REVIEW

Arch. Immunol. Ther. Exp., 2007, **55**, 247–259
PL ISSN 0004-069X

DOI 10.1007/s00005-007-0027-5

Endothelial progenitor cells as a new agent contributing to vascular repair

Ewa Miller-Kasprzak and Paweł P. Jagodziński

Department of Biochemistry and Molecular Biology, Karol Marcinkowski University of Medical Sciences, Poznań, Poland

Received: 2006.10.18, **Accepted:** 2007.04.26, **Published online first:** 2007.07.23

Abstract

A special type of stem cells, defined as endothelial progenitor cells (EPCs), has been found in the bone marrow and peripheral blood. These EPCs are incorporated into injured vessels and become mature endothelial cells during re-endothelialization and neovascularization processes. Though a complete phenotypic description of EPCs remains unclear, these cells express several surface markers, the most relevant including CD34 and CD133 antigens. Furthermore, EPCs derived from other sources could also give rise to mature endothelial cells, which makes this group of cells more diverse. The recruitment of EPCs from the bone marrow to homing sites of vasculogenesis is subject to regulation by many factors, including chemokines and growth factors. The precise mechanism of EPC mobilization and differentiation is not entirely elucidated and is still under investigation. Recent studies have suggested that EPCs may promote local angiogenesis by secreting angiogenic growth factors in a paracrine manner. The number and function of EPCs can be affected during pathological conditions, including diabetes mellitus, cardiovascular risk factors for ischemic disease, and graft vasculopathy. Additionally, EPC number and migration capacity could be improved by such factors as drugs, physical exercise, and growth factors. Transplantation of EPCs into ischemic tissues may emerge as a promising approach in the therapy of diseases associated with blood vessel disorders.

Key words: endothelial progenitor cells, angiogenesis, colony-forming unit, surface markers.

Corresponding author: Dr. Paweł P. Jagodziński, Department of Biochemistry and Molecular Biology, University of Medical Sciences, Święcickiego 6, 60-781 Poznań, Poland, fax: +48 61 865-95-86, e-mail: pjagodzi@am.poznan.pl

INTRODUCTION

Both vasculogenesis and angiogenesis are defined as processes responsible for new blood vessel formation [5, 38]. Consistent with the definitions proposed by Risau [48], vasculogenesis occurs during early embryogenesis, while angiogenesis takes place during both embryonic development and postnatal life. Under physiological conditions, angiogenesis occurs during the female reproductive cycle and vascular regeneration after wound healing, whereas under pathological conditions this process can be engaged in the creation of tumor vasculature [30, 48].

For several years, vascular growth and remodeling in postnatal life were traditionally explained as results of the proliferative and migratory capacity of mature endothelial cells. According to this hypothesis, fully differentiated endothelial cells were proposed to participate by sprouting from existing blood vessels and giving rise to new vasculature [50]. The discovery of bone marrow (BM)-derived endothelial progenitor cells (EPCs) circulating in the peripheral blood (PB) of adults extended the view of the angiogenic process in humans [5]. There is a growing body of data that the cells defined as EPCs play a significant role in the re-endothelialization and neovascularization of injured endothelium [58]. Asahara et al. [5] provided the first evidence that the PB is a reservoir of BM-derived EPCs circulating in the blood vessel system and exhibiting reparative properties. Umbilical cord blood (CB) also contains EPCs with even greater proliferative potential than EPCs from sources such as BM or PB [43].

The number of EPCs as well as their proliferative potential may change under pathological conditions (Fig. 1), including cardiovascular diseases, diabetes mellitus, and rheumatoid arthritis (RA) [19, 21, 23, 44, 52, 57, 58]. It has been shown that smoking tobacco is accompanied by a low level of EPCs, which increases after cessation of smoking [31]. Several factors are believed to be involved in the regulation of the number and functionality of circulating EPCs (Fig. 1). One of them, vascular endothelial growth factor (VEGF), was

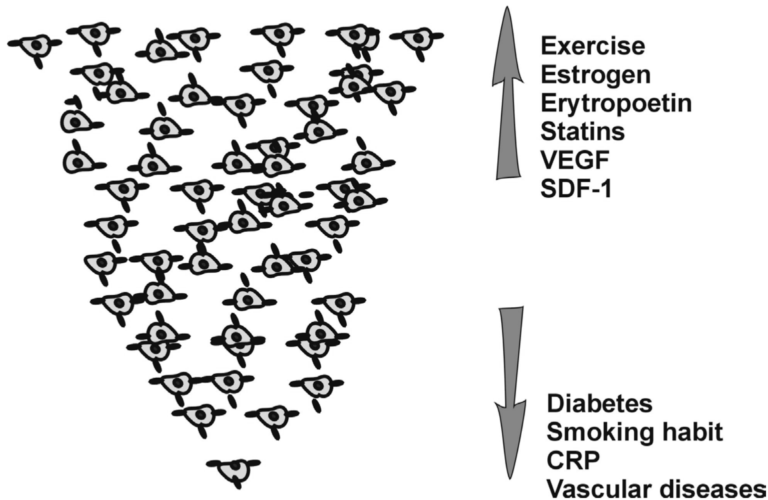

Fig. 1. The counts of EPCs in the peripheral blood and their proliferative potential can change under various conditions. Pathological stages, including diabetes, smoking, C-reactive protein (CRP), up-regulation of some factors, and vascular diseases, reduce EPC counts and their reparative ability. However, factors including physical exercise, estrogen, erytropoetin, statins, vascular endothelial growth factor (VEGF), and stromal-derived factor-1 (SDF-1) increase EPC count and function.

proposed to regulate EPC proliferation and differentiation [8, 13, 23, 58]. Patients with acute myocardial infarction (AMI) exhibited significantly increased VEGF plasma levels compared with a control group [52]. Bahlmann et al. [6, 7] discovered that erythropoietin is another factor able to regulate EPC count in PB. C-reactive protein (CRP) is involved in the negative regulation of EPC function during the vascular reparative process [61]. Recent studies reported the presence of the leptin receptor in EPCs and proposed leptin as a factor which could affect EPC function [63]. Interesting candidates for EPC regulation are also statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, that are used for the treatment of hypercholesterolemia. These compounds are able to induce EPC differentiation via activation of the phosphatidylinositol 3 kinase (PI3K) pathway [13]. Pistrosch et al. [44] demonstrated that treatment with rosiglitazone, an agonist of peroxisome proliferator-activated receptor γ (PPARγ), causes an increase in the count and migratory activity of cultivated EPCs obtained from type 2 diabetic patients. Estrogens and physical exercise were also able to increase EPC number in human and animal models [1, 24, 25, 27, 36, 46, 56].

We decided to highlight the characteristics and properties of EPCs, which can be promising agents in the therapy of vascular disorders.

ORIGIN OF EPCs

It has been proposed that EPCs may originate from a BM hematopoietic stem cell population defined as hemangioblasts [23, 50, 58]. Furthermore, the BM-derived myeloid cell line and non-BM cells can also give rise to mature endothelial cells [23, 58]. Stem cells that can differentiate into EPCs exist in a quiescent state associated with BM stromal cells. Transformation from the quiescent to the proliferative state in the BM depends on specific factors preceding the mobilization of stem cells to the PB. Recruitment of EPCs from the BM quiescent niche has been found to be associated with the activation of proteinases such as elastase, cathepsin G, and matrix metalloproteinases (MMPs) [20]. These enzymes proteolytically cleave the extracellular matrix- or cell membrane-bound molecules responsible for EPCs' adhesive bonds on BM stromal cells. These cells express membrane-bound Kit ligand (mKitL), which binds to the EPC membrane receptor c-kit when the ligand is in its soluble form (sKitL). MMP-9 proteolytically cleaves mKitL to sKitL, which then interacts with the EPC c-kit receptor to conduct the signal essential for BM EPC differentiation and migration to the PB (Fig. 2) [20, 23].

One of the models used in studying the recruitment of BM hematopoietic EPCs uses BM cell suppression by cytotoxic agents. This suppression does not affect hematopoietic stem cells in the G0 phase of the cell cycle. Therefore, these cells may serve as a cell population to reconstitute hematopoiesis and EPC release. The introduction of cytotoxic suppression in the BM of MMP-$9^{+/+}$ and MMP-$9^{-/-}$ mice resulted in poor recruitment and differentiation of hematopoietic cells only in the latter group of animals [20]. Treatment of MMP-$9^{+/+}$ mice with VEGF, stromal-derived factor-1 (SDF-1), and granulocyte colony-stimulating factor (G-CSF) caused a marked increase in the concentration of plasma sKitL compared with untreated animals [20]. The results of this experiment proved that VEGF, SDF-1, and G-CSF play significant roles in the induction of MMP-9 precursor biosynthesis, secretion, and further mobilization of BM EPCs to the PB [8, 23, 33, 58].

GROWTH FACTORS THAT EFFECT EPC FUNCTION

SDF-1 and VEGF have been considered crucial in the differentiation and migration of EPCs [8, 23, 33, 58].

