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FORUM REVIEW ARTICLE

Endothelial Progenitor Cells and Exercise-Induced Redox Regulation

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Abstract

Endothelial progenitor cells (EPCs) are thought to participate in endothelial cell regeneration and neovascularization in either a direct or an indirect way. The number of circulating EPCs is influenced by many factors like disease status, medication, age, and fitness level and is an independent predictor of disease progression and cardiovascular events. Experimental as well as clinical studies during the last 10 years clearly demonstrated that physical exercise training has a beneficial effect on endothelial function, which is a clear predictive value for cardiovascular mortality. Over the last years mainly clinical studies provided solid evidence for an exercise training induced mobilization of EPCs from the bone marrow, thereby possibly influencing the regeneration of the endothelial cell layer. This review will discuss the mechanisms how exercise induces mobilization of EPCs from the bone marrow with a focus on the influence on the redox balance. *Antioxid. Redox Signal.* 15, 997–1011.

Introduction

Since the first description of endothelial progenitor cells (EPCs) by Asahara and coworkers in 1997 (9), a huge amount of literature has been published on EPCs in cardiovascular (CV) diseases. Adult bone marrow (BM) (9) as well as other organs (6) are reservoirs of tissue-specific stem and progenitor cells. Among these, a scarce population of cells described as EPCs can be mobilized by various stimuli, including exercise training into the circulation to contribute to the neoangiogenic process or the repair of a damaged endothelial cell layer. Circulating levels of EPCs independently predict CV disease progression and exhibit a negative correlation with the Framingham risk factor score, CV events, and endothelial function (38, 93, 121). There has been no systematic study of the number of EPCs present in healthy individuals. However, several investigations have been reported showing that the number and function of EPCs decline with age (39), physical inactivity (107), and the occurrence of several diseases like diabetes (106) and chronic kidney disease (55). Before we go into the discussion of how exercise training by regulating the redox balance leads to a mobilization of EPCs, we would like to give a short introduction into the cell type EPCs and how we can detect this specific cell type with high accuracy.

EPCs: Definition and Detection

Even 13 years after the first description of EPCs (9) we have not a clear and definitive marker set to characterize and to quantify circulating EPCs with high accuracy. There is an ongoing debate whether these cells represent a structurally and functionally homogeneous population (43). Although the BM has been shown to be a principal source of EPC, it is probably not the only one, as stem/progenitor cells with endothelial cell-like properties have been isolated from different tissues, including peripheral blood, adipose, and cardiac muscle tissue (112). So, we are still trying to answer the question about the phenotype of a true EPC. This point makes it really difficult to standardize and compare the quantification of EPCs among different published studies. Another critical point in the definition of the cells involved in repairing damaged endothelium is the nomenclature for the cells used in different studies. A summary of the different names is given in Table 1. Some names are based purely on cell surface markers, whereas other names deviated from properties observed in cell culture.

Nevertheless, the most often used techniques for evaluating EPCs are flow cytometry and the quantification of colony forming units after cell culture.

Flow cytometry

The beauty of this methodology is that it can characterize cells at the single-cell level with the use of antibodies directed against specific cell surface markers and that a quantitative analysis of cells in solution can be rapidly achieved. In most cases a combination of a hematopoietic stem cell marker, like CD34, CD117 (cKit), or CD133, with a marker for endothelial cells, like vascular endothelial growth factor (VEGF) receptor 2 (KDR) or Ve-cadherin, is used to identify EPCs [summarized in (1, 40, 111)]. In general, the best approach to characterize and

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Table 1. Subtypes of Putative Endothelial Progenitor Cells, and Angiogenic and Vasculogenic Cells

Acronym	*Meaning*	*Comment*
EPC (9)	Endothelial progenitor cells	Population of rare cells that circulate in the blood with the ability to differentiate into endothelial cells. A pool of mixed cells.
CFU-ECs (123)	Colony-forming unit endothelial cells	CFU-ECs, derived from the hematopoietic system, with no ability to form secondary endothelial colonies *in vitro* or new vessels *in vivo*.
CACs (85)	Circulating angiogenic cells	In distinction from circulating EPCs culturing of mononuclear cells (MNCs) on fibronectin yields CACs. This cell population has been recently redefined to distinguish the cells from EPCs, as the latter do not appear to express monocyte/macrophage markers.
CEPs (25)	Circulating endothelial precursors	Has to be regarded as the equivalent to EPCs, based on cell surface marker expression.
ECFC (44)	Endothelial colony-forming cells	ECFCs belong to the endothelial lineage, which are also referred to as blood outgrowth endothelial cells (BOECs) (62).
LPP-ECFC (44)	Low proliferative potential ECFC	A unique population of LPP-ECFC in human cord blood that can achieve 20–30 population doublings, but they do not form secondary colonies on replating.
HPP-ECFC (44)	High proliferative potential ECFC	A unique population of HPP-ECFC in human cord blood that can achieve at least 100 population doublings, replate into at least secondary and tertiary colonies, and retain high levels of telomerase activity.
Early outgrowth EPC (38)	Early outgrowth endothelial progenitor cells	Plating of MNCs into endothelial-specific medium, and these cells arise after 5–7 days in culture.
Late outgrowth EPC (38)	Late outgrowth endothelial progenitor cells	Plating of MNCs into endothelial-specific medium, and these cells arise after 14 days in culture.

quantify EPCs would be to include as many markers as possible. Nevertheless, this is limited by the low abundance of EPCs in the peripheral blood, which represent between 0.01% and 0.0001% of mononuclear cells depending on several factors like age, state of health of the individual, as well as medication.

Another factor complicating the characterization of EPCs is that the expression of the often used stem cell marker CD34 is not only restricted to stem cells, but is also expressed on mature microvascular endothelial cells (21, 91). Therefore, CD133, a more immature hematopoietic stem cell marker that is absent on mature endothelial cells and monocytic cells (32), was introduced as a better marker for EPCs. It is postulated that $CD133^{pos}/KDR^{pos}$ cells more likely reflect immature progenitor cells, whereas $CD34^{pos}/KDR^{pos}$ may also represent shedded cells of the vessel wall. In addition, it is suggested by several authors that more immature EPCs in the BM are $CD133^{pos}/CD34^{pos}/KDR^{pos}/Ve\text{-}cadherin^{neg}$, whereas circulating EPCs lose the early marker CD133 and start to express more endothelial cell marker [review in (40)]. Therefore, they are defined as $CD133^{neg}/CD34^{pos}/KDR^{pos}/Ve\text{-}cadherin^{pos}$. In conclusion, the proper identification of EPCs by flow cytometry is still uncertain, but for practical reasons the definition $CD34^{pos}/KDR^{pos}$ is most often used.

