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Review

Post-natal endothelial progenitor cells for neovascularization in tissue regeneration

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Abstract

The isolation of endothelial progenitor cells (EPCs) derived from bone marrow (BM) was an outstanding event in the recognition of 'de novo vessel formation' in adults occurring as physiological and pathological responses. The finding that EPCs home to sites of neovascularization and differentiate into endothelial cells (ECs) in situ is consistent with 'vasculogenesis', a critical paradigm well described for embryonic neovascularization, but proposed recently in adults in which a reservoir of stem or progenitor cells contributes to vascular organogenesis. EPCs have also been considered as therapeutic agents to supply the potent origin of neovascularization under pathological conditions. This review provides an update of EPC biology as well as highlighting their potential use for therapeutic regeneration.

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1. Introduction

Tissue regeneration by somatic stem/progenitor cells has been recognized as a maintenance or recovery system of many organs in adult. The isolation and investigation of these somatic stem/progenitor cells has described how these cells contribute to postnatal organogenesis. On the basis of the regenerative potency, these stem/progenitor cells are expected to develop as a key strategy of therapeutic applications for the damaged organs.

Recently endothelial progenitor cells (EPCs) have been isolated from adult peripheral blood (PB). EPCs are considered to share common stem/progenitor cells with hematopoietic stem cells and have been shown to derive from bone marrow (BM) and to incorporate into foci of physiological or pathological neovascularization. The finding that EPCs home to sites of neovascularization and differentiate into endothelial cells (ECs) in situ is consistent with 'vasculogenesis', a critical paradigm well described for embryonic neovascularization, but recently proposed in adults in which a reservoir of stem/progenitor cells contributes to post-natal vascular organogenesis. The discovery of EPCs has therefore drastically changed our understanding of adult blood vessel formation. The following review provides an update of EPC biology as well as highlighting their potential utility for therapeutic vascular regeneration.

2. Post-natal neovascularization

Through the discovery of EPCs in PB [1,2], our understanding of post-natal neovascularization has been expanded from angiogenesis to angio/vasculogenesis. As previously described [3], post-natal neovascularization was

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originally recognized to be constituted by the mechanism of 'angiogenesis', which is neovessel formation, operated by in situ proliferation and migration of preexisting endothelial cells. However, the isolation of EPCs resulted in the addition of the new mechanism, 'vasculogenesis', which is de novo vessel formation by in situ incorporation, differentiation, migration, and/or proliferation of BM-derived EPCs [4] (Fig. 1). More recently, tissue specific stem/progenitor cells with the potency of differentiation into myocytes or ECs were isolated in skeletal muscle tissue of murine hindlimb, although the origin remains to be clarified [5]. This finding suggests that the origin of EPCs may not be limited to BM, e.g. tissue specific stem/progenitor cells possibly provide 'in situ EPCs' as other sources of EPCs than BM.

In the event of minor scale neovessel formation, i.e. slight wounds or burns, 'in situ preexisting ECs' causing post-natal angiogenesis may replicate and replace the existing cell population sufficiently, as ECs exhibit the ability for self-repair that preserves their proliferative activity. Neovascularization through differentiated ECs, however, is limited in terms of cellular life span (Hayflick limit) and their inability to incorporate into remote target sites. In the case of large scale tissue repair, such as patients who experienced acute vascular insult secondary to burns, coronary artery bypass grafting (CABG), or acute myocardial infarction [6,7], or in physiological cyclic organogenesis of endometrium [4], BM-derived or in situ EPC kinetics are activated under the influence of appropriate cytokines, hormones and/or growth factors through

the autocrine, paracrine, and/or endocrine systems. Thus the contemporary view of tissue regeneration is that neighboring differentiated ECs are relied upon for vascular regeneration during a minor insult, whereas tissue specific or BM-derived stem/progenitor cells bearing EPCs/ECs are important when an emergent vascular regenerative process is required (Fig. 1).

