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Endothelial progenitor cells in neovascularization of infarcted myocardium

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Abstract

Historically, revascularization of ischemic tissue was believed to occur through the migration and proliferation of endothelial cells in nearby tissues; however, evidence accumulated in recent years indicates that a subpopulation of adult, peripheral-blood cells, collectively referred to as endothelial progenitor cells (EPCs), can differentiate into mature endothelial cells. After ischemic insult, EPCs are believed to home to sites of neovascularization, where they contribute to vascular regeneration by forming a structural component of capillaries and by secreting angiogenic factors; new evidence indicates that EPCs can also differentiate into cardiomyocytes and smooth-muscle cells. These insights into the molecular and cellular processes of tissue formation suggest that cardiac function may be preserved after myocardial infarction by transplanting EPCs into ischemic heart tissue, thereby enhancing vascular and myocardial recovery. This therapeutic strategy has been effective in animal models of ischemic disorders, and results from randomized clinical trials suggest that cell-based strategies may be safe and feasible for treatment of myocardial infarction in humans and have provided early evidence of efficacy. However, the scarcity of EPCs in the peripheral blood and evidence that several disease states reduce EPC number and/or function have prompted the development of several strategies to overcome these limitations, such as the administration of genetically modified EPCs that overexpress angiogenic growth factors. To optimize therapeutic outcomes, researchers must continue to refine methods of EPC purification, expansion, and administration, and to develop techniques that overcome the intrinsic scarcity and phenotypic deficiencies of EPCs.

¹*Alphabetical list of abbreviations:* Ang1, angiopoietin-1; BDNF, brain-derived neurotrophic factor; BM, bone marrow; CABG, coronary artery bypass graft surgery; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HGF, hepatocyte growth factor; HSCs, hematopoietic stem cell; IGF-1, insulin-like growth factor-1; iNOS, inducible nitric oxide synthase; KDR, kinase insert domain protein receptor; MAPC, multipotent adult progenitor cell; MNC, mononuclear cell; PDGF-CC, platelet-derived growth factor CC; PEDF, pigment epithelium-derived factor; PlGF, placental growth factor; Ptc1, Patched-1; SDF-1, stromal cell-derived factor 1; Shh, Sonic hedgehog; TSP-1, thrombospondin-1; VEGF, vascular endothelial growth factor

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Keywords

Cardiac regeneration; Cell therapy; Cell differentiation; Cytokines; Growth factors; Myocardial infarction; Endothelial progenitor cells; Stem cells

Introduction

Over the last several years, our understanding of blood-vessel formation after tissue ischemia has evolved considerably. In the traditional view, revascularization of ischemic tissue was thought to occur through the migration and proliferation of mature endothelial cells in nearby tissues – a process called “angiogenesis”; however, mature endothelial cells are terminally differentiated and have a low proliferative potential, so their capacity to replace damaged endothelial cells and create new vessels is limited. Thus, effective endothelial repair and angiogenesis appear to require the involvement of other angiogenic processes. Evidence accumulated in recent years indicates that the peripheral blood of adults contains bone marrow (BM)-derived cells with properties similar to those of embryonic angioblasts [1–4]. These precursor cells have the potential to differentiate into mature endothelial cells and are collectively referred to as endothelial progenitor cells (EPCs). It is now believed that BM-derived EPCs home to sites of neovascularization after ischemic insult [5,6], where they differentiate into endothelial cells in a process that mimics the well-described mechanism of embryonic blood vessel formation called “vasculogenesis.”

Emerging evidence indicates that vasculogenesis contributes significantly to postnatal neovascularization. Studies in animals transplanted with BM expressing a traceable marker have shown that the contribution of BM-derived EPCs to new vessel formation may range from 5% to 25% during the formation of granulation tissue [7] or during growth factor-induced neovascularization [8]; their contribution is even greater (35% to 45%) during tumor neovascularization [9]. These novel insights into the molecular processes that contribute to the formation of blood vessels suggest a potential strategy for treatment of ischemic disease, namely, the transplantation of EPCs to improve vascular recovery. The remainder of this article describes the characterization of EPCs, their contribution to adult neovascularization, and their therapeutic potential for treatment of myocardial ischemia.