Cell culture

Classical isolation methods include adherence culture of total peripheral mononuclear cells (9) and the use of magnetic beads coated with anti-CD34 (9), anti-CD133 (82), or anti-CD14 (96, 113, 124) antibodies. After the isolation, the cells are plated on cell culture dishes coated either with fibronectin (17, 106, 116), collagen I (62), or gelatin (2, 89, 98) and cultured in cell culture media ranging from EBM-2 (46, 85, 117), medium-199 (38, 76, 98), to x-vivo-20 (25) as basal media and 20% fetal calf serum (9, 76), brain extract (9, 76), to commercial available single quots of VEGF, insulin-like growth factor, and basic fibroblast growth factor (FGF) (46, 85, 116) as supplements. An identification criterion often used for EPCs in the culture of mononuclear cells for 4 or 7 days is the double-positive staining of EPCs for endothelial-specific lectin (*e.g.*, Ulex europaeus agglutini-1) and Dil-labeled acetylated low-density lipoproteins (LDL) (51, 76, 87). Early EPCs appear within 4–7 days of culture, are spindle-shaped, and express both endothelial (von Willebrand factor) and monocytic (CD14) markers (41). Late EPCs develop after 2–3 weeks of culture and have the characteristics of endothelial lineage-like cobblestone pattern (2, 9) and the expression of endothelial nitric oxide synthase (eNOS) (2).

Another approach to culture EPCs is a preplating procedure. This procedure claims to avoid contamination for early adherent cells such as differentiated monocytic or possible mature endothelial cells (97). After replating nonattached cells onto fibronectin in endothelial-specific medium, typical clusters of round cells centrally with spindle-shaped cells sprouting at the periphery appear. Some, but not all, of the cells inside the colony-forming unit are indeed double positive for acetylated LDL and lectin staining (38). For quantitative analysis the number of colonies finally appearing on the

culture plate are counted (colony forming units assay) (38). Nevertheless, one has to be aware that also the colonies formed by this procedure have to be considered as a mixed population of cells originating mainly from the lymphocyte/monocyte lineage (88, 114).

Mobilization of EPCs from the BM

The microenvironment of the BM, consisting of macrophages, fibroblasts, and endothelial cells, is very important for the mobilization and differentiation of stem/progenitor cells (57). When the body is developing new blood vessels (such as during the developmental stage), or affected by injury, EPCs are activated and migrate into the vascular zone of the BM where proliferation is increased. Various injuries such as ischemia, atherosclerotic lesions, traumatic wound, tumor angiogenesis (25, 37, 113), and myocardial infarction (54) cause the frequency of EPCs in the peripheral blood to increase up to 50-fold (25). At least in ischemia, this mechanism appears to be mediated by the hypoxia-inducible factor 1α (HIF-1α)–mediated upregulation of VEGF in the periphery, which plays a crucial role in vasculogenesis, angiogenesis, and EPC kinetics (75). Other molecules that have been shown to facilitate EPC mobilization from the BM to the periphery are angiopoietin-1, FGF1, stromal cell-derived factor-1 (SDF-1) (100), granulocyte colony-stimulating factor (CSF) (81), granulocyte-macrophage CSF (15), hydroxymethylglutaryl–coenzyme A synthase inhibitor (19, 66, 75), erythropoietin (EPO) (7), peroxisome proliferator-activated receptor-gamma agonists (119), 17β-Estradiol (E2) together with FGF2 (22), and reactive oxygen species (ROS) generated by nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) and Rac1 (74).

The principal mechanism of EPC mobilization from the BM seems to depend on the activation of eNOS in the presence of several mobilizing factors such as VEGF (10), or placental growth factor (61). Gene targeting studies using either matrix metalloproteinase-2 (MMP-2) (14) or MMP-9 (42) knockout mice demonstrated that the presence of MMPs are crucial in ischemia-induced mobilization of EPCs and hence neovascularization. At the molecular/cellular level the following scenario for the mobilization is proposed (Fig. 1): Under steady state conditions EPCs reside in a niche of the BM, bound to stroma cells *via* adhesion molecules such as VCAM/VLA4 (57). Signal induced upregulation of MMP-9 results in the conversion of KitL from a membrane-bound molecule to a soluble survival (soluble Kit-ligand), conferring signals that enhances mobility of EPCs into a vascular-enriched niche favoring liberalization of the cells into the circulation (36).

Another model (50), based on the observation that stem cell release from the BM is regulated by circadian oscillations (13, 72), and the fact that a chemokine gradient of SDF-1 between the BM and the peripheral circulation plays a key role for stem cell mobilization (60), proposes the following mechanism: Activation of peripheral neurons and a subsequent release of norepinephrine leads to osteoblast suppression, thereby reducing the synthesis of SDF-1 in the BM. This generates a greater SDF-1 gradient between the BM and the circulation, thereby mobilizing the progenitor cells into the circulation (Fig. 2).

Endothelial Dysfunction

Impairment of endothelium-dependent vasomotion not only precedes the development of significant coronary artery stenosis, but has also been identified as a general phenomenon predicting future CV events in patients with CV risk factors (59, 92). There are several conditions that have been associated with endothelial dysfunction: (i) the presence of coronary risk factors like hypertension, hypercholesterolemia,

