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TOPIC HIGHLIGHT

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Endothelial progenitor cells as factors in neovascularization and endothelial repair

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Abstract

Endothelial progenitor cells (EPCs) are a heterogeneous population of cells that are provided by the bone marrow and other adult tissue in both animals and humans. They express both hematopoietic and endothelial surface markers, which challenge the classic dogma that the presumed differentiation of cells into angioblasts and subsequent endothelial and vascular differentiation occurred exclusively in embryonic development. This breakthrough stimulated research to understand the mechanism(s) underlying their physiologic function to allow development of new therapeutic options. One focus has been on their ability to form new vessels in injured tissues, and another has been on their ability to repair endothelial damage and restore both monolayer integrity and endothelial function in denuded vessels. Moreover, measures of their density have been shown to be a better predictor of cardiovascular events, both in healthy and coronary artery disease populations than the classical tools used in the clinic to evaluate the risk stratification. In the present paper we review the effects of EPCs on revascularization and endothelial repair in animal models and human studies, in an attempt to better understand their function, which may lead to potential advancement in clinical management.

Key words: Atherosclerosis; Bone marrow; Endothelial dysfunction; Endothelial progenitor cells; Neovascularization; Stem cells

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INTRODUCTION

Stem cells are primal cells found in all multicellular organisms. The biologic hallmark of stem cells is their ability to

renew through mitotic cell division and differentiate into a different specialized cell types. The three broad categories of mammalian stem cells are (1) embryonic stem cells derived from blastocysts; (2) intermediate stem cells isolated from fetal tissue and extra-embryonic membranes; and (3) adult progenitor cells found in adult tissues. Stem cells can be cultured *in vitro* and transformed into specialized cells, potentially offering treatment for a variety of diseases which were previously considered incurable. Other types include manually-manipulated stem cells such as human induced pluripotent stem cells[1,2], nuclear transfer stem cells[3] and pluripotent adult unipotent germline stem cells[4].

We begin by clarifying our terminology in order to better address this topic. The term progenitor cell is used in cell and developmental biology to refer to an immature or undifferentiated cell, typically found in post-natal animals. While progenitor cells share many common features with stem cells, these two terms are often incorrectly used as synonymous. Stem cells have unlimited self-renewal ability, while the self-renewal ability of progenitor cells is limited. Another differentiating feature is that stem cells are *pluripotent* (can differentiate into cells derived from any of the three germ layers) while adult progenitor cells are *unipotent* (can produce only one cell type, but have the property of self-renewal, which distinguishes them from non-stem cells) or *multipotent* (can produce only cells of a closely related family of cells, e.g. hematopoietic stem cells differentiate into red blood cells, white blood cells, platelets, *etc.*). Embryonic stem cells can differentiate into all of the specialized embryonic tissues that form the organism. In adults, progenitor cells act as a repair system for the body, replenishing specialized cells as and when needed.

The focus of this review is endothelial progenitor cells (EPCs), a population coming from mobilization and differentiation of precursors present in the bone marrow (BM) or other tissues such as fat, adventitia and skeletal muscles. EPCs have the ability to elicit neovascularization in response to ischemia, and to repair injured or damaged endothelium.

