Regulation of Bone Marrow–Derived Vascular Progenitor Cell Mobilization and Maintenance

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Abstract—Cell therapy is a promising option for treating ischemic diseases and heart failure. Bone marrow–derived vasculogenic cells, including progenitor cells and proangiogenic cells, have been shown to augment the functional recovery after ischemia. However, cardiovascular diseases affect the functional activity of the endogenous progenitor cell pools. The local microenvironment, also termed the stem cell niche, provides essential cues that maintain stem and progenitor cell functions and direct cell fate decisions in the bone marrow. A disturbed niche might lead to cell dysfunction (eg, by exhaustion). In addition, the niche controls mobilization of the cells into the circulation. This review will discuss the impact of cardiovascular disease on stem cell niches and summarize strategies targeting the niche for mobilization of vasculogenic cells.


Key Words: angiogenesis ■ progenitor cells

Various experimental and clinical studies demonstrate that vasculogenic cells improve the functional recovery after ischemia and moderately restore heart function in patients with heart failure. The bone marrow contains different types of stem cells. Hematopoietic stem cells (HSC)/hematopoietic progenitor cells (HPC), defined as CD34\(^+\) cells in humans or c-kit\(^+\) Sca-1\(^-\) lin\(^-\) cells in mice, and mesenchymal stem cells have been successfully used to improve neovascularization and functional recovery in ischemic models. In addition, circulating hematopoietic or endothelial progenitor cells (EPC), which can be mobilized from the bone marrow, were shown to give rise to new blood vessels and provide beneficial effects in vivo.\(^1,2\) EPC were originally defined as cells expressing hematopoietic markers (such as CD34 or CD133) and the vascular endothelial growth factor (VEGF) receptor 2 (KDR); however, the true identity of EPC is still under debate. Several studies also used culture assays to ex vivo expand circulating EPC. Although most cells isolated with the short-term culture assays (“early” EPC assay; colony-forming unit assays by Hill et al, see review\(^3\)) express myeloid markers (and are subsumed as “proangiogenic cells” in the present article), a few cells can be expanded and resemble more mature endothelial cells (for review, see Yoder\(^4\)). Despite the discussions regarding the characterization and origins of the EPC, various different subsets of cells were shown to augment neovascularization after ischemia when applied therapeutically. However, risk factors for cardiovascular diseases such as diabetes and heart failure itself affect endogenous proangiogenic cells, as well as progenitor cells (for review, see Dimmeler and Leri\(^4\)), thereby reducing the efficacy of patient-derived cells for therapeutic purposes. Moreover, increasing evidence suggests that a decrease in stem cell function plays a primary role in the pathogenesis of multiple diseases.\(^5\) The stem cell microenvironment, also termed the stem cell niche, provides essential cues that maintain stem cell function and direct cell fate decisions in the bone marrow but also in organs containing tissue-resident stem cells. A disturbed stem cell niche might lead to stem cell dysfunction (eg, by exhaustion). In addition, the niche controls mobilization of the cells into the circulation. This review discusses the impact of cardiovascular diseases on stem cell niches and summarizes strategies targeting the niche for mobilization of vasculogenic cells.

Regulation of the Bone Marrow Stem Cell Niche

In the adult bone marrow, HSC are localized in 2 niches: the osteoblastic niche and the vascular niche (see Figure 1). The osteoblastic niche (also referred to as endosteal niche) is composed of osteoblasts and other mesenchymal-derived stromal cells (eg, reticular cells, fibroblasts, adipocytes), which create a supportive environment for stem cells to maintain their quiescence.\(^6\) The crucial regulatory function of the osteoblastic niche is supported by the findings that the depletion of osteoblasts reduced the number of HSC in the bone marrow, whereas stimulation of osteoblasts increases HSC numbers.\(^7\) Additional studies report that osteoblasts regulate the mobilization of HPC.\(^8\) The vascular niche is less well defined, but the sinusoidal endothelium within the bone marrow is believed to be specialized to guide HPC prolifer-
Progenitor cell maintenance and mobilization in the bone marrow are controlled by various cytokines, including colony-stimulating factors and various angiogenic cytokines. Cytokines can induce the release of progenitor cells by activating proteases (including, for example, metalloproteases such as matrix metalloprotease (MMP)-2 or -9, cathepsins, and neutrophil elastase) that cleave receptors mediating the retention of the cells in the bone marrow (Figure 2). Important receptor interactions include the integrin very late antigen 4, which interacts with vascular cell adhesion molecule 1. In addition, cytokine gradients mediate retention of the cells in the niche, as has been shown for the adhesion molecule 1. In addition, cytokine gradients mediate retention of the cells in the bone marrow circulation. 