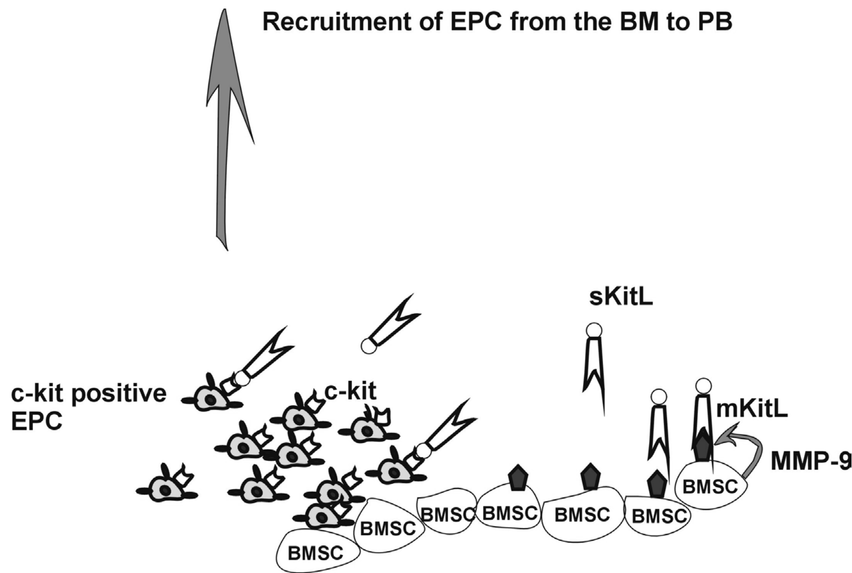

Fig. 2. Recruitment of EPCs from the bone marrow (BM) to the peripheral blood (PB). Stem cells that can differentiate into EPCs exist in a quiescent state associated with bone marrow stromal cells (BMSCs). Recruitment of EPCs from the BM quiescent niche is associated with the activation of proteinases such as elastase, cathepsin G, and matrix metalloproteinases (MMPs). These enzymes proteolytically cleave the extracellular matrix or cell membrane-bound molecules responsible for EPCs' adhesive bonds on BMSCs. These cells express membrane-bound Kit ligand (mKitL), which is proteolyticaly split by MMP-9 to its soluble form (sKitL). This sKitL form binds to the EPC membrane receptor c-kit and conducts signals essential for BM EPC differentiation and migration to the PB.

Increased plasma levels of SDF-1 and VEGF are eventually accompanied by the mobilization of hematopoietic cells, among them EPCs, into the circulation (Fig. 3). SDF-1 is a chemokine that binds specifically to the receptor designated as CXCR4 [33]. This receptor is expressed on the surface of hematopoietic stem cells, including EPCs [33, 34]. SDF-1 is expressed in various tissues, including lung, lymph nodes, liver, and BM [41]. The expression of SDF-1 is increased during stress conditions such as hypoxia or the presence of toxic agents. Recently it was reported that up-regulation of SDF-1 expression leads to the recruitment of cells expressing CXCR4 into ischemic tissue [33]. Binding of SDF-1 to the CXCR4 receptor activates the PI3K-AKT kinase--nuclear factor κB (PI3K-AKT-NF-κB) pathway, leading to mitogen-activated protein kinase (MAPK, p42/44) phosphorylation, intracellular calcium efflux, and fibronectin and fibrinogen adhesion properties [33].

Binding of VEGF to VEGF receptor 2 (VEGFR2) also mediates cell proliferation, migration, and survival via the PI3K-AKT-NF-κB or MAPK p42/44 signal transduction pathways. The expression of VEGFR2 was confirmed in the population of BM-derived cells, including EPCs. The physiological function of VEGF is focused mainly on the female reproductive cycle and the wound healing process [8]. However, an increased plasma level of VEGF was observed in various pathological conditions, including hypoxia, RA, diabetes, and tumor vascularization. Recent data has provided evidence for VEGF autocrine action in hematopoietic cells, including apoptosis protection and survival effect [8].

Granulocyte macrophage colony-stimulating factor (GM-CSF) is also proposed as a possible candidate for EPC function regulation [9]. In a study performed by Cho et al. [9], recombinant GM-CSF was able to mobilize EPCs and accelerated the re-endothelialization process in hypercholesterolemic rabbits.

EPC MARKERS EXPRESSION PATTERN

Identification of EPCs is based on the cell surface expressions of various protein markers. There is no straightforward definition of an EPC marker because these cells seem to be a heterogeneous group associated with different cell surface antigen expression profiles. The most commonly described molecules that serve as biomarkers for recognition of an EPC population include CD34, CD133, and VEGFR2 (Fig. 3 and 4). The pioneer study performed by Asahara et al. [5] recognized EPCs as CD34-positive mononuclear cells (MNCs). Hematopoietic stem cells that serve as a source of EPCs express CD34; however, this marker is also present on the surface of mature endothelial cells [15].

Human CD133 antigen is a membrane glycoprotein whose expression is related to hematopoietic stem cell differentiation into EPCs [58, 65]. This attribute explains the significance of using this marker to isolate EPCs [66]. The third marker proposed for EPC identification is VEGFR2, a protein predominantly expressed on the endothelial cell surface [5, 8]. Urbich and Dimmeler [58] claim that EPCs are positive for the CD34, CD133, and VEGFR2 markers. However, EPCs in culture have a slightly altered marker expression profile. Hristov and Weber [23] proposed a schema for human hemangioblast differentiation into EPCs throughout the early and late EPC stages. Differentiation was associated with changes in the marker expression profile. BM $CD133^+$, $CD34^+$, and $VEGFR2^+$ hemangioblasts giving rise EPCs were designated as angioblasts by Hristov and Weber [23]. In contrast to the subgroup of late EPCs, early EPCs showed expression of the CD133 and CD31 markers and were negative for von Willebrand factor, vascular endothelial cadherin (VE-cadherin), E-selectin, and endothelial nitric oxide synthase (eNOS) expression. Late EPCs still display expression of the CD34 marker and reveal no CD133 protein (Fig. 4) [23]. Among other biomarkers used for EPC