Identifying EPCs

Since the discovery of EPCs in the peripheral circulation [1,10], investigators have used a variety of methods to identify and isolate this cell population [1–3,10–15]; consequently, the consensus definition of EPCs is somewhat imprecise. EPCs comprise a group of cells that exist in a variety of developmental stages ranging from hemangioblasts to fully differentiated endothelial cells. In the bone marrow, it is difficult to distinguish between developmentally immature EPCs and hematopoietic stem cells (HSCs) because the two cell types express similar surface markers, such as CD133, CD34, and kinase insert domain protein receptor (KDR, also referred to as vascular endothelial growth factor receptor 2). Commitment to the hematopoietic lineage is signaled by the loss of CD133 and KDR expression, whereas circulating EPCs retain expression of all three markers [3]. As circulating EPCs mature, the expression of endothelial-specific markers (e.g., VE cadherin, E-selectin) increases, and CD133 expression may decline. Accordingly, circulating EPCs can be characterized by the expression of CD133 (for developmentally immature EPCs), CD34, KDR, and/or VE cadherin. To maximize the number of collected cells while minimizing costs, human EPCs can be efficiently isolated by selecting for the expression of a single marker; CD34 is perhaps the most frequently used marker for isolating human EPCs from the peripheral blood (Table 1) [16–22] and may be considered the

most widely applied method of antigen-based EPC selection. Several markers have been used for EPC selection in mice and other animals.

Origins of EPCs

During embryonic development, circulating stem cells (i.e., HSCs) and the progenitor cells that form vascular structures (EPCs and angioblasts) evolve from cells in blood islands that fuse to form the capillary network surrounding the yolk sac [23,24]. HSCs descend from cells located in the blood-island interior, whereas EPCs and angioblasts develop from cells near the perimeter. A number of cell-surface antigens, including CD34, KDR, Tie-2, CD117 (C-kit), and Sca-1 [25], are expressed by both HSCs and EPCs, and EPCs have been successfully isolated from populations of circulating mononuclear cells (MNCs) in adults by selecting for KDR, CD34, and CD133 expression [1,3,26–30].

EPCs differentiate into endothelial-lineage cells *in vitro*, and heterologous, homologous, and autologous EPCs are recruited to the foci of angiogenesis in animal models of ischemia, where they contribute to neovascularization. EPCs isolated from human umbilical cord blood have displayed similar properties both *in vitro* and *in vivo* [12,13,31,32]. Lin, *et al.* studied the cell growth *in vitro* of cultivated, peripheral MNCs obtained from patients who received BM transplants from gender-mismatched donors and identified a population of highly proliferative BM-derived endothelial cells [11] that were likely to be EPCs. Reyes, *et al.* isolated multipotent adult progenitor cells (MAPCs) from BM MNCs, then induced them to differentiate into EPCs during culture, indicating that MAPCs are another source of EPCs [33]. In patients with chronic myelogenous leukemia, Gonsky *et al.* found BM-derived EPCs in the walls of vessels that did not undergo neovascularization, suggesting that BM-derived EPCs may be involved in the turnover of endothelial cells in quiescent vessels [34]. Putative EPCs have also been identified in the interstitial spaces of mouse skeletal muscle. These observations support the presence and participation of BM-derived EPCs during neovascularization, and the existence of both donor and recipient sex chromosomes in endothelial cells of donor hearts after sex-mismatched heart transplantations provides strong inferential evidence for the contribution of circulating cells to the vascular endothelial cell population [35]. The presence of other “*in situ*” EPCs, potentially derived from tissue resident stem/progenitor cells, remains to be investigated [35].

EPC mobilization

Tissue trauma mobilizes progenitor cells from the hematopoietic system [36]. Evidence from animal studies has been reproduced in studies of EPC mobilization in patients recovering from burns, coronary artery bypass graft surgery (CABG), or acute myocardial infarction [37]. In addition, experiments performed in a mouse BM transplant model indicated that the incorporation of BM-derived EPCs into the foci of ocular neovascularization after corneal micropocket surgery was greater in mice with hindlimb ischemia than in nonischemic mice [38]. Thus, the mobilization of BM-derived EPCs appears to be a natural response to tissue ischemia, and the mobilized cells become incorporated into sites of vessel growth.