EPCS

The close regional and functional development of peripheral blood and vascular wall cells from the angioblast during embryonic development suggested the existence of a common origin, the hemangioblast. However, differentiation of these mesodermal cells to angioblasts and subsequent endothelial differentiation was believed to exclusively occur in embryonic development. This belief was first challenged by Asahara *et al*[5] in 1997, who isolated an angioblast from peripheral blood of adult humans, which differentiated *in vitro* into endothelial cells and contributed to *in vivo* neoangiogenesis in response to tissue ischemia. These cells were named EPCs[6,7]. They express various surface markers, hematopoietic CD34 surface marker and endothelial phenotype marker vascular endothelial growth factor receptor 2 (VEGFR2). Further observations also reported the existence of "circulating BM-derived EPCs" in adults, a subset of the CD34 blood-derived cell population, which was shown to differentiate into the endothelial lineage and express endothelial marker proteins. Because CD34 was not exclusively expressed on hematopoietic stem cells, further studies used a more immature stem cell marker CD133 and demonstrated that purified CD133 cells can differentiate to endothelial cells *in vitro*[8]. CD133+/CD34- EPCs have a higher vascular regeneration potential compared to CD133+/CD34+ EPCs[9]. Peripheral blood-derived EPCs can also form 'late-outgrowth colony-forming unit endothelial cells, and this ability characterizes the "true" EPCs *in vitro*, being a marker of their clonogenic potential[10]. Thus, CD133/VEGFR2-positive cells more likely reflect immature EPCs, whereas CD34/VEGFR2-positive cells represent circulating endothelial cells both derived from EPC differentiation and/or shed from the vessel endothelium into the blood[11]. Importantly, EPCs have been shown to differentiate *in vitro* into vascular smooth muscle cells[12].

We speculate that heterogeneity in cell markers may reflect different developmental stages of EPCs during the maturational process from the BM residual cell to the mature vascular wall cell. In addition to the BM (myelomonocytic)-derived cells, spleen-derived mononuclear cells, cord blood derived mononuclear cells[13], fat tissue derived stem cells[14], adventitial stem cells[15] and skeletal muscle progenitor cells[13] contribute to the pool of progenies of the endothelial cell lineage. Therefore, EPCs are a heterogeneous population of cells from BM or other adult tissue that share a common phenotype which when properly stimulated *in vivo* and *in vitro*, give rise to endothelial cells.

EPCS AND NEOVASCULARIZATION

The finding that BM-derived cells can mobilize to sites of ischemia and express endothelial marker proteins have been demonstrated in animal models and in humans. This suggests that isolated EPCs may be used in therapeutic vasculogenesis as a way to rescue tissue from critical ischemia. Infusion of various distinct cell types, either isolated from the BM or *ex vivo* cultivation, was shown to augment capillary density and neovascularization in ischemic tissue. In animal models of myocardial infarction, injection of *ex vivo* expanded EPCs or stem and progenitor cells significantly improved blood flow, improved cardiac function and reduced left ventricular scarring[16,17]. Similarly infusion of *ex vivo* expanded EPCs derived from peripheral blood mononuclear cells in athymic nude mice or rats improved neovascularization in hind limb ischemia models[18-21].

Initial human trials indicate that BM-derived or circulating blood-derived progenitor cells are useful for therapeutically improving blood supply to ischemic tissue. Autologous implantation of BM mononuclear cells in patients with ischemic limbs significantly augmented ankle-brachial index and reduced rest pain. In addition, transplantation of *ex vivo* expanded EPCs significantly im-

proved coronary flow reserve and left ventricular function in patients with acute myocardial infarction[22,23]. The role of EPCs in neovascularization remains to be elucidated. Basal incorporation of EPCs in non-ischemic tissues is very low[24] but the incorporation of EPCs in ischemia-injured tissues shows contradictory results. Data showing a wide range of EPC incorporation rates have been published, but other studies detected BM-derived cells only adjacent to the vessel, and they did not express endothelial markers[25-29]. This heterogeneity may be due to differences among models of ischemia that may significantly influence the incorporation rate. A homogenous finding among these studies was that the incorporation rate in an ischemic injured tissues model was quite low, or at least not enough to explain the observed effective increase in the neovascularization process. The challenge is to explain how such a low number of endothelial stem cells can improve neoangiogenesis. One possible explanation is that the efficiency of neovascularization may combine the incorporation of EPCs in newly formed vessels and the release of proangiogenic factors in a paracrine manner.