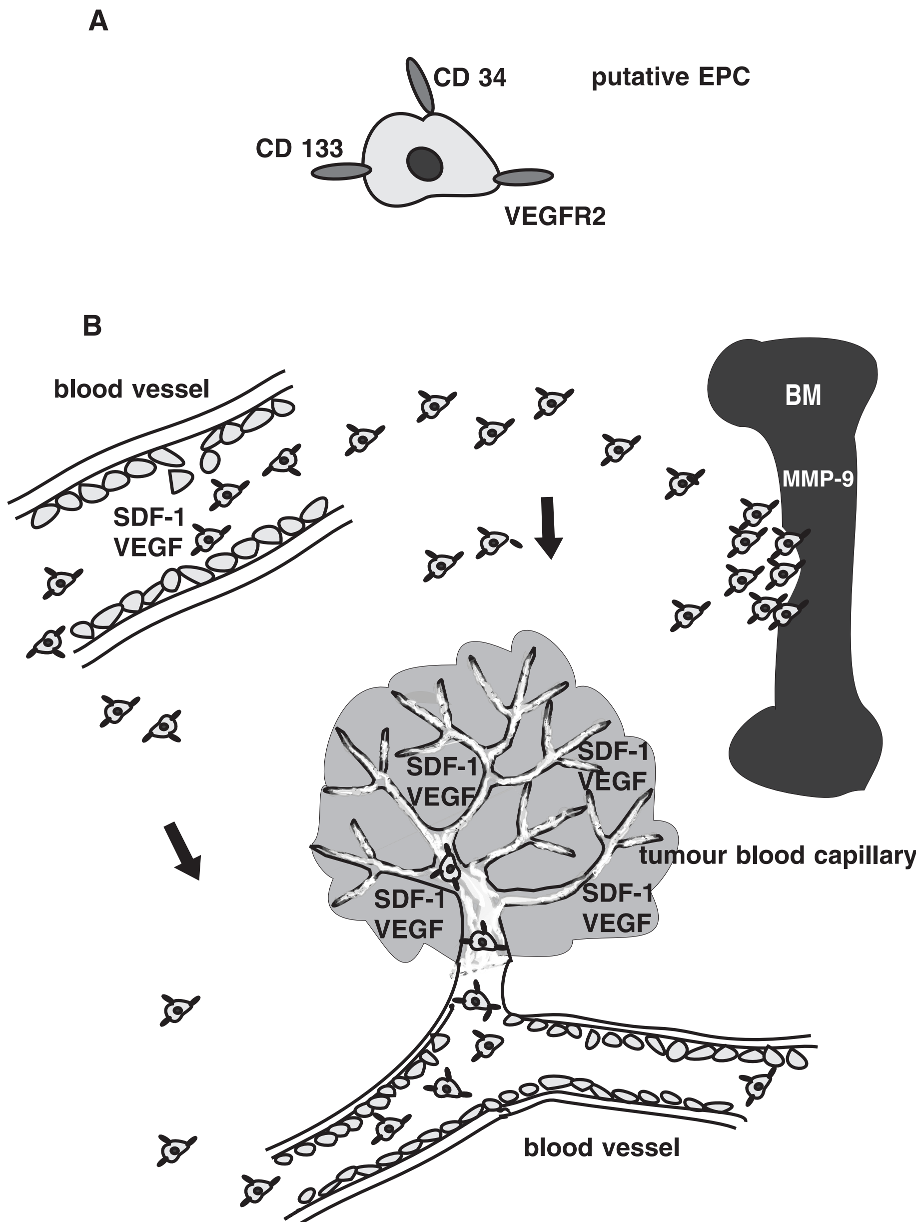

Fig. 3. EPCs are involved in blood vessel repair and tumor angiogenesis. **A** – CD34, CD133, and vascular endothelial growth factor receptor 2 (VEGFR2) are considered common markers of EPCs. **B** – increases in the expressions of matrix metalloproteinase-9 (MMP-9), vascular endothelial growth factor (VEGF), and stromal-derived factor-1 (SDF-1) are responsible for the mobilization and attraction of EPCs from the bone marrow (BM) into the peripheral blood (PB). PB EPCs are incorporated into injured vessel walls during the repair of distinct tissue vasculature. Under pathological conditions, tumor hypoxia leads to the up-regulation of VEGF and SDF expression, which attract EPCs and augment the formation of tumor vasculature. Tumor VEGF and SDF-1 are also able to stimulate the proliferation of endothelial cells in a paracrine manner.

identification is tyrosine kinase, with immunoglobulin and epidermal growth factor homology domains-2 (Tie-2) [5, 23]. On the other hand, Pistrosch et al. [45], in their study performed on MNC culture, recognized the EPC population as cells staining positively for ulex europeaus lectin (UEA-I) and able to uptake DiL-labeled acetylated low-density lipoprotein [44]. In a similar study, Simper et al. [54] termed PB endothelial cells positive for UEA-I and DiLDL markers as circulating endothelial cells (CECs). Remarkably, CECs originate from mature blood vessel wall cells, whereas EPCs are derived from circulating angioblasts [18, 54, 58].

Furthermore, blood-derived monocyte/macrophages were also found to be positive for UEA-I and showed DiLDL uptake and can secrete angiogenic growth factors such as VEGF, GM-CSF, and G-CSF [47, 59]. Rehman et al. [47] proposed the term circulating angiogenic cells (CACs) as more suitable for PB-derived UEA-I- and DiLDL-positive cells. However, they did not exclude the possibility that CACs involve a small population of true stem/progenitor cells and endothelial cells. In contrast, in the study performed by Urbich et al. [59] both $CD14^+$ and $CD14^-$ cultured cells were positive for UEA-I and DiLDL markers, expressed comparable levels of endothelial proteins, and had similar colony-forming capacity. According to the authors, the origin of blood-derived EPCs is not necessarily associated with the monocytic lineage marker CD14 [59].

EPC ISOLATION AND EVALUATION METHODS

Two main methods for EPC isolation from MNCs have been described so far (Fig. 5). The first technique

for EPC evaluation includes flow cytometry analysis with an array of antibodies to EPC markers. EPCs can be pre-selected from the PB by the immuno-magnetic technique using microbeads coated with antibodies against various surface markers, chiefly CD34 or CD133. This protocol is often performed on MNCs obtained by gradient density centrifugation of PB cells through ficoll. The second method of EPC evaluation encompasses *ex vivo* cultivation of MNCs or pre-selected $CD34^+$ cells on fibronectin or gelatin-coated dishes in the presence of a specific medium. Cultured EPCs appear as an adherent type of colony-forming units (CFUs). EPC CFUs obtained from a culture of 5–7 days are named early EPC CFUs, whereas a 1- to 4-week culture gives rise to late CFUs. The colony is defined as a group of "round cells" accompanied by thin cells of endothelial phenotype sprouting from the core [5, 22, 23, 58, 60]. Evaluation of the angiogenic properties of EPCs includes a migration assay and matrix assay for tube formation [43, 44]. The migration assay is based on EPC chemotactic ability. For example, in the experiment performed by Pistrosch et al. [44], after cultivation EPCs were harvested, counted, and resuspended in fresh medium. The cells were moved into a modified Boyden chamber that was placed in the culture dish with recombinant VEGF. During incubation, cells migrated to the lower side of the filter. The filter was washed, and after staining the nuclei the cells were counted [44]. In the matrix gel assay, EPCs obtained after culture are placed on a basement membrane matrix gel. In the work performed by Naruse et al. [43], after 3–7 days of culture on the matrix gel the number of endothelial cell networks, designated as tubes, was detected and counted using a fluorescence microscope.

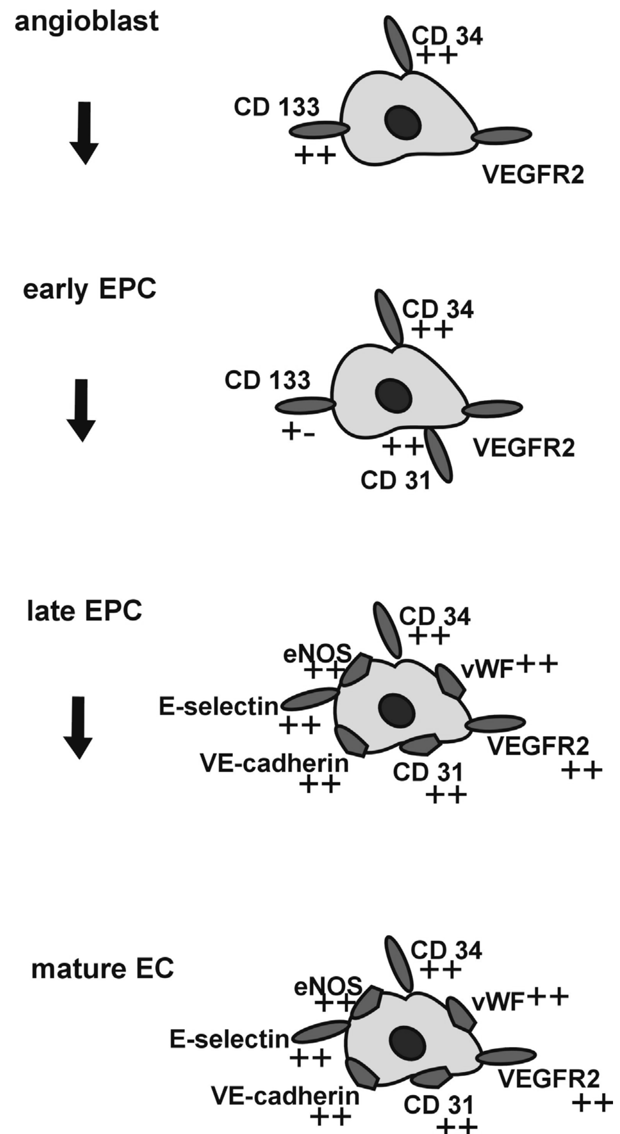

Fig. 4. Hemangioblast origin of EPCs. Proposed schema of angioblast differentiation into mature endothelial cells (ECs). Bone marrow hemangioblasts giving rise to EPCs, designated by Hristov and Weber [23] as $CD133^+$, $CD34^+$, and $VEGFR2^+$ angioblasts [23, 58]. Hristov and Weber proposed the schema of human angioblast differentiation into EPCs throughout the early and late EPC stages. Conversely to the subgroup of late EPCs, early EPCs showed expression of CD133 and CD31 markers and were negative for von Willebrand factor (vWF), vascular endothelial cadherin (VE-cadherin), endothelial selectin (E-selectin), and endothelial nitric oxide synthase (eNOS) expression. Late EPCs still display expression of CD34 marker and reveal no CD133 protein expression. vWF, eNOS, VE-cadherin, E-selectin, CD31, and VEGFR2 proteins are also expressed in mature EPCs. + + and + represent higher and lower protein marker expression, respectively.

THE INVOLVEMENT OF THE ONSET OF VARIOUS DISEASES IN EPC BIOLOGY

Cardiovascular diseases

It has been well proven that dysfunction of endothelium is associated with the course of cardiovascular diseases. Hill et al. [22] reported a strong inverse correlation between PB EPC level measured by colony-forming assay and the risk score of coronary artery disease (CAD) in healthy individuals. The authors found that EPC CFUs were fewer in volunteers with coronary artery risk compared with those without risk factors [22].

Vasa et al. [60] showed that in patients with CAD, the number of EPCs and their migration capacity were impaired. The number of PB-derived $CD34^+/VEGFR2^+$ cells evaluated by flow cytometric assay was found to be reduced by 48% in CAD patients compared with healthy controls. Similarly, $UEA\text{-}I^+$ and $DiLDL^+$ EPCs cultured from CAD patients showed a reduction by 40% and revealed significantly impaired migratory capacity in response to VEGF compared with a control group. The reduction in EPC level and the migratory capacity of these cells inversely correlated with the numbers of risk factors predisposing to CAD [60].

