Endothelial Progenitor Cells: Therapeutic Perspective for Ischemic Stroke

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SUMMARY

Endothelial progenitor cells (EPCs), which can be cultured in vitro from mononuclear cells in peripheral blood or bone marrow, express both hematopoietic stem cell and endothelial cell markers on their surface. They are believed to participate in endothelial repair and postnatal angiogenesis due to their abilities of differentiating into endothelial cells and secreting protective cytokines and growth factors. Mounting evidence suggests that circulating EPCs are reduced and dysfunctional in various diseases including hypertension, diabetes, coronary heart disease and ischemic stroke. Therefore, EPCs have been documented to be a potential biomarker for vascular diseases and a hopeful candidate for regenerative medicine. Ischemic stroke as the major cause of disability and death still has limited therapeutics based on the approaches of vascular recanalization or neuronal protection. Emerging evidence indicates that transplantation of EPCs is beneficial for the recovery of ischemic cerebral injury. EPC-based therapy could open a new avenue for ischemic cerebrovascular disease. Currently, clinical trials for evaluating EPC transfusion in treating ischemic stroke are underway. In this review, we summarize the general conceptions and the characteristics of EPCs, and highlight the recent research developments on EPCs. More importantly, the rationale, perspectives and strategies for using them to treat ischemic stroke will be discussed.

Keywords

Endothelial progenitor cells; Angiogenesis; Ischemia stroke; Transplantation; Stem cell therapy

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Conflict of Interest

The authors have no conflicts of interest.
Introduction

Stroke is the fourth leading cause of death in the United States. According to the updated statistics reported by American Heart Association, there are about 795,000 new and recurrent stroke patients and 134,100 deaths each year in the United States [1]. The burden of stroke is even higher in China, Africa, and South America [2]. Ischemic stroke accounts for about 85% of all stroke events. Thrombogenesis and embolism in the intracranial artery are the two major causes of ischemic stroke. Earlier recanalization with following reperfusion constructs the foundation for conserving brain tissue under acute ischemia. Current recanalization therapies for acute ischemic stroke mainly include intravenous or intra-arterial fibrinolysis [3, 4] and interventional treatments, such as percutaneous transluminal angioplasty and stenting (PTAS) and thrombectomy [5, 6]. Although the fibrinolytics and interventional managements have achieved certain benefits, these therapies have several limitations. Intravenous thrombolysis with recombinant tissue-type plasminogen activator (rt-PA) or alteplase has a narrow therapeutic time window (3-4.5 hours) [3, 7]. The interventional PTAS has a high rate (20.0%) of re-stroke within the first year [5]. On the other hand, antiplatelets are also commonly used for treating ischemic stroke.

Although numerous animal studies on neuroprotective drugs have shown promising data in treating ischemic stroke, clinical trials testing these drugs revealed disappointing results [8]. Current treatments for acute ischemic stroke mainly rely on vascular recanalization. However, approaches for promoting cerebral recovery following ischemic stroke are limited. Emerging studies document the beneficial role of different stem/progenitor cells in accelerating cerebral recovery after ischemic stroke, such as bone marrow (BM) stem cells [9], mesenchymal stem cells (MSCs) [10], neural stem cells [11] and endothelial progenitor cells (EPCs) [12]. EPCs probably have a great potential in cerebrovascular disease because of their unique characteristics [13-15]. This article reviews the general conceptions and recent research progress of EPCs. Furthermore, the rationale, perspectives and strategies of EPC-based therapy for ischemic stroke will be discussed.