Various studies have been conducted to evaluate the extent of neovascularization after infusion of EPCs or monocyte/macrophages lines. EPCs additionally incorporated into the newly formed vessel structures show endothelial marker protein expression *in vivo*[30,31]. Infusion of macrophages (which are known to release growth factors but are not incorporated into vessel-like structures), induces only a slight increase in neovascularization after ischemia, much less that the one induced by injection of EPCs. These studies indicate that the capacity of EPCs to physically contribute to vessel-like structures leads to their potent capacity to improve neovascularization[18,32,33]. Human studies performed to demonstrate the usefulness of EPCs gave mixed results. The TOPCARE-AMI[34] trial showed the safety and feasibility of intracoronary infusion of EPCs (either BM-derived cells or circulating progenitor cells) in patients successfully revascularized by stent implantation post-acute myocardial infarction. EPCs and BM mononuclear cells have been clinically evaluated for their benefits in limb ischemia[35-37], acute myocardial infarction, and dilated cardiomyopathy[38-42]. These cell types showed modest cardiovascular benefits with 2%-8% improvement in left ventricular ejection fraction[38] compared to significant improvement in limb ischemia. Many questions remain unanswered, and further studies are needed to elucidate the contribution of physical incorporation, paracrine effects and possible effects on vessel remodeling and facilitation of vessel branching to obtain EPC-mediated improvement of neovascularization.

EPCS AND ENDOTHELIAL REPAIR

Previously, the regeneration of the injured endothelium was believed to come only from the migration and proliferation of neighboring endothelial cells. However, accumulating evidence shows that additional mechanisms may exist, and may be mediated by the circulating pool of EPCs. Rafii *et al*[11] showed that a subset of CD34-positive cells (hematopoietic marker) have the capacity to differentiate into endothelial cells *in vitro* in the presence of basic fibroblast growth factor, insulin-like growth factor-1, and VEGF. These differentiated endothelial cells stained for von Willebrand factor (vWF), and incorporate acetylated low-density lipoprotein (LDL), therefore showing both hematopoietic (CD34) and endothelial phenotype (LDL and vWF). This also suggests the existence of a BM-derived precursor endothelial cell. To demonstrate this phenomenon *in vivo*, the authors further used a canine BM transplantation model, in which the BM cells from the donor and recipient were genetically distinct. After BM transplantation, a Dacron graft was implanted in the descending thoracic aorta, and they found that only donor alleles were detected in DNA from cells on the Dacron graft, indicating that re-endothelialization was mediated by circulating EPC derived from donor BM[43].

Xu *et al*[44] demonstrated that endothelial repair of a vein grafted into an artery is mediated by a circulating pool of endothelial cells provided by the recipient. They used TIE2-LacZ mice, which are transgenic mice with a promoter (TIE2) placed before an intron fragment containing the enhancer for LacZ gene. This combination allows LacZ gene expression (β-galactosidase) specifically on vascular endothelial cells. Thus TIE2-LacZ endothelial cells can be recognized by β-galactosidase staining. When they grafted the TIE2-LacZ vena cava into the carotid artery of wild-type mice, endothelial cells of freshly harvested vena cava from TIE2-LacZ mice showed β-galactosidase staining, whereas the intensity of blue color of β-gal cells in vein grafts was decreased 1 d after surgery, and almost disappeared 3 d after the implantation. Hence, these results confirmed that the endothelial cells of vein segments implanted in an artery are totally destroyed by the acute exposure to mechanical stress due to increased blood pressure. When they grafted the vena cava from wild-type mice into the TIE2-LacZ carotid artery, no β-galactosidase staining was observed on the surface of the freshly-harvested vena cava from the wild-type mouse, which in contrast appeared 24 h after grafting into the carotid artery of a TIE2-LacZ mouse and increased in number to reach a monolayer at 4 wk after the graft. These results showed that the endothelial cells on the grafted vein were coming from the recipient mice and not from the donor. To show that those cells were coming from the BM of the recipient, they further created chimeric mice by transplanting the BM from TIE2/LacZ mice to wild-type animals that were previously irradiated: these chimeric mice expressed B-galactosidase activity only on endothelial cells that were actually provided by its BM. The investigators then grafted a vena cava coming from the wild-type into the carotid artery of the chimeric mice, and 3 d after transplantation, β-galactosidase activity was detected on the vein. Since the chimeric mice expressed B-galactosidase activity only on endothelial cells provided by the BM, these B-galactosidase-positive cells could only come from their BM[44-46]. However, in a model of transplant arteriosclerosis, BM-