3. Profiles of EPCs in adults

3.1. The evidence of circulating EPCs in adults

In the embryo, evidence suggests that hematopoietic stem cells (HSCs) and EPCs [8,9] are derived from a common precursor (hemangioblast) [10,11]. During embryonic development, multiple blood islands initially fuse to form a yolk sac capillary network [12], which provides the foundation for an arteriovenous vascular system that eventually forms following the onset of blood circulation [8]. The integral relationship between the cells which circulate in the vascular system (the blood cells) and those principally responsible for the vessels themselves (ECs) is suggested by their spatial orientation within the blood islands; those cells destined to generate hematopoietic cells are situated in the center of the blood island (HSCs) while EPCs or angioblasts are located at the periphery of the blood islands. In addition to this arrangement, HSCs and EPCs share common antigens, including CD34, Vascular



Fig. 1. Post-natal neovascularization in the physiological or pathological events is consistent with neovessel formation contributed by angiogenesis and vasculogenesis at the various rates between their two mechanisms. Angiogenesis and vasculogenesis are due to the activations of in situ ECs and BM-derived or in situ EPCs, respectively.

endothelial growth factor receptor-2 (VEGFR2), Tie-2, CD117, and stem cell antigen-1 (Sca-1) [13].

The existence of HSCs in the PB and BM, and the demonstration of sustained hematopoietic reconstitution with HSC transplantation led to the idea that a closely related cell-type, namely EPCs, may also exist in adult tissues. Recently, EPCs were successfully isolated from circulating mononuclear cells (MNCs) using VEGFR2, CD34, and CD133 antigens shared by both embryonic EPCs and HSCs [1,14,15]. In vitro, these cells differentiate into endothelial lineage cells, and in animal models of ischemia, heterologous, homologous, and autologous EPCs have been shown to incorporate into the foci of neovasculature, contributing to neovascularization. Recently, similar studies with EPCs isolated from human cord blood have demonstrated their analogous differentiation into ECs in vitro and in vivo [16–19].

These findings have raised important questions regarding fundamental concepts of blood vessel growth and development in adults. Does the differentiation of EPCs in situ (vasculogenesis) play an important role in adult neovascularization, and would impairments in this process lead to clinical diseases? There is now a strong body of evidence suggesting that vasculogenesis in fact significantly contributes to postnatal neovascularization. Recent studies with animal BM transplantation (BMT) models in which BM (donor)-derived EPCs could be distinguished have shown that the contribution of EPCs to neovessel formation may range from 5 to 25% in response to granulation tissue formation [20] or growth factor-induced neovascularization [21]. Also, in the tumor neovascularization, the range is approximately 35-45% higher than the former events [22]. The degree of EPC contribution to post-natal neovascularization is predicted to depend on each neovascularizing event or disease.

3.2. Isolation of EPCs in circulation

Under the current status, it is impossible to differentiate 'immature EPCs' from primitive HSCs, as those cells share common surface markers, i.e. AC133, CD34, or VEGFR2 as described above. In circulation, the cell population with the capacity of differentiation to EPCs is considered to be included in the cell population expressing AC133 and VEGFR2 markers in the subset of CD34 positive cells [15]. Circulating EPCs are constitutively expressing stem/progenitor markers, i.e. CD34 or VEGFR2 except AC133, and start expressing endothelial lineage specific markers, VE cadherin or E-selectin. On the other hand, following the commitment and differentiation to hematopoietic stem/progenitor cells, the surface markers of AC133 and VEGFR2 are extinguished. Such stem/progenitor cell markers do not express on the differentiated hematopoietic cells. Alternatively, kinds of surface markers are expressed to characterize individual hematopoietic cell populations. AC133 is a marker to differentiate immature EPCs or primitive HSCs from circulating EPCs. To differentiate EPCs from hematopoietic stem/progenitor cells, VEGFR2, VE cadherin, or E-selectin are useful. Also, circulating EPCs do not express monocyte or myeloid markers, such as CD14 or CD15. Accordingly, circulating EPCs may be isolated via selection by the antigenicity of CD34, VEGFR2, and/or VE cadherin and also circulating immature EPCs by AC133 (Fig. 2).

3.3. Diverse identification of human EPCs and their precursors

Since the initial report of EPCs [1,2], a number of groups have set out to define this cell population better. Because EPCs and HSCs share many surface markers, and no simple definition of EPCs exists, various methods of EPC isolation have been reported [1,2,15–18,23–31]. The term EPC may therefore encompass a group of cells that exist in a variety of stages ranging from hemangioblasts to fully differentiated ECs. Although the true differentiation lineage of EPCs and their putative precursors remains to be determined, there is overwhelming evidence in vivo that a population of EPCs exists in human.

