. In vivo evidence for endothelial cell origin

Before classifying cells as EPCs, it is essential to validate their homing and differentiation potential in vivo. The ability to produce cells with endothelial characteristics in vitro is indicative, but the capacity to incorporate into an endothelial lining and conduct EC functions is a far more stringent criterion which cannot be tested outside the living organism. If EPCs exist and undertake physiologically relevant repair of the endothelium, it should be possible to detect their contribution by labelling circulating cells with a genetic tracking marker and analyse their fate using specific markers for phenotype and high-resolution microscopy of the blood vessel wall (Figure 2).38,39 This approach also has the advantage that it bypasses the errors involved with the isolation of EPCs, if they exist, and the confusion regarding appropriate phenotypic markers of these cells.

5. EPCs in models of arterial disease

Most arterial diseases involve injury to or death of ECs.4 During the development of atherosclerosis, ECs are continuously injured and replaced (i.e. turnover) while under other circumstances, EC injury may be more pronounced and cover a larger area to be repaired (i.e. regeneration). The process of EC turnover may differ from the one involved in endothelial regeneration.

**Figure 2** Plaque ECs are derived from the local arterial wall. Revised from Hagensen et al.38 (A) Experiment to investigate whether or not BM-derived EPCs contribute to plaque ECs during atherogenesis. An aortic root plaque from an apoE/−/− mouse transplanted with BM from an eGFP+/apoE/−/− mouse. No eGFP+/vWF+ double-positive cells are present. (B) An experiment to investigate whether or not any types of blood-borne EPCs contribute to plaque ECs during atherogenesis. A common carotid artery (CCA) segment from an apoE/−/− mouse was orthotopic transplanted into eGFP+/apoE/−/− mice (isotransplantation except for the eGFP transgene). None of the vWF+ cells is eGFP+. Green indicates eGFP; red, vWF; blue, nuclei; grey, DIC; L, lumen; BM, bone marrow; AA, aortic arch; CCA, common carotid artery; TCCA, transplanted common carotid artery. Scale bars = 50 μm.
Circulating EPCs may contribute to the generation of microvascular ECs at sites of neovascularization, and to relining of the endothelium after various kinds of arterial injuries, including mechanical removal, and AV. As well as to vein graft atherosclerosis. Although atherosclerosis is by far the most important arterial disease worldwide, only a few studies have been conducted on the role of putative EPCs in the EC turnover associated with this disease.

5.1 EPCs in atherosclerosis

Only indirect evidence exists supporting the prevailing understanding that circulating EPCs provide protection against atherosclerosis by their ability to replace dysfunctional ECs. The few studies in which athero-sclerotic mice received BM- or blood-derived cells assumed to contain EPCs intravenously yielded discrepant results. Thus, Silvestre et al. and George et al. reported that transplantation of BM-derived cells accelerated atherosclerosis, whereas Zoll et al. found that injection of EPCs had no effect on the development of the disease. In yet another study, the iv injection of BM-derived progenitors from young non-atherosclerotic into mature atherosclerotic apoE−/− mice exerted a vascular protective effect. Some of the injected cells adhered to the surface of atherosclerosis-prone areas of the aorta, an observation that lead to the suggestion that they may reduce atherosclerosis by replacing senescent ECs in plaques. Others, however, observed that the injection of fluorescently labelled BM- and spleen-derived EPCs in apoE−/− mice, resulted in the predominant accumulation of these cells within the lipid core of the plaques and not within the endothelium.

