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Endothelial progenitor cells and atherosclerosis

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1. ABSTRACT

Atherosclerosis is due to inflammation and endothelial dysfunction and damage caused by a variety of factors. Dysfunction of endothelial progenitor cells (EPCs) that differentiate into mature endothelial cell contributes to the development of atherosclerosis. Both the number and functionality of EPCs are regulated, particularly in vascular repair. Further elucidation of the role of EPCs in atherosclerosis could potentially enable the development of novel strategies for prevention and treatment of pathological changes in atherosclerosis.

2. INTRODUCTION

Atherosclerosis is a pathological inflammation of the arteries characterized by mononuclear cell infiltration, smooth muscle proliferation, and matrix protein accumulation in the intima; it is accompanied by endothelial dysfunction. Following endothelial injury, the arterial wall becomes lipid-laden and undergoes characteristic morphologic changes through a process similar to wound-healing (1-3). Although therapies targeting inflammation and cholesterol homeostasis have proven effective in preventing thromboembolic sequelae of atherosclerosis, atherosclerosis remains a serious health problem worldwide. Vascular endothelial dysfunction or loss is a crucial event in the formation of atherosclerosis (4). Endothelial damage may be induced by oxidized lipids, free radicals, cytokines, haemodynamic stress, and high concentrations of blood cholesterol, resulting in native atherosclerosis. All of these events lead to acute stress injury, which results in apoptosis or necrosis of the endothelial cellular layer accompanied by a loss of antithrombotic properties (5). Because endothelial dyfunction and damage plays a key role in the atherosclerosis, a greater understanding of endothelial reparation is needed and could potentially aid in the development of novel strategies for the prevention and therapy of atherosclerosis.

Following injury, adequate endothelial regeneration is crucial for diminishing arterial stenosis. Although mature endothelial cells repair the endothelium by migration and proliferation from surrounding areas, they are terminally differentiated and exhibit a low proliferative potential; thus, their capacity is limited. Therefore, stem cells are important in enabling endothelial regeneration. Endothelial progenitor cells (EPCs) exhibit the potential to differentiate into mature endothelial cells, a unique subtype

of bone marrow-derived cells with properties similar to embryonic angioblasts (6-8). Generally, EPCs are identified by flow cytometric characteristics, namely expression of CD34, CD133, or VEGFR2. Importantly, CD34+KDR+ combination is the only putative EPC phenotype that has been demonstrated repeatedly and convincingly to be an independent predictor of cardiovascular outcomes (9, 10). Accumulating evidence indicates that blood EPCs are able to repair injured vessels of dying endothelial cells in animal models (11, 12). EPCs counteract ongoing risk-factor induced endothelial cell injury and participate in endothelial cell repair and regeneration (13). In this review, we will focus on the changes of EPCs in atherosclerosis.

3. EPC AND ATHEROSCLEROSIS

A deficit of EPCs potentially contributes to the development of atherosclerosis. It has been previously reported that chronic treatment with bone marrow-derived progenitor cells from young non-atherosclerotic apolipoprotein E-deficient (ApoE-/-) mice prevents atherosclerosis progression in ApoE-/- recipients despite persistent hypercholesterolemia (14). During the development of atherosclerosis in ApoE-/- mice, endothelial turnover and repair by progenitor cells could potentially be derived from bone marrow (15).

Population-based studies demonstrate the relationship between EPCs and atherosclerosis. In the population-based Bruneck Study, a significant inverse association was found between EPC number and extent of carotid atherosclerosis (16). In a middle-aged, general population, peripheral blood CD34+KDR+ EPCs were found to be determinants of subclinical atherosclerosis (17). This relationship has also been studied in different ethnicities; the number of EPCs are reduced in European and South Asian men with atherosclerosis independent of other risk factors (18).