Lin et al. cultivated peripheral MNCs from patients receiving gender-mismatched BMT and studied their growth in vitro. In this study, they identified a population of BM (donor)-derived ECs with high proliferative potential (late outgrowth); these BM cells likely represent EPCs [24]. Gunsilius et al. investigated a chronic myelogenous leukemia model and disclosed that BMderived EPCs contribute to postnatal neovascularization in human [26]. Interestingly, in the report, BM-derived EPCs could be detected even in the wall of quiescent vessels without neovascularization events. This finding suggests that BM-derived EPCs may relate even to the turnover of ECs consisting of quiescent vessels.

Reyes et al. have recently isolated multipotent adult progenitor cells (MAPCs) from BM MNCs, differentiated them into EPCs and proposed MAPCs as an origin of EPCs [22]. These studies therefore provide evidence to support the presence of BM-derived EPCs that take part in neovascularization. Also, as described above, the existence of 'in situ EPCs' as derived from tissue specific stem/ progenitor cells in murine skeletal muscle remains to be investigated also in the other tissues [5] (Fig. 2).

4. EPC kinetics in adults

4.1. EPC kinetics effected by endogenous agents

The incorporation of BM-derived EPCs into foci of physiological and pathological neovascularization has been demonstrated through various animal experiments. One well-established model that allows the detection of BM-



Fig. 2. Origin and differentiation of EPCs in adult BM. EPCs are thought to differentiate not only from putative hemangioblasts, common precursor cells with HSCs, as previously described, but also from MAPCs. Representative antigenicities to stem/progenitor cells are shown (+, positive; -, negative).

derived EPCs includes transplanting wild-type mice with BM cells harvested from transgenic mice in which LacZ expression is regulated by an EC lineage-specific promoter, flk-1 or Tie-2 (flk-1/lacZ/BMT, Tie-2/lacZ/BMT). Using such mice, flk-1- or Tie-2-expressing endothelial lineage cells derived from BM (EPCs) have been shown to localize to vessels during tumor growth, wound healing, skeletal and cardiac ischemia, corneal neovascularization, and endometrial remodeling following hormone-induced ovulation [4].

Tissue trauma causes mobilization of hematopoietic cells as well as pluripotent stem or progenitor cells from the hematopoietic system [32]. Consistent with the notion that EPCs and HSCs share a common ancestry, recent data from our laboratory have shown that mobilization of BMderived EPCs constitutes a natural response to tissue ischemia. The aforementioned murine BMT model also provided direct evidence of enhanced BM-derived EPC incorporation into foci of corneal neovascularization following the development of hindlimb ischemia [33]. This finding indicates that circulating EPCs are mobilized endogenously in response to tissue ischemia and can incorporate into neovascular foci to promote tissue repair. These results in animals were recently confirmed by human studies illustrating EPC mobilization in patients following burns [6], CABG, or acute myocardial infarction [7].

As previous studies demonstrated, endogenous mobiliza-

tion of BM-derived EPCs, we considered exogenous mobilization of EPCs as an effective means of augmenting the resident population of EPCs/ECs. Such a strategy is appealing for its potential to overcome the endothelial dysfunction or depletion that may be associated with older, diabetic, or hypercholesterolemic patients. Granulocyte macrophage colony-stimulating factor (GM-CSF) is well known to stimulate hematopoietic progenitor cells and myeloid lineage cells, but has recently been shown to exert a potent stimulatory effect on EPC kinetics. The delivery of this cytokine induced EPC mobilization and enhanced neovascularization of severely ischemic tissues and de novo corneal vascularization [33].

The exact mechanism by which EPCs are mobilized to the peripheral circulation remains unknown, but may mimic aspects of embryonic development. Vascular endothelial growth factor (VEGF), critical for angio/vasculogenesis in the embryo [34–36], has recently been shown to be an important stimulus of adult EPC kinetics. Our studies carried out first in mice [37] and subsequently in patients undergoing VEGF gene transfer for critical limb or myocardial ischemia [38] established that a previously unappreciated mechanism by which VEGF contributes to neovascularization is in part by mobilizing BM-derived EPCs. Similar modulation of EPC kinetics has been observed in response to other hematopoietic stimulators, such as granulocyte-colony stimulating factor (G-CSF) and stroma-derived factor-1 (SDF-1) [39].