In a transgenic TIE2-lacZ mouse model of vein graft atherosclerosis expressing β-galactosidase positive (β-gal+) in ECs, the endothelium was partly repaired by BM-derived EPCs. In vein grafts from TIE2-LacZ mice transplanted into the carotid artery of wild-type mice, the number of β-gal+ cells was reduced markedly while such cells were evenly distributed on the surface of wild-type vein segments when transplanted into TIE2-LacZ mice. Moreover, β-gal+ cells were seen on wild-type veins transplanted into mice carrying BM cells from TIE2-LacZ mice. These data are in line with the observation that, in areas prone to lesion development in apoE−/− mice transplanted with BM from TIE2-lacZ mice, 3–4% of cells displaying progenitor markers were β-gal+.5

Only one study so far has analysed the origin of the plaque endothelium during the actual development of atherosclerosis in hyperlipidaemic apoE−/− mice. Not a single circulatory EPC-derived EC was present at the plaque surface. This finding was further corroborated by studies of endothelial regeneration after mechanical injury to advanced plaques in old apoE−/− mice, where the regenerated endothelium was derived exclusively from the local blood vessel wall, presumably through the proliferation of flanking ECs (Figure 2). Plaque neovascularization may contribute to plaque growth and increase plaque vulnerability. However, the lack of reliable animal models featuring plaque neovessels makes it difficult to determine the contribution of EPCs to this process.

5.2 EPCs in allograft vasculopathy

In 2002 Quaini et al. reported that recipient-derived cardiomyocytes, smooth muscle cells, and ECs were common in sex-mismatched (female-to-male) heart transplants. Multiple studies have been conducted to study this phenomenon in simplified rodent models where vessel allografts are transplanted into the arterial system of recipients without immunosuppression to protect the donor cells. In these systems, the endothelium of the allograft efficiently regenerates with recipient-derived cells. The hypothesis that the majority of these recipient-derived cells originates from circulating EPCs that home and differentiate into mature ECs in the allografts has attracted particular interest. Using a mouse model in which the expression of β-gal was regulated by the endothelial-specific TIE2 promoter, Hu et al. observed that ECs of neointimal lesions in allografts of aortic segments are derived from β-gal+ circulating EPCs with ≏30% of ECs being of BM origin. In contrast, Hillebrands et al. reported that the contribution of BM-derived EPCs was only minimal (1–3%) when investigating the origin of ECs by performing orthotopic aortic allografting in BM-chimeric recipient rats; they did, however, not determine the potential contribution from non-BM-derived EPCs. Taken in conjunction, these studies point to a contribution of non-BM- and BM-derived EPCs in transplant atherosclerosis rather than ECs from the nearby blood vessel wall, as inferred mainly from the absence of observable inward migration fronts of ECs at selected early time points after allografting. In contrast, the majority of the reported data favours the view that ECs in allografts are derived from migrating ECs from the flanking vasculature. When an allografted blood vessel or heart is interpositioned in a recipient, it is not obvious to ascertain whether cells originate, from the flanking vasculature vs. circulating EPCs. However, the use of an approach that distinguishes between different cell sources (Figure 2) demonstrated that migrating vascular ECs rather than circulating EPCs are the source of recipient-derived ECs in a murine model of hyperlipidaemia-accelerated AV.

5.3 EPCs in endothelial regeneration

Re-endothelialization after mechanical vascular injury, including PCI, is essential for the inhibition of neointima formation and restoration of vascular homeostasis. Several studies in experimental models have concluded that circulating EPCs may be involved in this process. Mobilization of EPCs has also been implicated in the promoting effect of several other interventions on endothelial regeneration, including oestrogen therapy, exercise, and systemic haeme oxygenase 1-overexpression. In contrast, diabetes was found to impair EPC function. Thus, it was reported that the transplantation of diabetic BM into non-diabetic mice resulted in delayed re-endothelialization after wire-induced carotid denudation presumably due to impaired incorporation of BM-derived ECs.

