1. Introduction

Sufficient supply of oxygen and nutrients by the blood vessels is essential for tissue survival. In the adult, blood vessel formation occurs through angiogenesis, arteriogenesis, or vasculogenesis. Although angiogenesis refers to the growth process of mature endothelial cells sprouting from pre-existing postcapillary venules through migration and proliferation, the term arteriogenesis is used to describe the maturation of vessels via recruitment of mural cells. Until recently, the term vasculogenesis described the process of blood vessel formation in the embryo. This occurs via differentiation of precursor cells (angioblasts) into endothelial cells, which then assemble into a primitive vascular network. However, this process has now also been shown to contribute to adult blood vessel formation. Endothelial progenitor cells (EPCs) play a major role in new vessel formation. Isolated primarily in 1997 by Asahara et al. as CD34⁺ haematopoietic progenitors from peripheral blood, these cells represented a heterogeneous population that expresses CD34 or VEGFR2 markers. EPCs share common properties and function, and they can proliferate and differentiate in vitro to endothelial cells. Thus they provide an ideal target for ex vivo expansion and transplantation into ischaemic areas. In vivo experiments have shown that they can improve neovascularization. Both in mice and humans, EPCs have been shown to contribute to adult blood vessel formation. Endothelial progenitor cells (EPCs) play a major role in new vessel formation. Isolated primarily in 1997 by Asahara et al. as CD34⁺ haematopoietic progenitors from peripheral blood, these cells represented a heterogeneous population that expresses CD34 or VEGFR2 markers. EPCs share common properties and function, and they can proliferate and differentiate in vitro to endothelial cells. Thus they provide an ideal target for ex vivo expansion and transplantation into ischaemic areas. In vivo experiments have shown that they can improve neovascularization. Both in mice and rabbits with hind limb ischaemia, mobilization of EPCs can remarkably promote new blood vessel formation in the injured areas, enhance perfusion, and lead to recovery of ischaemic tissue. Ex vivo expanded EPCs, isolated from peripheral blood mononuclear cells, can also incorporate into the foci of myocardial neovascularization, whereas intracoronary infusion of peripheral blood or bone marrow-derived progenitors in patients with acute myocardial infarction was shown to associate with significant benefits in post-infarction remodelling. EPC numbers can also be used as a predictive biomarker for cardiovascular risk and vascular function. In a large clinical study, Hill et al. reported that high-risk individuals have fewer EPCs compared with their low-risk counterparts, whereas Werner et al. identified a significant association between increasing numbers of EPCs and decreased risk of a major cardiovascular event and hospitalization in patients with coronary artery disease. EPC numbers can also predict severe endothelial dysfunction in patients with coronary heart disease. Additionally, reduced EPC numbers were reported in diabetic patients and in individuals who suffered a stroke. Finally, in a case study of approximately 550 patients, results indicated that EPC number is inversely correlated with the serum cytokine levels of stromal-cell-derived factor-1α (SDF-1α) and the extent of carotid atherosclerosis; however, this relationship did not extend to femoral artery atherosclerosis.

Conditions associated with cardiovascular diseases have an effect on the functional activity of progenitors. EPCs from type II diabetes patients exhibit impaired proliferation, adhesion, and reduced angiogenic potential in vitro.
Similar functional alterations have been reported in EPCs isolated from aged individuals\textsuperscript{16} and from patients with coronary artery disease or ischaemic cardiomyopathy.\textsuperscript{17,18} However, controversial issues on EPC phenotypes, origins, and functions of endothelial repair exist. The present review focuses on the role of EPCs in repairing the vessel wall during the development of atherosclerosis. We describe the latest developments in EPC classification and the impact of EPCs on the maintenance of endothelial integrity, neointima formation, and angiogenesis within the arterial wall. The mechanisms of cell homing and vascular cell differentiation are also discussed.

