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Cell Therapy and Gene Therapy Using Endothelial Progenitor Cells for Vascular Regeneration

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Abstract The isolation of endothelial progenitor cells (EPCs) derived from adult bone marrow (BM) was an epoch-making event for the recognition of "neovessel formation" occurring as physiological and pathological responses in adults. 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. Considering the regenerative implications, gene modification of stem cells has advantages over conventional gene therapy. Ex vivo gene transfection of stem cells may avoid administration of vectors and vehicles into the recipient organism. Stem cells isolated from adults may exhibit age-related, genetic, or acquired disease-related impairment of their regenerative ability. Transcriptional or enzymatic gene modification may constitute an effective means to maintain, enhance, or inhibit EPCs' capacity to proliferate or differentiate. This chapter provides an update of EPC biology as well as EPCs' potential use for therapeutic regeneration.

Keywords Endothelial progenitor cells · Gene therapy · Therapeutic vasculogenesis · Vascular regeneration

1 Introduction

Tissue regeneration by somatic stem/progenitor cells has been recognized as a maintenance or recovery system of many organs in adults. The isolation and investigation of these somatic stem/progenitor cells have led to descriptions of how these cells contribute to postnatal organogenesis. On the basis of the regenerative potency, these stem/progenitor cells are expected as a key in strategies aimed toward therapeutic applications for 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 postnatal vascular organogenesis. The discovery of EPCs has therefore drastically changed our understanding of adult blood vessel formation. The following issue provides the update of EPC biology as well as highlights their potential utility for therapeutic vascular regeneration.

2 Postnatal Neovascularization

Through the discovery of EPCs in PB (Asahara et al. 1997; Shi et al. 1998), our understanding of postnatal neovascularization has been expanded from angiogenesis to angio/vasculogenesis. As previously described (Folkman and Shing 1992), postnatal neovascularization was 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 (Asahara et al. 1999a). Furthermore, tissue-specific stem/progenitor cells with the potency to differentiate into myocytes or ECs were isolated in skeletal muscle tissue of murine hindlimb, although the origin remains to be clarified (Tamaki et al. 2002). This finding suggests that the origin of EPCs may not be limited to BM; tissue-specific stem/progenitor cells possibly provide "in situ EPCs" as a source of EPCs other than BM.

In the events of minor-scale neovessel formation, i.e., slight wounds or burns, "in situ preexisting ECs" causing postnatal angiogenesis may replicate and replace the existing cell population sufficiently, as ECs exhibit an ability

at preserves their proliferative activity. Neovascularization through differentiated ECs, however, is limited in terms of cellular lifespan (Hayflick limit) and their inability to incorporate into remote target sites. In the case of large-scale tissue repair—such as is seen with patients who experience acute vascular insult secondary to burns, coronary artery bypass grafting (CABG), or acute myocardial infarction (Gill et al. 2001; Shintani et al. 2001), or in physiological cyclic organogenesis of endometrium (Asahara et al. 1999a)—BM-derived or in situ EPC kinetics are activated under the influence of appropriate cytokines, hormones, and growth factors through the autocrine, paracrine, and 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.

3
Profiles of EPCs in Adults

3.1
The Isolation of EPCs in Adults

In the embryo, evidence suggests that hematopoietic stem cells (HSCs) and EPCs (Pardanaud et al. 1987; Risau et al. 1988) are derived from a common precursor (hemangioblast) (Flamme and Risau 1992; Weiss and Orkin 1996). During embryonic development, multiple blood islands initially fuse to form a yolk sac capillary network (Risau and Flamme 1995), which provides the foundation for an arteriovenous vascular system that eventually forms following the onset of blood circulation (Risau et al. 1988). 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) versus EPCs or angioblasts, which are located at the periphery of the blood islands. In addition to this arrangement, HSCs and EPCs share certain common antigens, including CD34, KDR, Tie-2, CD117, and Sca-1 (Choi et al. 1998).