Other studies, however, have questioned that differentiation of ECs from circulating EPCs is the underlying mechanism explaining these findings. Tsumuki et al. concluded to the absence of contribution of BM-derived cells to re-endothelialization in a wire-induced endothelial denudation model of the common carotid artery of mice transplanted with green fluorescence protein positive (GFP+) BM. Furthermore, Itoh et al. observed that re-endothelialization after injury of a confined area of the pial artery occurred by stepwise re-growth of the endothelium from the edges of the injured area, a pattern that does not permit to accept homing of circulating EPCs as a major mechanism for endothelial regeneration. Several studies reporting important contributions of EPCs have observed similar
patterns of regrowth of the endothelium from the edges as demonstrated by Evans blue injection.\textsuperscript{59,61,64} Using a mechanical arterial injury in TIE2-GFP\textsuperscript{+} transgenic mice, to establish whether circulating non-BM-derived EPCs or the nearby healthy endothelium contributed to EC regeneration, revealed that endothelial regeneration does not involve homing and differentiation of circulating EPCs into ECs.\textsuperscript{65} Instead, the endothelium regenerated by GFP\textsuperscript{+} cells as a function of time, evolving from the anastomosis sites towards the centre of the transplant. Thus, endothelial regeneration is mediated by migration and possible proliferation of ECs from the adjacent healthy vessel wall.

## 5.4 EPCs and neovascularization

Circulating cells can be incorporated into and facilitate neovascularization of ischaemic tissues.\textsuperscript{40,66} In ischaemic hind limbs of mice transplanted with BM cells expressing lacZ under the regulation of an EC lineage-specific promoter (Fik-1 or Tie-2), Asahara et al.\textsuperscript{40} found lacZ\textsuperscript{+} cells to be incorporated into capillaries between skeletal myocytes. Moreover, after ligation of the left coronary artery, EPCs were incorporated into foci of neovascularization at the border of the infarction.\textsuperscript{40} Circulating EPCs also contribute to the endothelial lining of microvessels during wound healing,\textsuperscript{67} tumour growth,\textsuperscript{40} and corneal neovascularization.\textsuperscript{68} The observation that BM-derived EPCs may facilitate and be incorporated into neovessels in ischaemic muscle offers hope to use this mechanism to treat chronic refractory myocardial ischaemia or to improve functional recovery after myocardial infarction.\textsuperscript{69,70} Hence, the effect of transplanting autologous BM-derived cells to the suffering myocardium has been tested in clinical studies. Some of these, but not all studies,\textsuperscript{69} have shown significant improvements in specified endpoints such as angina frequency or exercise tolerance.\textsuperscript{71} Whether or not the positive effects, when present, reflect stable integration of the transplanted cells and their differentiation to ECs is a matter of debate. Putative EPCs facilitate neovascularization by secreting paracrine factors.\textsuperscript{66,72,73} For example, injecting EPC-conditioned medium alone into a rat model of chronic hind limb ischaemia increased capillary density, blood flow, and muscle performance significantly.\textsuperscript{73} Whether mediated by paracrine signalling or not, experiments in animal models of both myocardial infarction and limb ischaemia demonstrate that the persistence of the transplanted EPCs and their differentiation to endothelial nitric oxide synthase (eNOS) expressing cells are necessary for functional improvement.\textsuperscript{74,75}

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**Figure 3** Endothelium in allograft vasculopathy (AV) lesions in a murine model is derived from ECs migrating into the lesion from the adjacent arterial wall. Revised from Hagensen et al.\textsuperscript{39} To locate the source of the recipient-derived cells, carotid artery segments (1.TCCA) were first isografted from apoE\textsuperscript{−/−} B6 mice into eGFP\textsuperscript{+}apoE\textsuperscript{−/−} B6 mice (A) or from eGFP\textsuperscript{+}apoE\textsuperscript{−/−} B6 mice into apoE\textsuperscript{−/−} B6 mice (B). Four weeks later, the isograft was transected, and a BALB/c carotid allograft was inserted end-to-end (2.TCCA). (A) No co-localization of eGFP\textsuperscript{+} and vWF\textsuperscript{+} to individual cells was identified. The recipient-derived eGFP\textsuperscript{+} cells were most likely macrophages. (B) Almost all vWF\textsuperscript{+} cells were also eGFP\textsuperscript{+}. Green indicates eGFP; red, vWF; blue, nuclei; grey, DIC; L, lumen; CCA, common carotid artery; TCCA, transplanted common carotid artery. Scale bars = 25 \textmu m.
6. Methodological challenges