Definition, Identification and Characterization of EPCs

EPCs were first isolated from human peripheral blood in 1997, and defined as bone marrow (BM)-derived immature cells with the ability to differentiate into mature endothelial cells (ECs) [16, 17]. They are believed to originate from hematopoietic lineage, whereas their nonhematopoietic lineage origin is still in debate [18]. EPCs have been identified through several methods, such as colony formation assay in combination with specific biomarkers, fluorescence detection of acetylated low-density lipoprotein uptake and lectin binding, as well as flow cytometry technique based on their surface markers [16, 19]. The biomarkers used for characterizing EPCs include both hematopoietic stem cell markers (CD34 and CD133) and endothelial cell markers, such as CD31, kinase insert domain receptor (KDR, VEGFR2), Von Willebrand factor (vWF), vascular endothelial cadherin (VE-cadherin or CD 144), Tie2, c-kit/CD117 and CD62E (E-selectin) [19-22]. In addition, CD45, CXCR4, CXCR2 and CCR2 are also expressed on EPCs [23]. The CD34+KDR+ antigenic combination appears to be of high sensitivity and specificity and has been often used for
EPC identification [20]. It was noticed that EPCs from different sources express different surface markers. For example, both bone-marrow derived EPCs (BMEPCs) and cord-blood derived EPCs (CB-EPCs) have been shown to express the CD105, CD73 and CD34 markers [24]. The markers CD31, CD144, CD146 and KDR are positive on CB-EPCs, but are negative or weak on BM-EPCs.

Another study showed that peripheral blood derived EPCs (PB-EPCs) expressed KDR, CD144, vWF, Tie-2, CD31, CD11b and CD14 [13]. Nevertheless, for therapeutic and diagnostic purposes, more exact identification of EPCs might be desired.

Based on the culture characters, EPCs are mainly divided into two types: early EPCs and late EPCs [20, 21]. Early EPCs appear after short-term (4-10 days) culture of mononuclear cells (MNCs) from peripheral blood. They are similar to colony-forming unit endothelial cells (CFU-ECs). Early EPCs are spindle shape and display peak growth at 2-3 weeks and live up to 4 weeks. The late EPC or endothelial colony forming cells (ECFCs) can be found after long-term culture (>14 days) of MNCs. Late EPCs exhibit cobblestone shape, rapid growth at 4-8 weeks, and survive until 12 weeks. Studies suggest that EPCs promote angiogenesis and neovascularization by producing diverse growth factors which may mainly be secreted by early EPCs [21, 25-27]. The late EPCs have a higher expression level of VE-cadherin and KDR and are able to physically contribute to vascular regeneration [21, 28]. Genome-wide transcriptional profiling and protein electrophoresis methods reveal that these two types of EPCs have different gene expression signatures [29]. Early EPCs display a molecular phenotype linked to monocytes, whereas late EPCs highly express vascular development and angiogenesis-related signaling genes (Tie2, eNOS, Ephrins).

**EPCs Generation, Mobilization and Homing**

Generally, EPCs are adult stem cells generated from BM [17]. Most of EPCs quiescently lodge in a microenvironment within the BM, termed the stem cell niche [30]. They can be mobilized into the circulation and are able to colonize in endothelium [31, 32]. The mechanisms for this process have not been fully understood. The chemokine stromal-derived factor 1 (SDF-1)/CXCR4 axis has been well documented to play a key role in EPC mobilization in response to hypoxia or injury [33, 34]. At basal conditions, the level of SDF-1 is low in circulation, BM and other tissues [31, 35]. Upon tissue ischemia, hypoxia-inducible factor-1 (HIF-1) is up-regulated, which can activate its downstream factors, SDF-1 and vascular endothelial growth factor (VEGF) [33, 36]. Then, EPCs are mobilized from BM to circulation and migrate towards ischemic tissue following SDF-1 gradients. VEGF also induces SDF-1 expression which further promotes the process of EPC mobilization [37]. On the other hand, matrix metalloproteinase-9 (MMP-9) which is up-regulated by SDF-1 and VEGF partakes in the transformation of EPCs from quiescent to proliferative state in BM [38]. MMP-9 also promotes the mobilization of EPCs into the circulation by inducing the release of soluble Kit Ligand (sKitL), which can bind with the c-Kit expressed on EPC for facilitating the mobilization [31]. Granulocyte colony-stimulating factor (G-CSF) has been used to mobilize functional EPCs into the circulation of patients with coronary artery disease [39]. G-CSF induced EPC mobilization is associated with increased level of neutrophils in circulation, which could release VEGF [40]. Another study
showed that G-CSF stimulates the mobilization of hematopoietic progenitor cells through BM-neutrophils released elastase and cathepsin G, which trigger proteolytic cleavage of vascular cell adhesion molecule-1 expressed by BM stromal cells [41]. In addition, numerous physiopathological and pharmacological stimuli have been shown to mobilize EPCs (Table 1).