A recent report by Werner et al. [62] concerned the relationship between EPC level and the occurrence of vascular events in patients with CAD. Results obtained

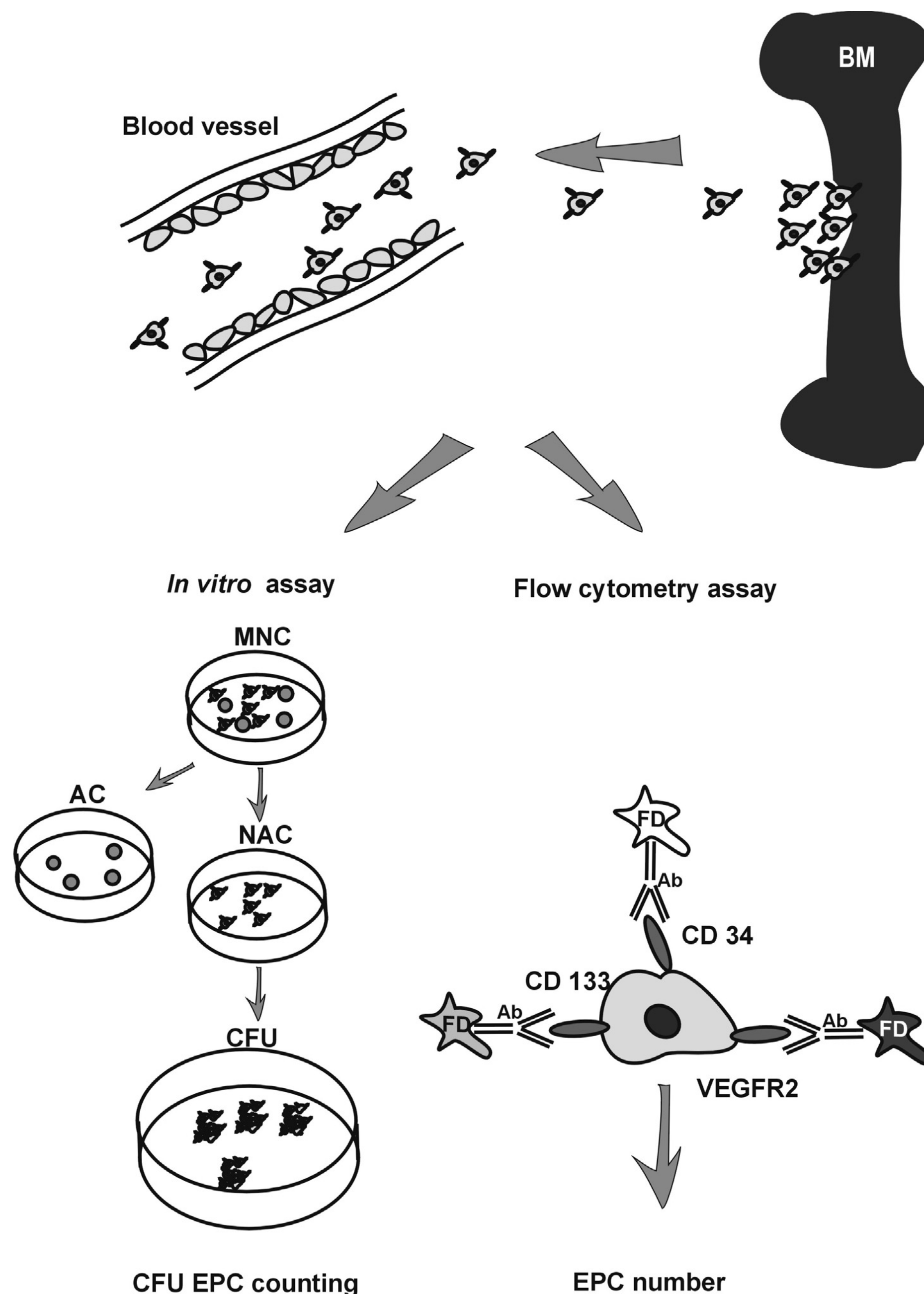

Fig. 5. EPCs can be isolated from bone marrow or peripheral blood. Two main methods are exploited to purify and determine EPC counts in mononuclear cells (MNCs) obtained from bone marrow and peripheral blood. The first technique of EPC evaluation is *ex vivo* cultivation of MNCs or CD34+ cells on fibronectin or gelatin-coated dishes in the presence of specific medium. After 2–3 days of culture, adherent cells (ACs) that contain monocytes, macrophages, and mature endothelial cells are discarded. Non-adherent cells (NACs) containing EPCs are harvested and transferred into fresh medium. Cultured EPCs appear as an adherent type of colony-forming units (CFUs) and are counted using a light microscope. EPC CFUs are usually obtained after 5–7 days of culture and are designated as early EPC CFUs, whereas those CFUs formed after 1–4 weeks of culture are designated as late. The second method of EPC evaluation in PB is flow cytometry analysis with an array of antibodies (Ab) directed against EPC markers and conjugated with fluorescent dyes (FD).

in this study clearly indicated that increased levels of $CD34^{+}$/$VEGFR2^{+}$ EPCs and EPC CFUs were accompanied by decreasing risk of death from cardiovascular cause, revascularization, hospitalization, and the occurrence of a first major cardiovascular event. These findings suggest that the level of PB EPCs may serve as an indicator of cardiovascular outcome in patients with CAD [62].

Shintani et al. [52] found significantly increased numbers of circulating $CD34^{+}$ cells in patients with AMI compared with control subjects. The highest count of $CD34^{+}$ cells measured by flow cytometric assay was observed

seven days after the onset of an acute ischemic event of the heart. The authors also found that EPCs cultivated from MNCs obtained at day 7 of the experiment were more numerous than EPCs derived from MNC probes obtained at day 1 from AMI patients. Conversely, no differences in cultured EPC count between days 1 and 7 were found in controls. These studies showed that the onset of human AMI is associated with BM mobilization and gradual differentiation of PB $CD34^+$ cells to EPCs [52].

Diabetes mellitus

Endothelial cell dysfunction associated with reduced reparative neovascularization is also a characteristic feature of the permanent hyperglycemia in diabetes mellitus [40]. The influence of glucose itself on the reduction of the function of EPCs was investigated by Kränkel et al. [32]. MNCs from healthy donors were cultivated with a glucose concentration resembling the condition observed in diabetic patients. Decreased count, increased apoptosis rate, and impaired migratory and integrative capacities of EPCs were observed after culture under hyperglycemic conditions compared with normal-condition controls [32]. The study performed by Pistrosch et al. [44] on ten patients with type 2 diabetes revealed a decreased migratory ability of EPCs in the examined group. However, the number of EPCs did not differ between patients and healthy controls. These researchers, after flow cytometry evaluation of EPC baseline counts, treated diabetes patients with rosiglitazone. After 12 weeks of rosiglitazone treatment, both migratory ability and counts of EPCs were ameliorated in the type 2 diabetic patients. Rosiglitazone activation of PPARγ receptor eventually improved endothelial cell function in type 2 diabetic patients [45]. Similar results were also obtained with respect to type 1 diabetes patients. Loomans's group observed 44% lower counts of cultured EPCs and impaired *in vitro* angiogenic activity of EPCs isolated from type 1 diabetic patients compared with controls [40]. They proposed that EPCs secreted angiogenic factors to activate mature endothelial cells. They suggested that the dysfunction of diabetic EPCs may be a consequence of secreted putative factors functioning as angiogenesis inhibitors *in vitro* [40].

Rheumatoid arthritis

RA is a disease accompanied by a risk of cardiovascular disorder. Compared with control subjects, reduced counts of EPCs and their migration impairment were documented in patients with RA [21]. Grisar et al. [19] reported that in the active stage of RA, flow cytometry-determined EPC counts in PB were significantly decreased compared with healthy controls. In this study, EPCs were defined as $CD34^+$, $CD133^+$, and $VEGFR2^+$ PB cells [19].

Transplant vasculopathy

In renal transplant recipients, EPC counts correlated significantly with graft function, and in stable renal graft recipients, EPC counts resembled those observed in healthy controls [11].

Atherosclerotic plaque formation within the graft of a cardiac transplant is associated with the chronic form of vascular rejection. Simper et al. [54] completed the quantification of both EPCs and CECs in cardiac transplantation patients. These subjects differed in angiographic evidence of vasculopathy, defined as >20% stenosis in a main, branch epicardial, or intramyocardial coronary artery [54]. They demonstrated that recipient-derived endothelial cells were present in areas of transplant atherosclerotic plaque and in coronary arteries of patients with transplant vasculopathy. Additionally, an *in vitro* assay revealed that EPC colonies from cardiac transplantation subjects with transplant vasculopathy were significantly reduced compared with those cultured from cardiac transplantation patients without angiographic evidence of vasculopathy. No such differentiation in these patients was observed with PB CEC count [54]. This suggests that BM EPC mobilization has a significant function in the development of coronary vasculopathy and the successful function of vascular grafts.