Physiological and pathophysiological events that require neovascularization induce the mobilization of EPCs from the BM *via* the release of a variety of cytokines, growth factors, and hormones from the target tissue. Granulocyte macrophage colony-stimulating factor (GM-CSF) is known to stimulate the release of hematopoietic progenitor cells and myeloid-lineage cells, and has proven to be a potent stimulant of EPC mobilization. The administration of GM-CSF induced EPC mobilization and enhanced both neovascularization in severely ischemic tissues and *de novo* corneal vascularization [38]. Vascular endothelial growth factor (VEGF) plays a critical role in embryonic angio/vasculogenesis [39–41] and is also an important participant during adult EPC-mediated neovascularization. Studies performed first in mice [6] and subsequently in patients undergoing VEGF gene transfer therapy for myocardial

ischemia [42] identified the previously unrecognized contribution of VEGF to neovascularization *via* the mobilization of BM-derived EPCs. Similar modulation of EPC activity has been achieved by the administration of other hematopoietic factors (e.g., granulocyte-colony stimulating factor [G-CSF], stromal cell-derived factor 1 [SDF-1] [43]), growth factors (e.g., platelet-derived growth factor CC [PDGF-CC], brain-derived neurotrophic factor [BDNF], placental growth factor [PIGF] [44–46]), and hormones (e.g., estrogen, erythropoietin [47,48]). The mechanism(s) of EPC mobilization continues to be elucidated [49–57].

In addition to natural hematopoietic and angiogenic stimulants, some pharmacological agents can also induce EPC mobilization. Statins (i.e., 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors) are known to rapidly activate the Akt-signaling pathway, which stimulates both endothelial cell activity [58] and mobilization of BM-derived EPCs [52,53,59,60], thereby enhancing angiogenesis. This newly appreciated role of statins, as well as their well-established safety when used to treat hypercholesterolemia, suggests that they may be beneficial for treatment of a variety of vascular diseases.

The role of EPCs during post-natal neovascularization

In response to events requiring small-scale neovascularization, such as minor wounds or burns, local endothelial cells retain enough capacity for self-repair and proliferation to replace the lost cells. However, this mechanism of neovascularization is limited because the ability of mature endothelial cells to replicate (i.e., the Hayflick limit) and to incorporate into remote sites is restricted. After injuries requiring large-scale tissue repair, such as acute vascular insult secondary to burns, CABG, or acute myocardial infarction [37,61], or during physiological cyclic organogenesis of endometrium [5], BM-derived EPCs are activated by cytokines, hormones, and/or growth factors through autocrine, paracrine, and/or endocrine mechanisms. Thus, tissue regeneration is now believed to occur primarily through the activity of nearby, mature endothelial cells after a minor insult, but the involvement of locally resident or BM-derived stem/progenitor cells, including EPCs, is crucial for recovery from emergent or large-scale vascular injury [1–4] (Figure 1).

The incorporation of BM-derived EPCs into foci of physiological and pathological neovascularization has been demonstrated in a variety of animal models. One well-established method for detecting BM-derived EPCs in newly formed vessels uses wild-type mice transplanted with BM cells from transgenic mice that express LacZ from an endothelial cell lineage-specific promoter, such as Flk-1 (Flk-1/LacZ/BMT mice) or Tie-2 (Tie-2/LacZ/BMT mice). With this model, LacZ-expressing endothelial lineage cells descended from the BM have been identified in vessels involved in tumor growth, wound healing, skeletal and cardiac ischemia, corneal neovascularization, and endometrial remodeling following hormone-induced ovulation [5,62–64]. Other cells with the capacity to differentiate into myocytes or endothelial cells have been identified in the hindlimb skeletal muscle of mice [34] and in the circulation, but are descended from non-BM sources [65]. Although the origin of these “tissue-resident” stem/progenitor cells remains unclear, this finding suggests that BM may not be the only source of EPCs; stem/progenitor cells could reside in a variety of tissues and function as *in situ* EPCs.

Apart from the structural role of EPCs in neovascularization, EPCs recruited to ischemic tissue may remain in the interstitial space and enhance angiogenesis by secreting cytoprotective proteins [66] or cytokines and growth factors that promote the proliferation and migration of local endothelial cells [67–70] (Figure 2). These paracrine effects represent an indirect mechanism by which EPCs contribute to neovascularization. A nonexhaustive list of factors known to be released by EPCs includes VEGF, hepatocyte growth factor (HGF), angiopoietin-1

(Ang1), endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), SDF-1 α , and insulin-like growth factor-1 (IGF-1). Both VEGF and HGF promote endothelial-cell proliferation and subsequent angiogenesis [71,72], and Ang1 may stabilize immature vessels in ischemic tissue [73]. Nitric oxide synthase (either eNOS or iNOS) maintains tissue perfusion by a variety of mechanisms, including the facilitation of EPC mobilization and local effects in ischemic tissue that are still being elucidated [70,74]. Although endothelial cells in the coronary arteries express eNOS, little eNOS is expressed in cardiac capillaries, so the expression of eNOS by recruited EPCs may be important for recovery from short-term cardiac ischemia, as in ischemia-reperfusion injury [70]. iNOS is also expressed by EPCs, but substantial amounts are produced only when tissue hypoxia is sustained for a long period, as in cases of chronic myocardial ischemia [75]. Deletion of eNOS or iNOS in BM cells exacerbates myocardial infarction induced by ischemia-reperfusion injury (eNOS) or permanent vessel occlusion (iNOS) [70], suggesting that the prevention of ischemic myocardial injury may be critically dependent on the “imported” NOS activity provided by EPCs. EPCs reduce cardiac apoptosis during ischemia via the production of IGF-1, a potent anti-apoptotic factor, and subsequent activation of the Akt signaling pathway [69]. Lastly, SDF-1 α released from EPCs functions as a chemotactic factor for the recruitment of additional EPCs [76].