2. EPCs repair damaged vessels in vivo

Atherosclerosis is an inflammatory disease characterized by leucocyte infiltration, smooth muscle cell accumulation, and neointima formation.\textsuperscript{19} Activation and damage of the endothelial monolayer seem to trigger the development of the lesions. Initially, it was thought that the damaged endothelial cells were replaced by the adjacent intact endothelium. However, recent studies demonstrated the recruitment and incorporation of vascular progenitor cells into atherosclerotic lesions and thus provided evidence in support of the role of vascular cells in the development of the disease. Direct incorporation of circulating EPCs into the vessel wall was detected in mice. In a model of transplant atherosclerosis, regenerated endothelial cells from arterial grafts were found to originate from recipient circulating blood but not the remaining endothelial cells of the donor vessels.\textsuperscript{20} Similarly, it was found that the endothelial monolayer in a vein graft 3 days post-surgery was completely lost and subsequently replaced by circulating endothelial progenitors.\textsuperscript{21}

Importantly, these progenitors can mediate vascular repair and attenuate atherosclerosis progression even in the continued presence of vascular injury. Chronic treatment with bone marrow-derived progenitor cells from young non-atherosclerotic ApoE knockout (ApoE KO) mice prevented the development of the disease in aged ApoE KO recipients despite persistent hypercholesterolemia.\textsuperscript{22} Although the mechanisms involved are still not clear, EPCs seem to contribute to the restoration of the endothelial monolayer. In addition to bone marrow, spleen-derived EPCs also have the capacity to repair damaged endothelium. For example, intravenous infusion of spleen-derived mononuclear cells improves endothelium-dependent vasodilation in atherosclerotic mice, indicating that progenitor cells play an important role in repairing the vascular injury.\textsuperscript{23} EPCs derived from spleen homogenates also enhanced re-endothelialization and reduce neointima formation after induction of endothelial cell damage using the carotid artery model.\textsuperscript{24}

Additionally, using a balloon injury model, accelerated repair of the denuded endothelium along with decreased activation of medial smooth muscle cells and neointima formation occurred after mobilization of circulating EPCs.\textsuperscript{25}

Furthermore, autologous EPCs over-expressing endothelial nitric oxide (eNOS) can rapidly restore endothelial integrity when transplanted into mice after balloon carotid artery injury. Increased NO bioavailability significantly strengthens the vasculoprotective properties of the reconstituted endothelium, leading to inhibition of neointimal hyperplasia.\textsuperscript{26} Studies have also suggested that an increase in the number of circulating cells is directly linked to the restoration of the endothelial lining and a reduction in neointima formation after injury.\textsuperscript{23,27} However, transfer of progenitor cells is not always beneficial. ApoE KO mice that received bone marrow mononuclear cells following induced hindlimb ischaemia, displayed not only increased neovascularization to these oxygen deficient regions, but also accelerated atherosclerotic plaque formation and lesion size when compared with control groups.\textsuperscript{28} In an alternate study, EPC-treated mice also displayed accelerated atherosclerosis along with reduced plaque stability, which may be because of proinflammatory properties of these cells, as reduction in IL-10 levels in the atherosclerotic aortas was observed.\textsuperscript{29} Similarly, even though implantation of an arteriovenous anti-CD34-ePTFE graft in pigs led to enhanced recruitment of circulating CD34\textsuperscript{+} cells and coverage of the graft, it also stimulated intimal hyperplasia.\textsuperscript{30} A strong increase in cellular proliferation observed at the shoulder region of the venous outflow tract implied a proliferative effect of these cells; however, the release of potent mitogens for smooth muscle cells or differentiation of circulating CD34\textsuperscript{+} cells to smooth muscle cells cannot be excluded.\textsuperscript{31} Besides obvious differences in various experimental models, it is difficult to reconcile these findings. Overall, it seems that excessive mobilization of progenitor cells may lead to restenosis, whereas its absence may impair re-endothelialization.\textsuperscript{32} Nevertheless, one should bear in mind that the term EPC is loosely used to describe a vastly mixed cell population that is consisted of different progenitors (see below). Recent studies have highlighted the impact of cell isolation protocols on the functional capacity of these progenitors emphasizing the heterogeneity of EPC population, i.e. different phenotypes.\textsuperscript{33}