Physical exercise

A decrease in the blood supply to a bodily organ or tissue, caused by constriction or obstruction of the blood vessels, is a common cause of ischemia. This process is probably responsible for the use of EPCs in postnatal vascular growth and remodeling. In the study performed by Adams et al. [1], patients with stable CAD were subjected to the single-exercise stress test to compare PB EPC counts before and after the experiment. It was found that the PB EPC count was increased significantly in ischemic patients within 24–48 h after exercise. The authors observed that an increase in EPC levels was accompanied by an elevation of VEGF plasma concentration in these patients. This result confirmed that VEGF is a significant factor responsible for EPC mobilization from BM to PB [1].

Since the incidence of cardiovascular disorders decreases with physical training, this suggests a possible contribution of EPCs in the reduced development of cardiovascular disorders. Laufs et al. [35] studied the effect of physical training on EPC count in wheel-randomized and non-wheel-randomized mice. The authors observed that the EPC count in the PB of the trained mice was significantly increased compared with the untrained mice [35]. Human subjects undergoing exhaustive dynamic exercise also revealed an increased EPC counts in the PB [35, 46]. Laufs et al. [35] applied three protocols of a running exercise for healthy human volunteers. The experiment proved that, in contrast to a moderate short-term run of 10 min, an intensive and moderate 30-minute run increased the EPC count in the PB of healthy persons. Consistent with the authors' findings, the migratory and *in vitro* colony-forming capacities of EPCs were also elevated after the physical exercise therapy [36].

The effects of exercise and ischemia on the mobilization and functional activation of PB EPCs was examined by Sandri et al. [51]. Three groups encompassing patients with peripheral arterial occlusive disease (PAOD), successfully revascularized patients with PAOD, and patients with CAD compared with three suitable control groups were enrolled in the randomized study. Patients with PAOD took part in ischemic training, successfully revascularized patients with PAOD were enrolled in a non-ischemic training study, and CAD patients underwent a sub-ischemic training option. After the experiment an increased fraction of CD34$^+$/VEGFR2$^+$ cells accompanied by an increased number of MNC-cultured DiLDL$^+$/UEA-I$^+$ cells were observed in the first group of patients compared with the control group. In contrast, in the remaining two groups of patients, changes in EPC level during the experiment were not observed. Nevertheless, the capacity of EPCs for vascular network formation assessed by the Matrigel assay was found to be significantly increased with respect to all three examined groups. The data obtained by the authors suggest that symptomatic ischemia during exercise is related to EPC release and mobilization into the PB in patients with POAD. Moreover, sub-ischemic exercise training performed in successfully revascularized PAOD patients and CAD patients also exerted benefits by improving the capacity of EPC incorporation into the vasculature [51].

FACTORS INFLUENCING EPC BIOLOGY

Estrogen

Imanishi et al. [24, 25] examined the effect of 17-β-estradiol on the senescence of human PB EPCs in culture. The authors demonstrated that 17-β-estradiol dose-dependently inhibited the senescence of cultured EPCs by increasing the catalytic activity of telomerase. Moreover, the mitogenicity of EPCs after 17-β-estradiol treatment was greater compared with the untreated controls [24].

Another research group reported that estrogen accelerated the re-endothelialization process via an increase in EPC mobilization from the BM in mice. These authors suggested that EPC mobilization might be dependent on nitric oxide-mediated signaling. They observed that estradiol treatment had no impact on re-endothelialization or EPC mobilization in eNOS$^{-/-}$ mice [27]. Additionally, the study performed by Strehlow et al. [56] reported that women with increased plasma levels of estrogen, resulting from ovarian hyperstimulation, had increased EPCs compared with controls.

Leptin

Wolk et al. [63] proposed leptin as a factor regulating EPC function. These authors showed that the leptin receptor was expressed in late EPC cultures. Leptin is an adipocyte-derived hormone whose overexpression has been found in obese humans [39]. The authors demonstrated that leptin had no effect on EPC proliferation, but affected EPC migration when present at a high concentration [63].

Statins

Inhibitors of cholesterol biosynthesis, statins, are the next factors which are able to increase EPC counts *in vitro* and in animal models *in vivo* [13]. Statin supplementation increased the number of differentiated EPCs isolated from human MNCs cultured in endothelial basal medium. Similar effects were obtained with respect to human recombinant VEGF treatment alone in this study. To determine the signaling pathway involved in the action of statins, cultured MNCs were transfected with a vector containing a mutant AKT gene encoding inactive enzyme or treated with pharmacological PI3K inhibitors. Overexpression of this mutant AKT form, as well as treatment with PI3K inhibitors, resulted in the hampering of beneficial effects of statins and VEGF on EPCs [13]. These results suggest that the beneficial effects of statin on EPC biology is primarily mediated by the PI3K/AKT pathway.

Erythropoietin

Erythropoietin is a cytokine produced by the kidney and is responsible for erythrocyte differentiation. Erythropoietin production is effected by hypoxia and this cytokine is considered a factor regulating the levels of EPCs [7]. De Groot et al. [12] observed that PB EPC counts were significantly decreased in patients with advanced renal failure. Furthermore, Bahlmann et al. [6, 7] examined patients with advanced renal failure who received human recombinant erythropoietin (rhEPO) for treatment of renal anemia. Treatment of renal anemia patients with rhEPO resulted in a significant release of BM CD34$^+$ cells to the PB. Administration of rhEPO also caused an increase in the number of cultivated EPCs from the MNCs of patients and healthy individuals [6]. It has been suggested that rhEPO acts in EPCs via AKT protein kinase activation. These investigations suggest that rhEPO uses the same EPC signaling pathway as VEGF and that both can be promising agents in the treatment of vascular disorders.

C-reactive protein

Increasing evidence suggests that circulating high-sensitivity CRP contributes to the process of atherogenesis and may serve as a key biomarker of cardiovascular diseases. Verma's research group investigated the influence of CRP on EPC biology [61]. In this study, PB-derived EPCs were cultured in the absence or presence of CRP and treated with rosiglitazone. The authors observed that at a dose-dependent concentration ($\geq$15 μg/ml), CRP led not only to a reduction in EPC counts, but also exhibited an inhibitory effect on the expressions

of endothelial cell markers, such as Tie-2, UEA-I, and VE-cadherin. CRP treatment also caused impaired EPC survival, increased EPC apoptosis, and a reduction of angiogenesis *in vitro*. Administration of CRP also significantly decreased eNOS transcription and destabilized eNOS mRNA in cultured EPCs. However, these negative effects of CRP on cultured EPCs were attenuated by PPARγ agonist treatment, which confirmed the beneficial rosiglitazone effect on the biology of EPCs [61].

EPCs AND TUMOR VASCULARIZATION

Besides the beneficial effects exerted by EPCs under pathological conditions, the possibility of negative EPC properties, particularly tumor-derived neovascularization, should be emphasized (Fig. 3) [23]. Opposite to normal physiological vessels, those derived from tumors are irregularly shaped, often leaky, and form chaotic networks [2, 8].

Asahara et al. [4] generated during BM transplantation experiments two mouse models expressing VEGFR2/β-galactosidase (LacZ) and Tie-2/LacZ fused genes. To induce tumor in these mice models, the researchers injected them with mouse syngeneic colon cancer cells. Fusion transcripts for VEGFR2/LacZ and Tie-2/LacZ were found in the tumor cells. Moreover, histological samples stained for 5-bromo-4-chloro-3--indolyl-beta-D-galactopyranosidase (X-gal) revealed the presence of VEGFR2/LacZ or Tie-2/LacZ fusion proteins in the tumor vasculature [4]. This observation provides evidence for the significance of EPCs in the development of tumor vasculature, which supports the proliferation of malignant cells.

Stoll et al. [55] proposed a mathematical model of the contribution of EPCs to tumor vasculature. According to them, EPCs play an important role in the growth and angiogenesis of tumors at early stages, whereas at late stages, EPCs concentrate in the peripheral areas of the tumor [55].

It has been noted that tumor hypoxia leading to an up-regulation of VEGF expression resulted in the promotion of angiogenesis. Tumor cells are able to secrete VEGF in a paracrine manner to stimulate the proliferation of endothelial cells [8]. Complete understanding of the neovascularization processes in tumor cell growth is also highly relevant in designing new anti-cancer drugs.