EPCs produce not only pro-angiogenic cytokines but also anti-angiogenic cytokines such as thrombospondin (TSP) [77] and pigment epithelium-derived factor (PEDF) (Ii et al., unpublished data, 2007). Whether TSP-1 promotes or inhibits angiogenesis in ischemic tissue is controversial; however, we have demonstrated that TSP-1 is associated with EPC dysfunction and delayed re-endothelialization in diabetic mice [77]. EPCs transplanted from diabetic mice displayed elevated TSP-1 expression and reduced adhesion to the injured vessel wall, suggesting that TSP-1 is a key negative regulator of EPC function. Upregulated TSP-1 expression in EPCs could contribute to some of the severe complications experienced by patients with diabetes, such as intractable skin ulcers and necrosis, that require neovascularization to heal. Our results also indicated that cytokine production in EPCs can be affected by disease or other physiological circumstances. TSP-2 is structurally similar to TSP-1 and appears to be a crucial regulator of cardiac matrix integrity [78]. The expression of TSP-2 was significantly elevated in hypertrophied human hearts with systolic dysfunction and in failing rat hearts [79], and unusually high rates of cardiac rupture have been reported after myocardial infarction in TSP-2-knockout mice [80,81].

EPCs for therapeutic angiogenesis

Our research has focused on the regenerative potential and unique characteristics of stem/progenitor cells. *In vitro*, stem/progenitor cells display the capacity for self-renewal and for differentiation into organ-specific cell types. *In vivo*, these cells differentiate into appropriate tissue types and contribute to organ regeneration. These observations indicate that EPC transplantation may enable a paradigm shift in cell therapy by targeting the microvasculature, which would deviate from the classic paradigm of angiogenesis developed by Folkman and colleagues [82].

Our laboratory and others have demonstrated that cell therapy performed with culture-expanded EPCs can successfully promote neovascularization in ischemic tissue without co-administration of angiogenic growth factors. This “supply-side” strategy for therapeutic neovascularization, in which a substrate (e.g., EPCs, endothelial cells) rather than a ligand (e.g., growth factors) is the therapeutic tool, was first performed by intravenously transplanting human EPCs into immunodeficient mice with hindlimb ischemia [14]. The findings from this initial study indicated that exogenously administered EPCs improved neovascularization and enhanced functional recovery. Human peripheral blood-derived EPCs transplanted in a rat model of myocardial ischemia localized to the area of neovascularization, differentiated into

mature endothelial cells, and were associated with greater preservation of left ventricular function and a reduction of infarct size [83]. Similar findings, including enhanced neovascularization in ischemic myocardium, have been reported with transplanted human umbilical cord blood-derived EPCs after myocardial infarction in nude rats [22].

Although the relationship between the number of mobilized or transplanted cells and the extent of myocardial repair is unclear, the number of EPCs in the peripheral circulation is positively correlated with recovery. Werner et al. found that higher endogenous levels of EPCs (identified via expression of CD34 and KDR) were associated with a lower risk of death from cardiovascular causes and with fewer occurrences of a first major cardiovascular event, revascularization surgery, or hospitalization [84]. In the FIRSTLINE-AMI (i.e., “Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction”) trial, EPC mobilization (via G-CSF administration) improved cardiac function, reduced ventricular remodeling, and was not associated with leukocytoclastic effects, accelerated restenosis, or delayed adverse events during the 1-year follow-up period [85]; however, enrollment in the MAGIC trial was ended early because of an unexpectedly high rate of in-stent restenosis in patients treated with G-CSF to mobilize stem/progenitor cells from the bone marrow [86]. Outcomes in subsequent trials were improved by using drug-eluting stents to decrease restenosis [87]. In-stent restenosis is perhaps the most common serious side effect of cell therapy in patients recovering from myocardial infarction, and patients must be carefully monitored for this condition.