derived cells appeared to contribute only to a minor extent to endothelial regeneration by circulating cells[47]. These data indicated that there might be at least two distinct populations of circulating cells that principally are capable of contributing to re-endothelialization, namely mobilized cells from the BM and non-BM derived cells. The latter may arise from circulating progenitor cells released by non-BM sources (e.g. tissue resident stem cells) or represent vessel wall-derived endothelial cells.

We emphasize here that the source of transfused endothelial cells after *in vitro* expansion is critical for interpreting the results. The observation that EPCs directly influence lesion formation and progression comes from experimental models using progenitor cell transfusion. The systemic application of healthy wild-type EPCs in atherosclerotic apolipoprotein E-knockout mice has been shown to improve endothelial function and to inhibit atherosclerotic lesion progression independent of high serum cholesterol levels[48]. However, these beneficial effects were not observed in a study conducted by George *et al*[49] in which aortic sinus lesion size was significantly increased in mice receiving EPCs compared with controls. Mice receiving EPCs showed plaques with larger lipid cores, thinner fibrous caps, and a higher number of infiltrating CD3 cells, suggesting an effect on plaque stability. An important aspect of this study was that intravenously transfused spleen-derived cells were administered without splenectomy of the recipient animals, and the tendency of spleen-derived cells to migrate back to the organ of origin may have affected the results.

Fujiyama *et al*[50] showed that infusion of EPCs leads to regeneration of a functionally active endothelium confirmed by release of nitric oxide (NO). They also noted a significant reduction in neointima formation. Similarly, Griese *et al*[51] showed that infused peripheral blood monocyte-derived EPCs deposit on bioprosthetic grafts and balloon-injured carotid arteries, with significantly reduced neointima deposition. These studies indicate that administration of EPCs not only facilitates re-endothelialization but also helps with recovery of endothelial function, while inhibiting neointima deposition.

MOBILIZATION, CHEMOTAXIS, ADHESION, TRANSMIGRATION AND DIFFERENTIATION

The mobilization of stem cells in the BM is determined by the local microenvironment, the so-called "stem cell niche," which consists of fibroblasts, osteoblasts, and endothelial cells[38,52]. Basically, mobilizing cytokines [VEGF, stromal-derived factor (SDF)-1] hamper the interactions between stem cells and stromal cells, which finally allow stem cells to leave the BM *via* trans-endothelial migration. Thereby, activation of proteinases such as elastase, cathepsin G, and matrix metalloproteinase (MMP) cleave adhesive bonds on stromal cells, which interact with integrins on hematopoietic stem cells. MMP-9 was additionally shown to cleave the membrane-bound Kit ligand and induce the release of soluble KitL (also known as stem cell factor). Physiologically, ischemia is believed to be the predominant signal to induce mobilization of EPCs from the BM. Ischemia is thus believed to upregulate VEGF or SDF-1, which in turn are released into the circulation and induce mobilization of progenitor cells from the BM *via* a MMP-9 dependent mechanism. Several mediators increase the number of circulating EPCs in the blood of both humans and animal models. Granulocyte-colony stimulating factor, a cytokine that is typically used for mobilization of CD34 cells, has been shown to increase the levels of circulating EPCs. A related cytokine, the granulocyte monocyte-colony stimulating factor, also increases EPC levels[53].