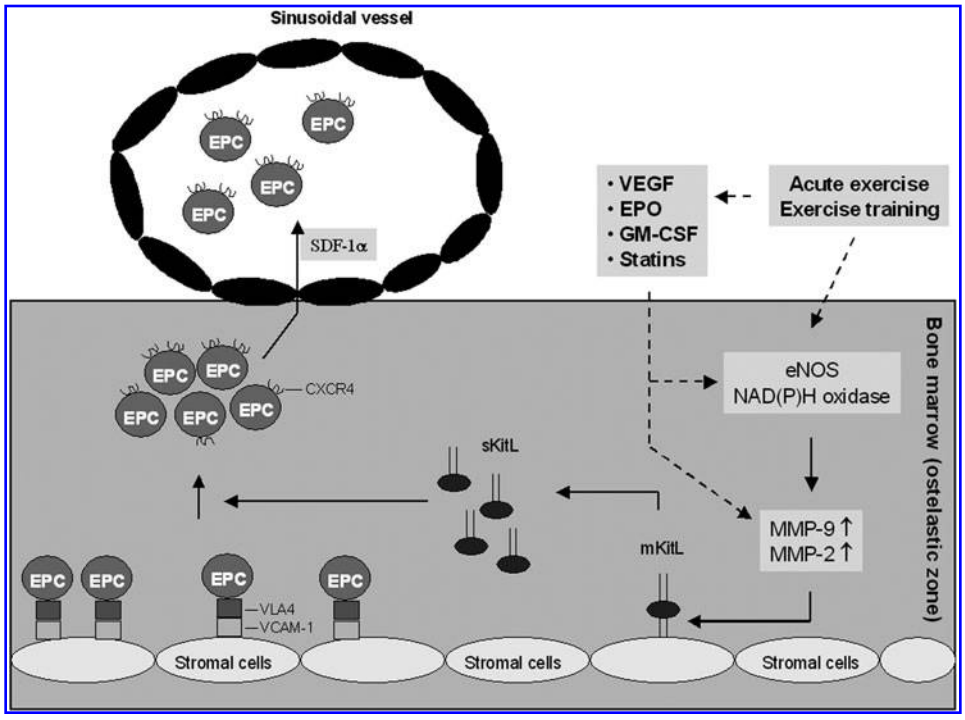

FIG. 1. A model for mobilization of circulating endothelial progenitor cells (EPCs) from the bone marrow by increased shear stress induced by exercise or by several different soluble molecules. Activation matrix metalloproteinases (MMP-9 and MMP-2) either directly or *via* the activation of NAD(P)H oxidase or endothelial nitric oxide synthase (eNOS) results in the release of soluble Kit-ligand (sKitL). SkitL confers signals enhancing mobility of EPCs. Along an SDF-1 gradient the EPCs are mobilized into the peripheral circulation. GM-CSF, granulocyte-macrophage colony-stimulating factor; NADPH, nicotinamide adenine dinucleotide phosphate. SDF-1, stromal cell-derived factor-1.

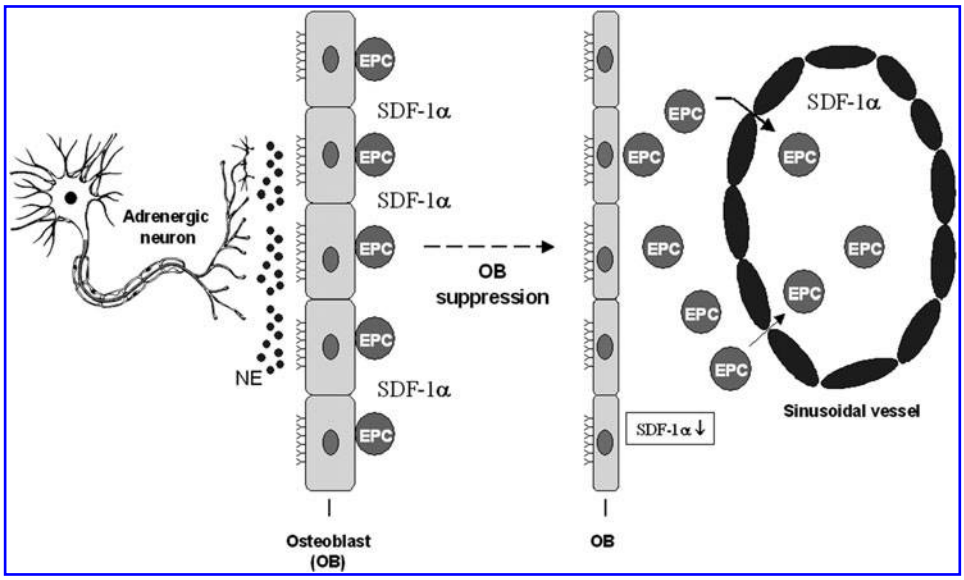

FIG. 2. Model describing the release of EPCs regulated by adrenergic neurons as described by Katayama and colleagues (50). After the activation of the adrenergic neuron by soluble factors like granulocyte CSF, norepinephrine (NE), and other yet unidentified factors mediate osteoblast (OB) suppression. This generates a greater SDF-1 gradient between the bone marrow and the circulation, thereby mobilizing the EPCs into the sinusoidal vessels.

diabetes mellitus, overweight, obesity, and cigarette smoking; (ii) a significant coronary artery disease (CAD), (iii) the presence of congestive heart failure (CHF), and (iv) the presence of inflammation and advanced age. More important, endothelial dysfunction can occur years before a makroangiopathy, for example, CAD, becomes evident. Therefore, endothelial dysfunction has been identified as an important treatment target.

Endothelial dysfunction and EPCs

As mentioned above, the maintenance of an intact and functional competent endothelial cell layer is very important for preventing CAD. Since mature endothelial cells possess only a limited regenerative capacity (20), there is growing interest in EPCs, especially in their role for the maintenance of endothelial integrity. Insufficient numbers of EPCs are related to endothelial dysfunction and adverse clinical events, suggesting that endothelial injury in the absence of sufficient repair by circulating progenitor cells promotes the development of vascular disease. But what is exactly the role of EPCs for the repair of a damaged endothelial cell layer? Currently, it is believed that mature endothelial cell and EPCs contribute to the rejuvenation of the endothelium (9, 97). It is thought that EPCs are attracted to the damaged endothelium by specific cell surface markers and after attaching to the neighboring endothelial cells fill in the gap and differentiate into a mature functional endothelial cell (90). This concept was supported by studies using transplantation of *ex vivo* expanded and labeled EPCs in a hindlimb mouse model (49). This first study clearly demonstrated an improved angiogenesis after cell transplantation, and an incorporation of the cells into the endothelial cell layer. Nevertheless, this concept of a direct involvement in endothelium repair by filling in the gap has been challenged in recent years (78). First hints for an indirect involvement of EPCs in endothelial regeneration came from studies demonstrating that EPCs secrete various cytokines such as SDF-1, VEGF and other factors enhancing the performance of mature endothelial cells in migration assays (110). Further, coculture experiments with endothelial cells and fibroblasts demonstrated that they enhanced tubulogenesis in a paracrine manner (99). In addition to this cell culture experiments, animal experiments using chimeric mice also showed that circulating EPCs do not participate directly in endothelial homeostasis and thus do not repair chronic, systemic endothelial dysfunction in young mice (78). Therefore, it is speculated at the moment that EPCs play an important role in regeneration and vasculogenesis either as cellular placeholder or as cytokine factories stimulating mature endothelial cells to proliferate and fill the gap in the damaged endothelial cell layer (Fig. 3).