The existence of HSCs in the PB and BM, and the demonstration of sustained hematopoietic reconstitution with HSC transplantation, led to an 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 KDR, CD34, and CD133 antigens shared by both embryonic EPCs and HSCs (Asahara et al. 1997; Peichev et al. 2000; Yin et al. 1997). 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 (Crisa et al. 1999; Kalka et al. 2000; Murohara et al. 2000; Nieda et al. 1997).

These findings have raised important questions regarding fundamental concepts of blood vessel growth and development in adult subjects. 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 does in fact make a significant contribution 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 (Crosby et al. 2000) or growth factor-induced neovascularization (Murayama et al. 2002). Also, in tumor neovascularization, the range is approximately 35%–45% higher than the former events (Reyes et al. 2002). The degree of EPC contribution to postnatal neovascularization is predicted to depend on each neovascularizing event or disease.

3.2 Diverse Identifications of Human EPCs and Their Precursors

Since the initial EPC report (Asahara et al. 1997; Shi et al. 1998), a number of groups have set out to better define this cell population. Because EPCs and HSCs share many surface markers, and no simple definition of EPCs exists, various methods of EPC isolation have been reported (Asahara et al. 1997; Boyer et al. 2000; Fernandez Pujol et al. 2000; Gehling et al. 2000; Gunsilius et al. 2000; Harraz et al. 2001; Kalka et al. 2000; Kang et al. 2001; Lin et al. 2000; Murohara et al. 2000; Nieda et al. 1997; Peichev et al. 2000; Quirici et al. 2001; Schatteman et al. 2000; Shi et al. 1998). The term EPC may therefore encompass a group of cells that exists 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 that a population of human EPCs exists in vivo.

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 (Lin et al. 2000). Gunsilius et al. investigated a chronic myelogenous leukemia model and disclosed that BM-derived EPCs contribute to postnatal neovascularization in human (Gunsilius et al. 2000). Interestingly, in the report, BM-derived EPCs could be detected even in the wall of quiescent vessels without neovascularization events. The finding suggests that BM-derived EPCs may be related even to the turnover of ECs consisting of quiescent vessels.

Reyes et al. have isolated multipotent adult progenitor cells (MAPCs) from BM MNCs, differentiated them into EPCs, and proposed MAPCs as an origin of EPCs (Reyes et al. 2002). 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 namely "in situ EPCs" as derived from tissue-specific stem/progenitor cells in murine skeletal muscle remains to be investigated even in the other tissues (Tamaki et al. 2002).

4 Cell Therapy Using EPCs

4.1 The Potent of EPC Transplantation

The regenerative potential of stem/progenitor cells is currently under intense investigation. In vitro, stem/progenitor cells possess the capabi ity 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 (Kalka et al. 2000). 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 (Kawamoto et al. 2001). Murohara et al. reported similar findings in which human cord blood-derived EPCs also augmented neovascularization in hindlimb ischemic model of nude rats, followed by in situ transplantation (Murohara et al. 2000).

Other researchers have more recently explored the therapeutic potential of freshly isolated human $CD34^+$ cells (EPC-enriched fraction). Schatteman et al.

