Original Article

Development of Angiogenic Cell and Gene Therapy by Transplantation of Umbilical Cord Blood with Vascular Endothelial Growth Factor Gene


Endothelial progenitor cells (EPCs) are present in the mononuclear cells (MNCs) of umbilical cord blood and peripheral blood. To establish the efficiency of angiogenic cell and gene therapies, we transfected the human vascular endothelial growth factor (hVEGF) gene into cord blood MNCs to enhance endothelialization. MNCs from cord blood and peripheral blood were isolated and transfected with pCR3 expressing hVEGF165 or GFP by the Hemagglutinating Virus of Japan (HVJ)-envelope and the cells were cultured in endothelium basal medium-2. The number of attached cells from cord blood was higher than that from peripheral blood. Attached cells expressed Flk-1, VE-cadherin, PECAM-1, CD34, and Tie-2. The increase in the number of attached cells was transient with the transfection of vascular endothelial growth factor (VEGF) gene early in the experimental period. Flt-1 mRNA was not expressed early in the culture period, but was expressed at 2 weeks after separation. VEGF gene transfer into MNCs at 12 days after separation, i.e., when Flt-1 mRNA was expressed continuously, increased the number of attached cells. We evaluated the effects of the transplantation of cord blood MNCs expressing the hVEGF gene on regional blood flow in an ischemic area in a rat model of chronic hindlimb ischemia. Blood flow was significantly improved in nude rats that received transplanted control MNCs. Transplantation of cord blood MNCs transfected with the hVEGF gene yielded greater improvements in blood flow. These results indicate that the hVEGF gene enhances endothelialization of EPCs, and that the transplantation of cord blood MNCs transfected with the VEGF gene may be feasible for the treatment of ischemic diseases as a type of angiogenic cell and gene therapy. (Hypertens Res 2004; 27: 119–128)

Key Words: cord blood, gene and cell therapy, vascular endothelial growth factor, angiogenesis, regenerative medicine

Introduction

Two major processes, vasculogenesis and angiogenesis (1), contribute to the development of vascular tissue. Vasculogenesis refers to the in situ formation of blood vessels from endothelial progenitor cells (EPCs) or angioblasts (2), whereas angiogenesis occurs by the sprouting of new capillaries from pre-existing mature endothelial cells (ECs). Until recently, vasculogenesis was thought to be restricted to the embryo...
(2), and neovascular formation in adults was thought to be the consequence of angiogenesis alone. However, EPCs have been identified in mononuclear cells (MNCs) from adult bone marrow (3). These EPCs produce cytokines with cell-cell interactions, which then lead to differentiation into mature ECs in vitro (4). EPCs in MNCs from bone marrow are mobilized from the peripheral blood to areas of organ ischemia, where the EPCs accumulate in vivo (5). Thus, EPCs are thought to contribute to neovascularization in adults.

Bone marrow-derived MNC transplantation (BMT) has been performed to generate therapeutic angiogenesis, which can reduce pain or improve ischemic ulcers due to limb ischemia by improving blood flow (6). BMT can also be used to treat ischemic heart diseases, and could possibly be used to improve left ventricular function as well.

However, BMT requires a large amount of bone marrow, and marrow extraction under general anesthesia is associated with a large risk when performed on patients with severe heart diseases. To address these problems, transplantation of cord blood, which is rich in EPCs, has been carried out (7). Moreover, transplantation of peripheral blood MNCs transfected with the vascular endothelial growth factor (VEGF) gene has been shown to improve neovascularization and blood flow recovery in vivo (8).

To establish the efficiency of both angiogenic cell therapy and gene therapy, we transferred the human VEGF (hVEGF) gene into cord blood MNCs in order to enhance endothelialization. We then examined the effects of transplantation of these transfected MNCs on chronic hindlimb ischemia in nude rats.

Methods

Source of Cord Blood

Written informed consent was obtained from all mothers participating in the study to obtain cord blood samples prior to labor and delivery.