Endothelial Dysfunction and Exercise

As mentioned above the maintenance of an intact endothelial cell layer is of great importance. In the last decade it became evident by several animal and clinical studies that exercise training is able to improve endothelial function in patients exhibiting an endothelial dysfunction [reviewed in Refs. (33, 64, 104)].

More than a decade ago it was observed that exercise training increases endothelium-dependent vasodilation in dog epicardial arteries (120). This observation was confirmed many times, and accumulating evidence suggest that exercise-induced activation of the NO/cGMP pathway is an important mechanism mediating beneficial vascular effects of exercise training. Numerous clinical studies in primary and secondary prevention of CV disease clearly revealed that mild to moderate exercise training in patients with endothelial dysfunction has the potential to correct endothelial dysfunction.

Hambrecht and colleagues were the first who prospectively assessed the effect of 4 weeks of exercise training on coronary vasomotion in patients with CAD (31). At 4 weeks follow up, acetylcholine-induced coronary vasoconstriction had declined and adenosine mediated flow reserve improved. Similar effects were seen in patients with CHF in response to an aerobic exercise training program (30). Besides systemic aerobic exercise training, also a 8-weeks resistance training of the forearm (68) and a lower limb exercise program (65) was able to improve local as well as systemic endothelial function in patients with CHF.

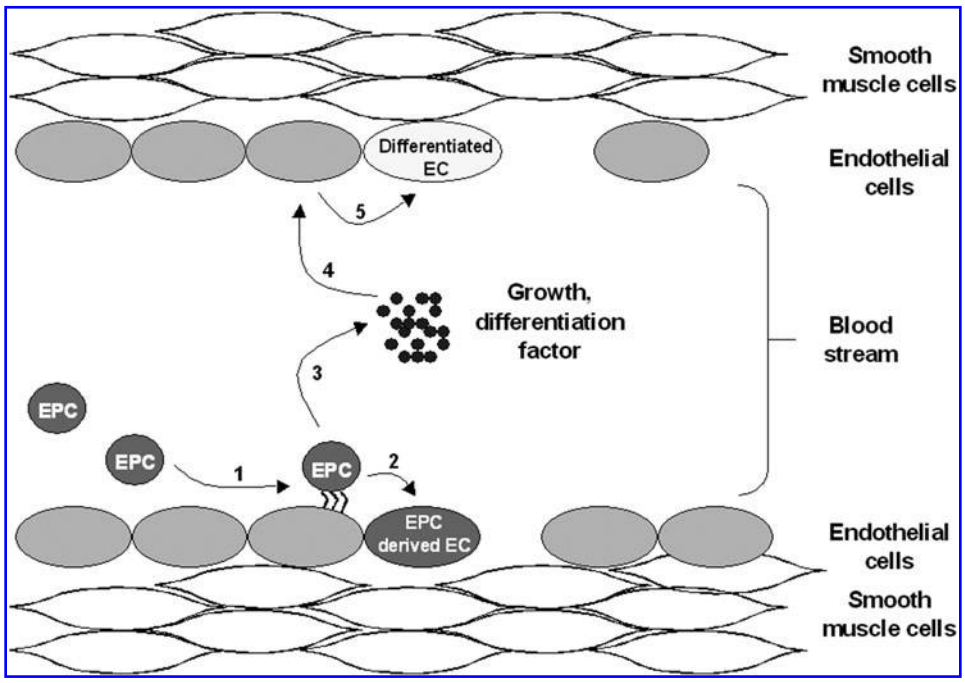

FIG. 3. Potential role of EPCs in maintaining an intact endothelial cell layer. EPCs bind to mature endothelial cells (EC) *via* specific cell surface marker (1). After binding to EC the EPC may have to possible pathways to repair the damage in the endothelial cell layer. First, it fills in the gap and differentiates into a mature endothelial cell (2) or second it secretes growth differentiation factors (3), which in turn stimulate mature endothelial cells (4) to proliferate and thereby closing the gap in the endothelial cell layer (5).

What are the molecular mechanisms behind these improvements in endothelial dysfunction? Experimental studies in animals and cell culture suggest that repetitive increases in shear stress enhance eNOS expression and eNOS phosphorylation, thereby contributing to an augmented nitric oxide (NO) bioavailability (16, 84). It has been shown in animals as well as in humans that shear stress-mediated activation of the phosphatidylinositol 3-kinase and subsequently of protein kinase A and B results in eNOS phosphorylation at Ser^{1177} with the consequence of an increase in eNOS activity and NO production (Fig. 4) (29, 125). Besides a direct activation of eNOS in endothelial cells, exercise training also reduces the expression of NAD(P)H oxidase thereby reducing the generation of ROS (4). In conjunction with an increased expression of radical scavenger enzymes (63) this finally leads to an increased concentration of bioavailable NO (Fig. 4).

The beneficial effects of exercise training on endothelial function as described above dependents on exercise intensity and time. As elegantly demonstrated in a study by Goto and colleagues only 12 weeks of moderate (50% VO_2max)