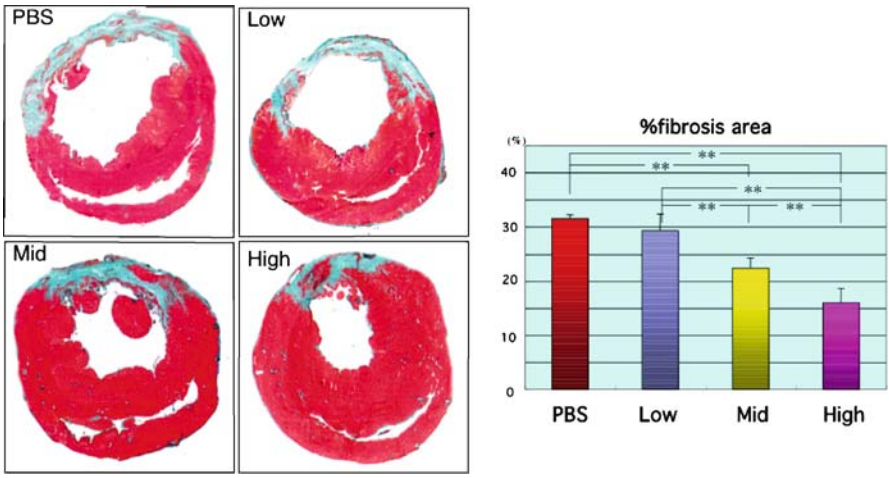

Fig. 1 Roadmap of EPC research. EPC biology research starts from the identification of endothelial progenitor cells in peripheral blood published by Asahara et al., inspired by evolving knowledge in developmental biology, hematology, and vascular biology. The development of this field is contributing to medicine through plentiful translational research throughout the world

conducted local injection of freshly isolated human $CD34^+$ cells into diabetic nude mice with hindlimb ischemia, and showed an increase in the restoration of limb flow (Schatteman et al. 2000). Similarly Kocher et al. attempted intravenous infusion of freshly isolated human $CD34^+$ cells into nude rats with myocardial ischemia, and found preservation of LV function associated with inhibition of cardiomyocyte apoptosis (Kocher et al. 2001). 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 in Sect. 5.2, will likely require further optimization techniques to acquire the ideal quality and quantity of EPCs for EPC therapy.

Very recently, Iwasaki et al. have demonstrated a $CD34^+$ cell dose-dependent contribution to recovery of LV function and neovascularization in ischemia tissues in models of myocardial ischemia (Iwasaki et al. 2006; Fig. 1). Furthermore, $CD34^+$ cells in higher dose groups differentiated into not only vasculogenic (endothelial and mural) lineage but also myocardial lineage cells. Clinical trials using mobilized $CD34^+$ may be effective in terms of vasculogenesis and myocardiogenesis.

4.2 Future Strategy of EPC 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 (Kalka et al. 2000) suggest that heterologous transplantation requires systemic injection of 0.5×10^4 to approximately 2.0×10^4 human EPCs/g body weight of the recipient animal to achieve satisfactory reperfusion of an ischemic hindlimb. Rough extrapolation of these data to humans suggests that a blood volume of as much as 12 l 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 may help to overcome the primary scarcity of a viable and functional EPC population. These should include: (1) local delivery of EPCs; (2) adjunctive strategies (e.g., growth factor supplements) to promote BM-derived EPC mobilization (Takahashi et al. 1999; Asahara et al. 1999b); (3) enrichment procedures, i.e., leukapheresis or BM aspiration, or (4) enhancement of EPC function by gene transduction (gene modified EPC therapy, vide infra); and (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 above, allogenic EPCs derived from umbilical cord blood or culture-expanded from human embryonic stem cells (Murohara et al. 2000; Levenberg et al. 2002) may be available as the sources supplying EPCs.

5
Gene-Modified EPC Therapy

5.1
Gene Modification of Stem/Progenitor Cells

Considering stem cells' regenerative ability, the gene modification of stem/progenitor cells exerts several advantages over conventional gene therapy. Ex vivo gene transduction of stem/progenitor cells may avoid the direct administration of vectors and vehicles into the recipient organism. Here are possible target mechanisms of gene-modified stem/progenitor cells for medicine demonstrated in Fig. 2.

1. Phenotype modification of stem/progenitor cell. Stem/progenitor cell target
   Stem/progenitor cells isolated from adults may exhibit a variety of impairments in regenerative ability, these being related to aging and genetic or acquired diseases. Certain properties of stem/progenitor cells can be functionally recovered by gene modifications. Transcriptional or enzymatic