In the elderly, the relationship between EPCs and atherosclerosis is more obvious, particularly in patients with hypercholesterolaemia or cardiovascular disease. In patients with hypercholesterolaemia, the number of EPCs is inversely correlated with total cholesterol. The functional activities of isolated EPCs, such as proliferative, migratory, adhesive, and in vitro vasculogenesis capacity, were also impaired (19). In patients with stable coronary artery disease and patients with acute coronary syndrome, reduced levels of circulating EPCs independently predicts atherosclerotic disease progression, thus supporting an important role for endogenous vascular repair to modulate the clinical course of coronary artery disease (20). Patients undergoing percutaneous coronary intervention to the subsequent development of in-stent restenosis exhibit a higher number of subpopulations of EPCs that play a role in arteriogenesis compared with controls and patients with either progression of coronary atherosclerosis or stable disease (21). In summary, the number and functionality of EPCs is associated with the development of atherosclerosis both in the general population and in high-risk groups.

4. MODULATION OF EPC IN ATHEROSCLEROSIS

EPCs are modulated via several paths in atherosclerosis. The phosphoinositide 3-kinase (PI3K)/Akt pathway plays a key role in the modulation of EPCs. Atherothrombosis and its risk factors are associated with endothelial dysfunction (22), one manifestation of which is inadequate production of bioactive endothelial nitric oxide (NO). Remnant like particles (RLPs) inhibit nitric oxide synthase (eNOS) and telomerase activity, thus inducing atherosclerosis by promoting EPC senescence via focal adhesion kinase (FAK) and its downstream PI3K/Akt pathway via an oxidative mechanism (23). Meanwhile, the activation of the PI3K/Akt pathway promotes the protection of hypoxia against apoptosis in EPCs (24). The apoptosis of EPCs via PI3K/Akt pathway is down-regulated by stromal cell-derived factor-1alfa (SDF-1alfa)/CXCR4 (25) (Figure 1).

EPCs shed increased microparticles, which reduce circulating EPC levels and thus contribute to increased aortic stiffness in addition to traditional risk factors (26). The semi-essential amino acid L-arginine is the principal substrate of the NO synthases. The modulation of EPC levels potentially leads to the beneficial effects of L-arginine in the prevention of atherosclerosis in hypercholesterolemic rabbits (27).

5. EPC AND VASCULAR DAMAGE

EPCs are involved in vascular damage; circulating EPCs potentially represent biological markers of occult vascular damage in offspring with hereditary risk of coronary artery disease (CAD) (28). Paracrine factors secreted by EPCs prevent oxidative stress-induced apoptosis of mature endothelial cells. Thus, EPCs also release angiogenic factors; when EPCs are infused into ischaemic limbs of immuno-compromised mice a remarkable improvement in perfusion and recovery from injury is observed (29-32). This supportive function of EPCs may be crucial to ensure the survival of tissue-residing cells during vascular damage.

Furthermore, EPCs express proinflammatory paracrine factors and adhesion molecules that are involved in atherosclerosis (33). Soluble factors secreted by EPC, potentially via broad synergistic action, exert strong cyto-protective properties on differentiated endothelium through modulation of intracellular antioxidant defensive mechanisms and pro-survival signals (34). However, in patients with early coronary atherosclerosis retention of osteoblastic marker osteocalcin (OCN) (+) EPCs within the coronary circulation potentially lead to progressive coronary calcification rather than normal repair (35). OCN+ EPCs do not appear to lead to ideal reparation following vascular damage. Thus, the mechanisms underlying EPCs during vascular damage in atherosclerosis are complex; proinflammatory molecules are expressed on the one hand and protective factors are secreted on the other hand. We hypothesize that the expression of proinflammatory molecules is the response to damage,