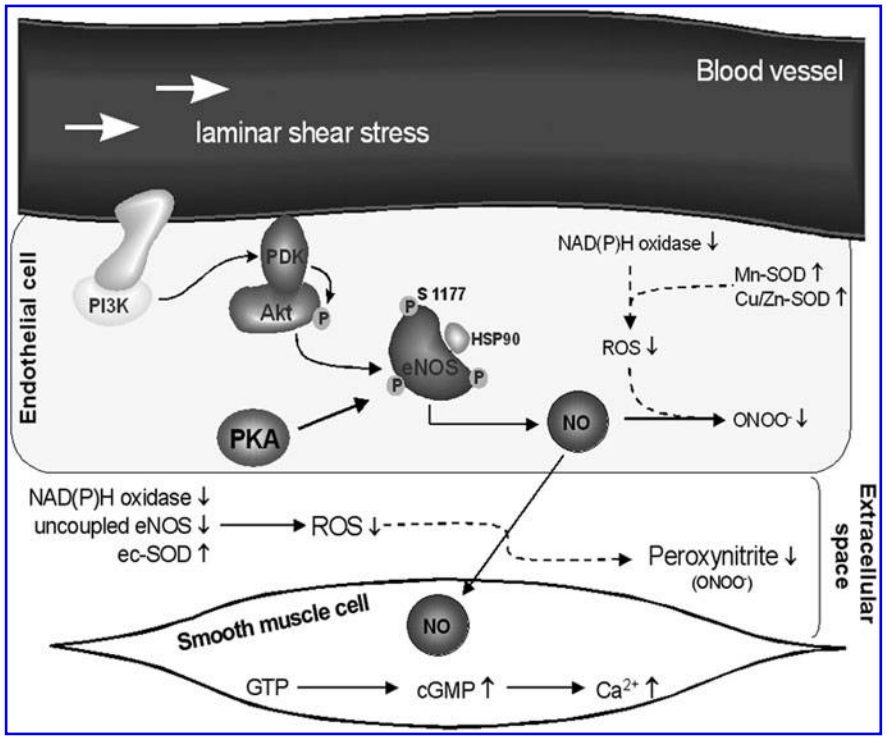

FIG. 4. Schematic diagram on the influence of exercise *via* increased laminar shear stress influences the concentration of NO bioavailability. Repetitive increase shear stress enhances eNOS phosphorylation at position serine-1177 *via* the activation of phosphatidylinositol 3-kinase (PI3K) and AKT. This activation leads to a higher concentration of NO. This increased NO concentration finally results in a smooth muscle-mediated dilation. The concentration of bioavailable NO is further stabilized by the reduced production of reactive oxygen species (ROS) due to downregulation of NAD(P)H oxidase. NO, nitric oxide.

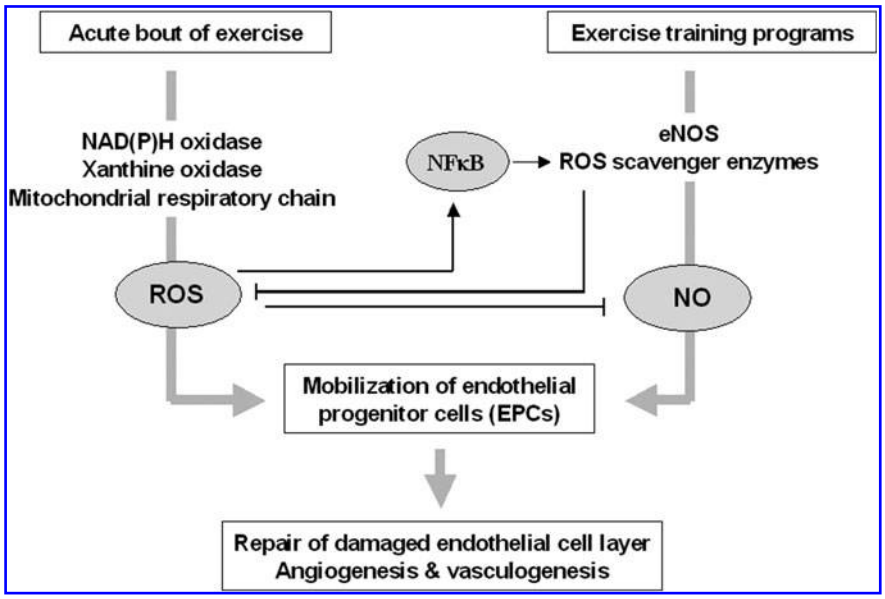

FIG. 5. Schematic diagram on the influence of exercise, either as an acute bout of exercise or as exercise training programs on the mobilization of EPCs. Acute exercise leads to the mobilization of EPCs *via* an activation of NAD(P)H oxidase, leading to an increase of ROS. On the other hand long-term exercise training upregulates eNOS and radical scavenger enzymes, leading to an elevation of bioavailable NO. Both mechanisms increase the concentration of circulating EPCs finally resulting in repair of damaged endothelial cells and angiogenesis and vasculogenesis.

exercise augmented endothelium-dependent vasodilation, whereas mild (25% VO_2max)- or high-intensity (75% VO_2max) exercise showed no effect (27). The reason for this dosage effect is probably due to the rate of ROS production. High-intensity exercise increases plasma concentrations of 8-hydroxy-2′-deoxyguanosine and serum concentrations of malondialdehyde-modified LDL, whereas moderate exercise tended to decrease both indices of oxidative stress. However, in a recent study from Tjonna *et al.* the authors clearly demonstrated in patients with metabolic syndrome that high intensity interval training (95% VO_2max) was superior to moderate exercise with respect to endothelial function (108).

There is increasing evidence that ROS are not only toxic, but also modifies gene expression. As demonstrated by Gomez-Cabrera, the inhibition of xanthine oxidase by allopurinol also prevented the nuclear factor-kappaB mediated elevation in antioxidative enzymes like manganese superoxide dismutase (MnSOD) or glutathione peroxidase (GPX) (26) (Figs. 5 and 6). Therefore, exercise itself can be considered also as an antioxidant.