4.2. EPC kinetics effected by exogenous agents

EPC mobilization has recently been implicated not only by natural hematopoietic or angiogenic stimulants but also by pharmacological agents. For instance, 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are known to rapidly activate Akt signaling in ECs, thereby stimulating EC bioactivity in vitro and enhancing angiogenesis in vivo [40]. Recent studies by Dimmeler et al. and our laboratory have demonstrated a novel function of statins by mobilizing BM-derived EPCs through the stimulation of the Akt signaling pathway [41–44]. Therefore this newly appreciated role of statins, along with their already well-established safety and efficacy on hypercholesterolemia, suggests that they can offer benefit in treating various forms of vascular diseases. On the other hand, some antiangiogenic agents, i.e. angiostatin or soluble flk-1, have been shown to inhibit BM-derived EPC kinetics, leading to tumor regression [45], as BMderived EPC kinetics is a critical factor for tumor growth, in terms of tumor neovascularization [46].

4.3. Clinical profile of EPC kinetics

There is a strong body of evidence to suggest that impaired neovascularization results in part from diminished cytokine production. However, endogenous expression of cytokines is not the only factor leading to impaired neovascularization. Diabetic or hypercholesterolemic animals—like clinical patients—exhibit evidence of dysfunction in mature endothelial cells. While the cellular dysfunction does not necessarily preclude a favorable response to cytokine replacement therapy, the extent of recovery in limb perfusion in these animals fails to reach that of control animals; this suggests another limitation imposed by a diminished responsiveness of EPCs/ECs [47–49].

The aging characterized by impaired neovascularization [50,51] might be associated with dysfunctional EPCs and defective vasculogenesis. Indeed, preliminary results from our laboratory indicate that transplantation of BM (including EPCs) from old mice into young mice led to minimal neovascularization in a corneal micropocket assay, relative to transplantation of young BM. We also demonstrated that EPCs from older patients with clinical ischemia had significantly less therapeutic effect in rescuing ischemic hindlimb of mice compared with those from younger ischemic patients [52]. These studies provide evidence to support an age-dependent impairment in vasculogenesis (as well as angiogenesis) that is heavily influenced by the EPC phenotype. Moreover, analysis of clinical data from older patients at our institution disclosed a significant reduction in the number of EPCs at baseline, as well as that in response to VEGF165 gene transfer [38]. Thus impaired EPC mobilization and/or activity in response to VEGF may contribute to the age-dependent defect in postnatal neovascularization. Recently Vasa et al. have further investigated EPC kinetics and their relationship to clinical disorders, showing that the number and migratory activity of circulating EPCs inversely correlates with risk factors for coronary artery disease, such as smoking, family history and hypertension [53]. On the basis of these findings, monitoring of BM-derived EPC kinetics in the patients with vascular diseases is expected to be valuable in the evaluation of lesion activity and/or therapeutic efficacy.

5. Therapeutic vasculogenesis

5.1. The potential of EPC transplantation

The regenerative potential of stem/progenitor cells is currently under intense investigation. In vitro, stem/ progenitor cells possess the capability of self-renewal and differentiation into organ-specific cell types. When placed in vivo, these cells are then provided with the proper milieu that allows them to reconstitute organ systems. The novel strategy of EPC transplantation (cell therapy) may therefore supplement the classic paradigm of angiogenesis developed by Folkman and colleagues. Our studies indicated that cell therapy with culture-expanded EPCs can successfully promote neovascularization of ischemic tissues, even when administered as 'sole therapy,' i.e. in the absence of angiogenic growth factors. Such a 'supply-side' version of therapeutic neovascularization in which the substrate (EPCs/ECs) rather than ligand (growth factor) comprises the therapeutic agent, was first demonstrated by intravenously transplanting human EPCs to immunodeficient mice with hindlimb ischemia [25]. These findings provided novel evidence that exogenously administered EPCs rescue impaired neovascularization in an animal model of critical limb ischemia. Not only did the heterologous cell transplantation improve neovascularization and blood flow recovery, but also led to important biological outcomes-notably, the reduction of limb necrosis and auto-amputation by 50% in comparison with controls. A similar strategy applied to a model of myocardial ischemia in the nude rat demonstrated that transplanted human EPCs localize to areas of myocardial neovascularization, differentiate into mature ECs and enhance neovascularization. These findings were associated with preserved left ventricular (LV) function and diminished myocardial fibrosis [54]. Murohara et al. reported similar findings in which human cord blood-derived EPCs also augmented neovascularization in a hindlimb ischemic model of nude rats, followed by in situ transplantation [17].