In a clinical environment, the effects of VEGF and erythropoietin (EPO) on EPC mobilization have been evaluated, and both demonstrated an augmentation of EPC levels in humans[40,41,54,55]. Moreover, the correlation between EPO serum levels and the number of CD34 or CD133 hematopoietic stem cells in the BM of patients with ischemic coronary artery disease (CAD) further supports an important role of endogenous EPO levels as a physiologic determinant of EPC mobilization. Some athero-protective drugs can also positively modulate the number of circulating EPCs. Statins increase the number and the functional activity of EPCs *in vitro*, in mice, and in patients with stable CAD. This increase in EPC numbers was associated with increased BM-derived cells after balloon injury and accelerated endothelial regeneration[56]. Other factors that augment the circulating EPCs are estrogen[57] and exercise[58,59].

The molecular signaling pathways have not, as yet, been identified. However, several studies indicate that the activation of the PI3K/Akt pathway may play an important role in the statin-induced increase in EPC levels[60]. Likewise, EPO[61], VEGF[62], estrogen[63] and exercise (shear stress)[64] are also well known to augment the PI3K/Akt-pathway. Thus, these factors may share some common signaling pathways. Recent data shows that endothelial NO synthase is essential for mobilization of BM-derived stem and progenitor cells[65], and we speculate that these stimuli may increase progenitor cell mobilization by PI3K/Akt-dependent activation of the NO synthase within the BM stromal cells.

Factors that drive the EPCs to the site of endothelial injury (chemotaxis) may be the same that normally stimulate engraftment of hematopoietic cells to the BM, such as SDF-1 or sphingosine-1-phosphate. SDF-1 has been proven to stimulate recruitment of progenitor cells to the ischemic tissue. SDF-1 protein levels increase during the first days after induction of myocardial infarction[66]. Integrins are known to mediate the adhesion of various cells including hematopoietic stem cells and leukocytes to extracellular matrix proteins and to endothelial cells[67,68]. Integrins capable of mediating cell-cell interactions are the β2-integrins and α4β1-integrin. Various cell types including endothelial cells and hematopoietic cells express

β1-integrins, whereas β2-integrins are found preferentially on hematopoietic cells[69]. Because adhesion to endothelial cells and transmigration events are involved in the *in vivo* homing of stem cells to tissues with active angiogenesis, integrins such as the β2-integrins and the α4β1-integrin may be involved in the homing of progenitor cells to ischemic tissues. However, the data regarding physiologic mobilization, chemotaxis and differentiation of EPCs at the site of endothelial injury is limited.

EPCS AND CARDIOVASCULAR RISK FACTORS, RISK STRATIFICATION AND PROGNOSTIC VALUE

Endothelial cell number and function is a valuable surrogate biologic marker for vascular function and cumulative cardiovascular risk and a strong predictor of the risk of cardiovascular events[70-73]. Hill *et al*[74] started from Ross's classic paradigm stating that endothelial cell injury is the stimulus for the development of atherosclerotic plaque. This model argues that seemingly disparate risk factors act on a final common pathway that culminates in endothelial-cell injury, including both direct endothelial damage and endothelial dysfunction. They speculated that indicators of cumulative risk, such as the Framingham score, or function, such as brachial reactivity, represent useful composite measures of overall vascular status. In 45 healthy adult subjects, with different associations of cardiovascular risk but with no symptoms of atherosclerosis or active organ ischemia, the correlation between EPC count in the peripheral blood, and both Framingham risk score and brachial vascular reactivity was evaluated. The number of colony-forming units was negatively correlated with Framingham risk score and positively correlated with brachial reactivity. Interestingly, when the subjects were divided according to the number of EPCs circulating into "high" and "low", activity of EPCs was a stronger predictor of flow-mediated brachial reactivity than the presence or absence of conventional cardiovascular risk factors. Tepper *et al*[75] isolated EPCs from human type Ⅱ diabetics and age-matched control subjects and found that the proliferation of diabetic EPCs relative to control subjects was significantly decreased and inversely correlated with patient levels of glycosylated hemoglobin. Diabetic EPCs had normal adhesion to fibronectin, collagen, and quiescent endothelial cells but a decreased adherence to human umbilical vein endothelial cells (HUVEC) activated by tumor necrosis factor (TNF)-α. These authors conclude that type Ⅱ diabetes may alter EPC biology in processes critical for new blood vessel growth and, furthermore, EPC monitoring may identify a population at high risk for morbidity and mortality after vascular occlusive events.