3. EPC phenotypes

In most studies, EPCs are identified by flow cytometric characterization, namely expression of CD34, CD133, or VEGFR2. As these cells may originate from multiple precursors including the haemangioblast, non-haematopoietic mesenchymal precursors such as the bone marrow, monocyctic cells, and also tissue resident stem cells, their accurate characterization is very difficult. Two methods for isolation of EPCs from the peripheral blood have been described.\textsuperscript{34} First, isolated monocyte cells are seeded onto fibronectin-coated plates and cultured in the presence of growth factors that promote EPC outgrowth and form colonies after 5–7 days. These colonies consist of a central cluster of round cells surrounded by multiple spindle-shaped cells (endothelial cell colony-forming units, CFU-EC). Further adding to the complexity of the EPC population, the presence of a subset of T cells at the centre of the EPC colony was recently reported. These angiogenic T cells play a pivotal role in colony formation and could serve as a therapeutic target for ischaemic cardiovascular diseases.\textsuperscript{35} Second, monocyteic cells from peripheral blood plated onto collagen-I-coated plates in endothelial growth media (EGM-2) can give rise to CFU-EC after 14–21 days. Interestingly, the origin of EPCs is not restricted to the monocyteic lineage marker CD14. Both CD14\textsuperscript{+} and CD14\textsuperscript{−} cells (also known as early and the late outgrowth EPCs; see below), isolated from peripheral blood and expanded in vitro, exhibit
similar expression of endothelial marker proteins, functional characteristics, and improve neovascularization after hind-limb ischaemia. Additional studies demonstrated that the expression of VEGFR2 on peripheral blood monocytes is essential for their endothelial-like function.  

As stated above, two distinct phenotypes of EPCs have been described, the early and the late outgrowth EPCs which are distinguishable based on their proliferation potential. The early outgrowth EPCs that are derived from monocytic cells have low proliferative capacity and adopt characteristics of ECs such as expression of eNOS. Importantly, although these cells may incorporate into the endothelial monolayer, they fail to form perfused vessels in vivo. Alternatively, the late outgrowth EPCs have a high proliferation rate and can be maintained in culture extensively. These cells may play a key role in neoangiogenesis in vivo as experimental data indicated that they are vessel-forming EPCs. Recent studies further identified these cells as CD34⁺CD45⁻ precursors and clarified their origin from the peripheral blood monocytes. CD14⁺ cells seem to give rise to early EPCs, whereas late EPCs develop exclusively from the CD14⁻ subpopulation. Interestingly, although infusion of EPCs into ischaemic limbs of immunocompromised mice can remarkably improve perfusion and recovery from injury, only low numbers of EPCs incorporated into the new capillaries can be identified. This suggests that EPCs may release angiogenic factors in a paracrine manner. This supportive function of EPCs may be crucial in ensuring the survival of tissue-residing cells and enhancing blood vessel formation and tissue repair. Of note early outgrowth EPCs appear to produce higher levels of growth factors compared with late outgrowth EPCs, suggesting a diverse role in neovascularization for the two phenotypes. Consequently, it could be suggested that although early outgrowth EPCs exhibit a low proliferative capacity by themselves, they may act to secrete angiogenic growth factors stimulating the proliferative capability of the late outgrowth EPCs or resident mature endothelial cells. In addition, a controversial report recently showed that human CD34⁺AC133⁺VEGFR-2⁺ cells are not EPCs but distinct, primitive haematopoietic progenitors, indicating that a uniform definition of EPCs is required to clarify their origins. An acceptable concept that could be suggested for EPC clarification is that the phenotype of these cells may vary depending on their origin and although similar in terms of cell markers, they may display variable functions, including immature EPCs that have proliferative ability, material EPCs that can physically engraft into neo-endothelial layer, and supportive EPCs that produce growth factors to promote endothelial repair.

4. Non-bone marrow-derived EPCs

There is no doubt that bone marrow can release progenitor cells that may differentiate into EPCs. However, it was also reported that a large proportion of EPCs in circulating blood is of non-bone marrow origin. For example, the spleen is an organ particularly rich in EPCs. Isolated spleen-derived mononuclear cells, pre-selected with an endothelial cell medium, demonstrated endothelial cell characteristics and formed tubular-like structures. These cells could sufficiently enhance re-endothelialization and diminish neointima formation after carotid artery injury. Of note, intravenous transfusion of spleen-derived EPCs in splenectomized mice revealed exclusive homing to the injured area. However, this was only observed when the host organ was removed. It was suggested that removal of the spleen prolonged the EPC time in circulation, which may result in a change of surface markers on the cell (because of homing signals of the injury site), favouring recruitment to the ischaemic area rather than preferential homing to the organ of origin.