The seminal descriptions of circulating EPCs have inspired a large number of studies investigating various aspects of this cell population, including their phenotypic characterization, mobilization and homing, and their value as a blood biomarker of vascular health.\(^15,17,18,21,22,29 – 31,41\) A number of those, however, have fundamentally questioned the theory that these ‘EPCs’ act as a source of arterial endothelium in vivo and have pointed out a number of potential methodological pitfalls that may have led to incorrect conclusions.\(^14,16,20,21,38,39,65,76 – 79\)

The key methodological issues in the EPC field are probably not different from those initiating other controversies in the progenitor cell field being rooted in the failure to reach clear single-cell resolution, and in the use of unspecific cell markers or detection systems.\(^38,80\)

The detection of EPC contribution to the endothelium has been based on models in which a population of cells, e.g. BM or circulating cells, assumed to be enriched for EPCs, is tagged with a transgene encoding a reporter molecule, such as eGFP or β-gal. Incorporation of tagged cells in the endothelial lining and their expression of characteristic endothelial proteins is the criterion used to detect EPC-derived arterial ECs. In many vascular diseases, homing of BM-derived cells is an inherent part of the pathophysiological process. After vascular injury, haematopoietic cells may transiently cover the denuded area before being replaced by regenerated endothelium,\(^16\) and such cells may easily be mistaken for BM-derived ECs early after injury. Furthermore, in atherosclerosis, there is an ongoing passage of BM-derived inflammatory cells through the endothelial layer. Moreover, some of these cells may reside in the subendothelial space in close proximity to ECs.\(^38,81\) Choi et al.\(^82\) have shown that dendritic cells are abundant in the normal mouse aorta and localized beneath the endothelium in the subintimal space. Moreover, dendritic cells are abundant in the intima of atherosclerosis-predisposed regions of the wild-type C57BL/6 mouse aorta.\(^83\) If clear single-cell resolution is not reached, a dendritic or other BM-derived cell lying close to an EC may appear as a single BM-derived EC. Furthermore, the use of widefield rather than confocal microscopy can contribute to the above problem since it is not possible to visualize thin optical sections of cells in tissue sections when using the former. A cell with a tracking marker overlying a cell with a phenotypic marker can therefore be misinterpreted as ‘co-localization’.

The choice of markers to establish the EC type is equally important. A number of studies have used CD31 as an EC marker, but since this protein is also expressed by monocytes\(^84\) the detection of BM-derived CD31\(^+\) cells is not surprising. More specific markers, such as vascular endothelial (VE)-cadherin, eNOS, and vWF, should be used as markers for ECs. Even though vWF is also found in platelets and in the subendothelial extracellular space, it is specific for ECs when identified within nucleated cells in the arterial wall, typically with a characteristic granular cytoplasmic staining pattern.\(^85\)

Reporter transgenes driven by the endothelial-specific angiopoietin receptor TIE2 promoter have often been used to track BM-derived ECs.\(^3,40,42,46,59,66,87\) The use of a reporter molecule that is only expressed in cells of the endothelial lineage obviates the need for single-cell resolution, but puts great dependence on the endothelial specificity of the promoter. However, a subpopulation of monocytes that circulates in the peripheral blood and is recruited to sites of angiogenesis and in regenerating tissues also expresses TIE2.\(^88,89\) These cells localize in close proximity to ECs and can therefore easily be mistaken for BM-derived ECs.