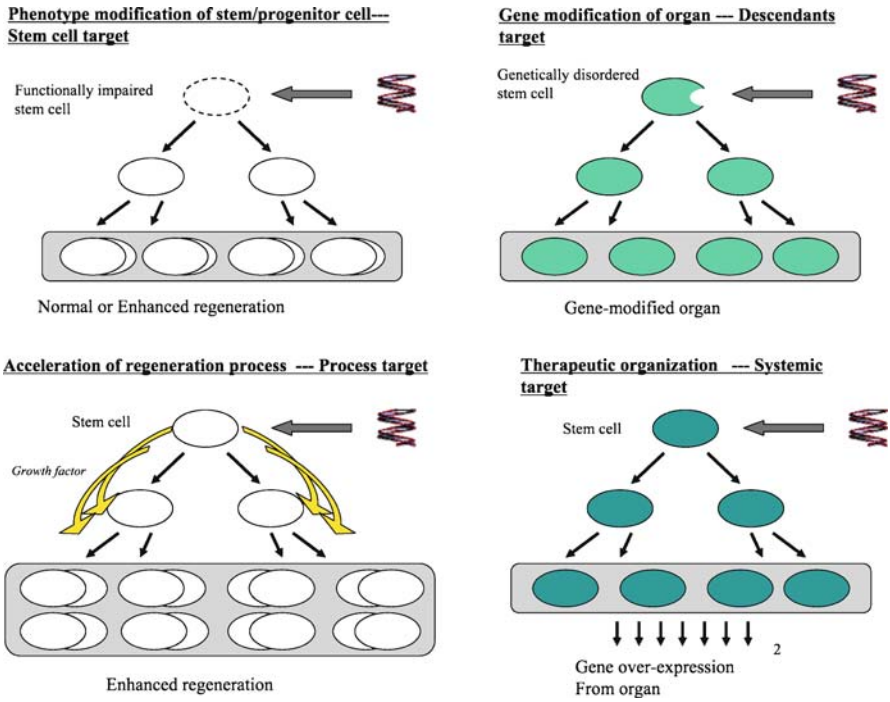

Fig. 2 The strategies of stem/progenitor cell gene modification

gene modification may constitute an effective means to maintain, enhance, or inhibit stem/progenitor cell bioactivities to proliferate or differentiate.

2. Phenotype modification of organ. Progeny target

   Genes introduced into stem cells are inherited by their progeny through differentiation cascades. Specifically, expression of the inserted gene may persist through the life of the organ system that is reconstructed by the gene-modified stem cells. Genetically disordered organs, especially in the case of hematopoietic pathology, could be replaced by this strategy following bone marrow transplantation (Cowan et al. 1999; Dunbar et al. 1998; Karlsson 1991; Reisner and Segall 1995; Wong-Staal et al. 1998).

3. Acceleration of regeneration process. Process target

   Regeneration of injured tissues is in part or primarily achieved via stem cell expansion and differentiation. Examples include endothelial progenitor cells for neovascularization, neural stem cells for neurogenesis, and HSCs and mesenchymal stem cells (MSCs) for bone marrow reconstruction. Natural reparatory processes are often too impaired from unexpectedly severe injury, basic diseases, or aging to accomplish regeneration. Gene modification of stem cells to supplement mitogens (Iwaguro et al. 2002) or to deliver

inhibitory factors for negative control might accelerate retarded regenerative processes following stem cell proliferation, incorporation, and gene expression in situ.

4. Therapeutic organization. Systemic target

   To supply target molecules for therapeutic use, transplantation of genetically modified stem cells might generate expressional tissues or pseudo-organs. The established tissues or organs will continuously express a certain number of molecules locally or generally for the short- or long-term by means of vector selection and conditioning. This strategy could represent an alternative approach for therapeutic delivery in lieu of standard drug distribution and uptake.