The homing or recruitment of circulating EPCs (cEPCs) into injury or ischemic sites is an important process for executing their angiogenic and repairing function [42]. Both tissue factors and EPC surface receptors are involved in homing of EPCs (Table 1). For example, the SDF-1/CXCR4 axis plays a significant role in mediating EPC homing in ischemic tissue [34, 43, 44]. CXCR2 and its ligands, CXCL1 and CXCL7, have been shown to mediate EPC homing to injured arteries [23, 45]. Recently, the interaction of chemokine ligand CCL5 and its receptor CCR5 is suggested as a signal for EPC recruitment into wounded tissue [46]. The mobilization and homing of EPCs to injured blood vessels and ischemic tissue are important for them to participate in endothelial repair and contribute to postnatal angiogenesis (see below). Although there is no evidence showing that EPCs directly induce malignant tumorigenesis, EPC migrating to tumor tissue may have a risk in supporting tumor vascularization [47, 48]. The potential adverse effects of EPC-based therapy are detailed in the section of “Safety Respects of EPC-based Therap”.

Several technologies have been developed for tracking EPCs in vivo. For example, EPCs stained with DiI-Ac-LDL or radiolabeled with $^{111}$In-oxine have been used for tracking EPCs after injecting them into animals [13, 14, 49]. A recent study has used Dex-DOTA-Gd$^{3+}$ as a magnetic resonance imaging contrast agent for monitoring the anatomical migration and the survival period of transplanted EPCs in a rat model of hindlimb ischemia [50]. Hence, these methods provide useful approaches for supporting pre-clinical and clinical research on EPC-based therapy.

**Functional Characteristics of EPCs**

**EPCs Participate in Endothelial Homeostasis and Repair**—The abilities of EPCs to differentiate into mature ECs and secrete different protective cellular factors indicate that they play a significant role in endothelial homeostasis and repair. This notion is supported by solid evidence. For one thing, EPCs presenting in both vascular intima and circulation have been shown to participate in endothelialization and replacement of dysfunctional ECs [32, 65, 88, 89]. Secondly, reduction of cEPCs can independently predict the progress of atherosclerotic disease [20, 88]. More directly, transfusion of EPCs has been reported to reduce neo-intima formation in a vascular injury model [90], and to inhibit platelet activation and thrombogenesis in an arterial thrombosis model [91].

**EPCs Contribute to Angiogenesis**—Angiogenesis is necessary for blood vessel reconstruction and collateral circulation establishment, which are important to deliver nutrients and protectants to the jeopardized tissue for repair. The first finding of EPCs by Asahara et al. has initiated a new era in angiogenesis research [16, 17]. Thereafter, mounting evidence confirms the role of EPCs in angiogenesis. Both early and late EPCs have been suggested to participate in the process of angiogenesis. Early EPCs are involved in
angiogenesis by secreting an array of growth factors and cytokines, such as VEGF, SDF-1, IGF-1 and G-CSF, which can enhance EC proliferation, reduce cell apoptosis, and recruit endogenous progenitor cells [13, 21, 26]. Later evidence suggests that late EPCs may also have ability to secrete soluble factors to contribute to these processes [14]. These findings help to explain the why EPC-conditioned medium promotes neovascularization [25]. Moreover, late EPCs contribute to neovasculogenesis by differentiating into ECs [20, 21]. EPCs have been shown to account for up to 26% of all ECs in neovascularization [92]. On the other hand, the contribution of EPCs in angiogenesis has also been documented in the recovery processes of various diseases, such as myocardial ischemia [93, 94], limb ischemia [16, 34], ischemic stroke [12, 13] and wounds [95]. All these researches in animal models prelude the physiological function of EPCs, and highlight the potential of EPCs as a cell candidate for regenerative therapy.