EPCs IN THERAPY

Animal experiments

The discovery of EPCs changed the view of the reparative mechanism involved in vascular injury and provided the possibility of exploiting EPCs as a powerful tool for vascular therapy. Asahara et al. [5] used athymic nude mouse and rabbit models of hind-limb ischemia to test $CD34^+$ MNCs for their angiogenic properties *in vivo*. Nude mice after femoral artery excision were injected with human $CD34^+$ MNCs labeled with the fluorescent dye 1,1'-dioctadecyl-3,3,3',3'--tetramethylindocarbocyanine perchlorate (DiL). This experiment revealed that DiL-positive cells were able to be incorporated into blood capillaries after six weeks of the study. This research group also isolated $VEGFR2^+$ MNCs from transgenic mice constitutively overexpressing β-galactosidase (β-GAL) and further injected them into mice with hind-limb ischemia. They found that cells expressing β-GAL were arranged into blood capillaries and small arteries. Injection of rabbits with autologous $CD34^+$ DiL-labeled MNCs resulted in accumulation of DiL cells in the neovascular zone of the ischemic limbs [5].

Kalka et al. [28] injected cultured *in vitro* human EPCs into mice and found these cells ameliorated the neovascularization area of blood vessels. In this experiment, athymic nude mice with impaired angiogenesis after femoral artery excision were injected with *in vitro* expanded DiL-labeled EPCs [28]. The researchers underlined that *in vitro* expanded EPCs were more efficient in improving neovascularization than freshly isolated $CD34^+$ cells.

Recently, Naruse et al. [43] carried out a study related to the therapeutic treatment of diabetic neuropathy by *in vitro* expanded human EPCs. In this experiment, streptozocin-induced diabetic nude rats were injected with human umbilical CB-derived EPCs into hind-limb skeletal muscles. Investigators demonstrated that rats injected with EPCs, compared with controls, developed augmented conduction velocity and ameliorated blood flow of the sciatic nerve. Moreover, an increased number of microvessels were observed on the side of EPC injection. The results of these experiments suggest that culture-expanded EPCs may serve as a potential healing tool for diabetic neuropathy [43]. EPC therapy has also been proposed for cerebrovascular disease treatment. Improvement of neurological function was reported in chronic cerebral ischemic rats injected intracerebrally with PB $CD34^+$ hematopoietic stem cells, including EPCs. Injected cells developed into glial cells, neurons, and vascular endothelial cells in the ischemic brain [53].

To avoid intimal hyperplasia (IH), which is the main cause of graft rejection, Rotmans et al. [49] carried out an experiment involving the application of special polytetrafluoroethylene grafts for hemodialysis. According to the authors, the occurrence of IH is closely related to the lack of a functional endothelial monolayer on the prosthetic polytetrafluoroethylene graft. Therefore they decided to attract $CD34^+$ cells to the surface of the prosthetic grafts covered with an antibody to the CD34 marker. These grafts were implanted between the carotid artery and internal jugular vein of pigs [49]. On day 3 of the experiment, the coverage of grafts by endothelial cells reached 95% compared with the surface of bare grafts. On day 28, bare graft endothelial cell coverage expanded to 32%, whereas antibody-covered grafts were seeded in 85% by endothelial cells.

However, after 28 days of the experiment, significant IH was observed in the grafts covered with antibody compared with the bare grafts [49]. Despite the fact that this experiment did not stop IH development after prosthetic polytetrafluoroethylene graft implantation, the involvement of $CD34^+$ EPCs in the healing of vascular grafts was again proved.

To determine the extent to which EPCs may participate in postnatal vascularization, Murayama et al. [42] performed an experiment on a mouse BM transplantation model. In this study, lethally irradiated mice were transplanted with BM from a mouse consecutively expressing β-GAL under the Tie-2 promoter. Four weeks after BM transplantation, the mice obtained either Matrigel with fibroblast growth factor-2 subcutaneusly or a VEGF pellet into the cornea. X-gal staining and anti-β-GAL antibody reaction elegantly demonstrated the incorporation of about 6–27% BM-derived EPCs into the newly formed vasculature of Matrigel and cornea [42].

In the context of this data, Ingram et al. [26] performed an interesting study indicating EPC existence in the walls of large vessels. Human umbilical vein endothelial cells (HUVECs), human aortic endothelial cells (HAECs), and CB-derived EPCs were cultured in endothelial growth medium. It was demonstrated that all three examined groups of cells shared a similar profile of endothelial-specific marker expression. The single-cell assay showed that about 50% of HUVECs and HAECs, similarly to 55% of CB EPCs, underwent at least one cell division. By replanting clonal progeny derived from single CB EPCs, HUVECs, and HAECs it was proved that HUVECs and HAECs contained highly proliferative potential colony-forming cells allowing the formation of secondary colonies or became confluent. The identification of EPCs in the HUVEC and HAEC monolayer may be an explanation for the different data on the level of *in vivo* incorporation of BM--derived EPCs into the vasculature [26].

On the other hand, the ability of EPCs to expand in culture under *in vitro* conditions raises another tentative prospect for the therapeutic usage of these cells. Genetically modified and *ex vivo* expanded progenitor cells may become new promising agents that will be able to appropriately rescue impaired neovascularization processes under disease conditions. It has recently been demonstrated in the rhesus model that $CD34^+$ cells may serve as vehicles supplying modified genes to the area of angiogenesis. It was observed that $CD34^+$ cell transfection *ex vivo* with recombinant, nonreplicative herpes virus vector and subsequent cell transplantation resulted in the expression of vector genes in angiogenic areas of skin autografts of rhesus macaques. Since $CD34^+$ cells possess a natural angiogenic tropism to injured endothelium, they may serve as ideal candidates for the delivery of genes into areas of angiogenesis [17]. Recently, Arafat et al. [3] showed that $CD34^+$ cells may be efficiently applied to deliver toxin genes into disseminated areas of tumor angiogenesis.

Active glycogen synthase kinase-3β (GSK3β) inhibits the activation of the transcription factor β--catenin, which was found to induce VEGF expression in endothelial cells and promote local angiogenesis *in vivo.* EPC transfection with a vector predominantly expressing a catalytically inactive mutant GSK3β-CI gene increases β-catenin activation, VEGF expression, and EPC survival [10, 29]. Choi et al. [10] transfected cultivated human PB EPCs with a vector containing GSK3β-CI. The engineered cells were then injected into the ischemic hind-limb model of athymic nude mice. The EPC expressing the GSK3β-CI gene localized into ischemic areas and secreted VEGF, resulting in an increase in blood capillary density and improvement of blood flow.

Human studies

The Yamamoto group presented a study of patients with chronic limb ischemia receiving an intramuscular injection of autologous BM-derived MNCs containing 1% $CD34^+$ cells [64]. At the beginning of the experiment and after injection, the expressions of EPCs and endothelial cell markers, such as CD133 and VE-cadherin, were quantitatively evaluated in patients and healthy controls. Before injection, the transcription of these molecules was undetectable in the PB cells of the patients, whereas a steady level of this molecule's mRNA was observed in the healthy individuals. Autologous MNC injection caused an elevation of EPC marker gene transcription in the PB cells of the patients, particularly significantly in a patient with Buerger disease [64]. This suggests that transplantation of bone morrow-derived autologous MNCs increases the EPC marker expression in PB cells and may be used in the therapy of patients with arterial diseases.

To assess the clinical efficacy of autologous EPC application, Lenk et al. [37] performed a study in patients with PAOD and critical limb ischemia. Blood--derived MNCs were cultured for four days in endothelial growth medium and then injected into the superficial femoral artery of each patient. The EPC treatment resulted in a 30-fold increase in pain-free walking distance, a decrease in the subjective pain index, and amelioration of the ankle-brachial pressure index in the examined patients [37].

In line with these data is the study performed by Erbs et al. [14] on patients who had undergone recanalization of chronic coronary total occlusion (CTO). After four days of culture in endothelial growth medium, EPCs were injected through a catheter into the patients' coronary vessels while control placebo patients received serum without EPCs. The treatment with EPCs improved coronary endothelial function and wall motion abnormalities and had a benefit influence on the metabolism in the target area in patients with symptomatic coronary atherosclerosis [7]. An improvement in global left ventricular ejection fraction was noted with respect to patients receiving EPCs. Conversely, in the

control placebo group receiving EPC-free serum, the above parameters remained unchanged. The authors surmised that EPCs are probably involved in the rescue of the hibernating myocardium that often remains after successful recanalization of CTO [14]. The results obtained by the authors in both phase I trial studies raise the promise of a safe and effective therapeutic method based on EPCs in the near future [14, 37].

Despite of growing data relating to the field of EPCs, current knowledge concerning the biology of these cells remains unclear. A recent study provided evidence that adipose tissue is also a source of cells able to differentiate into endothelial phenotype cells [16]. This indicates that EPCs represent a population that is heterogenous in nature and still undefined. EPC count and functionality may be affected by pathological or physiological conditions. These stages are associated with changes in the production and action of various factors, including hormones and growth factors, that determine EPC biology. Finally, numerous drugs are also able to regulate the function of EPCs, suggesting the use of these cells as targets in the therapy of blood vessel disorders and cancer treatment.

Acknowledgment: This work was supported by grant no. 501-01-1124182-08837 from the Poznan University of Medical Sciences. We would like to acknowledge Margarita Lianeri for editorial assistance.