5.2 Gene Modification of EPCs

EPC transplantation constitutes a novel therapeutic strategy that could provide a robust source of viable ECs to supplement the contribution of ECs resident in the adult vasculature that migrate, proliferate, and remodel in response to angiogenic cues, according to the classic paradigm of angiogenesis developed by Folkman and Shing (1992). Just as classical angiogenesis may be impaired in certain pathologic phenotypes, however, aging, diabetes, hypercholesterolemia, and hyperhomocysteinemia may likewise impair EPC function, including mobilization from the bone marrow and incorporation into neovascular foci. Gene transfer of EPCs during ex vivo expansion constitutes a potential means of addressing such putative liabilities in EPC function. Moreover, phenotypic modulation of EPCs ex vivo may also reduce the number of EPCs required for optimal transplantation post-ex vivo expansion, and thus serve to address a practical limitation of EPC transplantation, namely the volume of blood required to extract an optimal number of EPCs for autologous transplantation.

Iwaguro et al. have determined the impact of vascular endothelial growth factor (VEGF) gene transfer on certain properties of EPCs in vitro and the consequences of VEGF EPC transfer on neovascularization in vivo (Iwaguro et al. 2002; Fig. 3). In vitro, VEGF-gene transfer can be augmented by adenovirus vector for EPC-proliferative activity and enhanced incorporation of EPCs into endothelial cell monolayers. In vivo, transplantation of VEGF gene-transduced EPCs improved neovascularization and reduced limb necrosis by 63.7%. VEGF EPC gene transfer permitted a dose reduction of transplanted EPCs that was 30 times less than that required in previous experiments to achieve similar rates of limb salvage. These findings present one option to address the limited number of EPCs that can be isolated from peripheral blood prior to ex vivo expansion and subsequent autologous readministration for augmentation of neovascularization.

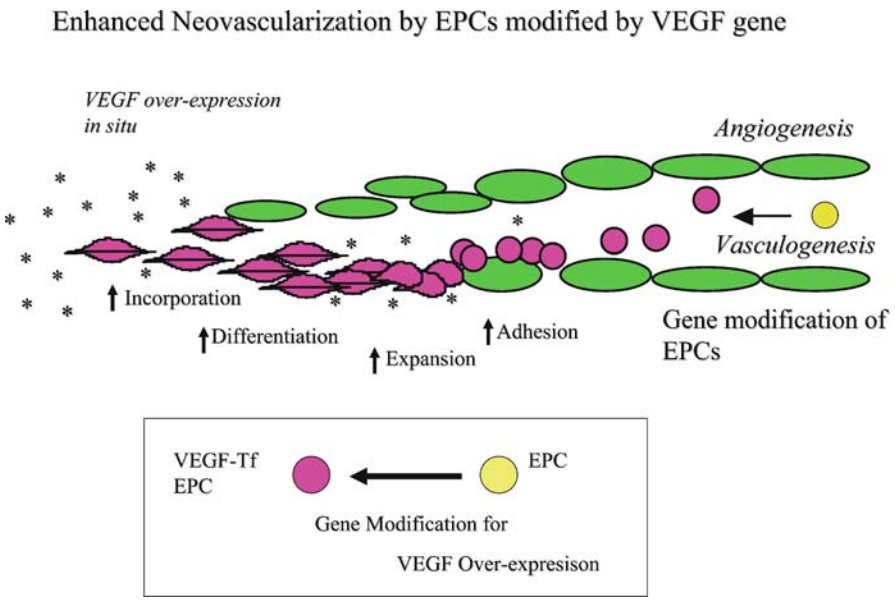

Fig. 3 Enhanced neovascularization by EPCs modified by the VEGF gene. The delivery of VEGF gene-transduced EPCs presents stimulated proliferation, migration, differentiation, and incorporation capability to the ischemic area for vasculogenesis

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.