EPCs for Treating Ischemic Stroke

1.) Pathophysiology of Ischemic Stroke

The pathophysiology of ischemic stroke involves complex processes such as energy failure, loss of cellular ion homeostasis, free radical-mediated and cytokine-mediated toxicities, inflammation, disruption of the blood-brain barrier (BBB) and infiltration of leukocytes. These events are interrelated and coordinated [96]. Upon ischemic stroke, cerebral damage occurs early and in a progressive fashion. Based on the time course, ischemic stroke can be roughly derived into acute (hours), subacute (hours to days) and chronic (days to months) phases [97]. The acute phase is manifested with BBB disruption and vascular tonus. Neutrophils adhere to the endothelium and produce superoxide anions by reacting with NO and can further trigger tissue damage and inflammation. Within the subacute phase, frank edema and injury appear. Multiple genes such as MMP-9, IL-1, VEGF, angiopoietin-2 are activated. In the chronic phase, limited endogenous angiogenesis and neurogenesis attempting for recovery are proceeding. Pathologically, ischemic areas include an infarct core and penumbra (peri-infarct area). Dead cells constitute the infarct core, which represents irreversible damage, whereas the penumbra is the rescuable area where the angiogenesis can develop [98, 99]. Thus, the penumbra is the target for reducing acute damage.

2.) Rationale for Using EPCs to Treat Ischemic Stroke

Level of cEPCs correlates with ischemic stroke

Mounting evidence advocates that the level of cEPCs is reduced in various stroke risk factors such as hypertension [76], hypercholesterolemia [79], diabetes [77, 78] and atherosclerosis [88]. The level of cEPCs has been manifested as an important biological marker to predict endothelial dysfunction, cardiovascular risk [88, 89, 100] and cerebrovascular events [101, 102]. Clinical studies show that acute stroke induces a transient increase of cEPCs [103] and the level of cEPCs negatively correlates with severity of ischemic damage [104, 105]. A higher level of CFU-ECs during the first week of stroke is shown to independently associate with a better outcome [106]. Current evidence supports
that EPCs not only serve as biomarker but also might offer a new therapeutic strategy for ischemic stroke [19, 42].

**EPCs Contribute to Neurovascular Protection, Angiogenesis and Neurogenesis**—As stated above, EPCs have been suggested to maintain endothelial protection/repair and angiogenesis. Further studies provide evidence that angiogenesis is coupled with neuroprotection and neurogenesis following ischemic injury [14, 107]. The underlying mechanisms include that the regenerated blood vessels provide nutritive blood flow and that EPCs, by secreting factors such as SDF-1 and VEGF, create a microenvironment for neural regeneration and survival [108, 109]. Furthermore, neuroblasts migrate along these regenerated vessels to achieve neurogenesis in peri-infarct area [107, 110, 111]. Therefore, suppression of angiogenesis substantially reduces migration of neuroblasts from the subventricular zone to the ischemic region [111].

3.) Transplantation of EPCs Accelerates Cerebral Repair Following Ischemic Stroke

The involvement of endogenous EPCs in cerebral neovascularization after ischemic stroke was first reported by Zhang et al. in 2002 [12]. However, EPCs are usually reduced in number and dysfunctional in disease conditions. Therefore, transfusion of exogenous EPCs could accelerate the repairing processes. Several transplantation studies on CD34+ cells (EPC-rich fraction) have shown their therapeutic effect in promoting new vessel formation and neurogenesis after ischemic stroke [112, 113]. Lately, injection of human ECFCs was shown to decrease cell apoptosis, promote angiogenesis and neurogenesis, and improve functional recovery [14]. It is also suggested that administration of EPCs can increase regional cortical blood flow, reduce infarct volume and neurological deficits in 2 days after stroke [114]. Our study demonstrates that EPCs are reduced in quantity and dysfunctional in db/db type-2 diabetic mice, which might account for decreased cerebral microvascular density and enlarged ischemic damage [15]. Infusion of functional EPCs reduces ischemic cerebral damage in db/db diabetic mice, which is associated with improvement in angiogenesis. A recent study demonstrates that labeled EPCs were found around microvessels in the cerebral ischemic boundary 24 hours after EPC transplantation, and improved long-term neurobehavioral outcomes of ischemic stroke [13]. Several studies demonstrated that EPCs could replace dysfunctional endothelium at the site of denuding injury [115-117]. All these studies indicate that EPCs could serve as a cellular reservoir for the replacement/repair of dysfunctional ECs in stroke and are promising stem cells for the treatment of ischemic stroke.