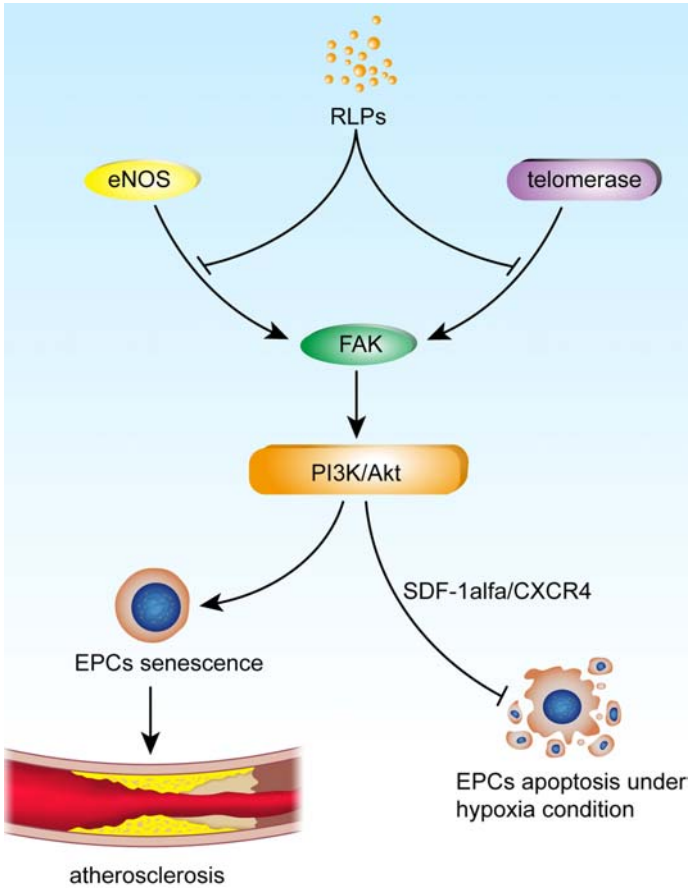

Figure 1. Inadequate production of bioactive endothelial nitric oxide (NO) in endothelial dysfunction. Remnant like particles (RLPs) inhibit nitric oxide synthase (eNOS) and telomerase activity, thus inducing atherosclerosis by promoting EPC senescence via focal adhesion kinase (FAK) and its downstream PI3K/Akt pathway through an oxidative mechanism. Meanwhile, activation of PI3K/Akt pathway participates in the protection of hypoxia against apoptosis in EPCs. The apoptosis of EPCs via PI3K/Akt pathway is down-regulated by stromal cell-derived factor-1alfa (SDF-1alfa)/CXCR4.

whereas secretion of protective factors is one of the primary functions of EPCs.

6. REGULATION OF EPC IN VASCULAR REPAIR

EPC number and functionality are considered to reflect the endogenous vascular repair capacity in atherosclerosis. During the process of vascular reparation, EPCs are regulated by several factors. Following ischemia, EPCs migrate from bone marrow to repair damaged sites either through direct incorporation of EPCs or by repopulating mature endothelial cells. Aging is associated with an increased risk for atherosclerosis. Age-dependent impairment of EPCs is corrected by a growth-hormone mediated increase of insulin-like growth factor 1 (36). Plasma cholesterol directly increases endothelial damage, and reduces endothelium repair capacity by endothelial progenitors in patients with hypercholesterolemia-related aortic stiffness (37). High-density lipoproteins enhance progenitor-mediated endothelium repair in mice (38).

Homocysteine (Hcy) is a risk factor for vascular dysfunction. High levels of Hcy may result in vascular injury, accelerating atherosclerosis and leading to ischemia. Alam and colleagues found that increased Hcy leads to a decrease in EPC numbers. This decrease in EPC by Hcy potentially occurs via increased apoptosis, and B vitamin (B(6), B(9)) intervention can attenuate such effects (39).

Aside from risk factors for atherosclerosis, protective factors are also involved in the regulation of EPCs in vascular reparation. Adiponectin protects against atherosclerosis and decreases risk in myocardial infarction. Lavoie and colleagues found that adiponectin protects certain EPC sub-populations against apoptosis, and therefore could potentially modulate the ability of EPCs to induce repair of vascular damage. (40)

