Endothelial Progenitor Cells: Therapeutic Target for Cardiovascular Diseases

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Abstract. Circulating endothelial progenitor cells (EPCs) derived from bone marrow were isolated for the first time in 1997 and characterized. Recent evidence has indicated that EPCs contribute to reendothelialization of injured vessels as well as neovascularization of ischemic lesions and that a decrease in the number of EPCs is an independent predictor of morbidity and mortality of cardiovascular diseases. These findings suggest that EPCs play a major role in the pathogenesis of atherosclerosis and cardiovascular diseases. Interestingly, the number and function of EPCs are regulated by not only various kinds of angiogenic cytokines and cardiovascular risk factors per se but also some interventions, including lifestyle modification (aerobic exercise, body weight loss, and smoking cessation) and pharmacological therapy (e.g., renin-angiotensin system inhibitor, statin, and erythropoietin). It is thought that regulation of the number and function of EPCs directly influences the maintenance and development of atherosclerosis. Therefore, it is clinically important to estimate the degree of EPC bioactivity and to increase the EPC bioactivity by appropriate interventions. In this review, we focus on the relationship between EPCs and cardiovascular risk factors and the role of EPCs in cardiovascular diseases.

Keywords: endothelial progenitor cell, cardiovascular disease, cardiovascular risk factor, nitric oxide

1. Introduction

In 1997, Asahara et al. (1) reported for the first time the existence of bone marrow-derived endothelial progenitor cells (EPCs) in peripheral circulation. EPCs contribute to reendothelialization of injured vessels as well as neovascularization of ischemic lesions, suggesting that EPCs play a major role in the pathogenesis of atherosclerosis and cardiovascular diseases. The number and function of EPCs are regulated by not only various kinds of angiogenic growth factors, cytokines, and chemokines in response to ischemia or vascular injury and cardiovascular risk factors but also lifestyle modification, such as aerobic exercise, and smoking cessation and pharmacological therapy (e.g., renin-angiotensin system inhibitor, statin, erythropoietin). An increase in the number of EPCs induced by ischemic stimuli contributes to reduction in ischemic damage through neovascularization. Recently, Werner et al. (2) have demonstrated that a reduced number of EPCs is an independent predictor of morbidity and mortality of cardiovascular diseases. These findings suggest that regulation of the number and function of EPCs directly influence the maintenance and development of cardiovascular diseases.

Surgical bypass, percutaneous transluminal angioplasty, and one of these in combination with pharmacological therapy are options for revascularization and improvement in limb ischemic symptoms in patients with peripheral arterial diseases (PAD). Unfortunately, patients with PAD with no other treatment option are subjected to amputation. Recent studies have shown that therapeutic transplantation of bone marrow-derived mononuclear cells, including EPCs, increases collateral vessel formation in ischemic limb models (3, 4) and in patients with limb ischemia who have no other treatment options.
option (5, 6). Impairment of the function of donor EPCs under various clinical conditions, such as diabetes mellitus, coronary artery disease (CAD), and ischemic cardiomyopathy, may result in poor revascularization.

Although a precise definition of EPCs is lacking, unique surface markers of hematopoietic and endothelial lineages such as CD34, AC133, and endothelial growth factor receptor 2 (VEGFR-2) or kinase domain receptor (KDR) are used for identification of EPCs. Combinations of the following markers: CD34⁻KDR⁺, CD34⁺AC133⁺, AC133⁺KDR⁻, CD34⁺AC133⁺KDR⁻, and CD34⁺KDR⁻ have been used to identify EPCs in several studies (7, 8). However, there is no gold standard marker, resulting in confusion regarding the definition of EPCs. In addition, in functional assays of EPCs by using culture methods, different culture conditions produce different EPC phenotypes such as early EPCs and late outgrowth EPCs or mixed ones. The fact that a method for analysis of EPCs has not been established should be taken into account when considering results of EPC studies.