More recently, other researchers have explored the therapeutic potential of freshly isolated human CD34+ MNCs (EPC-enriched fraction). Shatteman et al. conducted local injection of freshly isolated human CD34+ MNCs into diabetic nude mice with hindlimb ischemia, and showed an increase in the restoration of limb flow [29].

Similarly Kocher et al. attempted intravenous infusion of freshly isolated human CD34+ MNCs into nude rats with myocardial ischemia, and found preservation of LV function associated with inhibition of cardiomyocyte apoptosis [55]. Thus two approaches of EPC preparation (i.e. both cultured and freshly-isolated human EPCs) may provide therapeutic benefit in vascular diseases, but as described below, will likely require further optimization of techniques to acquire the ideal quality and quantity of EPCs for EPC therapy (Fig. 3).

5.2. Future strategy of EPC cell therapy

Ex vivo expansion of EPCs cultured from PB-MNCs of healthy human volunteers typically yields 5.0×10^6 cells per 100 ml of blood on day 7. Our animal studies [25] suggest that heterologous transplantation requires systemic injection of $0.5-2.0 \times 10^4$ human EPCs/g body weight of the recipient animal to achieve satisfactory reperfusion of an ischemic hindlimb. Rough extrapolation of these data to human suggests that a blood volume of as much as 12 1 may be necessary to obtain adequate numbers of EPCs to treat critical limb ischemia in patients. Therefore, the fundamental scarcity of EPCs in the circulation, combined with their possible functional impairment associated with a variety of phenotypes in clinical patients, such as aging, diabetes, hypercholesterolemia, and homocyst(e)inemia (vide infra), constitute major limitations of primary EPC transplantation. Considering autologous EPC therapy, certain technical improvements that may help to overcome the primary scarcity of a viable and functional EPC population should include: (1) local delivery of EPCs, (2) adjunctive strategies (e.g. growth factor supplements) to promote BM-derived EPC mobilization [33,37], (3) enrichment procedures, i.e. leukapheresis or BM aspiration, or (4) enhancement of EPC function by gene transduction (gene modified EPC therapy, vide infra), (5) culture-expansion of EPCs from self-renewable primitive stem cells in BM or other tissues. Alternatively, unless the quality and quantity of autologous EPCs to satisfy the effectiveness of EPC therapy may be acquired by the technical improvements described above, allogenic EPCs derived from umbilical cord blood or culture-expanded from human embryonic stem cells [17,56], may be available as the sources supplying EPCs.

5.3. Gene modified EPC therapy

A strategy that may alleviate potential EPC dysfunction in ischemic disorders is considered reasonable, given the findings that EPC function and mobilization may be impaired in certain disease states. Genetic modification of EPCs to overexpress angiogenic growth factors, to enhance signaling activity of the angiogenic response, and to



Fig. 3. EPC therapy using autologous EPCs derived from BM for vascular regeneration. Transplantation of BM- or mobilized PB-MNCs are considered 'crude EPC therapy', as EPCs are not selected. BM-MNCs have already been under clinical application. Following the manipulation to acquire the optimized quality and/or quantity, e.g. sorting by surface markers, ex vivo culture-expansion and/or gene transfection, EPC therapy is expected to be the useful strategy for vascular regeneration.

rejuvenate the bioactivity and/or extend the life span of EPCs, can constitute such potential strategies.

We have recently shown for the first time that genemodified EPCs rescue impaired neovascularization in an animal model of limb ischemia [57]. Transplantation of heterologous EPCs transduced with adenovirus encoding human VEGF165 not only improved neovascularization and blood flow recovery, but also had meaningful biological consequences, i.e. limb necrosis and auto-amputation were reduced by 63.7% in comparison with controls. Notably, the dose of EPCs needed to achieve limb salvage in these in vivo experiments was 30 times less than that required in the previous experiments involving unmodified EPCs [25]. Thus, combining EPC cell therapy with gene (i.e. VEGF) therapy may be one option to address the limited number and function of EPCs that can be isolated from peripheral blood in patients.