Several investigators have explored the therapeutic potential of an EPC-enriched fraction of cells that express the CD34 antigen. Kocher, *et al.* intravenously infused freshly isolated human CD34+ cells into nude rats with myocardial ischemia and observed greater preservation of left ventricular function, inhibition of cardiac apoptosis, and evidence that the infused EPCs induced neovascularization at the myocardial infarct zone [88]. Iwasaki et al. found that intramyocardial transplantation of human CD34+ cells dose-dependently (1×10^3 to 5×10^5 cells) improved vasculogenesis, cardiomyogenesis, and the preservation of cardiac function in a rat model of myocardial infarction. Furthermore, a subpopulation of cardiomyocytes and smooth-muscle cells were stained positively by human-specific antibodies, and the number of positively stained cells increased with the cell dose, suggesting that transplanted EPCs can differentiate into functional cardiomyocytes and smooth muscle cells [89] (Figure 2). This interpretation is also supported by evidence that cultured, mobilized stem/progenitor cells express the cardiac transcription factor GATA-4 [66]; however, the concept of “transdifferentiation” remains somewhat controversial, and the number of cardiomyocytes generated via this mechanism is unlikely to adequately replace the cells damaged by infarction.

Both cultured and freshly isolated EPCs have been used in multicenter placebo-controlled clinical trials. In the TOPCARE-AMI (i.e., “Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction”) trial [90], intracoronary infusion of cultured human EPCs in patients with recent myocardial infarction was associated with improvements in global left ventricular function and microvascular function. We have recently published results from a randomized trial (i.e., “Injection of Autologous CD34-Positive Cells for Neovascularization and Symptom Relief in Patients with Myocardial Ischemia”) [91] that demonstrated the feasibility, safety, and bioactivity of freshly isolated, autologous CD34+ cells administered intramyocardially via an electromechanical mapping system to patients with intractable angina [92]. Enrollment in a larger, phase IIb study has been completed, and results are expected in late 2008.

Limitations of EPC therapy

The proportion of EPCs is much lower in unselected BM MNCs than in cells selected for CD34 [93] or CD133 (Li et al., unpublished data, 2007) expression. Theoretically, the heterogeneity of the MNC population could lead to greater variability in treatment outcomes, which might also provide clues to the factors that determine the potency of cell-based therapy. These observations may explain, at least in part, why the benefits of cell therapy in some of the clinical trials, most of which were performed with unselected BM MNCs (Table 2), have not consistently replicated the results of preclinical studies with EPCs (Table 3); however, there are many other factors that may also contribute to this inconsistency. Furthermore, one must bear in mind that there have been no definitive (i.e., phase 3) studies of any cell-based therapy to date.

Researchers have chosen to use MNCs, rather than EPCs, in clinical studies for a variety of reasons, including convenience, and because the most effective subpopulation of cells has not been identified [94]. EPCs obtained from healthy human volunteers produce approximately 5.0×10^6 cells per 100 mL of peripheral blood after 7 days in culture, but our animal studies suggest that satisfactory improvement of myocardial ischemia requires *systemic* injection of approximately 1.0×10^5 human EPCs per kilogram of body weight [83]. Taken together, these results indicate that as much as 6–12 liters of blood may be needed to treat patients *via* systemic infusion of EPCs. In addition, the number and/or function of circulating EPCs is reduced by many conditions that are common among patients requiring cardiovascular regeneration, such as aging [95], diabetes [77,96], hypercholesterolemia [96], hypertension [96,97], and smoking [98,99], so systemic EPC delivery is not likely to be a feasible therapeutic option for many patients.

The limited availability EPCs can be overcome with a number of techniques, including: 1) transplantation of EPCs directly into the ischemic tissue, 2) administration of cytokines or growth factors to mobilize EPCs from the BM before collection [6,38], 3) enrichment procedures such as leukapheresis or BM aspiration, 4) enhancement of EPC function with concomitant gene therapy, or 5) culture expansion of EPCs from self-renewable primitive stem/progenitor cells obtained from the BM or other sources. If autologous EPCs of sufficient quantity and quality cannot be obtained through these techniques, allogenic EPCs derived from umbilical cord blood or culture-expanded from human embryonic stem cells [13,100] may be another option.

There are two feasible approaches for transplanting EPCs directly into ischemic myocardium (Figure 3): intracoronary infusion and intramyocardial injection. Results from a few small clinical trials suggest that both techniques can provide mild or moderate benefits, but a conclusive evaluation will require more large-scale studies. Intramyocardial injection can be administered through the endocardium by using an electromechanical mapping system [101–103] or through the pericardium during CABG surgery; however, myocardial injections could potentially increase the degree of myocardial damage if performed during the acute phase of myocardial infarction. Moreover, EPC transplantation in ApoE-knockout mice increased aortic valve plaque formation [104], so patients with uncontrolled hyperlipidemia may require special attention.