The beneficial effects of EPC-based therapy might come from several aspects (as shown in Figure 1). At the early stage of ischemic stroke, both injected and endogenous EPCs could protect cells (ECs and neurons) from ischemia-induced death/damage because EPCs secrete various growth factors such as VEGF, SDF-1, IGF-1. These factors also assist to recruit more EPCs and support their survival, while alleviating acute injury via protecting the function of neurovascular units and/or existing collateral blood vessels. In the later stage, EPCs working together with their secreted factors promote neovascularization and
neurogenesis, which functionally and structurally rebuild the BBB, blood vessels and neuron networks; in turn, contributing to the recovery.

Strategies of EPC-based Therapy for Ischemic Stroke

Administration of EPCs

The optimal starting time point for administration of EPCs following ischemic stroke may be important for the therapeutic efficacy. However, there is limited research on this aspect. Based on the ability of EPCs to secrete various growth factors which have protective effects on ECs and neurons, their application at the earlier stage of stroke may have better efficacy. However, it should be pointed out that inflammation, free radical-mediated and cytokine-mediated toxicities occurring in the acute phase of stroke may limit the function and survival of transplanted EPCs [96, 97, 118]. EPCs obtained from patients in the subacute phase of ischemic stroke have showed greater vasculogenic capacity than those from patients in the acute phase [119]. It remains to be determined whether administration of autologous EPCs in the subacute period is more effective. In regards to EPC administration in clinical settings, intravenous infusion should be the optimal route because intra-arterial infusion is inconvenient and could cause embolism and direct injection of stem cells into the brain is complex and might cause local hemorrhaging [120]. As for the dosing, administration of EPCs with the range of 0.2–3.0×10^4 per gram body weight have shown satisfactory efficacies in various animal models [13, 14, 18, 112]. The first on-going clinical trial on EPC-based therapy for ischemic stroke (Identifier: NCT01468064) is designed to intravenously apply 2.5×10^6 EPCs per kilogram body weight. It is also unclear regarding the ideal frequencies of EPC administration. The current clinical trial adopts two EPC transplantations one week after initial dosing.

A recent study on late EPCs raises perspective for the use of late EPCs as an optimal EPC-based therapy [14]. However, in this study, transplantation of early EPCs also led to similar improvement in modified neurological severity score and somatosensory scores up to 14 days after stroke. Another study showed infusion of early EPCs significantly reduced ischemic infarct volume at 3 days following stroke and enhanced the long-term outcome [13]. Which type of EPCs is more effective should be further investigated since the data from comparison of early or late EPCs is still elusive. Currently, co-administration of different types of progenitor/stem cells may constitute a novel therapeutic strategy for ischemic diseases [121].

Ex Vivo Modification of EPCs before Administration

In order to enhance the therapeutic effect, EPC modifications such as gene transfection, ischemia preconditioning and pretreatment have been investigated. EPCs transduced with vectors over-expressing diverse genes such as CXCR4 [122], VEGF [123], IGF-1 [52], HIF-1 [124] and eNOS [125] have shown positive results. In a carotid artery injury model, transplantation of EPCs over-expressing CXCR4 was able to further enhance the reendothelialization capacity of EPCs [122]. In a hind limb ischemic model, combination of intravenous infusion of EPCs over-expressing VEGF with local SDF-1 application showed to be more efficient in improving local blood supply than either of them used alone [123].