7. EPC or only putative EPC

As said above, in most arterial diseases inflammation takes place, in which mobilization, circulation, homing, and local differentiation of BM-derived leucocytes play important roles in disease progression. Since many EPC populations contain monocytes, it therefore can be expected that, when injecting putative EPC populations into animals, some of these cells will home to sites of vascular damage. Dil-Ac-LDL uptake and lectin binding together with endothelial marker expression is the phenotypic profile that is commonly used for EPC identification. However, monocytes express a similar phenotypic profile when cultured under specific conditions in vitro. When Rohde et al.\(^77\) cultured monocytes for 4 to 6 days under angiogenic conditions, the cells lost CD14/CD45 and displayed a commonly accepted EPC phenotype (including LDL uptake, lectin binding, CD31/CD105/CD144 expression, and formation of cord-like structures) that made them indistinguishable from putative EPCs. These characteristics of monocytes/macrophages have also been reported by others.\(^78,90\) Notably, monocytes already express most tested endothelial genes and proteins at even higher levels than the so-called EPCs, and the formation of colony-forming unit ECs (CFU-EC) is strictly dependent on the presence of monocyte.\(^77\) The putative EPC population of colony-forming mononuclear cells in human peripheral blood, similar to those described by Asahara et al.\(^13,40\) in mice, later appeared to be composed mainly of inflammatory and immune mononuclear cells rather than true EPCs\(^20,26,76,77,91,92\) and such cells home to sites of arterial injury and atherosclerosis.

8. Paracrine effect

An alternative explanation for the association between EPCs and endothelial regeneration may be that some of the cells contained in the ‘EPC’ cell populations exert pro-angiogenic paracrine effects without actually differentiating into ECs.\(^20,81,89,91\) They may adhere and migrate through the endothelium into the blood vessel wall and release growth factors and chemokines that stimulate endothelial regeneration by resident ECs. Putative ‘EPCs’ obtained in short-term culture assays (early EPCs) express monocyte/macrophage markers and secrete multiple potent angiogenic growth factors\(^76\) that enhance EC migration\(^73\) and protect against oxidative stress-induced apoptosis.\(^94\) Consistent with such paracrine function, serial injections of conditioned media harvested from peripheral blood-derived EPCs into an ischaemic hind limb ameliorated local perfusion by promoting neovascularization and vascular maturation.\(^73\) TIE2-expressing monocytes have been reported to exert similar functions. Indeed, during tumour angiogenesis, TIE2\(^+\) monocytes are located adjacent to new blood vessels without integrating into the endothelium.\(^89\) Induced apoptosis of these cells through the activation of a suicide gene blocked tumour angiogenesis, indicating that they stimulate angiogenesis through paracrine signalling.\(^89\)

The term EPC was initially used in the literally correct way for immature precursor cells capable of differentiating into mature ECs in vivo.\(^13,40 – 44,46\) Now the term has gradually been redefined to include circulating angiogenic cells without an endothelial fate. Thus, an alternative explanation for the conflicting results reported in the literature could be due to paracrine signalling to local ECs by blood-
derived pro-angiogenic cells. However, to avoid confusion, the literally correct definition of a progenitor cell as an immature precursor cell capable of differentiating into a mature cell type in vivo should be retained.

9. Conclusion

After more than 10 years of enthusiastic research in EPC biology, researchers worldwide still do not agree on how this cell population should be defined. Despite the fact that a number of studies show an inverse correlation between the number and functional activity of EPCs in the blood and the development of cardiovascular disease, this correlation does not imply causation.

The present review has identified limitations and pitfalls in the EPC literature and also pointed out methodological challenges. Although there have been numerous attempts to define the exact EPC phenotype, it has become obvious that one deals with a heterogeneous population of cells with different phenotypes and in vitro potential. Instead of being cells that differentiate into ECs, these putative EPCs rather represent a population of monocytes sharing many characteristics of ECs in vitro and in vivo affecting existing cells through paracrine mechanisms. These putative EPCs do not contribute directly to regeneration of the endothelium in arterial diseases. Thus, as of today, the paradigm from the 1970s, which states that ECs in arterial disease are repaired and regenerated by proliferation and migration of ECs from the local and adjacent vasculature, remains valid. The time probably has come to abandon the term EPC and instead to focus more on investigating the paracrine potential of this heterogeneous population of cells and the mechanisms responsible for EC proliferation and migration from the existing endothelium.

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References


Endothelial progenitor cells in arterial regeneration