Future strategies for EPC therapy

Given the findings that EPC function and mobilization may be impaired in certain disease states, strategies to recover potential EPC dysfunction are being developed. Combining EPC therapy with local gene therapy [105] is one option, and a second approach involves genetic modification of EPCs. In addition to increasing EPC activity and viability, modifications could be designed to augment their recruitment and proliferation or to enhance cytokine secretion

from EPCs, fibroblasts, vascular smooth muscle cells, and cardiomyocytes (Figure 4). Accordingly, if gene transfection is done successfully, the use of genetically modified EPCs could be considered the “second generation” of EPC therapy. Genetically modified EPCs could also be used as vectors for gene therapy, and unlike naked vectors, the incorporated cells will remain active at the site of neovascularization for an extended period and can be engineered to overexpress several angiogenic factors simultaneously. Furthermore, injection volumes could be lower with cell-based gene therapy than with traditional gene or protein therapies, which may prevent pressure-induced enlargement of the damaged area.

Iwaguro et al. published the first evidence that genetically modified EPCs can rescue impaired neovascularization in an animal model of hindlimb ischemia [106]. Transplantation of heterologous EPCs transduced with an adenovirus encoding human VEGF₁₆₅ improved neovascularization and blood flow recovery, and reduced limb necrosis and auto-amputation rate, with an EPC dose that was 1/30 of the dose required in previous experiments with unmodified EPCs [14]. A variety of other genes have been targeted in similar investigations [67,106–112]. Our laboratory has focused on Sonic hedgehog (Shh), an embryonic morphogen that plays a significant role in organogenesis. Our results indicate that human Shh protein has an indirect but robust angiogenic effect in mouse models of acute and chronic myocardial ischemia through upregulation of multiple angiogenic cytokines from interstitial mesenchymal cells such as fibroblasts [113]. These findings suggest that the embryonic Shh pathway can be reactivated postnatally in response to ischemia. Moreover, because EPCs express Patched-1, the receptor for Shh, Shh could influence EPCs through autocrine as well as paracrine effects. Accordingly, we have begun investigations to determine whether genetic modification of EPCs to express Shh will augment neovascularization in ischemic tissue.

Conclusion

Accumulating evidence suggests that BM-derived EPCs have the potential to promote postnatal vasculogenesis in the setting of adult myocardial ischemia through both direct and indirect effects. Clinical investigations of EPCs for regenerative medicine are now under way. To optimize therapeutic outcomes, researchers must continue to expand our understanding of the mechanisms by which EPCs contribute to ischemic tissue repair, to refine methods of EPC purification, expansion, and administration, and to develop techniques that overcome the scarcity and dysfunction of EPCs associated with many disease states. Techniques that combine autologous EPC therapy with local gene therapy or that enhance EPC phenotype (e.g., by administering genetically modified EPCs) are promising strategies for increasing the therapeutic potential of cell therapy and may advance the clinical use of this treatment modality.

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Figure 1. Angiogenesis and vasculogenesis

During angiogenesis (left), pre-existing endothelial cells proliferate and migrate to form a new vessel in response to tissue injury. During vasculogenesis (right), a variety of factors released from the jeopardized tissue or surrounding area induce mobilization of EPCs from the BM into the peripheral circulation. EPCs are recruited to the site of vascularization, differentiate, proliferate, migrate, and participate in vascular growth. Abbreviations: EC, endothelial cell; EPC, endothelial progenitor cell.

Figure 2. Mechanisms of EPC-mediated neovascularization

EPCs contribute to neovascularization indirectly by secreting a variety of factors that induce angiogenesis/vasculogenesis, vasodilation, and stem/progenitor cell mobilization and recruitment, or reduce apoptosis. Direct EPC contributions to neovascularization occur through differentiation into endothelial cells or transdifferentiation into vascular smooth muscle cells and cardiomyocytes to form the structural components of capillaries, arterioles, and the myocardium. Abbreviations: Ang-1, angiopoietin 1; eNOS, endothelial nitrous oxide synthase; EPC, endothelial progenitor cell; G-CSF, granulocyte colony stimulating factor; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; SDF-1 α , stromal cell-derived factor-1 α ; VEGF, vascular endothelial growth factor.