7. REGULATION OF CIRCULATING EPC LEVELS

Atherosclerosis develops in an environment of endothelial injury and inflammation. Griese and colleagues (41) demonstrated that peripheral blood monocyte-derived EPCs home to bioprosthetic grafts and balloon-injured carotid arteries; the latter is associated with a significant reduction in neointima deposition. Likewise, infusion of

bone marrow-derived CD34-/ CD14+ mononuclear cells contributes to endothelial regeneration (42). Direct incorporation of circulating EPCs into the vessel wall has been reported in mice. In a model of transplant atherosclerosis, regenerated endothelial cells from arterial grafts have been found to originate from recipient circulating blood but not from the remaining endothelial cells of the donor vessels (43). Similarly, it has been reported that the endothelial monolayer in a vein graft three days post-surgery was completely lost and subsequently replaced by circulating endothelial progenitors (44). Taken together, these findings suggest that circulating EPCs contribute significantly to re-endothelialization. When released from bone marrow, EPCs are mobilized via cytokines such as stromal cell-derived factor (SDF)-1, nitric oxide, and VEGF, and then they are retained on the vascular surface by binding to adhesion molecules such as P/E-selectin and ICAM-1 (45). Once attached to the surface of the injured endothelium/vessel, EPCs differentiate into ECs. Next, the injured endothelial monolayer is regenerated by these circulating bone marrow derived EPCs that accelerate re-endothelialization and limit atherosclerotic lesion formation; the level of circulating EPCs potentially determines the capacity to repair.

The level of circulating EPCs can be determined by the number of precursors EPCs in the bone marrow. The maintenance and mobilization of the precursors of EPCs in the bone marrow is determined by the local microenvironment, the "stem cell niche", which consists of stromal cells (46). The direct influence of the overall risk factors for coronary artery disease on the bone marrow microenvironment remains unclear. However, the effect of aging has been extensively studied. The capacity to react to stress-induced mobilization gradually declines with increased age, whereas basal hematopoiesis is maintained during aging. (47).

In addition to the number of precursors of EPCs in the bone marrow, many factors directly impact the level of circulating EPCs. Classical risk factors for atherosclerosis such as age are associated with reduced numbers of circulating CD34/KDR+ and CD133/KDR cells (48). Likewise, elderly patients exhibit a limited response toward EPC mobilization/differentiation stimuli and therefore exhibit a reduced number of circulating EPCs (49). Population-based data have served to confirm the decline of EPC number with advancing age (16). A lower increase in circulating EPCs has also been found in elderly patients following surgery (50); circulating EPC levels are higher during childhood compared to adult life (51). Studies in ApoE-/- mice (52) as well as in patients with coronary artery disease (53) have demonstrated that age significantly reduces circulating EPCs whereas the overall number of hematopoietic stem cells or mature endothelial cells remains unchanged. Thus, age itself potentially interferes with the functional activity of stem cells and progenitor cells.

In addition to age, several factors are involved in the regulation of circulating EPC levels. A common feature of the characterization of various EPC subtypes is endothelial nitric oxide synthase (eNOS) expression. Deficiency for eNOS can cause impairment of VEGF-induced mobilization of EPCs and blunt hematopoietic recovery following myelosuppression (54). Reduction in exercise-induced EPC mobilization has been observed in eNOS-/- mice (55). In addition, hyperhomocysteinemia (HHcy) contributes to atherosclerosis and coronary artery disease by inducing endothelial cell injury and dysfunction. Though EPC number and functional capacity has been found to be impaired in patients with Hhcy (56), the peripheral EPC population was not significantly altered in HHcy mice (57). Low-density lipoprotein apheresis also influences circulating EPCs in familial hypercholesterolemia. Hypercholesterolemic patients exhibit a lower percentage of EPCs compared to controls (58), whereas low dose endotoxemia in humans leads to a significant decrease in peripheral EPCs (59).

The chromosome 9p21 locus was discovered in 2007 by independent genome-wide association studies for coronary artery disease. This genomic region contains a gene encoding cyclin-dependent kinase 2A, a regulator of proliferation and differentiation of EPCs that has been implicated in vascular repair and protection against cardiovascular disease (60). However, the chromosome 9p21 variant does not influence the risk of coronary heart disease and stroke via circulating EPCs (61).