In addition to what was shown in the healthy population, in patients with CAD the number of cardiovascular risk factors negatively correlated with progenitor cell counts. Vasa *et al*[76] determined the number and functional activity of EPCs in 45 patients with CAD and in 15 healthy volunteers. The number of isolated and circulating EPCs was significantly reduced in patients with CAD. To determine the influence of atherosclerotic risk factors, a risk factor score including age, sex, hypertension, diabetes, smoking, positive family history of CAD, and LDL cholesterol levels was used. The number of risk factors was significantly correlated with a reduction of EPC levels. Analysis of the individual risk factors demonstrated that smokers and patients with a family history of CAD had significantly reduced levels of EPCs. EPCs isolated from patients with CAD also revealed an impaired migratory response, which was inversely correlated with the number of risk factors. They concluded that patients with CAD show reduced levels and functional impairment of EPCs, which correlated with risk factors for CAD. Another potentially attractive marker for risk stratification in patients with atherosclerotic disease seems to be the so-called "endothelial cell-derived microparticles (EMP)": endothelial cell damage mediated by chemical or mechanical injury leads to endothelial cell apoptosis, which is associated with conformational changes of the cell's plasma membrane leading to the release of membrane microparticles, in which antigens derived from their mother cell and can be quantified *in vivo* by flow cytometry[77]. Elevated EMP levels have been described in all conditions of severe endothelial cell damage (e.g. thrombotic thrombocytopenic purpura[78], diabetes[79], arterial hypertension[80], acute coronary syndromes[81], and myocardial infarction[82]) and microparticles themselves have been shown to elicit direct effects on endothelium-dependent vasorelaxation *in vitro*. Microparticles derived from patients with acute coronary syndromes or preeclampsia directly impaired endothelial function in rat aortic rings or myometrial arteries[83], and in humans, increased apoptotic microparticle counts positively correlated with the impairment of coronary endothelial function[84]. In a study investigating coronary endothelial function in 50 patients with CAD, multivariate analysis revealed that increased apoptotic microparticle counts predict severe endothelial dysfunction independent of classical risk factors such as hypertension, hypercholesterolemia, smoking, diabetes, age, and gender[84]. In the context of human atherogenesis, it may be pivotal to evaluate the current status of regeneration and endothelial cell apoptosis in each individual. EPC and EMP may be valuable biomarkers in patients with atherosclerotic disease.

Werner *et al*[85] performed a clinical study to evaluate the prognostic value of circulating EPCs and their potentially vasculoprotective role. The number of EPCs was measured in 519 patients with angiographically documented CAD and correlated with cardiovascular outcomes. Primary end points included cardiovascular mortality, the occurrence of a first major cardiovascular event (myocardial infarction, hospitalization, revascularization, and cardiovascular death), revascularization, hospitalization, and all-cause mortality after 1 year. The cumulative event-free survival increased stepwise across tertiles of baseline EPC levels for cardiovascular mortality, first major cardiovascular event, revascularization, and hospitalization. After adjustment for