Figure 3. Therapeutic angiogenesis/vasculogenesis with EPC transplantation

Clinical trials of EPC therapy have used EPCs cultured from bone marrow or peripheral blood MNCs or EPCs freshly isolated from the peripheral blood after G-CSF mobilization for treatment of patients with acute myocardial infarction or chronic myocardial ischemia. Administration of EPCs from either source is safe and feasible. Abbreviations: BMMNC, bone marrow mononuclear cell; EPC, endothelial progenitor cell; G-CSF, granulocyte colony stimulating factor; PBMNC, peripheral blood mononuclear cell.

Figure 4. The use of genetically engineered EPCs for treatment of ischemic heart disease EPCs are transduced to express angiogenic proteins, and the released proteins stimulate neighboring fibroblasts by binding to their specific receptors. *Paracrine effects:* The fibroblasts secrete a variety of cytokines to promote angiogenesis and recruit endogenous EPCs from the BM. *Autocrine effects:* EPCs express receptors for many angiogenic proteins, so the proteins produced by genetically modified EPCs could promote EPC proliferation, migration, tube formation, and cytokine production. Moreover, the secreted proteins may recruit vascular smooth muscle cells and induce cardiomyocyte proliferation in ischemic myocardium. Abbreviations: Ang-1, angiopoietin 1; CM, cardiomyocyte; EPC, endothelial progenitor cell; IGF-1, insulin-like growth factor-1; SDF-1 α , stromal cell-derived factor-1 α ; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell.

Table 1

Human and Mouse EPC Markers

Source of EPCs	Human		References	Source of EPCs	Mouse Marker(s)	References
	Marker(s)					
PB	CD34+/KDR+		[95,114–129]	PB	Sea-1+/Flk-1+	[47,76,130–135]
PB	CD3-/CD34+/KDR+		[136]	PB	Sea-1+/Flk-1+/C-kit+	[137]
PB	CD133+/KDR+		[95,126,138–140]	PB, BM	CD45-/CD133+/Sea-1+/Flk-1+	[141]
PB	CD133+/KDR+		[117,142]	PB	CD34+/Flk-1+	[48,143–146]
PB	CD34+/CD133+/KDR+		[27–30,147–155]	PB	CD45-/CD34+/Flk-1+	[156]
PB	CD34+/CD133+		[118,140,157–162]	PB	Flk-1+/CD11b-	[163–165]
PB	CD34+		[16–18,93]	PB	C-kit+/Flk-1+/Cd11b-	[166]
PB, CB	CD34+ (expanded*)		[19–22]	PB	C-kit+/Flk-1+/CD45+	[167]
PB	CD133+		[163]	PB	Tie2+/Flk-1+	[168]
PB	CD34+/CD133+/CD45+		[169]	PB	CXCR4+/Flk-1+	[168]
PB	CD45(dim-)/CD133+/CD144+/KDR+		[170]	PB, BM	CD34+/CD133+	[171]
PB	CD45(low)/CD34+/CD133+/KDR+		[98]	BM	Sea-1+/C-kit+	[164]
PB	CD34+/CD31+		[115]	PB	C-kit+/CD31+	[135]
PB	CD34+/CD31+ (CFU)		[172]	PB	C-kit+/Tie2+	[146]
PB	CD34+/P-selectin+		[115]	PB	CD45-/CD3-/CD31+/Tie2+	[173]
PB	CD34+/C-kit/KDR+		[28]	ESCs	Flk-1+/E-cadherin-	[174]
PB	CD34-/CD133+/KDR+		[30]			

CB indicates cord blood; CFU, colony forming unit; EPCs, endothelial progenitor cells; ESCs, embryonic stem cells; PB, peripheral blood; VEGFR, vascular endothelial growth factor receptor.