Angiotensin II receptor antagonism and aspirin exhibit an effect on the level of circulating EPCs. Pelliccia and colleagues (62) found that Angiotensin II receptor antagonism with telmisartan increases the number of regenerative EPCs and improves endothelial function in normotensive patients with coronary artery disease (CAD). In addition, aspirin has been found to exert a time-dependent effect on circulating EPCs; however, the short-term exposure to differing doses of aspirin exhibits an indeterminate effect on EPC levels (63). The regulation of circulating EPC levels is complex, and the precise underlying mechanism remains unclear. Erythropoietin has been shown to be correlated to vasculoprotective effects, such as enhanced nitric oxide production in endothelial cells and mobilization of EPCs. Darbepoetin, administered in addition to optimal medical treatment, can result in significantly improved endothelial function in patients with coronary artery disease, indicating that it could serve as a promising new atheroprotective treatment option (64).

8. THERAPEUTIC POTENTIAL OF EPC

EPCs have been extensively studied in atherosclerosis, and accumulating evidence indicates their importance in vascular repair and tissue remodelling. Researchers have investigated EPC therapy for atherosclerosis. The EPC therapy for atherosclerosis has been discussed for the aging population, and EPC therapy could potentially increase life expectancy in this population (65). In vivo and in vitro studies have demonstrated the effect of EPC therapy for atherosclerosis. Intravenous transfusion of spleen-derived mononuclear cells improves endothelium-dependent vasodilation in atherosclerotic ApoE-/- mic, which indicates the important role played by

circulating progenitor cells for ongoing vascular injury repair (66). The beneficial therapeutic effect on atherosclerosis following only EPC transplantation has been demonstrated by transplantation of bone endothelial progenitor cells that over-express paraoxonase-1 gene by recombinant adeno-associated virus in rats (67); this finding suggests that recombinant adeno-associated virus (rAAV)-mediated paraoxonase-1 (PON1) gene-transfected endothelial progenitor cells (EPCs) could serve as a potentially valuable new tool in the treatment of atherosclerosis.

Furthermore, therapies that address EPC impairment would be helpful in atherosclerosis treatment. A greater understanding of the mechanisms in diseases that drive EPC senescence and apoptosis could potentially result in new treatment approaches for atherosclerosis. For instance, the therapy based on the PI3K/Akt/eNOS pathway may regulate senescence in EPCs. Physical exercise and other factors that increase EPC levels are well established activators of the PI3K/Akt pathway (55), suggesting that this may be a common pathway to support EPC survival. Although this clearly does not rule out the possible importance of other signaling pathways, such as Janus kinase/signal transducer and activator of transcription; activation of Akt may be useful in improving stem/progenitor cell therapy. Interestingly, overexpression of Akt recently has been shown to increase the efficiency of mesenchymal stem cell therapy after myocardial ischemia in mice (68). In addition, the use of NOS3 knockout mice has demonstrated that the beneficial effects were dependent on a functional eNOS. Interestingly, while transient statin therapy exhibits a positive effect on EPC numbers, chronic continuous treatment with a high amount of statins inversely correlates with the EPC number in patients with coronary artery disease (69).

9. CONCLUSION

Atherosclerosis is a progressive disease caused by endothelial dysfunction and damage by various risk factors. EPC deficit contributes to the development of atherosclerosis. In atherosclerosis, EPC number and functionality are regulated, particularly during the process of vascular reparation. Further elucidation of the role of EPCs in atherosclerosis could potentially permit the development of novel strategies for prevention and therapy of pathological and vascular changes in atherosclerosis.

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Abbreviations: EPCs: Endothelial progenitor cells; ApoE-/-: apolipoprotein E-deficient; PI3K: phosphoinositide 3-kinase; RLPs: Remnant like particles; SDF-1alfa: stromal cell-derived factor-1alfa; CAD: coronary artery disease; OCN: osteoblastic marker osteocalcin; Hcy: Homocysteine; SDF: stromal cell-derived factor; eNOS: endothelial nitric oxide synthase; HHcy: hyperhomocysteinemia; rAAV: recombinant adeno-associated virus; PON1: paraoxonase-1

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