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Interestingly, VEGF over-expression on EPCs could increase the expression of CXCR4 which could further enhance EPC homing. Transplantation of EPCs over-expressing IGF-1 has led to inhibition of cardiac apoptosis, enhancement of cardiomyocyte proliferation and increment of capillary numbers in the peri-infarct area [52]. On the other hand, hypoxia preconditioning enhances VEGFR2 expression on EPCs, and accordingly, augments the neovascularization efficacy of EPCs after administration [126]. In addition, pre-incubating EPCs with SDF-1 enhances their pro-angiogenic potential in treating hindlimb ischemia [127]. The mechanism is mainly due to the up-regulation of α4 and αM integrin subunits, which are involved in the homing of EPCs, and secretion of FGF-2 and MMP-2 which are involved in enhancing cell invasion. All these studies indicating the advantages of modified EPCs advocate the new directions of EPC-based therapy for ischemic stroke.

**Modulation of Endogenous EPC Mobilization and Function by Drugs**

Drugs that can affect endogenous EPC behavior are summarized in Table 1. GCSF is one of the early drugs discovered to be able to enhance EPC mobilization into the circulation and augments EPC colony-forming capacity after venous administration [39]. Afterwards, Ang II was shown to induce proapoptotic signaling pathways through Ang II type 1 receptor (AT1-R) expressed on EPCs, and impairs colony-forming and migratory capacities of EPCs [65]. By decreasing Ang II production or blockade of AT1-R, the drugs targeting the renin-angiotensin system such as ACEI and ARB are shown to increase the number and functional activity of EPCs in vitro or in vivo [56, 57]. Furthermore, statins have also been shown to promote the mobilization, clonal growth ability of cEPCs, and may consequently increase myocardial capillary density in the chronically ischemic heart [55, 128]. The underlying molecular mechanisms may relate to the activation of AKT signaling and inhibition of TNF-alpha-induced apoptosis pathway. Since these drugs are commonly used in clinical treatment of cardiovascular diseases, all these data may help to interpret the beneficial effects of these drugs on top of their known pharmacological actions. Further studies in this area could facilitate the discovery of new drugs targeting EPCs.

**Risk Factor Management**

The risk factors for stroke such as hypertension, diabetes or hypercholesterolemia could reduce the number and biological activity of EPCs (Table 1). It can be logically speculated that environment of circulation is essential for the living of EPC, which would raise the perspective on the demand in managing the risk factors of stroke.

**Promising Strategies Relate to EPCs**

A recent study showed that a collagen patch seeded with EPCs promotes angiogenesis and arteriogenesis when placed on cryo-injured rat heart [129]. This may offer a new strategy to increase the local number of EPCs in ischemic area through interventional therapy for stroke. In addition, application of a bio-engineered EPC-capture stent, which accelerates re-endothelialization and reduces thrombogenicity, may reduce the rate of restenosis after PTAS in the future [130].
Safety Respects of EPC-based Therapy

Translational research (from laboratory to clinic) on stem cell-based therapeutics for stroke has been explored in recent years. The studies have been guided by the research recommendations from STEPS (Stem Cell Therapeutics as an Emerging Paradigm for Stroke) in order to enhance therapeutic safety and efficacy [131]. The pioneering pilot studies have been conducted in stroke patients to explore the feasibility and safety of autologous BM stem cell and MSC transplantations [9, 10, 120, 132, 133]. Intravenous infusion of autologous human MSCs has not shown any treatment-related abnormal cell growth or tumorigenesis, neurological deterioration and venous thromboembolism during 1-5 years follow-up [10, 120]. Intra-arterial transplantation of BM stem cells at 5-9 days after stroke onset has also been demonstrated to be safe and has a trend to improve the Barthel Index, positively correlating with the number of CD34+ cells [133]. However, these pilot studies had a relatively small size of samples. Larger clinical trials are in need to further warrant the results of those studies.

The safe aspects of EPC transfusion have been explored in recent years. The level of cEPCs has been found higher in patients with lung, hepatocellular, breast and colorectal cancers [47]. BM-EPCs have been shown to present in the early phase of tumor angiogenesis and ablation of EPCs results in delay of tumor growth which is associated with decreased vessel density [48]. This evidence indicates that EPCs participate in the neovascularization of tumors and that EPC transfusion to patients with tumors should be avoided. In addition, EPCs might aggravate ischemia by increasing the ischemic inflammation because they could produce inflammatory factors such as interleukin-8, monocyte chemotactic protein-1, and recruit monocytes and macrophages [14, 21, 134]. By contrast, transplantation of EPCs was shown to decrease inflammation and enhance neovascularization in a rat model of myocardial infarction [135]. A study of EPC transfusion in patients with acute myocardial infarction showed that EPC therapy did not affect the serum levels of C-reactive protein and leukocytes [136], and did not cause any tumorigenesis during the five-year follow-up [137]. Currently, two clinical trials (clinicaltrials.gov identifier: NCT01468064; NCT00535197) are undergoing to evaluate the safety and efficacy of autologous EPC and CD34+ stem cell transplantation for treating ischemic stroke.