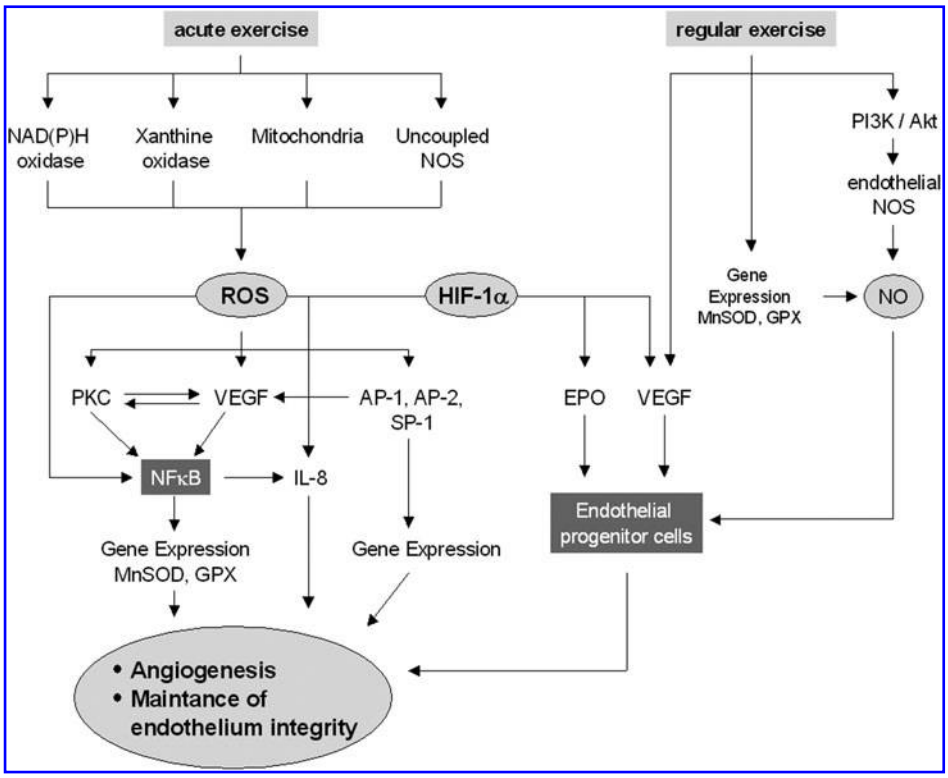

FIG. 6. Possible signaling pathway of acute and regular exercise-induced angiogenesis and maintenance of an intact endothelial cell layer. Partially modified from Maulik (71).

Exercise and EPC Mobilization

Exercise training as a nonpharmacologic intervention has the potency to increase the number and function of circulating EPCs. This concept is based on the findings that exercise intervention programs are able to improve endothelial function in patients with CAD and chronic heart failure as shown in the previous chapter, and that EPCs are thought to participate in the repair of damaged endothelial cell layers (52, 111). Both mesenchymal stem cells and EPCs can be mobilized from the BM by ischemia, neurohormonal factors (*e.g.*, VEGF, placental growth factor), and exercise. It is still a matter of continuing debate whether training-induced flow changes or ischemia induction is required to stimulate EPC release. In two clinical studies we demonstrated that an increase in circulating EPCs was only achieved in response to exercise-induced ischemia (2, 89). Other authors showed exercise-related EPC increase also in the absence of ischemia (Table 2). Prolonged strenuous exercise (half-marathon, marathon or a spartathlon), however, showed no change or a significant increase in circulating progenitor cells (Table 2). What are possible explanations for these discrepancy among the different studies? Several reasons may account for these differences:

1. Subjects investigated in the studies. Looking at Table 2 it is evident that different study populations were analyzed in the studies ranging from healthy subjects (39, 86, 115) to patients with CAD (2, 58, 77) peripheral artery occlusion disease (89, 95) and patients requiring dialysis (69).
2. Different training conditions—intensity and time. Intensity of the training program varied significantly ranging from 2 × /day walking for 10 min (69) to training several times per day at 60%–80% of VO_2max (39, 58, 89). In addition the training program lasted from 4 weeks (58, 89) up to 6 month (69).
3. Age of the subjects. At least in one study only school children were analyzed (118), whereas most other studies investigated middle-aged (39, 95) or older subjects (3, 39, 95).

With respect to the molecular mechanisms regulating the exercise-mediated mobilization of EPCs, we have to discriminate between the effect of an acute bout of exercise and a regular physical exercise program over a certain period. In the setting of acute exercise, ROS seems to play an important role, whereas in the situation of regular exercise training, NO is the major molecule regulating EPC mobilization (Fig. 5).

Acute Exercise and EPC Mobilization

As pointed out above and summarized in Table 2, several studies clearly demonstrated that an acute bout of exercise in either patients with CAD (2) or healthy subjects (77, 115, 122) resulted in the mobilization of EPCs from the BM. A consistent finding in the literature is that metabolic genes increase transiently after an acute bout and returning to near pre-exercise levels 24 h after the end of exercise (79, 80). Besides the induction of the peroxisome proliferator-activated receptor gamma coactivator-1 alpha, an important regulator of mitochondrial biogenesis, HIF-1, is activated after a single exercise bout (Fig. 6) (67). HIF-1 is a heterodimer consisting of the constitutively expressed HIF-1β protein and the HIF-1α protein. HIF-1 responsive genes include VEGF, SDF-1, and EPO, all important molecules inducing EPC mobilization (Fig. 6) (10, 35) Based on a recently published animal study by the group of Dr. Brandes in Frankfurt, Germany, it is reasonable to assume that ROS generated by the NAD(P)H oxidase are essential for hypoxia or EPO-induced mobilization of EPCs (94). In that study the authors used C57Bl6 and Nox2 knockout mice. The exposure of these mice to hypoxia or EPO resulted in a significant mobilization in the wild-type animals but failed in the Nox2 knockout mice. Mechanistically, they proposed the following mechanism: EPO binds to the EPO receptor, which then dimerizes and allows binding of Janus-kinase 2. Janus-kinase 2 creates SH2 binding sites by phosphorylating tyrosine residues at the receptor. Phosphorylation of the receptor activates Rac1 and phosphatidylinositol 3-kinase, which leads to the assembly of Nox2-containing NADPH oxidase subsequently producing superoxide anions. Superoxide anions are dismuted to hydrogen peroxide (H_2O_2), which inactivates the protein-tyrosine phosphatase SHP-2. Meanwhile, STAT5 binds to the SH2 domains of the EPO-receptor, where it gets phosphorylated, dimerizes, and eventually translocates to the nucleus to induce transcription of target genes, leading to EPC mobilization. The importance of Nox2-based NAD(P)H oxidase for the mobilization of stem/progenitor cells was also evident in a hind-limb ischemia model (109). In this model the induction of a hind-limb ischemia in C57Bl6 mice resulted in a significant increase of Nox2 expression in BM, which is associated with an increase in circulating stem/progenitor cells. Mice lacking Nox2 showed a significant reduction of ischemia-induced flow recovery, ROS levels in BM cells, as well as EPC mobilization from the BM. This defect in Nox2-deficient mice could be rescued by the transplantation of BM from normal mice.

Other factors possibly involved in the mobilization of EPCs after an acute bout of exercise are certain growth factors such as VEGF, SDF-1, or granulocyte CSF. Our group could demonstrate that in patients with symptomatic CAD a single maximal exercise stress test a significant rise in VEGF could be documented, which exhibited a positive correlation to the amount of mobilized EPCs (2).

