Role of Circulating Vascular Progenitors in Angiogenesis, Vascular Healing, and Pulmonary Hypertension
Lessons From Animal Models
Masataka Sata

Abstract—Accumulating evidence suggests that circulating progenitors contribute to vascular healing and remodeling under physiological and pathological conditions. Although there is growing enthusiasm for therapeutic and diagnostic application of bone marrow–derived progenitors, there are concerns that transplanted precursors or bone marrow cells may participate in the pathogenesis of unfavorable diseases such as cancer, retinopathy, and atherosclerosis. This review summarizes recent findings obtained from animal models to examine the roles of circulating vascular progenitor cells in angiogenesis, pulmonary hypertension, and vascular healing. (Arterioscler Thromb Vasc Biol. 2006;26:1008-1014.)

Key Words: stem cell ■ angiogenesis ■ cancer ■ regeneration ■ progenitor

In contrast to conventional assumption that damaged organs are repaired only by migration and proliferation of adjacent cells, accumulating evidence suggests that ectopic stem cells are mobilized into systemic circulation and recruited into the site of tissue regeneration. Recent reports documented that bone marrow–derived endothelial progenitor cells (EPCs) significantly contributed to neovascularization and re-endothelialization after acute vascular injury.1 Several animal and clinical studies demonstrated that transplantation of autologous EPCs or unfractionated bone marrow cells is effective for the treatment of ischemic cardiovascular diseases.2 On the other hand, others suggested that bone marrow cells or circulating progenitor cells could participate not only in maintenance of vascular homeostasis but also in the pathogenesis of various diseases. This review is intended to summarize recent findings on the roles of circulating vascular progenitor cells in angiogenesis, vascular remodeling, and pulmonary hypertension under physiological and pathological conditions. Because bone marrow–derived progenitors show great promise as therapeutic and diagnostic tools, several issues need to be clarified before these cells are widely used as an established strategy to treat patients with cardiovascular diseases.

Discovery of Putative Circulating EPCs
The integrity of the endothelial lining of the vasculature is essential for vascular homeostasis and normal organ function. Endothelial cells regulate vascular form and function and...
provide antithrombotic interface. Endothelial injury or dysfunction is assumed as an early event in the development of atherosclerosis. On endothelial injury, adjacent endothelial cells migrate, proliferate, and re-endothelialize the denuded luminal surface. At the sites of tissue ischemia or local inflammation, new blood vessels develop from the pre-existing vasculature. In addition to this traditional concept, recent evidence suggests that adult peripheral blood contains progenitors of endothelial cells. When peripheral circulating cells were cultured on fibronectin-coated dish in the presence of vascular endothelial growth factor, they changed morphology and started to express endothelial cell markers. Those EPCs were successfully incorporated into the site of angiogenesis under physiological and pathological conditions. EPCs were also shown to participate in re-endothelialization after vascular injury. After the discovery of putative EPCs, numerous studies have been done highlighting therapeutic applications of EPCs or bone marrow–derived progenitor cells to treat various cardiovascular diseases.

**Potential of EPCs to Accelerate Endothelial Healing**

First, therapeutic application of EPCs has been shown in acceleration of re-endothelialization. Seeding of autologous endothelial progenitors dramatically improved graft patency in decellularized vascular grafts and inhibited neointimal hyperplasia in prosthetic grafts. When spleen-derived EPCs were infused intravenously into the splenectomized mice after wire-mediated vascular injury, systemically applied EPCs homed to the injured artery, resulting in an enhanced re-endothelialization associated with decreased neointima formation. Similarly, human bone marrow–derived CD34−/CD14+ monocyte lineage cells activated by monocyte chemoattractant protein-1 adhered onto the luminal side of the injured artery, differentiated into endothelial-like cells, and inhibited neointimal hyperplasia. Local delivery of cultured EPCs to the balloon-injured carotid artery was associated with accelerated re-endothelialization, enhanced endothelium-dependent vasoreactivity, and reduced neointima formation. Notably, only 5% of endothelial cells were derived from the injected cells, although the re-endothelialized area was increased from 67±6% to 91±7% by administration of EPCs. It was hypothesized that transplanted EPCs might secrete several proangiogenic cytokines that stimulated migration and proliferation of adjacent endothelial cells in a paracrine manner.

Atheroprotective effects of bone marrow–derived EPCs were also demonstrated in hyperlipidemia-induced atherosclerosis. Chronic treatment with bone marrow–derived progenitor cells from young apolipoprotein E–deficient (apoE−/−) mice prevented atherosclerosis progression in apoE−/− recipients. Intravenously injected cells persistently and predominantly expressed an endothelial cell marker at atherosclerotic aorta. Together, these results suggest that local or systemic administration of EPCs or bone marrow cells may prevent vascular diseases by accelerating restoration of the endothelial lining and maintenance of vascular homeostasis.