In the intestine and the liver, high levels of mobilized tissue-residing progenitor cells have been discovered recently using a rat model of intestine and liver transplantation. Systemic infusion of progenitor cells derived from the perivascular niche in the liver incorporated into vascular structures and subsequently enhanced neovascularization and improved blood flow recovery in ischaemic hindlimbs. Moreover, in the human adipose tissue, the presence of stem cells that can differentiate in vitro to endothelial cells has been reported. These cells are capable of incorporating into the ischaemic leg, increase the capillary density, and improve postnatal neovascularization. They could represent a good source of stem cells for vascular tissue engineering, as they are available in relatively high numbers and autologous. However, further experiments are required to fully characterize these cells and identify the mechanisms involved in their differentiation.

Another unexpected finding in the adventitia of ApoE KO mice is the abundant presence of progenitor cells close to the aortic root. To determine the origin of these cells, chimeric mice that express LacZ transgene only in bone marrow cells were used. No β-gal-positive cells were identified, suggesting that these cells are not derived from the bone marrow. Further experiments revealed that these sca1⁺ cells can differentiate in vitro to endothelial and smooth muscle cells. In vivo when applied to the adventitia of irradiated vein grafts they differentiated to smooth muscle cells and migrated to the neointima of the grafts in atherosclerotic lesions. Their function is still unknown but these data imply that they could putatively contribute to the population of circulating progenitors. The presence of progenitor cells in the adult vascular wall was also demonstrated in humans. In an area between the medial and adventitia layer, high numbers of EPCs were identified. Furthermore, Ingram et al. demonstrated a complete hierarchy of EPCs can be derived from human vessel walls and discriminated by their clonogenic and proliferative potential. This study provides evidence that a diversity of EPCs exists in human vessels and provides a conceptual framework for determining both the origin and function of EPCs in maintaining vessel integrity. Thus, many organs contain progenitor cells that may serve as a circulating pool of EPCs (Figure 1).

To provide direct evidence that non-bone marrow-derived EPCs contribute to endothelial repair in vessel grafts, our early work using two different models of vessel graft atherosclerosis revealed that the reconstituted endothelial mono-layer is formed from recipient-circulating progenitors cells. Importantly, when Balb/c aorta was allografted into the carotid artery of chimeric mice with bone marrow derived from Tie2-LacZ mice, β-gal activity was seen on the surface of allografts 4 weeks after surgery. Quantification of the obtained data indicated that more than 70% of the regenerated endothelial cells were derived from non-bone marrow tissues. Interestingly, a separate study showed that in rat aortic but not in cardiac allografts,
recipient-derived endothelial cells replaced the damaged donor endothelium on the graft. However, the observed contribution of bone marrow-derived endothelial cells in the late stages of transplant atherosclerotic lesions was very low, reaching just 3% of the total number of endothelial cells. The variability seen between these studies may be because of differences in species and techniques used i.e. mouse vs. rat or section vs. en face analyses, and consequently should be taken into consideration when analysing similar animal models.

5. Angiogenesis in the vessel wall

As mentioned above, the vessel wall contains progenitor cells that may contribute to the endothelial repair as well as neointimal formation. How do the vessel wall progenitor cells migrate to the endothelial and intima layer of the vessel? We hypothesized that the vasa vasorum in the vessel wall may play a significant role in transporting cells to the intimal region. Support to this notion is provided by the fact that microvessels within the vessel wall were positively correlated with the development of atherosclerosis. In the absence of atherosclerosis, normal vessel walls have a microvasculature confined to the adventitial layers also known as vasa vasorum. In atherosclerotic lesions, however, abundant microvessels can be observed. They are considered to significantly contribute to atherosclerosis progression and plaque instability, but there is also evidence to support the opposite. Decreased blood supply through the adventitial vasa vasorum can trigger atherogenic intima thickening. Using the Lac-Z mice in a transplantation model of atherosclerosis, Hu et al. provided unique insights into the formation of these microvessels. It was clearly demonstrated that endothelial cells of microvessels within allografted vessels were derived from bone marrow progenitor cells (Figure 2). Taken together with previous findings that indicate the incorporation of circulating endothelial progenitors to the regenerated endothelium of the lesion, these results suggest a potentially dual role of EPCs in transplant atherosclerosis, both protective through the repair of the denuded endothelium and detrimental through promoting plaque angiogenesis. This dual function may also account for some of the conflicting data in the literature. Intriguingly, progenitor cells have also been reported in tumour angiogenesis. Studies have suggested a correlation between circulating EPC numbers and the risk of multiple myeloma, whereas an increased number of CD133+ cells that contribute to capillary formation were identified in lung cancer. Additional experiments are required to fully delineate the functional significance of stem cell incorporation into the microvasculature and define the role of progenitors in tipping the balance between atheroprotection and atherogenesis.