Table 2

Clinical trials of unselected MNCs in myocardial ischemia

Trial	Disease or Disorder	Cells	Delivery Route	Mean Cell Dose ($\times 10^6$)	Patients (treated:control*)	Design	Mean Duration of Follow-up (months)	Outcome
Assmus et al. [175]	OMI	BM-MNCs	Intracoronary	205	28:23	RC	3	Effective
ASTAMI trial [176,177]	AMI	BM-MNCs	Intracoronary	68	50:50	R	12	Ineffective
Beeres et al. [101]	IHF	BM-MNCs	Intramyocardial (NOGA)	94	15:0	NR	3-6	NA
BOOST [178,179]	AMI	BM-MNCs	Intracoronary	2460	30:30	R	18	Ineffective
Chen et al. [180]	AMI	BM-MNCs	Intracoronary	8000	34:34	R	6	Ineffective
Fernandez-Aviles et al. [181]	AMI	BM-MNCs	Intracoronary	78	20:13	NR	6	Effective
HFBE [182]	AMI	BM-MNCs	Intracoronary	246	26:0	Pilot	4	NA
Hendricks et al. [183]	IHF	BM-MNCs	Intramyocardial (during CABG)	60	10:10	R	4	Ineffective
Janssens et al. [184]	AMI	BM-MNCs	Intracoronary	172	33:34	RC	4	Ineffective
Koethe et al. [185]	AMI	BM-MNCs	Intracoronary	39	5:0	NR	12	NA
MAAGIC [86]	AMI	PB-MNCs (+GCSF)	Intracoronary	1000	10:10:7*	R	6	Effective
Meulzin et al. [186,187]	AMI	BM-MNCs	Intracoronary	high: 90-200 low: 9-20	20:20:20**	R	12	Effective
Mocini et al. [188]	RMI	BM-MNCs	Intramyocardial (during CABG)	292	18:18	NR	12	Effective
Perin et al. [102,189]	IHF	BM-MNCs	Intramyocardial (NOGA)	28	11:9	NR	12	Effective
REPAIR-AMI [190-192]	AMI	BM-MNCs	Intracoronary	236	100:101	R/MC	12	Effective
Ruan et al. [193]	AMI	BM-MNCs	Intracoronary	—	9:11	R	6	Effective
Strauer et al. [194]	AMI	BM-MNCs	Intracoronary	28	10:10	R	4	Effective
Strauer et al. [195]	AMI	BM-MNCs	Intracoronary	15	18:18	R	3	Effective
Tatsumi et al. [196]	AMI	PB-MNCs	Intracoronary	4920	36:18	Cohorts	6	Effective
TEC-STAMI [197]	AMI	BM-MNCs	Intracoronary	40	10:10	R/MC	6	Effective
TOPCARE-AMI [198-200]	AMI	PB-MNCs or BM-MNCs	Intracoronary	213	30:29***	R	12	Effective
Tse et al. [201]	IHF	BM-MNCs	Intramyocardial (NOGA)	—	8:0	NR	3	NA

AMI indicates acute myocardial infarction; BM-MNCs, bone marrow-mononuclear cells; CABG, coronary artery bypass graft surgery; GCSF, granulocyte colony stimulating factor; IHF, ischemic heart failure; MC, multicenter; NA, not applicable (uncontrolled trial); NR, nonrandomized; OMI, old myocardial infarction; PB-MNCs, peripheral blood-mononuclear cells; R, randomized; RC, randomized and controlled; RMI, recent myocardial infarction

*Cell infusion+GCSF; GCSF alone: control

high dose:low dose:control

*** PB-MNCs: BM-MNCs

**** With elevated rate of in-stent restenosis.

Table 3

Clinical trials of selected cells in myocardial ischemia

Trial	Disease or Disorder	Cells	Delivery Route	Mean Cell Dose ($\times 10^6$)	Patients (treated:control *)	Design	Mean Duration of Follow-up (months)	Outcome
Ahmadi et al. [202]	RMI	CD133+	Intramyocardial (during CABG)	1.89	18:9	NR	6	Effective
Balogh et al. [94]	RMI	CD34+	Intracoronary	(1-15)	8:18	NR	6	Inconclusive *
Bartunek et al. [203]	AMI	CD133+	Intracoronary	12.6	19:16	R	4	Effective
Boyle et al. [204]	OMI	CD34+ (+GCSF)	Intracoronary	67	5:0	NR	12	NA
Erbs et al. [205]	OMI	Cultured PB-EPCs	Intracoronary	69	13:13	R	3	Effective
Li et al. [206]	AMI	PB-EPCs (+GCSF)	Intracoronary	73	35:35	Cohorts	6	Effective
Losordo et al. [207]	AP	CD34+	Intramyocardial (NOGA)	0.3	18:6	R	6	Safe and feasible
Stamm et al. [208, 209]	RMI	CD133+	Intramyocardial (during CABG)	5.8	46:9	NR	6	Effective

AMI indicates acute myocardial infarction; AP, angina pectoris; CABG, coronary artery bypass graft surgery; GCSF, granulocyte colony stimulating factor; NA, not applicable (uncontrolled trial); NR, nonrandomized; OMI, old myocardial infarction; PB-EPCs, peripheral blood endothelial progenitor cells; R, randomized; RC, randomized and controlled; RMI, recent myocardial infarction.

* EPC therapy was associated with a trend toward improvement, but the trend did not reach statistical significance.