**EPC Insufficiency as a Possible Cause of Vascular Diseases**

The reduced number of EPCs and consequent delayed vascular repair have been implicated in the pathogenesis of vascular diseases. Impairment of circulating EPCs has been documented under pathological conditions. Human EPCs from type II diabetic patients exhibited impaired proliferation, adhesion, and incorporation into vascular structures. Similarly, it was reported that active rheumatoid arthritis is associated with a depletion of circulating EPCs, which might be one of several factors contributing to the increased cardiovascular risk in rheumatoid arthritis. In human cardiac recipients, decrease in circulating EPCs was associated with allograft vasculopathy. Rauscher et al reported that circulating cells with vascular progenitor potential are decreased in the bone marrow of aged apoE−/− mice. Although chronic treatment with bone marrow–derived progenitor cells from young nonatherosclerotic apoE−/− mice prevented atherosclerosis progression in apoE−/− recipients despite persistent hypercholesterolemia, treatment with bone marrow cells from older apoE−/− mice having atherosclerosis was much less effective. These results suggest that circulating endothelial progenitors normally repair and rejuvenate the arteries and that progressive progenitor cell deficits and consequent delayed vascular healing may account for the pathogenesis of atherosclerosis.

**Therapeutic Application of EPCs to Maintain Pulmonary Vasculature**

Pulmonary hypertension is a refractory disease characterized by progressive increase in pulmonary artery pressure and resistance. Although the etiology of pulmonary hypertension appears to be heterogeneous, it is generally accepted view that the pulmonary vasculature initially undergoes persistent vasoconstriction and structural remodeling, leading to increased medial thickness of muscular arteries, peripheral extension of arterial muscularization, and increased matrix deposition. It was hypothesized that endothelial dysfunction or damage may trigger the pathogenesis of hypoxia-induced pulmonary hypertension. Consistently, transplantation of EPCs has been demonstrated to restore microvascular structure and function in rats and dogs. Nagaya et al reported that intravenously administered EPCs were incorporated into pulmonary arterioles. Transplantation of adrenomedullin–gene-transduced EPCs significantly improved pulmonary hypertension. Together, these results suggest that administration of EPCs can inhibit pulmonary hypertension and pulmonary arterial remodeling by accelerating endothelial healing of the damaged pulmonary arterioles.

**Enhancement of Collateral Development to Ischemic Tissues by EPCs**

The development of a vascular supply is essential not only for organ development but also for wound healing, tissue growth, and reproductive functions in the adult. Much effort has been devoted to understanding of molecular pathways that regulate angiogenesis. After the discovery of EPCs, a number of studies documented that transplantation of exogenous EPCs...
isolated from adult peripheral blood, cord blood, or total bone marrow cells augments collateral development to ischemic tissues. The number of incorporated cells with an endothelial phenotype into ischemic tissues is generally quite low. Ziegelmöller et al reported that bone marrow-derived cells do not incorporate into vessels. Nevertheless, the authors observed accumulations of bone marrow-derived cells around growing collateral arteries with expression of several growth factors and chemokines. The authors suggested that bone marrow-derived cells do not promote vascular growth by incorporating into vessel walls but may function as supporting cells. Similarly, it was reported that mobilization of bone marrow-derived cells enhances the angiogenic response to hypoxia through paracrine release of growth factors but not transdifferentiation into endothelial cells. It was hypothesized that the release of proangiogenic factors may influence the efficacy of neovascularization in autologous bone marrow transplantation. The mechanistic clarity of the positive effects of transplantation of EPCs or bone marrow cells is valid before these strategies are applied to a wide range of patients with ischemic diseases.