vascular risk factors, drug therapy, and concomitant disease, increased EPC levels were independently associated with a lower risk of cardiovascular death, first major cardiovascular event, revascularization, and hospitalization. Primary and secondary prevention trials suggest that statins possess favorable effects on atherosclerosis development and progression and that these effects are independent of cholesterol reduction. Statins can also improve vascular perfusion by causing several positive side effects such as reduction of both hypertrophy and proliferation of smooth muscle cells[86-89], an increase in NO synthesis[90] and a decrease in production of adhesion molecules[91-93]. In a model of carotid balloon-injury, Walter *et al*[94] investigated whether statin therapy may also accelerate re-endothelialization after carotid balloon injury: they treated male Sprague-Dawley rats with simvastatin and found that this treatment accelerated re-endothelialization of the balloon-injured arterial segments and resulted in a dose-dependent significant reduction in neointimal thickening when compared with saline-injected controls. They further tried to elucidate the mechanism, and investigated the contribution of BM-derived EPCs by BM transplantation from Tie2/lacZ mice to background mice or athymic nude rats. As described earlier TIE2-LacZ endothelial cells can be recognized by β-galactosidase staining. β-galactosidase staining of mouse carotid artery specimens revealed a significant increase in the number of β-galactosidase-positive cells per mm^2 appearing on the carotid artery luminal surface of treated rats. In addition, statins increased circulating rat EPCs and induced adhesiveness of cultured human EPCs by upregulation of the integrin subunits α5, β1, α(v), and β5 of human EPCs. These findings showed physiological evidence that EPC mobilization represents a functionally relevant consequence of statin therapy[94]. Furthermore, Werner *et al*[95] investigated vascular lesion formation in mice after transplantation of BM transfected by means of retrovirus with enhanced green fluorescent protein; they induced carotid artery injury, resulting in neointimal formation. Fluorescence microscopy and immunohistological analysis revealed that BM-derived progenitor cells were involved in re-endothelialization of the vascular lesions. Treatment with rosuvastatin enhanced the circulating pool of EPCs, propagated the advent of BM-derived endothelial cells in the injured vessel wall, and, thereby, accelerated re-endothelialization and significantly decreased neointimal formation. These results also show that statin treatment promotes BM-dependent re-endothelialization and diminishes vascular lesion development. Estrogens increase EPC numbers in mice and humans, which contributes to repair mechanisms of the vascular wall[96]. Also physical activity, which is known to reduce cardiovascular morbidity and mortality by mainly unknown mechanisms, increases the number and function of EPCs in rodents and healthy humans[58].

INFLAMMATION, REACTIVE OXYGEN SPECIES AND STEM CELLS

Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal stem cells that can be isolated from many adult tissues. MSCs are multipotent stem cells that can differentiate into a variety of cell types. Cell types that MSCs differentiate into *in vitro* or *in vivo* include osteoblasts, chondrocytes, myocytes, adipocytes, endothelium, and, as described recently, β-pancreatic islets cells[97]. Interestingly, some studies disputed the differentiation potential of adult BM-derived stem cells[98-100]. MSCs can interact with cells of both the innate and adaptive immune systems, leading to the modulation of several effectors functions. Once MSCs are administered *in vivo* they may induce peripheral tolerance and migrate to the injured tissues, where they help damaged cells survive by inhibiting the release of pro-inflammatory cytokines[101]. The key role of myeloid dendritic cells (DCs) is to present antigen to naive T cells following DC maturation, induced by cytokines. As the DCs are maturing, they acquire expression of co-stimulatory molecules and upregulate expression of MHC class I and class II molecules together with other cell-surface markers[101-103]. MSCs have been shown to inhibit the maturation of monocytes, cord blood and CD34+ hematopoietic progenitor cells into DCs *in vitro*[101]. The final outcome of the immunomodulatory activity of MSCs is likely to be significantly influenced by the micro environmental cues encountered following *in vivo* administration. Micro environmental cues encountered following *in vivo* administration influences the immunomodulatory activity of MSCs including their effect on target cells, as exemplified by the opposite outcomes that can arise from the interaction of MSCs with DCs and natural killer cells in the presence of high or low concentrations of interferon-γ[101]. Stem and progenitor cells are critical for organogenesis during the fetal stage of development[104]. Recently the existence of somatic stem cells has been reported in adult organs[104]. Somatic stem cells and progenitor cells are thought to sense and repair damaged tissues and organs[104]. Reactive oxygen species accelerate the senescence of stem and progenitor cells[104].