It is recognized now a days that in addition to induce cellular damage, ROS also serve as secondary intracellular messengers and impact the overall redox status of a cell. Importantly, the intracellular redox environment has a critical role in controlling apoptosis, proliferation, self-renewal, senescence, and differentiation (5, 56). Although excess amount of ROS is toxic to EPCs and stem/progenitor cells, optimal levels of ROS, determined by a balance of ROS generating and antioxidative enzymes, are required for normal EPC function in neovascularization. What are possible sources for the elevated ROS concentration after an acute bout of exercise? As already discussed above, the NAD(P)H oxidase seems to be an important ROS source. The Nox2-based NAD(P)H oxidase is also localized in the BM, where its activation seems to be essential for EPC mobilization (94, 109). An other potential source is the electron transport chain of mitochondria (18).

Regular Exercise Training and EPC Mobilization

As discussed previously, exercise training has the potency to increase the concentration of bioavailable NO. This can be achieved either by increasing the expression and activity of eNOS (29, 58) or by decreasing the concentration of ROS (4).

Table 2. Summary of Human Studies Analyzing the Impact of Exercise on the Amount of Circulating Progenitor Cells

Study	*Study population*	*Description of study and exercise protocol*	*Progenitor cell definition*	*Study outcome*
Single exercise bout				
Adams *et al.* (2)	28 CAD points with or without exercise-induced ischemia and 11 healthy controls	Maximal stress test. Follow-up of cell concentration for up to 144 h	Flow cytometry: $CD34^+/KDR^+$ Cell culture: $Ac\text{-}LDL^+/lectin^+$	Time-dependent increase in ischemic points (max. after 24–48 h), no change in nonischemic points and controls
Rehman *et al.* (86)	22 healthy subjects	Symptom-limited treadmill or bicycle exercise test. Blood samples were collected 5 to 10 min after the test.	Flow cytometry: $AC133^+/VECadherin^+$ or $AC133^+/VECadherin^-$	Significant increase of both cell types after exercise test
Shaffer *et al.* (95)	9 young healthy subjects, 13 older healthy subjects, 15 older subjects with PAOD	Blood was collected from subjects before and 20 min after a graded exercise.	Flow cytometry: $CD34^+/KDR^+$ or $CD133^+/KDR^+$ or $CD34^+/CD133^+/KDR^+$	Level of EPCs tended to increase in all subjects after exercise.
Van Craenenbroeck *et al.* (115)	25 healthy subjects	Symptom-limited cardiopulmonary test. Blood was collected 10 min after peak exercise.	Flow cytometry: $CD34^+/KDR^+$	Significant increase of EPCs after exercise test
Training program				
Laufs *et al.* (58)	19 points with stable CAD	Moderate exercise training for 28 days at 60%–80% VO_2max, ergometer and strength training	Flow cytometry: $CD34^+/KDR^+$	Significant increase of EPCs after 28 days of an exercise program
Steiner *et al.* (101)	20 CAD points for training 20 age-matched sedentary controls	12 weeks training, 3 × per week individual training plus 2 endurance running sessions	Flow cytometry: $CD34^+/CD133^+/KDR^+$	Augmentation of circulating EPCs in the training group, no change in the sedentary control group
Sandri *et al.* (89)	18 points with ischemic PAOD 18 points with nonischemic PAOD 31 points with CAD	4 weeks, 6 × per day under close supervision in hospital. Randomization into a exercise training or sedentary group	Flow cytometry: $CD34^+/KDR^+$	Exercise training augments the amount of circulating progenitor cells, but ischemia seems to be necessary. No effect was observed in sedentary controls.

(*continued*)

Table 2. (Continued)

Study	*Study population*	*Description of study and exercise protocol*	*Progenitor cell definition*	*Study outcome*
Hoetzer *et al.* (39)	10 healthy middle-aged or older men	3-month home-based moderate aerobic training, 5–7 days/week, 40–50 min/day, 60%–75% VO_2max. Measurement before and after training period	Cell culture–CFU	Significant increase in CFU after 3 month
Paul *et al.* (77)	50 points with CAD	The points completed a 36-session cardiac rehabilitation program	Flow cytometry: $CD133^+/KDR^+$ Cell culture–CFU	Increase in EPCs either measured by flow cytometry or CFU after finishing the rehabilitation program
Walther *et al.* (118)	182 healthy children	Randomization into intervention class (daily exercise lessons) or control class (school sports twice weekly) for 1 year	Flow cytometry: $CD34^+/KDR^+/CD45^{low}$	Significant increase in progenitor cells in the intervention class but not in the control class after 1 year
Manfredini *et al.* (69)	32 dialysis patients	Randomization into exercise (2 × /day 10 min home-walking at moderate intensity) and sedentary control. Cell measurement at begin and after 6 month	Flow cytometry: $CD34^+/CD133^+/KDR^+$ Cell culture–CFU	Increase of CFU in training group but no change in $CD34^+/CD133^+/KDR^+$ cell number. No change in the control group
Extreme conditions				
Bonsignore *et al.* (12)	8 subjects running a marathon and 8 subjects running a half-marathon	Blood was collected before, after finishing the race and at the morning postrace.	Flow cytometry: $CD34^+$	No change in CD34+ cells after finishing the race, but a significant decrease in cell concentration the morning after the race
Adams *et al.* (3)	68 advanced-age marathon runners	Blood was collected before and after finishing the marathon race.	Flow cytometry: $CD133^+/KDR^+$	No change of EPC concentration was observed comparing before and after the race.
Möbius-Winkler *et al.* (73)	18 healthy young men	4h cycling at 70% of IAT. Blood sampling at beginning, during, and after finishing cycling.	Flow cytometry: $CD34^+/KDR^+$ or $CD133^+/KDR^+$	EPCs showed a significant time-dependent increase with a maximum after 210/240 min.
Goussetis *et al.* (28)	10 volunteer athletes	Blood was drawn before and after a 246-km foot race (Spartathon).	Cell culture–CFUs	EPCs increased significantly in the peripheral blood at the end of the race.

Ac-LDL, acetylated low-density lipoprotein; CAD, coronary artery disease; PAOD, peripheral artery occlusion disease.