5.4. BM-MNC transplantation

Nonselected total BM cells or BM-MNCs including immature EPC population have also been investigated for their potential to induce neovascularization. Several experiments have reported that autologous BM administration into rabbit [58] or rat [59] hindlimb ischemic model, and porcine myocardial ischemic model [60,61] could augment neovascularization in ischemic tissue mainly through the production of angiogenic growth factors and less through the differentiation of a portion of the cells into EPCs/ECs in situ. Although there are no long-term safety and efficacy data for local delivery of such cell population mostly composed of inflammatory leukocytes, these strategies have already been applied to clinical patients in some institutions and preliminary results are expected soon.

6. Other devices of EPCs for clinical application

EPCs have recently been applied to the field of tissue engineering as a means of improving biocompatibility of vascular grafts. Artificial grafts first seeded with autologous CD34+ cells from canine BM and then implanted into the aortae were found to have increased surface endothelialization and vascularization compared with controls [62]. Similarly, when cultured autologous ovine EPCs were seeded onto carotid interposition grafts, the EPCseeded grafts achieved physiological motility and remained patent for 130 days versus 15 days in non-seeded grafts [63]. Alternatively, as previously reported, the cell sheets of cultured cardiomyocytes may be effective for the improvement of cardiac function in the damaged hearts, i.e. ischemic heart disease or cardiomyopathy [64,65]. The cell sheets consisting of cardiomyocytes with EPCs expected to induce neovessels may be attractive, as blood supply is essential to maintain the homeostasis of implanted cardiomyocytes in such cell sheets.

EPCs have also been investigated in the cerebrovascular field. Embolization of the middle cerebral artery in Tie2/lacZ/BMT mice disclosed that the formation of new blood vessels in the adult brain after stroke involves vas-culogenesis/EPCs [66]. Similar data were reported using gender-mismatched wild-type mice transplanted with BM from Green Fluorescein Protein transgenic mice [67]. However, whether autologous EPC transplantation would augment cerebral revascularization has yet to be examined.

To date, the role of EPCs in tumor angiogenesis has been demonstrated by several groups. Davidoff et al. showed that BM-derived EPCs contribute to tumor neovasculature and that BM cells transduced with an anti-angiogenic gene can restrict tumor growth in mice [45]. Lyden et al. recently used angiogenic defective, tumor resistant Id-mutant mice and showed the restoration of tumor angiogenesis with BM (donor)-derived EPCs throughout the neovessels following the transplantation of wild-type BM into these mice [46]. These data demonstrate that EPCs are not only important, but also critical, to tumor neovascularization. Given the findings, 'anti-tumor EPC mediated gene therapy' by transplantation of EPCs transferred genes to inhibit tumor growth may be developed in the near future.

Orlic et al. recently demonstrated that lineage marker negative (non-committed) and CD117 positive BM cells can regenerate de novo myocardium and ECs and improve cardiac function when they were locally delivered into murine myocardial infarction model [68]. They also reported that mobilization of BM cells by G-CSF and stem cell factor leads to a reduction in infarct size, improves cardiac function and decreases the mortality in this animal model [69]. Jackson et al. showed that BM-derived stem cells (side population cells defined by dye exclusion) can differentiate into cardiomyocytes and ECs at a very low rate in murine cardiac reperfusion injury model following BMT [70]. These studies suggest a clinical use of BM for cardiovascular diseases other than EPCs/therapeutic vasculogenesis. Given the extensive plasticity of BM cells differentiating into neural, hepatic and mesenchymal lineages, BM-derived EPCs may also exhibit such a potential, as seen in the report suggesting the transdifferentiation of endothelial lineage cells into cardiomyocytes [71].

7. Conclusion

As the concepts of BM-derived EPCs in adults and postnatal vasculogenesis are further established, clinical applications of EPCs to regenerative medicine are likely to follow. To acquire the more optimized quality and quantity of EPCs, several issues remain to be addressed, such as the development of a more efficient method of EPC purification and expansion, the methods of administration and senescence in EPCs. Alternatively, in the case of impossible utility of autologous BM-derived EPCs in the patients with impaired BM function, an appreciable number of EPCs isolated from umbilical cord blood or differentiated from tissue specific stem/progenitor or embryonic stem cells need to be optimized for EPC therapy. However, the unlimited potential of EPCs along with the emerging concepts of autologous cell therapy with gene modification suggests that they may soon reach clinical fruition.

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