**Impact of Cardiovascular Diseases on the Bone Marrow Niche**

Despite ample evidence that cardiovascular diseases affect cell functionality, the primary cause underlying the cell dysfunction is still unclear. The dysfunction of the cells may be due to a cell intrinsic defect, for example, one induced by high levels of oxidative stress. As such, the impairment of vasculogenic cells may have similar reasons as the endothelial dysfunction. Indeed, various risk factors well known to harm endothelial function also impair vasculogenic cell activity in part via similar molecules and signaling pathways. For example, reduced nitric oxide (NO) bioavailability contributes to both endothelial and progenitor cell dysfunction. However, proangiogenic cells and bone marrow–derived progenitor cells may also be affected by extrinsic factors provided by the local environment imposed by the niche or by systemic factors. Indeed, lack of NO in the niche was sufficient to induce a defective neovascularization response in vivo, although the transplanted bone marrow progenitor cells expressed endothelial NO synthase. Moreover, coculture of wild-type HPC with NO-deficient feeder layers reduced the number of repopulating HPC, indicating that NO provided by the local environment regulates progenitor cell functions. 

Acute ischemic diseases such as myocardial infarction or vascular trauma also affect the bone marrow stem cell niche and induce a transient mobilization of HPC, late out-growing EPC, and proangiogenic cells in animal models and in humans. The kinetic of CD34+ cell mobilization varies between the different studies, but overall a significant increase was seen, with a maximum between 24 hours and 7 days after onset of ischemia (for review, see Brunner et al). The ischemia-induced mobilization appears to be mediated by a variety of ischemia-induced cytokines that will be discussed in detail below. Furthermore, although only limited information is available, it might be that ischemic injury or cardiovascular disease affects the endosteal niche. Indeed, various stressors modulate bone remodeling and thereby provoke progenitor cell mobilization. In detail, adrenergic agonists were shown to indirectly stimulate osteoclast differentiation and promote the egress of stem/progenitor cells from bone marrow. This link might be particularly important during neurohumoral activation as it occurs in patients with heart failure. Patients with heart failure are characterized by dysregulated levels of circulating HPC and proangiogenic cells, as well as impaired progenitor cell functions in the bone marrow. Support for a modulation of the endosteal niche stems from epidemiological findings showing a higher incidence of osteoporosis in patients with heart failure. Therefore, one may speculate that a dysregulation of the osteoblast/osteoclast activity, which controls progenitor cell maintenance and mobilization in the endosteal stem cell niche, might causally contribute to the dysregulated levels and function of bone marrow–derived or circulating HPC and proangiogenic cells in heart failure. Candidates that may contribute to the dysregulated endosteal niche in heart failure may include the cytokine RANKL, which activates osteoclasts. However, heart failure also leads to changes of a variety of other circulating cytokines that might affect mobilization and maintenance of progenitor cells.
Mobilization of Cells From the Bone Marrow Niche

Colony-Stimulating Factors
Colony-stimulating factors such as granulocyte macrophage colony-stimulating factor (G-CSF) or granulocyte-monoocyte colony-stimulating factor are established mobilizers of HPC and are clinically used for reconstitution of hematopoiesis. G-CSF and granulocyte-monoocyte colony-stimulating factor also mobilize CD34⁺, CD133⁺ cells and cultured proangiogenic cells and improved the recovery after myocardial ischemia. On the basis of these experimental findings, clinical trials addressed whether G-CSF improves left ventricular ejection fraction in patients with acute myocardial ischemia. The results of these clinical trials have been variable and overall showed that G-CSF therapy appears rather safe but does not lead to a significant improvement of cardiac function (for review, see recent metaanalysis). The reason for the lack of clinical benefit may include that the design of the clinical trials might have been suboptimal because subanalysis indicated that very early treatment of patients with lower ejection fraction might provide some benefit. In addition, G-CSF was shown to reduce the activity of the homing receptor CXCR4, thereby potentially impairing the migration and retention of the mobilized progenitor cells and proangiogenic cells to the infarcted tissue. Despite the disappointing clinical results with G-CSF therapy, G-CSF was successfully used to mobilize CD34⁺ cells for cell therapy of patients with refractory angina, and the injection of G-CSF mobilized cells induced a better improvement of cardiac function in patients with acute myocardial infarction compared with G-CSF alone therapy. These trials indicate that G-CSF might be useful to augment the number of circulating progenitor cells for intravascular or intramyocardial injection. Furthermore, a recent experimental study showed that G-CSF effectively improved the recovery of cardiac function after myocardial infarction if coadmininstered with a CD26/DPP-IV inhibitor that reduces the cleavage of SDF-1 in the heart and thereby augments recruitment of progenitor cells. This approach is currently being tested in a first clinical trial.