2. EPCs and cardiovascular risk factors

Age, male gender, hypertension, diabetes mellitus, hyperlipidemia, smoking, obesity, and physical inactivity are recognized as cardiovascular risk factors that promote vascular injury. Hyperhomocysteinemia and hyperuricemia are also recognized as risk factors. It is well known that cardiovascular risk factors influence the number and function of EPCs. Hill et al. (9) reported an inverse correlation between number of EPCs and total number of risk factors. Interestingly, they found an inverse correlation between number of EPCs and endothelial function. Several investigators, including us, have confirmed the relationship between number of EPCs and cardiovascular risk factors (Fig. 1) (10, 11). Vasa et al. (11) demonstrated that EPCs in patients with CAD had an impaired migratory function, which inversely correlated with the number of risk factors. In addition, bone marrow-derived mononuclear cells from patients with CAD have reduced capacity of neovascularization. Apparently, cardiovascular risk factors have a negative correlation with both the number and function of EPCs. However, the mechanism underlying the decrease in number or function of EPCs in patients with cardiovascular diseases has not been fully elucidated. It is thought that peripheral circulating EPCs are predominately decreased by reduced mobilization from bone marrow rather than by depletion of stem cells. Mobilization of EPCs is regulated by various angiogenic cytokines, such as vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor, and stromal cell-derived factor-1, and by the bioavailability of nitric oxide (NO) in bone marrow. NO appears to be essential for regulation of EPCs in response to various stimuli through activation of metalloproteinase (MMP)-9. MMP-9 induces transformation of membrane-bounded Kit ligand to a soluble Kit ligand, triggering subsequent movement of cKit-positive stem cells, including common precursors of hematopoietic progenitor cells and EPCs (i.e., heman-

![Fig. 1. Relationship between endothelial progenitor cells (EPCs) and cardiovascular risk factors. Bar graphs show the numbers of EPCs in healthy subjects and patients with coronary artery diseases (CAD) (left). The number of EPCs was significantly smaller in patients with CAD than in healthy subjects. Scatterplots show the relationships between the number of EPCs and number of total cardiovascular risk factors (right). There was a significant relationship between the number of EPCs and number of total cardiovascular risk factors.](image-url)
relationship between plasma level of VEGF and aging subjects remains unknown. On the other hand, no enhanced, inducing mobilization and homing of EPCs from bone marrow. In ischemic tissue, VEGF expression is a cytokine that strongly induces mobilization of EPCs from bone marrow in elderly subjects, but the VEGF was associated with reduced mobilization of Scheubel et al. (14) reported that low plasma level of more than two-times higher than the baseline level. Ischemic tissue increases circulating EPCs to levels into the ischemic lesion. Gene transfer of VEGF into profound mobilization of EPCs (12). NO synthase (NOS) 3 \(^{-}\) mice displayed profoundly reduced mobilization of EPCs (12). NO bioavailability is influenced by cardiovascular risk factors, which probably cause the decrease in mobilization of EPCs. In addition, functions of EPCs such as migration and capability of incorporation into a tube-like structure also appear to be partly regulated by the endothelial NOS-NO pathway. Influence of each risk factor on proliferating potential, cell senescence, and apoptosis of EPCs is also in the process of being uncovered.

2.1. Age

Aging is a strong factor causing reduction in the number of EPCs and affects the function of EPCs. Our multivariate analysis showed that aging is an independent factor for reduction in the number of EPCs (10). Decrease in NO bioavailability in elderly individuals is well established. Decrease in NO bioavailability should contribute to the reduction in number of EPCs in relation to aging. Thum et al. (13) demonstrated that growth hormone treatment in middle-aged healthy males restored the reduction in number of EPCs and the colony forming capacity, migration capacity, and capability for incorporation into a tube-like structure though IGF-1-mediated up-regulation of the phosphoinositide (PI)-3- kinase/Akt/eNOS pathway. VEGF is known as a cytokine that strongly induces mobilization of EPCs from bone marrow. In ischemic tissue, VEGF expression is enhanced, inducing mobilization and homing of EPCs into the ischemic lesion. Gene transfer of VEGF into ischemic tissue increases circulating EPCs to levels more than two-times higher than the baseline level. Scheuebel et al. (14) reported that low plasma level of VEGF was associated with reduced mobilization of EPCs from bone marrow in elderly subjects, but the cause of decrease in circulating VEGF level in elderly subjects remains unknown. On the other hand, no relationship between plasma level of VEGF and aging has been reported. Cell damage and/or genome instability with aging promote senescence of EPCs probably through increase in oxidative stress. Indeed, EPCs from elderly subjects displayed reduced telomerase and increased cell senescence (15).

2.2. Gender

Male gender is regarded as one of cardiovascular risk factors. It is not clear whether the number and function of EPCs are decreased more in males than in females. Conversely, although estrogen both in vivo and in vitro increases the number of EPCs and functions of EPCs such as adhesion, migration, proliferation, and anti-apoptosis, the increased number and function of EPCs in females are not well recognized. Masuda et al. (16) demonstrated that the number of CD34\(^+\) cells and AC133\(^+\) cells including immature EPCs achieved a peak in the postovulatory phase following a peak in serum concentration of estrogen in the periovulatory phase, suggesting that the number of EPCs is regulated by the menstrual cycle. Further studies on the effects of male gender and female gender on the biological activity of EPCs that take into account these findings are needed.