6. Mobilization of EPCs

Although non-bone marrow-derived EPCs may form a part of the circulation pool of EPCs, it is unknown how these progenitor...
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cells can be released into blood. Since most available data concerning EPC mobilization come from the studies of bone marrow, in the present review, we focus only on the mechanism of EPC release from bone marrow. Stem cell niches are specific sites where stem cells reside in the bone marrow. In these microenvironments the cells can either remain in an undifferentiated and quiescent state or differentiate. Supporting cells in the niche interact with stem cells and regulate their self-renewal and differentiation. In response to vascular injury or physiological stress, stem cells have to be rapidly mobilized and recruited to the damaged area.

VEGF is an effective mobilizer of EPCs and a potent inducer of angiogenesis. It is an endothelial-specific growth factor and functions as an angiogenic switch. After tissue injury, when formation of new blood vessels is urgently required, VEGF mediates proliferation, differentiation, and chemotaxis of endothelial cells. A rapid elevation of circulating VEGF leads to the recruitment of EPCs to the site of injury and promotes neovascularization. Further studies demonstrated that this was at least partially mediated by MMP-9 activation. The activated MMP-9 catalyses the conversion of KitL from a membrane-bound molecule to a soluble survival factor (sKitL). The release of sKitL leads to enhanced mobility of endothelial progenitors, transferring them from the osteoblastic to the vascular-enriched niche and favouring differentiation and mobilization to the peripheral circulation.

The expression of proMMP-9 and active MMP-9 seems to be NO dependent. Expression and activity of MMP-9 is dramatically reduced in endothelial nitric oxide knockout (NOS3 KO) mice, both at baseline and after stimulation with VEGF or 5-FU, a cytotoxic agent that depletes cycling haematopoietic cells (Figure 3). These mice have impaired ischaemia-induced neovascularization and exhibit defective EPC mobilization and incorporation to the ischaemic areas. Treatment with sKitL can augment EPC numbers after 5-FU challenge, suggesting that release of sKitL by the activity of MMP-9 accounts for the defective haematopoietic recovery and progenitor cell mobilization. The crucial role of NO in tissue repair was further highlighted in bone marrow-derived progenitors from patients with ischaemic cardiomyopathy ex vivo. In comparison to control cells, treatment with AVE9488, a substance that can induce eNOS expression, increased NO production by EPCs along with enhancing their migratory capacity in vivo, and promoted neovascularization when the bone marrow progenitors were used in a mouse hindlimb model of ischaemia.

Increased NO bioavailability and activation of MMP-9 seems to mediate the angiogenic response that is initiated by other chemokines or reagents. The chemokine SDF-1 and its receptor CXCR4 play a major role in the recruitment and retention of stem cells to ischaemic areas. SDF-1 is constitutively expressed, but its levels are rapidly up-regulated by a range of stimuli such as inflammatory mediators, changes in the extracellular matrix, altered mechanical forces, and hypoxia. Intriguingly, it was recently reported that activated platelets secrete extremely high levels of SDF-1. As platelets adhere within minutes to the exposed subendothelial in injured vessels, this could provide a very effective mechanism of mobilization and homing of stem cells to the damaged area. However, increased levels of SDF-1 cannot induce neovascularization in the absence of injury. Intramuscular gene transfer of SDF-1 into ischaemic limbs enhanced neovascularization in mice by augmented mobilization and incorporation of EPCs into neo-vessels, but in the absence of ischaemia these effects were ablated, indicating that other signals from the ischaemic limb are required. VEGF may be this additional signal as inhibition of VEGF signalling abrogated any benefit from SDF-1 up-regulation. Similar results were obtained when NOS3 KO mice were used, suggesting that it involves VEGF/eNOS signalling. Additionally an interesting pattern of biphasic response in CXCR4 signalling has been recently reported. Inhibition of CXCR4 allows recruitment of readily available stem cells from the perivascular niche, while stimulation of CXCR4 results in mobilization of quiescent hibernating cells that requires the activation of proteases or stem cell active chemokines. Importantly, SDF-1 treatment could increase active MMP-9 in stromal and haematopoietic cells and up-regulate production of sKitL in mice, providing further support to the notion that the NO-MMP-9 signalling pathway is involved (Figure 3).