SDF-1/CXCR4
SDF-1/CXCR4 signaling has been implicated as important axis in the bone marrow niche, regulating retention but also migration and mobilization of HPC (for review, see Lapidot et al). SDF-1, which is produced by ischemic tissue, seems to have an important role for mobilization of proangiogenic cells and recombinant SDF-1 or SDF-1 overexpression by using adenovirus-mediated gene delivery increased circulating HPC in murine models. However, SDF-1 is also highly expressed in the bone marrow around sinusoids and near the endostium and acts as a retention factor to maintain progenitor or stem cells in the bone marrow and CXCR4 expression in HPC is essential for stem cell quiescence. Therefore, CXCR4 antagonists such as AMD3100 were developed that block SDF-1/CXCR4 interaction and thereby enhance mobilization of progenitor cells. The mobilized population of HSC exhibited different intrinsic characteristics compared with those of HSC mobilized with G-CSF alone. Current experiment studies address whether AMD3100 might be useful to augment the recovery after myocardial ischemia (D. Losordo, personal communication). The crucial question is whether systemic AMD3100 application may exert negative effects via blocking the protective SDF-1/CXCR4 signaling in the heart. Indeed, AMD3100 prevented the protective effect of SDF-1 in experimental myocardial infarction.

VEGF and Other Proangiogenic Growth Factors
Increasing the levels of circulating VEGF by plasmids or by infusing recombinant protein enhanced the levels of circulating HPC and KDR⁺ cells in experimental models. Moreover, gene therapy with VEGF increased the number of circulating ex vivo cultured proangiogenic cells. Interestingly, it has been reported recently that VEGF (but not G-CSF) in combination with CXCR4 antagonists specifically mobilizes EPC and stromal progenitor cells, whereas HPC were only slightly increased. The authors of this study suggest that VEGF differentially affects VEGF-R1 expressing HPC compared with VEGF-R2 expressing EPC. Via activation of the VEGF-R1, VEGF induces HPC proliferation that is associated with a block of migration, whereas VEGF-R2 expressing EPC did not proliferate and were mobilized.

Other angiogenic cytokines that were shown to mobilize bone marrow–derived progenitor cells include angiopoietins. Thus, angiopoietin-1 overexpression by adenovirus-mediated gene delivery induced a delayed mobilization of VEGFR2⁺ cells and HPC in murine models. Furthermore, placenta-derived growth factor stimulates mobilization of VEGF-R1 expressing CD34⁺ cells or mouse Lin-Sca-1+c-Kit+ bone marrow-repopulating stem cells.

Erythropoietin
An alternative cytokine with a lower proinflammatory profile is erythropoietin (Epo). One of the main functions of the cytokine Epo is to stimulate the proliferation of early erythroid precursors and the differentiation of later precursors of the erythroid lineage. However, Epo has been shown to perform more functions than erythropoiesis. Epo significantly increased mobilization of circulating proangiogenic cells and Sca-1⁺ Flk-1⁺ progenitor cells in experimental models and stimulated the mobilization of CD34⁺CD45⁺ circulating progenitor cells in humans. Epo elicits a similar potency for the improvement of EPC mobilization as VEGF. In addition, Epo is also protective for cardiac myocytes after ischemia/reperfusion in patients with heart failure, because it decreases the number of apoptotic myocytes, thereby limiting infarct expansion and attenuating the deterioration of hemodynamic function after acute myocardial infarction. Moreover, anemia has been recognized as important comorbidity in patients with heart failure. These beneficial effects of Epo, which may override potential Epo-related side effects, such as elevated blood pressure and the incidence of thrombosis, lead to the initiation of clinical trials using recombinant Epo for the treatment of patients with large infarcts or cardiac ischemia.