Exercise training, EPC, and NOS

That the eNOS plays a central role for the exercise-mediated increase in EPCs was first documented in eNOS knockout mice by Laufs and colleagues *et al.* (58). In these experiments voluntary running in a running wheel over 28 days resulted in a significant upregulation of circulating progenitor cells, defined as Sca-1^+/KDR^+ cells, only in wild-type mice, but was significantly blunted in eNOS knockout mice. The same effect could be mimicked in wild-type mice treated with the specific NOS inhibitor L-NG-Nitroarginine methyl ester (58).

How can we explain the NO-dependent exercise-induced mobilization of EPC? In several studies it could be demonstrated that exercise increases the concentration of NO (29), which in turn can activate MMP-9 in BM (47), leading to enhanced mobilization of progenitor cells as depicted above. As soon as the EPCs are circulating, the most important factors for tissue engraftment of the mobilized cells are the local concentration of SDF-1α, and its cell receptor CXCR-4 (11). This notion is further supported by the observation that mice lacking CXCR4 die *in utero* due to defects in vascular development (105). Although the animal data make shear-stress-induced NO generation a likely mechanism for NO-mediated EPC mobilization, animal experiments with ischemic exercise are still lacking. Therefore, the above-mentioned controversy still awaits experimental resolution.

Antioxidative enzyme, EPC, and exercise training

Stem cells express high levels of genes that are associated with DNA repair and protection from stress (45, 83). It has been shown that EPCs have lower levels of basal and stress-induced ROS than mature endothelial cells due to a high expression of catalase, MnSOD, extracellular superoxide dismutase (ecSOD), and GPX-1 (17, 34). Importantly, the collective inhibition of catalase, MnSOD, and GPX-1 increases levels of ROS in EPC and impair survival and function of these cells (17).

ROS and especially H_2O_2 play an important part in vascular oxidative stress and exert several deleterious effects in the CV system such as increased smooth muscle proliferation, stimulation of inflammation, promotion of atherosclerotic process, and alteration in vascular tone (8). Beside all these effects, it could recently be demonstrated that H_2O_2 also seems to inhibit exercise-induced increase of circulating EPCs (103). In this elegant study, Suvorava and colleagues used a transgenic animal model overexpressing catalase under the control of a Tie-2 promoter and a voluntary exercise training regime with excess to running wheels. They could clearly demonstrate that exercise training was only efficient in EPC mobilization in mice with a catalase overexpression. As molecular explanation the authors speculated that H_2O_2 diffuse from the vascular niche to the stem cell niche in the BM, where it may have an inhibitory effect on MMP-9, the essential molecule for EPC mobilization. That the expression of radical scavenger enzymes plays an important role for reparative neovascularization or angiogenesis was also shown in two other transgenic animal models (24, 53). In GPX-1-deficient mice ischemia-induced angiogenesis was significantly impaired (24). Analyzing the EPCs from these mice it became evident that they exhibit an impaired angiogenic capacity *in vivo* and *in vitro* and increased susceptibility to oxidative stress- and ROS-induced cell death *in vitro* (24). These findings could be confirmed in mice lacking the expression of ecSOD, the major anti-oxidative enzyme in the extracellular space (70, 102). Also in these mice, EPCs in both peripheral and BM are reduced compared to wild-type animals, and the ischemia-induced neovascularization is significantly impaired. This defective neovascularization could be rescued by transplantation of BM from wild-type animals. Collectively, these data suggest that an imbalance in ROS can contribute to EPC dysfunction and their lack of angiogenic function.

Does exercise training has any impact also on the antioxidative system? Acute aerobic and anaerobic exercise training increases vascular oxidative stress and subsequent damage to cellular proteins, lipids, and nucleic acids as well as changes to the glutathione system (48). While acute and exhaustive exercise leaves little time for cellular adaptation reactions based on altered gene expression, these adaptation reactions have been shown to occur in moderate exercise training for several weeks, for example, the upregulation of eNOS above. Interestingly, induction of eNOS expression by exercise training was shown to be followed by an induction of ecSOD protein (23).

Conclusion

The beneficial effects of exercise training on vascular function are well established: long-term physical activity significantly improves endothelium-dependent vasodilation in response to flow or acetylcholine infusion. An important cellular system for maintaining an intact endothelial cell layer as well as for the generation of new blood vessel by angiogenesis or vasculogenesis is EPCs. These cells are liberated in increasing concentration from the BM as a result of exercise—acute exercise as well as long-term exercise training. Based on the current data available in the literature, it seems that the molecular mechanisms regulating the exercise-induced mobilization of EPCs are different in acute exercise and exercise training. In acute exercise the increased generation of ROS *via* the NAD(P)H oxidase system seems to trigger the release of the cells from the BM, whereas in chronic exercise training the concentration of bioavailable NO is the major molecule. These two imported system are also influenced by the expression of radical scavenger enzymes like ecSOD and GPX-1. Still, a matter of debate is the exact function of the EPCs (direct mechanisms to repair the endothelial cell layer or paracrine effects) and the exact cellular definition *via* cell surface marker of EPCs.

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Abbreviations Used

Ac-LDL = acetylated low-density lipoprotein
BM = bone marrow
BOEC = blood outgrowth endothelial cell
CAC = circulating angiogenic cell
CAD = coronary artery disease
CFP = circulating endothelial precursor
CFU-EC = colony forming unit endothelial cells
CHF = congestive heart failure
CV = cardiovascular
ECFC = endothelial colony-forming cells
ecSOD = extracellular superoxide dismutase
eNOS = endothelial nitric oxide synthase
EPC = endothelial progenitor cell
EPO = erythropoietin
FGF = fibroblast growth factor
GM-CSF = granulocyte-macrophage colony-stimulating factor
GPX = glutathione peroxidase
H_2O_2 = hydrogen peroxide
HIF-1α = hypoxia-inducible factor 1α
HPP = high proliferative potential
LPP = low proliferative potential
MMP = matrix metalloproteinase
MNC = mononuclear cell
MnSOD = manganese superoxide dismutase
NADPH oxidase = nicotinamide adenine dinucleotide phosphate-oxidase
NF-κB = nuclear factor-kappaB
NO = nitric oxide
PAOD = peripheral artery occlusion disease
PI3K = phosphatidylinositol 3-kinase
ROS = reactive oxygen species
SDF-1 = stromal cell-derived factor-1
sKitL = soluble Kit-ligand
VEGF = vascular endothelial growth factor

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