REFERENCES

1. Adams V., Lenk K., Linke A., Lenz D., Erbs S., Sandri M., Tarnok A., Gielen S., Emmrich F., Schuler G. and Hambrecht R. (2004): Increase of circulating endothelial progenitor cells in patients with coronary artery disease after exercise-induced ischemia. Arterioscler. Thromb. Vasc. Biol., **24**, 684–690.
2. Aghi M. and Chiocca E. A. (2005): Contribution of bone marrow-derived cells to blood vessels in ischemic tissues and tumors. Mol. Ther., **12**, 994–1005.
3. Arafat W. O., Casado E., Wang M., Alvarez R. D., Siegal G. P., Glorioso J. C., Curiel D. T. and Gomez-Navarro J. (2000): Genetically modified CD34+ cells exert a cytotoxic bystander effect on human endothelial and cancer cells. Clin. Cancer Res., **6**, 4442–4448.
4. Asahara T., Masuda H., Takahashi T., Kalka C., Pastore C., Silver M., Kearne M., Magner M. and Isner J. M. (1999): Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ. Res., **85**, 221–228.
5. Asahara T., Murohara T., Sullivan A., Silver M., van der Zee R., Li T., Witzenbichler B., Schatteman G. and Isner J. M. (1997): Isolation of putative progenitor endothelial cells for angiogenesis. Science, **275**, 964–967.
6. Bahlmann F. H., De Groot K., Spandau J. M., Landry A. L., Hertel B., Duckert T., Boehm S. M., Menne J., Haller H. and Fliser D. (2004): Erythropoietin regulates endothelial progenitor cells. Blood, **103**, 921–926.
7. Bahlmann F. H., De Groot K., Duckert T., Niemczyk E., Bahlmann E., Boehm S. M., Haller H. and Fliser D. (2003): Endothelial progenitor cell proliferation and differentiation is regulated by erythropoietin. Kidney Int., **64**, 1648–1652.
8. Byrne A. M., Bouchier-Hayes D. J. and Harmey J. H. (2005): Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). J. Cell Mol. Med., **9**, 777–794.
9. Cho H. J., Kim H. S., Lee M. M., Kim D. H., Yang H. J., Hur J., Hwang K. K., Oh S., Choi Y. J., Chae I. H., Oh B. H., Choi Y. S., Walsh K. and Park Y. B. (2003): Mobilized endothelial progenitor cells by granulocyte-macrophage colony-stimulating factor accelerate reendothelialization and reduce vascular inflammation after intravascular radiation. Circulation, **108**, 2918–2925.
10. Choi J. H., Hur J., Yoon C. H., Kim J. H., Lee C. S., Youn S. W., Oh I. Y., Skurk C., Murohara T., Park Y. B., Walsh K. and Kim H. S. (2004): Augmentation of therapeutic angiogenesis using genetically modified human endothelial progenitor cells with altered glycogen synthase kinase-3beta activity. J. Biol. Chem., **279**, 49430–49438.
11. De Groot K., Bahlmann F. H., Bahlmann E., Menne J., Haller H. and Fliser D. (2005): Kidney graft function determines endothelial progenitor cell number in renal transplant recipients. Transplantation, **79**, 941–945.
12. De Groot K., Bahlmann F. H., Sowa J., Koenig J., Menne J., Haller H. and Fliser D. (2004): Uremia causes endothelial progenitor cell deficiency. Kidney Int., **66**, 641–646.
13. Dimmeler S., Aicher A., Vasa M., Mildner-Rihm C., Adler K., Tiemann M., Rutten H., Fichtlscherer S., Martin H. and Zeiher A. M. (2001): HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. J. Clin. Invest., **108**, 391–397.
14. Erbs S., Linke A., Adams V., Lenk K., Thiele H., Diederich K. W., Emmrich F., Kluge R., Kendziorra K., Sabri O., Schuler G. and Hambrecht R. (2005): Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: first randomized and placebo-controlled study. Circ. Res., **97**, 756–762.
15. Fina L., Molgaard H. V., Robertson D., Bradley N. J., Monaghan P., Delia D., Sutherland D. R., Baker M. A. and Greaves M. F. (1990): Expression of the CD34 gene in vascular endothelial cells. Blood, **75**, 2417–2426.
16. Fraser J. K., Schreiber R., Strem B., Zhu M., Alfonso Z., Wulur I. and, Hedrick M. H. (2006): Plasticity of human adipose stem cells toward endothelial cells and cardiomyocytes. Nat. Clin. Pract. Cardiovasc. Med., **3** (suppl. 1), S33–37.
17. Gomez-Navarro J., Contreras J. L., Arafat W., Jiang X. L., Krisky D., Oligino T., Marconi P., Hubbard B., Glorioso J. C., Curiel D. T. and Thomas J. M. (2000): Genetically modified CD34+ cells as cellular vehicles for gene delivery into areas of angiogenesis in a rhesus model. Gene Ther., **7**, 43–52.
18. Goon P. K., Boos C. J., Stonelake P. S., Blann A. D. and Lip G. Y. (2006): Detection and quantification of mature circulating endothelial cells using flow cytometry and immunomagnetic beads: a methodological comparison. Thromb. Haemost., **96**, 45–52.
19. Grisar J., Aletaha D., Steiner C. W., Kapral T., Steiner S., Seidinger D., Weigel G., Schwarzinger I., Wolozcszuk W., Steiner G. and Smolen J. S. (2005): Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. Circulation, **111**, 204–211.
20. Heissig B., Hattori K., Dias S., Friedrich M., Ferris B.,