2.3. Hypertension

Hypertension is one of the strong risk factors for reduction in the number and function of EPCs. Vasa et al. (11) demonstrated that the number and migratory capacity of EPCs were decreased in patients with hypertension. Accumulation of reactive oxygen species (ROS), especially due to the renin-angiotensin system (RAS), is thought to play an important role in the decrease in NO bioavailability as evidenced by the fact that endothelium-dependent vasodilation was diminished in patients with hypertension, probably leading to defective mobilization of EPCs from bone marrow (17). ROS also affect proliferation and senescence and/or apoptosis of EPCs. Accumulating findings suggest that angiotensin II accelerates the onset of EPC senescence by gp91 phox–mediated increase in oxidative stress in patients with hypertension (18). Interestingly, subjects who were treated with RAS inhibitors had an increased number of EPCs. Bahlmann et al. (19) showed that the angiotensin II–receptor antagonist olmesartan increases the number of EPCs in prospective studies in normoten-sive and moderate hypertensive individuals. These findings support the important role of RAS in regulation of EPC bioactivity in patients with hypertension. Unfortunately, there is little information on the function of EPCs in patients with hypertension.

2.4. Diabetes mellitus

The number of EPCs is decreased in both patients with type 1 diabetes and patients with type 2 diabetes. Tepper et al. (20) showed that EPCs from patients with diabetes mellitus have impaired function of adhesion, proliferation, and tubulization. Uncontrolled plasma glucose level represented by glycated hemoglobin levels and free plasma glucose levels was inversely correlated with the number of EPCs. Insulin resistance plays an important role in endothelial dysfunction and progression of atherosclerosis through down-regulation of the PI3 kinase/Akt/eNOS pathway in patients with diabetes mellitus. It is thought that inactivation of the PI3 kinase/Akt/eNOS pathway reduces mobilization of EPCs from bone marrow through a decrease in NO bioavailability. Krankel et al. (21) demonstrated that glucose
toxicity by hyperglycemia impaired the proliferation and augmented the apoptosis of EPCs through upregulation of p16\textsuperscript{Ink-4\textalpha} and p21\textsuperscript{Waf-1}. The induction of p16\textsuperscript{Ink-4\textalpha} and p21\textsuperscript{Waf-1} leads to cell cycle arrest. P38 mitogen-activated protein kinase also contributes to hyperglycemia-induced reduction of EPCs. Impairment of the migration and integrative capacity of EPCs in diabetes mellitus is involved in the altered NO bioavailability and metalloproteinase-9 activity. The degree of impairment of EPC function is related to the severity of diabetic vasculopathy such as PAD and carotid stenosis. These findings suggest that EPCs play an important role in the pathogenesis of diabetic vasculopathy.

2.5. Dyslipidemia

Dyslipidemia, especially high levels of low-density lipoprotein (LDL) cholesterol, has been shown to be as one of strongest factors for progression of atherosclerosis. Several studies have demonstrated that a high level of LDL reduced the number and function of EPCs (22). Oxidized LDL induces senescence and impairment of adhesive, migratory, and tube-formation capacities of EPCs through inactivation of the Akt/eNOS pathway. Llevadot et al. (23) showed that simvastatin augmented the mobilization of EPCs from bone marrow through activation of the Akt/eNOS pathway. Induction of telomerase inactivity by oxidized LDL was abolished by pretreatment with atorvastatin, leading to restoration of EPC senescence.

High-density lipoprotein (HDL) prevents the progression of atherosclerosis. Low HDL levels increase the incidence of cardiovascular diseases. Although the mechanisms by which HDL has beneficial effects on atherosclerosis remain unclear, HDL-induced increase in the number and function of EPCs would contribute to the vascular protective effect. Indeed, injection of reconstituted HDL increases the number of EPCs in patients with type 2 diabetes (24). It is likely that HDL enhances the mobilization and differentiation of EPCs through activation of the PI3-kinase/Akt/eNOS pathway and prevention of apoptosis.