The regulatory molecule for cell lifespan, telomerase was modified by human telomerase reverse transcriptase (hTERT) gene transfer to investigate its effect on the regenerative properties of endothelial progenitor cells (EPCs) in neovascularization (Murasawa et al. 2002). Along with high telomerase activity in hTERT-transduced EPCs, mitogenic capacity exceeded that of the control group. VEGF-induced cell migration in EPCs was also markedly enhanced by hTERT overexpression. hTERT overexpression has rescued EPCs from starvation-induced cell apoptosis, an outcome that was further enhanced in response to VEGF. The colony appearance of totally differentiated EC was detected prior to day 30 only by hTERT overexpression, whereas no EC colonies could be detected in the control group. In vivo transplantation of heterologous EPCs demonstrated that hTERT-expressing EPCs dramatically improved postnatal neovascularization in terms of limb salvage by fourfold in comparison with the control group's limb perfusion as measured by laser Doppler and capillary density. These findings provide the novel evidence that telomerase activity contributes to EPC angiogenic properties; mitogenic activity, migratory activity, and cell survival. This enhanced regenerative activity of EPC by hTERT transfer will provide a novel therapeutic strategy for postnatal neovascularization in severe ischemic disease patients.

6 Other Potential of EPCs

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 (Bhattacharya et al. 2000). Similarly, when cultured autologous ovine EPCs were seeded onto carotid interposition grafts, the EPC-seeded grafts achieved physiological motility and remained patent for 130 days vs 15 days in nonseeded grafts (Kaushal et al. 2001). 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 (Shimizu et al. 2002a, b). The cell sheets consisting of cardiomyocytes with EPCs expectedly inducing neovessels may be attractive, as blood supply is essential to maintain the homeostasis of implanted cardiomyocytes in such cell sheets.

7 Conclusion

As the concept of BM-derived EPCs in adults and postnatal vasculogenesis are further established, clinical applications of EPCs in 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 more efficient methods of EPC purification and expansion, along with the methods of administration and senescence in EPCs. Alternatively, in case of the impossible utility of autologous BM-derived EPCs in patients with impaired BM function, appreciable 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 these treatments may soon reach clinical fruition.

References

Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964–967

Asahara T, Masuda H, Takahashi T, Kalka C, Pastore C, Silver M, Kearney M, Magner M, Isner JM (1999a) Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ Res 85:221–228

Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner JM (1999b) VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J 18:3964–3972

Bhattacharya V, McSweeney P, Shi Q, Bruno B, Ishida A, Nash R, Storb R, Sauvage L, Hammond W, Wu M (2000) Enhanced endothelialization and microvessel formation in polyester graft seeded with $CD34^+$ bone marrow cells. Blood 95:581–585

Boyer M, Townsend L, Vogel L, Falk J, Reitz-Vick D, Trevor K, Villalba M, Bendick P, Glover J (2000) Isolation of endothelial cells and their progenitor cells from human peripheral blood. J Vasc Surg 31:181–189

Choi K, Kennedy M, Kazarov A, Papadimitriou J, Keller G (1998) A common precursor for hematopoietic and endothelial cells. Development 125:725–732

Cowan KH, Moscow JA, Huang H, Zujewski JA, O'Shaughnessy J, Sorrentino B, Hines K, Carter D, Schneider E, Cusack G, Noone M, Dunbar C, Steinberg S, Wilson W, Goldspiel B, Read EJ, Leitman SF, McDonagh K, Chow C, Abati A, Chiang Y, Chang YN, Gottesman MM, Pastan I (1999) Paclitaxel chemotherapy after autologous stem-cell transplantation and engraftment of hematopoietic cells transduced with a retrovirus containing the multidrug resistance complementary DNA (MDR1) in metastatic breast cancer patients. Clin Cancer Res 5:1619–1628

Crisa L, Cirulli V, Smith K, Ellisman M, Torbett B, Salomon D (1999) Human cord blood progenitors sustain thymic T-cell development and a novel form of angiogenesis. Blood 94:3928–3940

Crosby JR, Kaminski WE, Schatteman G, Martin PJ, Raines EW, Seifert RA, Bowen-Pope DF (2000) Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. Circ Res 87:728–730

Dunbar CE, Kohn DB, Schiffmann R, Barton NW, Nolta JA, Esplin JA, Pensiero M, Long Z, Lockey C, Emmons RV, Csik S, Leitman S, Krebs CB, Carter C, Brady RO, Karlsson S (1998) Retroviral transfer of the glucocerebrosidase gene into $CD34^+$ cells from patients with Gaucher disease: in vivo detection of transduced cells without myeloablation. Hum Gene Ther 9:2629–2640