Similar results were observed for a number of other bioactive proteins. Estrogen can induce rapid regeneration of the denuded endothelial monolayer after carotid artery injury by enhancing mobilization and proliferation of progenitor cells. These events, however, are diminished in NOS3 KO mice suggesting that they are NO mediated. Additional experiments using a myocardial infarction model further clarified the mechanism involved and revealed that MMP-9 activity is essential for EPC mobilization after estrogen treatment.

Erythropoietin, statin therapy and physical exercise can also increase mobilization of EPC numbers, augment neovascularization, and at the same time inhibit neointimal hyperplasia via an eNOS-dependent mechanism. The use of NOS3 KO mice showed that the beneficial effects were dependent on a functional eNOS. Interestingly, while...
transient statin therapy has a positive effect of EPC numbers, chronic continuous treatment with high amounts of statins inversely correlates with the EPC number in patients with coronary artery disease.\textsuperscript{89}

7. EPC homing

Maintaining the integrity of the endothelial monolayer is extremely important. Not only does the endothelium act as a barrier between the blood and subendothelial matrix proteins, it also prevents inflammatory cell infiltration, thrombus formation, modulates vascular tone, and controls vascular smooth muscle proliferation.\textsuperscript{90–92} A key issue in designing therapeutic interventions to combat endothelial dysfunction is to understand how the denuded endothelium is replaced and identify the possible sources of cells that can regenerate it. The recruitment of stem cells to the neovascularization sites strongly resembles that of an inflammatory response. Once in the vicinity of an injured vessel, progenitor cells interact with the damaged endothelial monolayer in a similar way as leucocytes interact with activated endothelial cells.

Adhesion molecules previously known to be involved in the phase of rolling and firm adhesion of leucocytes was thus identified as key regulators of EPC homing. P-selectin and E-selectin seem to mediate the initial steps of this process. Activation of EphB4 in EPCs leads to a higher expression of PGLS-1, a selectin ligand. Subsequently, increased adhesion to P-selectin and E-selectin is observed. siRNA for P-selectin abrogates this response indicating that PGLS-1 expression facilitates the recruitment of EPCs and thus enhances their proangiogenic capacity.\textsuperscript{93} Other studies have shown that E-selectin also potentiates angiogenesis in ischaemic hindlimbs, at least in part by mediating EPC-endothelial cell interactions.\textsuperscript{94} β2-integrins expressed on the cell surface of EPCs mediate the firm adhesion and transmigration of EPCs to the damaged endothelial monolayer. Activation of the β2 integrins was shown to improve the homing and the neovascularization capacity of EPCs in a mouse model of hindlimb ischaemia.\textsuperscript{95} Interestingly, high-mobility group box 1 (HMGB1) was recently reported to activate β1 and β2 integrins on the surface of endothelial progenitors. HMGB1 is released into the extracellular space by necrotic but not apoptotic cells and was shown to induce EPC adhesion and homing to ischaemic areas \textit{in vivo}.\textsuperscript{96} The importance of β2 integrins in homing of EPCs is also highlighted by studies focusing on ICAM-1. Up-regulation of ICAM-1 in the ischaemic muscle was shown to associate with enhanced EPC recruitment to ischaemic limbs.\textsuperscript{97} Moreover, integrin-linked kinase (ILK), a hypoxia-responsive gene was reported to control ICAM-1 expression. ILK overexpression induces both ICAM-1 activation and SDF-1 expression in endothelial cells. As a result EPC recruitment to ischaemic tissues was enhanced.\textsuperscript{98} α4 integrin also seems to play a crucial role in progenitor cell homing. Reports have indicated that it promotes homing of circulating endothelial progenitors to the sites of active tissue remodelling\textsuperscript{99} and improves blood flow recovery and tissue preservation.\textsuperscript{100} Thus, interaction between EPC surface molecules with their ligands on the dying endothelial cells or subendothelial matrix proteins plays a major role in EPC homing (Figure 4).