Clinical studies suggest that TNF levels in the serum are correlated negatively with the CD34+ stem cells and EPCs circulating in the peripheral blood in patients with congestive heart failure. This is thought to be related to the myelosuppressive effect of circulating TNF[55]. In a murine congestive heart failure model, elevated serum TNF levels and reduced BM progenitor cells have been reported[55]. An *in vitro* study indicated a causal relationship between TNF and suppression of hematopoietic stem cell growth, and that TNF directly inhibited stem cell factor-stimulated proliferation of CD34+ hematopoietic progenitor cells[103]. Human CD34+ myeloid leukemic cells and BM progenitor cells (CD34+CD38-) demonstrated similar results[103]. Similar results were also found in human CD34+ myeloid leukemic cells and primitive human BM progenitor cells (CD34+CD38-)[103]. Interestingly, the inhibitory effects of TNF in these studies were consistently mediated by TNFR-Ⅰ, but not TNFR-Ⅱ. To the contrary, the TNFR-Ⅱ signaling pathway shows a protective profile on stem cell function. Thus, distinct effects of TNF are mediated by different subtypes of TNF receptors in stem

cells while the overall effect might be dependent on the expression level and ratio of these two receptors. Apart from the direct effect, TNF is able to indirectly influence the fate of stem cells. TNF markedly stimulates production of granulocyte macrophage-colony stimulating factor, a strong mobilizer of stem cells from the BM[105]. Activation of the TNF/Fas pathway in lymphocytes in the BM may play a pathogenic role in suppressing hematopoiesis[103]. EPC adhesion to HUVEC, a process mediated by upregulation of E-selectin, was significantly increased by TNF pre-treatment of HUVEC in the peripheral circulation. Interestingly, EPC adhesion to HUVECs was not induced when pretreatment was carried out for EPCs instead of HUVECs[103]. TNF also has effects on stem cell differentiation: administration of TNF switched the differentiation of these cells from granulocytes to almost complete production of macrophages when mouse Lin-Sac+ hematopoietic progenitor cells were cultured with stem cell factor and IL-7[103]. In summary, TNF plays an important role in regulating stem cell-mediated vascular repair and remodeling. However, the overall effect of TNF on stem cell mobilization, proliferation and function is complicated, depending on the subtypes of the TNF receptors, the presence of other cytokines as well as other cells. Although stem cell-based treatments are effective in myocardial infarction, the vascular protective effects of stem cells in ischemia-reperfusion injury in coronary microcirculation have not been studied. Further studies will improve our understanding of the mechanisms and remediation of ischemia-reperfusion injury.

CONCLUSION

Stem and progenitor cells possess the ability to self-regenerate and differentiate into many cell types, and inflammation is involved in most cardiovascular diseases. An understanding of the communication and interaction between TNF and stem cells is important[103]. The mechanism underlying this function remains unclear because the number of endothelial cells incorporated in ischemic tissue is too low to create a new vessel just by incorporating themselves into it; we speculate they may act through two different mechanisms, which may consist of both physical incorporation and paracrine stimulation of another "*in loco*" population to stimulate their differentiation into vessel cells. This aspect needs further study. The molecular mechanisms for effective mobilization of stem cells are, however, poorly understood. We speculate that the functional properties of EPCs in cardiovascular disease are impaired and that regeneration by endogenous cells without further mobilization of cells is diminished or absent in the presence of cardiovascular disorders or risk factors. Consistently, impaired mobilization of EPCs has been associated with older age, the presence of cardiovascular risk factors, and the presence of atherosclerotic disease. The presence of cardiovascular risk factors may interrupt the delicate equilibrium between endothelial damage and repair, leading to manifestation of endothelial dysfunction and atherosclerosis. The fact that physiological mechanisms of EPC mobilization, homing adhesion and differentiation is poorly understood adds to the challenge of unraveling this complex problem. Further studies are needed to elucidate the complexities of stem cell mobilization, homing and differentiation to identify mechanisms and develop therapies suitable for clinical application.

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