Fernandez Pujol B, Lucibello FC, Gehling UM, Lindemann K, Weidner N, Zuzarte ML, Adamkiewicz J, Elsasser HP, Muller R, Havemann K (2000) Endothelial-like cells derived from human CD14 positive monocytes. Differentiation 65:287–300

Flamme I, Risau W (1992) Induction of vasculogenesis and hematopoiesis in vitro. Development 116:435–439

Folkman J, Shing Y (1992) Angiogenesis. J Biol Chem 267:10931–10934

Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M, Schuch G, Schafhausen P, Mende T, Kilic N, Kluge K, Schafer B, Hossfeld DK, Fiedler W (2000) In vitro differentiation of endothelial cells from AC133-positive progenitor cells. Blood 95:3106–3112

Gill M, Dias S, Hattori K, Rivera ML, Hicklin D, Witte L, Girardi L, Yurt R, Himel H, Rafii S (2001) Vascular trauma induces rapid but transient mobilization of $VEGFR2^+AC133^+$ endothelial precursor cells. Circ Res 88:167–174

Gunsilius E, Duba HC, Petzer AL, Kahler CM, Grunewald K, Stockha G, Gabl C, Dirnhofer S, Clausen J, Gastl G (2000) Evidence from a leukaemia model for maintenance of vascular endothelium by bone-marrow-derived endothelial cells. Lancet 355:1688–1691

Harraz M, Jiao C, Hanlon HD, Hartley RS, Schatteman GC (2001) $CD34^-$ blood-derived human endothelial cell progenitors. Stem Cells 19:304–312

Iwaguro H, Yamaguchi J, Kalka C, Murasawa S, Masuda H, Hayashi S, Silver M, Li T, Isner JM, Asahara T (2002) Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. Circulation 105:732–738

Iwasaki H, Kawamoto A, Ishikawa M, Oyamada A, Nakamori S, Nishimura H, Sadamoto K, Horii M, Matsumoto T, Murasawa S, Shibata T, Suehiro S, Asahara T (2006) Dose-dependent contribution of CD34-positive cell transplantation to concurrent vasculogenesis and cardiomyogenesis for functional regenerative recovery after myocardial infarction. Circulation 113:1311–1325

Kalka C, Masuda H, Takahashi T, Kalka-Moll WM, Silver M, Kearney M, Li T, Isner JM, Asahara T (2000) Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci USA 97:3422–3427

Kang HJ, Kim SC, Kim YJ, Kim CW, Kim JG, Ahn HS, Park SI, Jung MH, Choi BC, Kimm K (2001) Short-term phytohaemagglutinin-activated mononuclear cells induce endothelial progenitor cells from cord blood $CD34^+$ cells. Br J Haematol 113:962–969

Karlsson S (1991) Treatment of genetic defects in hematopoietic cell function by gene transfer. Blood 78:2481–2492

Kaushal S, Amiel GE, Guleserian KJ, Shapira OM, Perry T, Sutherland FW, Rabkin E, Moran AM, Schoen FJ, Atala A, Soker S, Bischoff J, Mayer JE Jr (2001) Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. Nat Med 7:1035–1040

Kawamoto A, Gwon HC, Iwaguro H, Yamaguchi JI, Uchida S, Masuda H, Silver M, Ma H, Kearney M, Isner JM, Asahara T (2001) Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. Circulation 103:634–637

Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkoff D, Wang J, Homma S, Edwards NM, Itescu S (2001) Neovascularization of ischemic myocardium by human bone marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 7:430–436

Levenberg S, Golub JS, Amit M, Itskovitz-Eldor J, Langer R (2002) Endothelial cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A 99:4391–4396

Lin Y, Weisdorf D, Solovey A, Hebbel R (2000) Origins of circulating endothelial cells and endothelial outgrowth from blood. J Clin Invest 105:71–77