Conclusion

To sum up, there is no doubt of the angiogenic ability of EPCs, which is probably the most distinguishable characteristic over other stem cells. Accumulating evidence suggests the great therapeutic potential of EPCs for ischemic stroke. It remains to clarify if EPC-based therapy is the safest and has the greatest efficacy over other types of stem/progenitor cells. How to improve the strategies in order to maximize the therapeutic application of EPCs deserves further investigation. Besides the hope of therapy, the potential of EPC-based prevention for ischemic stroke may also present a future direction.
Acknowledgments

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References


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Figure 1.
EPC function and therapeutic mechanism of EPCs for ischemic stroke
## Table 1

Factors affect the release, mobilization, and homing/recruitment of EPCs

<table>
<thead>
<tr>
<th>Mobilization and/or Release</th>
<th>Homing/Recruitment</th>
</tr>
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<tr>
<td><strong>Chemokines/growth factors (GF)</strong></td>
<td><strong>Chemokines, GF and/or their receptor</strong></td>
</tr>
<tr>
<td>HIF-1 [33], SDF-1 [34]</td>
<td>↑ SDF-1/CXCR4 [34]</td>
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<tr>
<td>VEGF [51], IGF-1 [52]</td>
<td>↑ CCL5/CCR5 [46]</td>
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<tr>
<td>G-CSF [39]</td>
<td>↑ CXCL1 and CXCL7/CXCR2 [45]</td>
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<tr>
<td>Angiopoietin-2 [53], PAR-1 [54]</td>
<td>↑ VEGF/VEGFR [81]</td>
</tr>
<tr>
<td><strong>Drugs/protein/hormone</strong></td>
<td>IL-8/Gro CXC chemokines [82]</td>
</tr>
<tr>
<td>Statin [55], ARB [56]</td>
<td>↑ IGF2/IGF2R [83]</td>
</tr>
<tr>
<td>ACEI [57]</td>
<td>↑ Other factors</td>
</tr>
<tr>
<td>Estrogen [58], EPO [59]</td>
<td>↑ Caspase-8 [84]</td>
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<tr>
<td>Phytoestrogen [60]</td>
<td>↑ Hyaluronic acid and thrombin [85]</td>
</tr>
<tr>
<td>Berberine [61]</td>
<td>↑ CD9 [86]</td>
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<tr>
<td>Heme oxygenase-1 [62]</td>
<td>↑ Alpha6 integrin subunit [87]</td>
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<td>NO and eNOS [63, 64]</td>
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<td>Ang II [65], Endostatin [66]</td>
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<tr>
<td>Morphine [67]</td>
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<td>Aldosterone [68]</td>
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<tr>
<td><strong>Physiologic/pathological factors</strong></td>
<td></td>
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<tr>
<td>Physical training [69]</td>
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<tr>
<td>Wound [70]</td>
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<tr>
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<tr>
<td>Aging [73], Obesity [74]</td>
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<td>Smoking [75]</td>
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<tr>
<td>Hypercholesterolemia [79]</td>
<td>↓</td>
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<tr>
<td>Homocysteine [80]</td>
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G-CSF, granulocyte-colony stimulating factor; IGF-1, insulin-like growth factor-1; PAR-1, protease-activated receptor-1; ARB, angiotensin II type 1 receptor blocker; ACEI, angiotensin-converting enzyme inhibitor; EPO, Erythropoietin; eNOS, endothelial nitric oxide synthase; Ang II, Angiotensin II; IL-8, Interleukin-8; IGF2R, insulin-like growth factor 2 receptor.