Statins
Statins are cholesterol-lowering 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors with additional pleitropic
activities. In experimental mouse models, as well as in clinical trials, the onset of statin treatment was shown to increase the number of circulating HPC and proangiogenic cells (including cultured myeloid EPC).47-50 The increase in circulating vasculogenic cells was seen within days after treatment, and some studies suggest that this response is transient.51 The effects of statins on circulating vasculogenic cells were independent on cholesterol lowering and were shown to be at least in part dependent on endothelial NO synthase.47 Statins not only mobilized vasculogenic cells but also improved the survival and endothelial commitment.49

Treatment of mice or patients with acute myocardial infarction with high-dose statins improves recovery after ischemia and prolongs event-free survival. However, because statins have multiple protective effects (eg, on endothelial function), the causal contribution of vasculogenic cell mobilization to repair and recover after ischemia is difficult to dissociate.52

Exercise

Physical exercise is an important atheroprotective factor, which was shown to enhance the level of circulating proangiogenic cells and outgrowing EPC by various groups.53–55 The mechanisms by which exercise increase vasculogenic cells are not entirely clear. One may speculate that the induction of ischemia in muscles enhances hypoxia-induced cytokine levels such as VEGF in the circulation that subsequently induces mobilization of bone marrow–derived progenitor cells.55 Alternatively, a direct effect of increased blood flow in the bone marrow might be relevant. The latter possibility is underscored by the fact that mice lacking endothelial NO synthase did not show an augmented EPC mobilization after exercise.54

Other Pharmacological Strategies

Various other pharmacological interventions were shown to enhance the levels of proangiogenic cells. Estrogen was shown to increase circulating levels of several subsets of vasculogenic cells via activation of an MMP-9-dependent mobilization by the bone marrow.56,57 In addition, the antidiabetic and antiinflammatory peroxisome proliferator-activated receptor-γ agonists promote differentiation and mobilization of angiogenic progenitor cells and improve reendothelialization after vascular intervention.58,59 The age-dependent decline in vasculogenic cell number and activity was further prevented by growth-hormone-mediated increase of insulin-like growth-factor-1.60

Modulators of the Endosteal Niche

The first evidence that stimulation of cells composing the endosteal niche influences HPC growth and mobilization was provided by Calvi et al, showing that mice expressing the activated parathyroid hormone (PTH) receptor in osteoblastic cells increased HPC.61 Consistently, treatment of mice with PTH was shown to expand resident HPC, resulting in enhancement of G-CSF-induced mobilization.10,61 In the context of cardiovascular disease, PTH augments the number of recruited progenitor cells in the heart and improves the recovery of function after acute myocardial infarction in mice.62 Of note, PTH exhibits several other effects (eg, it acts as vasodilator); thus, it remains to be deciphered to what extent progenitor cell mobilization contributes to the beneficial effects seen.

The wingless (Wnt) morphogen family of signaling molecules has been identified as prominent regulators of the endosteal stem cell niche (Figure 1). Wnts were initially shown to augment HPC proliferation via canonical β-catenin-dependent signaling. However, long-term activation with Wnts induced stem cell exhaustion, indicating that Wnts act in a highly context dependent manner. Surprisingly, overexpression of the Wnt inhibitor Dickkopf-1 in osteoblast (thereby increasing local Dkk-1 concentrations in the endosteal niche) activated HPC cell cycling and induced a progressive decline in regenerative functions.63 Interestingly, short-term systemic application of recombinant Dkk-1 preferentially induced the mobilization of vasculogenic progenitor cells and cultured proangiogenic cells but did not increase systemic levels of neutrophils.64 The reason underlying the rather specific mobilization is unclear but may be related to the induction of cycling in subsets of HPC that, in analogy to the study by Pitchford et al,42 may interfere with mobilization. On a molecular level, Dkk-1 stimulates osteoclast activity and increased the cytokine RANKL,64 which was previously shown to induce progenitor cell mobilization via activating osteoclasts8 (Figure 1). Consistently, angiogenesis was augmented by transient treatment with recombinant Dkk-1, as well as by its downstream mediator RANKL.64

Open Questions

Ample experimental studies suggest that factors that induce mobilization of proangiogenic and bone marrow–derived progenitor cells augment the recovery after ischemia. However, the few clinical trials that used mobilizing growth factors such as G-CSF have been disappointing and it remains to be shown that endogenous mobilization of progenitor cells can be therapeutically used in patients with myocardial infarction or heart failure. Of note, various atheroprotective strategies (eg, exercise, statins) augment the number of circulating proangiogenic cells indicating that the increased mobilization may contribute to the vasculoprotective effects of these therapies. However, most if not all of these approaches have additional direct effects on the vasculature, and it is difficult to determine the specific contribution of vasculogenic cell mobilization to the observed clinical benefits.

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