2.6. Obesity, insulin resistance, and metabolic syndrome

Obesity is known to promote endothelial dysfunction and atherosclerosis. Little is known about the relationship between obesity per se and EPCs. Fadini et al. (25) reported that patients with metabolic syndrome had a reduced number of EPCs. Accumulation of visceral fat induces up-regulation of TNFa and IL-6 and down-regulation of adiponectin, leading to endothelial dysfunction and high insulin resistance, which is negatively correlated with the number and function of EPCs. Peroxisome proliferator-activated receptor-\(\gamma\) (PPAR-\(\gamma\)) agonists, a new class of insulin sensitizers, have beneficial effects (e.g., decrease in vascular inflammation, improvement of endothelial function, and inhibition of smooth muscle cell hyperplasia) on vasculatures. In addition, it has been demonstrated that treatment with a PPAR-\(\gamma\) agonist, pioglitazone or rosiglitazone, improves the number and migratory function of EPCs in patients with CAD and in patients with type 2 diabetes. Redondo et al. (26) also confirmed an increase in number of EPCs by treatment with pioglitazone in a culture model, and they found that the increased number of EPCs contributed to augmented adhesion and differentiation of EPCs but not to up-regulation of proliferation and antiapoptosis of EPCs. These finding support the notion of an association between EPC bioactivity and insulin resistance.

2.7. Smoking

Kondo et al. (27) reported that smoking reduced the number of EPCs in healthy subjects and that smoking cessation restored the level of EPCs. Some other studies have also indicating that smoking reduces the number of EPCs (28). However, a significant relationship between smoking and reduced number of EPCs in patients with CAD has not been found. In our analysis, smoking did not reduce the number of EPC in patients with cardiovascular diseases. On the other hand, healthy smokers without cardiovascular risk factors had a reduced number of EPCs compared with that in non-smokers. The reason for the discrepancy in these results remains unclear. Determination of the effects of other cardiovascular risk factors and the effects of duration of smoking and smoking cessation on the number and function of EPCs would enable more specific conclusions concerning the role of smoking per se in EPC bioactivity to be drawn. Smoking-related ROS production may decrease bioavailability of NO, leading to inhibition of the mobilization of EPCs from bone marrow. An increase in ROS also alters function of EPCs. Michaud et al. (28) demonstrated that upregulation of ROS formation decreased adhesive, migratory, and tube formation capacities of EPCs in smokers. The magnitude of increase in EPCs after smoking cessation was smaller in heavy smokers than in light smokers, suggesting that excessive smoking induces irreversible damage to EPC bioactivity.

2.8. Physical inactivity

Moderate-intensity exercise improves endothelial function and reduces the morbidity and mortality of cardiovascular diseases. It is postulated that increased NO production and inactivation of ROS through
augmentation of antioxidant systems, such as superoxide dismutase and glutathione peroxidase, contribute to up-regulation of NO bioavailability, leading to protection against atherosclerosis. Although it is unclear whether physical inactivity per se decreases the number of EPCs, aerobic exercise training is known to increase the number and function of EPCs. Laufs et al. (29) demonstrated that 4-week controlled, standardized exercise training increases the number of EPCs and reduces EPC apoptosis in patients with stable CAD, and they showed in an experimental animal model that exercise training for more than 7 days increases EPCs through upregulation of VEGF and activation of the NO-dependent pathway. Even single exercise-induced myocardial ischemia increased the number of EPCs with a maximum after 24 to 48 h (30).

2.9. Other risk factors

Interestingly, some studies, including our study, have shown that family history of cardiovascular disease tends to decrease the number of EPCs. Future studies are needed to evaluate the relationship between EPCs and genetic polymorphisms of factors involved in the regulation of EPCs. Likewise, homocysteine level is inversely correlated with the number of EPCs, and hyperhomocysteinemia impairs migratory and adhesional capacity of EPCs. Little is known about the relationship between EPCs and hyperuricemia. A transient surge in uric acid has been reported to promote mobilization of EPCs.

3. Summary and perspectives

Imbalance of injury and repair of vasculature promotes the progression of atherosclerosis. One possible mechanism by which cardiovascular risk factors cause the onset, maintenance, and progression of atherosclerosis is inactivation of the bioactivity of EPCs (Fig. 2). Assessment of the number and function of EPCs may be a good surrogating biomarker for atherosclerosis. Several interventions, such as lifestyle modification and pharmacological therapy, increase the number and function of EPCs. It is expected that these interventions prevent and restore vascular injury. Therefore, from a clinical perspective, it is important to select an appropriate intervention that is effective in increasing EPCs in patients with cardiovascular diseases or even in healthy subjects. Novel therapies, including cell implantation, are effective in patients with critical limb ischemia who have no other treatment option. However, we should take care that when performing cell therapy, including EPCs implantation, EPCs from patients with severe ischemic diseases who have inactivated EPCs may not induce effective angiogenesis in these patients. For clinical application, further investigations are needed to understand in more detail the biology of EPCs.

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