To deliver a beneficial effect on neovascularization, EPCs need to transmigrate to the injured tissue and thus their invasive capacity is crucial for tissue repair and restoration of organ function. Recently, the role cathepsin L in this process was revealed. This protease is highly expressed in EPCs and it seems to be essential for matrix degradation and invasion. Cathepsin L knockout mice displayed impaired recovery following hindlimb ischaemia and cathepsin L knockout EPCs neither homed to sites of ischemia nor augmented neovascularization.\textsuperscript{101} MMP-2 was also found to affect the invasive properties of endothelial progenitors. EPCs from MMP2\textsuperscript{−/−} mice exhibit reduced ECM degradation and as a result, MMP2\textsuperscript{−/−} mice respond poorly to hindlimb ischaemia because of reduced neoangiogenesis. Of note, these mice can be rescued by transplantation of MMP2\textsuperscript{+/+} bone marrow cells.\textsuperscript{102} These data suggest that MMP-2 is also an important enzyme in determining EPC homing.

8. Differentiation of stem cells towards endothelial lineage

On their way to the injured tissue, progenitor cells begin the process of differentiation into endothelial cells. Cytokines and mechanical forces seem to initiate a cascade of events that will lead to progenitor cells acquiring some phenotypic features of endothelial cells. VEGF and SDF-1 can strongly up-regulate the expression of endothelial cell marker on progenitor cells and thus increase the available cell population capable of repairing the endothelial monolayer and improving vascular function. Shear stress, the mechanical force generated by blood flow, can also effectively induce expression of endothelial-specific genes in stem cells. As recent studies revealed, laminar flow can enrich both adult and embryonic stem cell populations for endothelial progenitors.\textsuperscript{103–106} Interestingly, histone deacetylase (HDAC) activity is essential in this process, which activates transcription factor p53 and p21. Commitment to endothelial lineage is sharply diminished when HDAC activity is
inhibited, mainly because of down-regulation of HoxA9 expression. Forced expression of HoxA9 could rescue the endothelial differentiation imposed by HDAC inhibitors. In line with these findings, experiments using HoxA9 –/– mice elucidated a defect in circulating EPCs and an impaired postnatal neovascularization capacity after the induction of ischaemia. Interestingly, suppression of HoxA9 levels was also detected after knockdown of HDAC1, implying that this isoform may play a regulatory role.104 In embryonic stem cells on the other hand, HDAC3 was shown to be crucial in shear- and VEGF-induced differentiation towards endothelial cells.103,102 These data collectively imply that the members of HDAC family may serve as therapeutic targets in postnatal neovascularization.

9. Future perspectives

EPCs have been extensively studied in cardiovascular diseases and accumulating evidence highlights their importance in vascular repair and tissue remodelling. Despite these encouraging results, there are also several reports on adverse effects in various animal models and there are still many issues concerning the biology of the EPCs that need to be addressed. Numerous studies have underlined the need of increasing EPC numbers to allow sufficient neovascularization and recovery of the ischaemic tissue. Thus, identifying other sources of stem cells and understanding the molecular mechanisms involved in the mobilization, differentiation, and migration of these cells is essential to design effective therapeutic strategies.

Development of safe methodologies isolating sufficient numbers of EPCs that maintain their angiogenic potential which may be used to treat patients with damaged vasculature would be another challenge. Recent reports from two clinical studies investigated the effect of bone marrow cell transfer after myocardial infarction using similar protocols. One study indicated a modest improvement of left ventricular ejection fraction and improvement in cardiac function in patients receiving bone marrow cells,108 whereas the second found no effect.109 The reason for these conflicting results has yet to be determined. Characterization of the specific subpopulation of stem/bone marrow cells that harbours the most beneficial properties for vascular repair would allow us to establish safer and successful isolation protocols.

The functional impairment identified in EPCs derived from high-risk patients poses an additional hurdle in using autologous cell sources for transplantation. Delineating the regulatory mechanisms that are involved in sustaining protective properties would greatly enhance our ability to genetically modify these cells in vitro and improve vascular function following application in vivo.

EPCs represent a promising therapeutic approach that may transform the treatment of cardiovascular diseases. However, further understanding of the biology of these cells is essential to fully benefit for their regenerative properties and design novel ways to successfully intervene with the progress of the disease.

Funding

This work was supported by Grants from British Heart Foundation and Oak Foundation.

Conflict of interest: none declared.

References