**Potential Participation of EPCs in Pathological Angiogenesis**

Angiogenesis is also implicated in the pathogenesis of a variety of disorders including diabetic retinopathies, tumors, rheumatoid arthritis, and psoriasis. EPCs have been shown to contribute to those pathological angiogenesis. Tumor angiogenesis is associated with recruitment of hematopoietic and circulating endothelial precursor cells. Impaired recruitment of bone marrow–derived endothelial and hematopoietic precursor cells blocked tumor angiogenesis and growth. Kaplan et al demonstrated that hematopoietic progenitors expressing vascular endothelial growth factor receptor 1 are required for the regulation of tumor metastasis. Moreover, injection of bone marrow cells promoted injury-associated retinal angiogenesis. Hu et al reported that endothelial cells of neointimal lesions in allografts are derived from circulating progenitor cells and that bone marrow–derived progenitors are responsible for formation of microvessels in transplant arteriosclerosis. Thus, there is a possibility that transplantation of endothelial progenitors or bone marrow cells may promote tumors, diabetic retinopathy, and atherosclerosis by augmenting disease-associated unfavorable angiogenesis in some patients (Figure 1). However, clinical studies demonstrated that the number of circulating EPCs correlated inversely with risk factors for coronary artery disease. Moreover, it was reported that the level of circulation EPCs predicts the occurrence of cardiovascular events. Thus, it is likely that physiological levels of circulating EPCs function to prevent atherosclerosis without promoting unfavorable angiogenesis.

**Detection of Bone Marrow–Derived Smooth Muscle–Like Cells in Vascular Lesions**

We and others suggested that circulating vascular progenitor cells could potentially participate in the pathogenesis of vascular diseases. The contribution of bone marrow cells to vascular lesions was first investigated in graft vasculopathy, a robust form of atherosclerosis that develops rapidly in transplanted organs. The majority of the neointima was composed of recipient cells. It was also observed that some of the medial smooth muscle cells as well as endothelial cells had been replaced by recipient cells. These results indicated that the majority of the neointimal cells derived from the recipient cells but not from the medial cells of donor origin. Consistent with these observations, others independently reported that recipient cells were a major source of graft vasculopathy in aortic transplantation models. Moreover, it was demonstrated that most of the

**Contribution of Vasa Vasorum Neovascularization to Plaque Growth and Instability**

Recent evidence suggests that new vessel formation plays a pivotal role in the pathogenesis of atherosclerosis and in the neointima thickening. The vasa vasorum are microvasculature present in the adventitial layer of the vessel wall, presumably supplying nutrients to the vessel wall of large arteries. Extent of vasa vasorum neovascularization correlates with severity of atherosclerosis as determined by postmortem angiography and microcomputed tomography analysis. Microvessel density was increased in lesions with inflammation, intraplaque hemorrhage, and in thin-cap fibroatheromas. Microvessels at the base of the plaque independently correlated with plaque rupture, suggesting a contributory role for microvessels in plaque instability. These findings suggest that vasa vasorum neovascularization may account not only for plaque growth but also for plaque destabilization. Consistent with this hypothesis, Moulton et al reported that late-stage inhibition of angiogenesis with angiotatin reduced macrophage accumulation and progression of atherosclerosis, having beneficial effects on plaque stability.
neointimal cells and endothelial cells were derived from recipients in human transplant-associated arteriosclerosis after renal transplantation.56,57

To investigate the potential source of the recipient cells that contribute to graft vasculopathy, bone marrow chimeric mice were used as recipients of cardiac transplantation.5 We documented that recipient bone marrow cells substantially contributed to neointimal formation in transplanted cardiac allografts. Contribution of bone marrow cells was also documented in the process of vascular healing and lesion formation after mechanical injury.5,58 At 1 week after severe injury induced by insertion of a large wire, bone marrow–derived cells were observed to attach to the luminal side of the injured vessels. Bone marrow–derived cells did not express a marker for smooth muscle cells or that for endothelial cells at these time points. The dilated lumen gradually narrowed because of neointimal hyperplasia that contained bone marrow–derived cells,5 some of which expressed α-smooth muscle actin or CD31.5,58 In hyperlipidemia-induced atherosclerotic lesions, we also found that a significant amount of α-smooth muscle actin–positive cells derived from bone marrow.3 Similarly, Davie et al reported that circulating progenitor cells could be involved in vessel wall thickening in the setting of hypoxia-induced pulmonary hypertension.59 These results suggest that bone marrow–derived progenitors contribute not only to vascular healing but also to lesion formation under certain pathological conditions.