Murasawa S, Llevadot J, Silver M, Isner JM, Losordo DW, Asahara T (2002) Constitutive human telomerase reverse transcriptase expression enhances regenerative properties of endothelial progenitor cells. Circulation 106:1133–1139

Murayama T, Tepper OM, Silver M, Ma H, Losordo D, Isner JM, Asahara T, Kalka C (2002) Determination of bone marrow-derived endothelial progenitor cell significance in angiogenic growth factor-induced neovascularization in vivo. Exp Hematol 30:967

Murohara T, Ikeda H, Duan J, Shintani S, Sasaki K, Eguchi H, Onitsuka I, Matsui K, Imaizumi T (2000) Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. J Clin Invest 105:1527–1536

Nieda M, Nicol A, Denning-Kendall P, Sweetenham J, Bradley B, Hows J (1997) Endothelial cell precursors are normal components of human umbilical cord blood. Br J Haematol 98:775–777

Pardanaud L, Altmann C, Kitos P, Dieterlen-Lievre F, Buck CA (1987) Vasculogenesis in the early quail blastodisc as studied with a monoclonal antibody recognizing endothelial cells. Development 100:339–349

Peichev M, Naiyer AJ, Pereira D, Ahu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MAS (2000) Expression of VEGFR-2 and AC133 by circulating human $CD34^+$ cells identifies a population of functional endothelial precursors. Blood 95:952–958

Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL (2001) Differentiation and expansion of endothelial cells from human bone marrow $CD133^+$ cells. Br J Haematol 115:186–194

Reisner Y, Segall H (1995) Hematopoietic stem cell transplantation for cancer therapy. Curr Opin Immunol 7:687–693

Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM (2002) Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 109:337–346

Risau W, Flamme I (1995) Vasculogenesis. Annu Rev Cell Dev Biol 11:73–91

Risau W, Sariola H, Zerwes HG, Sasse J, Ekblom P, Kemler R, Doetschman T (1988) Vasculogenesis and angiogenesis in embryonic stem cell-derived embryoid bodies. Development 102:471–478

Schatteman GC, Hanlon HD, Jiao C, Dodds SG, Christy BA (2000) Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. J Clin Invest 106:571–578

Shi Q, Rafii S, Wu MH, Wijelath ES, Yu C, Ishida A, Fujita Y, Kothari S, Mohle R, Sauvage LR, Moore MA, Storb RF, Hammond WP (1998) Evidence for circulating bone marrow-derived endothelial cells. Blood 92:362–367

Shimizu T, Yamato M, Akutsu T, Shibata T, Isoi Y, Kikuchi A, Umezu M, Okano T (2002a) Electrically communicating three-dimensional cardiac tissue mimic fabricated by layered cultured cardiomyocyte sheets. J Biomed Mater Res 60:110–117

Shimizu T, Yamato M, Isoi Y, Akutsu T, Setomaru T, Abe K, Kikuchi A, Umezu M, Okano T (2002b) Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. Circ Res 90:e40

Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T (2001) Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation 103:2776–2779

Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner J, Asahara T (1999) Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 4:434–438

Tamaki T, Akatsuka A, Ando K, Nakamura Y, Matsuzawa H, Hotta T, Roy RR, Edgerton VR (2002) Identification of myogenic-endothelial progenitor cells in the interstitial spaces of skeletal muscle. J Cell Biol 157:571–577

Weiss M, Orkin SH (1996) In vitro differentiation of murine embryonic stem cells: new approaches to old problems. J Clin Invest 97:591–595

Wong-Staal F, Poeschla EM, Looney DJ (1998) A controlled phase 1 clinical trial to evaluate the safety and effects in HIV-1 infected humans of autologous lymphocytes transduced with a ribozyme that cleaves HIV-1 RNA. Hum Gene Ther 9:2407–2425

Yin A, Miraglia S, Zanjani E, Almeida-Porada G, Ogawa M, Leary A, Olweus J, Kearney J, Buck D (1997) AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 90:5002–5012