Hackett N. R., Crystal R. G., Besmer P., Lyden D., Moore M. A., Werb Z. and Rafii S. (2002): Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell, **109**, 625–637.
21. Herbrig K., Haensel S., Oelschlaegel U., Pistrosch F., Foerster S. and Passauer J. (2006): Endothelial dysfunction in patients with rheumatoid arthritis is associated with a reduced number and impaired function of endothelial progenitor cells. Ann. Rheum. Dis., **65**, 157–163.
22. Hill J. M, Zalos G., Halcox J. P., Schenke W. H., Waclawiw M. A., Quyyumi A. A. and Finkel T. (2003): Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N. Engl. J. Med., **348**, 593–600.
23. Hristov M. and Weber C. (2004): Endothelial progenitor cells: characterization, pathophysiology, and possible clinical relevance. J. Cell Mol. Med., **8**, 498–508.
24. Imanishi T., Hano T. and Nishio I. (2005): Estrogen reduces angiotensin II-induced acceleration of senescence in endothelial progenitor cells. Hypertens Res., **28**, 263–271.
25. Imanishi T., Kobayashi K., Hano T. and Nishio I. (2005): Effect of estrogen on differentiation and senescence in endothelial progenitor cells derived from bone marrow in spontaneously hypertensive rats. Hypertens Res., **28**, 763–772.
26. Ingram D. A., Mead L. E., Moore D. B., Woodard W., Fenoglio A. and Yoder M. C. (2005): Vessel wall-derived endothelial cells rapidly proliferate because they contain a complete hierarchy of endothelial progenitor cells. Blood, **105**, 2783–2786.
27. Iwakura A., Luedemann C., Shastry S., Hanley A., Kearney M., Aikawa R., Isner J. M., Asahara T. and Losordo D. W. (2003): Estrogen-mediated, endothelial nitric oxide synthase-dependent mobilization of bone marrow-derived endothelial progenitor cells contributes to reendothelialization after arterial injury. Circulation, **108**, 3115–3121.
28. Kalka C., Masuda H., Takahashi T., Kalka-Moll W. M., Silver M., Kearney M., Li T., Isner J. M. and Asahara T. (2000): Transplantation of *ex vivo* expanded endothelial progenitor cells for therapeutic neovascularization. Proc. Natl. Acad. Sci. USA, **97**, 3422–3427.
29. Kim H. S., Skurk C., Thomas S. R., Bialik A., Suhara T., Kureishi Y., Birnbaum M., Keaney J. F. Jr. and Walsh K. (2002): Regulation of angiogenesis by glycogen synthase kinase-3beta. J. Biol. Chem., **277**, 41888–41896.
30. Komarova N. L. and Mironov V. (2005): On the role of endothelial progenitor cells in tumor neovascularization. J. Theor. Biol., **235**, 338–349.
31. Kondo T., Hayashi M., Takeshita K., Numaguchi Y., Kobayashi K., Iino S., Inden Y. and Murohara T. (2004): Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. Arterioscler. Thromb. Vasc. Biol., **24**, 1442–1447.
32. Kränkel N., Adams V., Linke A., Gielen S., Erbs S., Lenk K., Schuler G. and Hambrecht R. (2005): Hyperglycemia reduces survival and impairs function of circulating blood--derived progenitor cells. Arterioscler. Thromb. Vasc. Biol., **25**, 698–703.
33. Kucia M., Jankowski K., Reca R., Wysoczynski M., Bandura L., Allendorf D.J., Zhang J., Ratajczak J. and Ratajczak M. Z. (2004): CXCR4-SDF-1 signalling, locomotion, chemotaxis and adhesion. J. Mol. Histol., **35**, 233–245.
34. Lataillade J. J., Clay D., Dupuy C., Rigal S., Jasmin C., Bourin P. and Le Bousse-Kerdiles M. C. (2000): Chemokine SDF-1 enhances circulating CD34(+) cell proliferation in synergy with cytokines: possible role in progenitor survival. Blood, **95**, 756–768.
35. Laufs U., Urhausen A., Werner N., Scharhag J., Heitz A., Kissner G., Bohm M., Kindermann W. and Nickenig G. (2005): Running exercise of different duration and intensity: effect on endothelial progenitor cells in healthy subjects. Eur. J. Cardiovasc. Prev. Rehabil., **12**, 407–414.
36. Laufs U., Werner N., Link A., Endres M., Wassmann S., Jurgens K., Miche E., Bohm M. and Nickenig G. (2004): Physical training increases endothelial progenitor cells, inhibits neointima formation, and enhances angiogenesis. Circulation, **109**, 220–226.
37. Lenk K., Adams V., Lurz P., Erbs S., Linke A., Gielen S., Schmidt A., Scheinert D., Biamino G., Emmrich F., Schuler G. and Hambrecht R. (2005): Therapeutical potential of blood-derived progenitor cells in patients with peripheral arterial occlusive disease and critical limb ischaemia. Eur. Heart J., **26**,1903–1909.
38. Liekens S., De Clercq E. and Neyts J. (2001): Angiogenesis: regulators and clinical applications. Biochem. Pharmacol., **61**, 253–270.
39. Lonnqvist F., Arner P., Nordfors L. and Schalling M. (1995): Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat. Med., **1**, 950–953.
40. Loomans C. J., de Koning E. J., Staal F. J., Rookmaaker M. B., Verseyden C., de Boer H. C., Verhaar M. C., Braam B., Rabelink T. J. and van Zonneveld A. J. (2004): Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes, **53**, 195–199.
41. Muller A., Homey B., Soto H., Ge N., Catron D., Buchanan M. E., McClanahan T., Murphy E., Yuan W., Wagner S. N., Barrera J. L., Mohar A., Verastegui E. and Zlotnik A. (2001): Involvement of chemokine receptors in breast cancer metastasis. Nature, **410**, 50–56.
42. Murayama T., Tepper O. M., Silver M., Ma H., Losordo D. W., Isner J. M., Asahara T. and Kalka C. (2002): Determination of bone marrow-derived endothelial progenitor cell significance in angiogenic growth factor-induced neovascularization *in vivo*. Exp. Hematol., **30**, 967–972.
43. Naruse K., Hamada Y., Nakashima E., Kato K., Mizubayashi R., Kamiya H., Yuzawa Y., Matsuo S., Murohara T., Matsubara T., Oiso Y. and Nakamura J. (2005): Therapeutic neovascularization using cord blood--derived endothelial progenitor cells for diabetic neuropathy. Diabetes, **54**, 1823–1828.
44. Pistrosch F., Herbrig K., Oelschlaegel U., Richter S., Passauer J., Fischer S. and Gross P. (2005): PPARgamma--agonist rosiglitazone increases number and migratory activity of cultured endothelial progenitor cells. Atherosclerosis, **183**, 163–167.
45. Pistrosch F., Passauer J., Fischer S., Fuecker K., Hanefeld M. and Gross P. (2004): In type 2 diabetes, rosiglitazone therapy for insulin resistance ameliorates endothelial dysfunction independent of glucose control. Diabetes Care, **27**, 484–490.
46. Rehman J., Li J., Parvathaneni L., Karlsson G., Panchal V. R., Temm C. J., Mahenthiran J. and March K. L. (2004): Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells. J. Am. Coll. Cardiol., **43**, 2314–2318.

47. Rehman J., Li J., Orschell C. M. and March K. L. (2003): Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation, **107**, 1164–1169.
48. Risau W. (1997): Mechanisms of angiogenesis. Nature, **386**, 671–674.
49. Rotmans J. I., Heyligers J. M., Verhagen H. J., Velema E., Nagtegaal M. M., de Kleijn D. P., de Groot F. G., Stroes E. S. and Pasterkamp G. (2005): *In vivo* cell seeding with anti-CD34 antibodies successfully accelerates endothelialization but stimulates intimal hyperplasia in porcine arteriovenous expanded polytetrafluoroethylene grafts. Circulation, **112**, 12–18.
50. Rumpold H., Wolf D., Koeck R. and Gunsilius E. (2004): Endothelial progenitor cells: a source for therapeutic vasculogenesis? J. Cell Mol. Med., **8**, 509–518.
51. Sandri M., Adams V., Gielen S., Linke A., Lenk K., Krankel N., Lenz D., Erbs S., Scheinert D., Mohr F. W., Schuler G. and Hambrecht R. (2005): Effects of exercise and ischemia on mobilization and functional activation of blood-derived progenitor cells in patients with ischemic syndromes: results of 3 randomized studies. Circulation, **111**, 3391–3399.
52. Shintani S., Murohara T., Ikeda H., Ueno T., Honma T., Katoh A., Sasaki K., Shimada T., Oike Y. and Imaizumi T. (2001): Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation, **103**, 2776–2779.
53. Shyu W. C., Lin S. Z., Chiang M. F., Su C. Y. and Li H. (2006): Intracerebral peripheral blood stem cell (CD34+) implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. J. Neurosci., **26**, 3444–3453.
54. Simper D., Wang S., Deb A., Holmes D., McGregor C., Frantz R., Kushwaha S. S. and Caplice N. M. (2003): Endothelial progenitor cells are decreased in blood of cardiac allograft patients with vasculopathy and endothelial cells of noncardiac origin are enriched in transplant atherosclerosis. Circulation, **108**, 143–149.
55. Stoll B. R., Migliorini C., Kadambi A., Munn L. L. and Jain R. K. (2003): A mathematical model of the contribution of endothelial progenitor cells to angiogenesis in tumors: implications for antiangiogenic therapy. Blood, **102**, 2555–2561.
56. Strehlow K., Werner N., Berweiler J., Link A., Dirnagl U., Priller J., Laufs K., Ghaeni L., Milosevic M., Bohm M. and Nickenig G. (2003): Estrogen increases bone marrow-derived endothelial progenitor cell production and diminishes neointima formation. Circulation, **107**, 3059–3065.
57. Tepper O. M., Galiano R. D., Capla J. M., Kalka C., Gagne P. J., Jacobowitz G. R., Levine J. P. and Gurtner G. C. (2002): Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation, **106**, 2781–2786.
58. Urbich C. and Dimmeler S. (2004): Endothelial progenitor cells: characterization and role in vascular biology. Circ. Res., **95**, 343–353.
59. Urbich C., Heeschen C., Aicher A., Dernbach E., Zeiher A. M. and Dimmeler S. (2003): Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. Circulation, **108**, 2511–2516.
60. Vasa M., Fichtlscherer S., Aicher A., Adler K., Urbich C., Martin H., Zeiher A. M. and Dimmeler S. (2001): Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ. Res., **89**, E1–7.
61. Verma S., Kuliszewski M. A., Li S. H., Szmitko P. E., Zucco L., Wang C. H., Badiwala M. V., Mickle D. A., Weisel R. D., Fedak P. W., Stewart D. J. and Kutryk M. J. (2004): C-reactive protein attenuates endothelial progenitor cell survival, differentiation, and function: further evidence of a mechanistic link between C-reactive protein and cardiovascular disease. Circulation, **109**, 2058–2067.
62. Werner N., Kosiol S., Schiegl T., Ahlers P., Walenta K., Link A., Böhm M. and Nickenig G. (2005): Circulating endothelial progenitor cells and cardiovascular outcomes. N. Engl. J. Med., **353**, 999–1007.
63. Wolk R., Deb A., Caplice N. M. and Somers V. K. (2005): Leptin receptor and functional effects of leptin in human endothelial progenitor cells. Atherosclerosis, **183**, 131–139.
64. Yamamoto K., Kondo T., Suzuki S., Izawa H., Kobayashi M., Emi N., Komori K., Naoe T., Takamatsu J. and Murohara T. (2004): Molecular evaluation of endothelial progenitor cells in patients with ischemic limbs: therapeutic effect by stem cell transplantation. Arterioscler. Thromb. Vasc. Biol., **24**, e192–196.
65. Yin A. H., Miraglia S, Zanjani E. D., Almeida-Porada G., Ogawa M., Leary A. G., Olweus J., Kearney J. and Buck D. W. (1997): AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood, **90**, 5002–5012.
66. Yu Y., Flint A., Dvorin E. L and Bischoff J. (2002): AC133-2, a novel isoform of human AC133 stem cell antigen. J. Biol. Chem., **277**, 20711–20716.