Characterization of Smooth Muscle Progenitor Cells

The molecular mechanism of mobilization, homing, and differentiation of putative smooth muscle–like progenitor cells remained to be clarified. There were few articles that described the phenotype of putative smooth muscle progenitor cells.17,60 It still remains unclear whether endothelial-like cells or smooth muscle–like cells differentiate from a common vascular progenitor. Deb et al reported that smooth muscle progenitors had high expression of β1 integrin, moderate expression of α1, low levels of αvβ3, and did not express αvβ5, β2, α2β1, or α4β1 integrins.61 In contrast, endothelial progenitors had high expression of α2β1, αv β3, αvβ5, β1, and α1 and minimal expression of α4β1. The authors suggested the potential importance of integrins in mediating adherence of smooth muscle progenitors to specific extracellular matrix both in vitro and in vivo. Given the diversity of smooth muscle–like cells observed in human lesions,62,63 it is likely that there are heterogeneous sources of smooth muscle–like progenitor cells.58,64 Future studies are required to identify the source of smooth muscle progenitors and the molecular signaling that dictates the recruitment of smooth muscle progenitors at the site of vascular repair and lesion formation.65,64

Injury-Dependent Recruitment of Progenitor Cells to Vascular Lesions

Numerous reports have demonstrated that neointimal cells are heterogeneous and that smooth muscle cells in vascular lesions are composed of cells of diverse origin.58,62,63 It was shown that the cellular constituents of a lesion differ depending on the type of vascular injury.58 In this study, 3 distinct types of mechanical injuries were compared in the same mouse whose bone marrow had been labeled. After wire-mediated endovascular injury, a significant number of the neointimal and medial cells derived from bone marrow. In contrast, marker-positive cells were seldom detected in the lesion induced by perivascular cuff replacement. Only a few bone marrow–derived cells could be detected in the neointima after ligation of the common carotid artery. These findings suggest that the mode of injury is crucial for the recruitment of bone marrow–derived cells to tissue remodeling and that bone marrow cells substantially contribute to lesion formation only when arteries are subjected to severe injuries. Therefore, circulating progenitors would be predicted to mainly contribute to vascular remodeling in humans when arteries are subjected to severe injuries, such as balloon angioplasty, transplantation, and plaque rupture. Consistent with this notion, an analysis of sex-mismatched bone marrow transplant subjects revealed that the recruitment of bone marrow–derived smooth muscle cells is more extensive in diseased compared with undiseased segments.65

Potential Contribution of Bone Marrow–Derived Cells to Vascular Diseases

Coronary angioplasty causes vessel wall injury followed by smooth muscle cell proliferation with subsequent abundant production of extracellular matrix. Transplant-associated arteriosclerosis is also considered a consequence of an immunologic attack against the allograft by the recipient. Similarly, various atherogenic substances, such as oxidized low-density lipoprotein,66 homocysteine,67 angiotensin II,68 and lipopolysaccharides,69 have been reported to induce vascular cell...
apoptosis, presumably initiating the earliest phase of lesion development in atherosclerosis.\textsuperscript{70} Therefore, neointima formation appears to be the sequence of healing process in response to vascular injuries. Bone marrow might be an additional source of vascular cells that contribute to vascular repair as suggested by numerous studies.\textsuperscript{5,12,58,71} However, putative progenitor cells may also participate in vascular lesion formation, when the abilities of bone marrow–derived cells to participate in vascular repair were impaired under the influence of various factors such as aging, diabetes mellitus, hypercholesterolemia, and smoking (Figure 2).\textsuperscript{46,47}

In this regard, several studies reported that unfractonated progenitors showed deleterious effects on atherosclerosis in some patients and animal models. A recent clinical trial with myocardial infarction patients has shown that granulocyte colony-stimulating factor (G-CSF) mobilization of stem cells and subsequent infusion of such cells improved cardiac performance and angiogenesis.\textsuperscript{72} However, this improvement was associated with an unexpectedly high rate of in-stent restenosis, which led to the premature termination of the trial. Rotmans et al reported that in vivo cell seeding with anti-CD34 antibodies successfully accelerated endothelialization but stimulated intimal hyperplasia in porcine arteriovenous expanded polytetrafluoroethylene grafts.\textsuperscript{73} Rauscher et al also noted that some of the transplanted bone marrow–derived progenitor cells were identified as nonendothelial cells, which potentially participate in inflammation and neointima formation when endothelial progenitors are depleted from bone marrow with aging.\textsuperscript{12} Similarly, transplantation of bone marrow cells or EPCs have been shown to accelerate atherosclerosis in apoE/−/− mice.\textsuperscript{74,75}

Conclusions

In summary, findings indicate potential utilities of EPCs or bone marrow–derived vascular progenitor cells for regenerative medicine. On the other hand, there remain difficulties to regulate their homing, differentiation, and proliferation to achieve optimal therapeutic benefits without serious adverse effects. Because the number and function of endothelial progenitors are potentially impaired in patients with vascular diseases,\textsuperscript{46,47} attention should be paid for transplantation of autologous bone marrow cells or circulating EPCs not to promote atherosclerosis. Because circulating progenitors or bone marrow cells afford great promise to regenerate damaged organs, cautions should be paid for their clinical use.

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