Cell Culture

MNCs from cord blood and peripheral blood were isolated with lymphoprep (Nycomed Pharma, Oslo, Norway). Thirty milliliters of blood was placed onto 15 ml of lymphoprep, and the sample was centrifuged at 800 µg for 30 min at room temperature. MNCs formed as a distinct band at the serum/lymphoprep interface. These MNCs were washed twice in endothelium basal medium (EBM-2, Clonetics, San Diego, USA), resuspended in EBM-2 supplemented with 2% fetal bovine serum, human fibroblast growth factor-B, hVEGF, insulin-like growth factor-1, ascorbic acid, and human epidermal growth factor, and the cells were plated in culture dishes coated with type I collagen. The cells were then observed every 4 days under a conventional light microscope.

Immunohistochemistry

Isolated MNCs were cultured in 8-chamber slides. Thirty days after isolation, the cells were washed twice with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 15 min and 70% ethanol for 5 min. After blocking with 5% normal horse serum and 3% bovine serum albumin in PBS, the cells were stained with primary antibody (Flk-1, VE-cadherin, PECAM-1, CD34, or Tie-2; Santa Cruz Biotechnology, Santa Cruz, USA) diluted 1 : 200 in PBS for 30 min at 37°C, and then they were stained with the appropriate biotinylated secondary antibody for 30 min at 37°C. The immunocomplexes were visualized with ABC reagent (Santa Cruz Biotechnology).

Gene Transfer

cDNA of hVEGF165 or cDNA of GFP was subcloned into a multicloning site of pCR3 (phVEGF165 or pGFP), as described previously (9), in which VEGF165 or GFP expression was under the transcriptional control of the cytomegalovirus promoter. Hemagglutinating Virus of Japan (HVJ)-envelope vector (Ishihara-Sanyo Inc., Osaka, Japan) containing phVEGF165 or pGFP was added to each culture dish (100 ng plasmid/well) and incubated at 37 ºC. To determine the transfection efficiency, cells transfected with pGFP were observed 2 days after transfection under a fluorescence microscope. Five visual fields were selected randomly, and the number of fluorescent cells, as well as that of attached cells, was counted. Transfection efficiency (%) was calculated as follows: (the number of cells expressing GFP/the number of attached cells) × 100.

Reverse Transcription–Polymerase Chain Reaction (RT-PCR) Analysis

Cord blood MNCs were inoculated at a density of 1.5 × 10^6 cells/cm² in 24-well culture plates. The total RNA was extracted at 1, 4, 8, 16, and 24 days after inoculation with Isogen (Nippon Gene, Toyama, Japan). Cells were lysed in 1 ml of Isogen and were incubated for 5 min at room temperature. Cell lysates were mixed with 0.2 ml chloroform, shaken vigorously for 15 s, incubated for 3 min at room temperature, and centrifuged at 12,000 × g for 15 min at 4°C in order to extract the total RNA. An aliquot (500 µl) of each aqueous phase was mixed with an equal volume of isopropanol, incubated for 10 min at room temperature, and centrifuged at 12,000 × g for 10 min at 4°C to precipitate the RNA. The RNA pellet was washed with 1 ml of 70% (vol/vol) ethanol by vortex mixing and centrifugation at 7,500 × g for 5 min at 4°C; then, the pellet was dried and dissolved in 10 µl of 10 mmol/l Tris-HCl (pH 8.0).

RT-PCR analyses of rat VEGF, Flt-1, and PECAM-1 mRNAs, and 18S ribosomal RNA (rRNA) as an internal control were performed with 1 µg of total RNA, as described
previously (10). The amplification conditions were 30 cycles of denaturing at 94°C for 1 min, primer annealing at 60°C for 1 min, and extension at 72°C for 1 min in a DNA thermal cycler (Perkin-Elmer-Cetus, Norwalk, USA). The VEGF sense primer (5:\textasciitilde CAGGGCAGAATCATCACGAGT-3)\  and antisense primer (5:\textasciitilde GTAGAGATCTGGTTCCCGAA\ AC-3)\ were derived from the coding region of human VEGF cDNA. The Flt-1 sense primer (5:\textasciitilde GATGGTCAGCTA\ CTGGGACAC-3)\  and antisense primer (5:\textasciitilde GTGCTTCACA\ GTCGAGAAGC-3)\ were derived from the coding region of the hVEGF receptor cDNA. The PECAM-1 sense primer (5:\textasciitilde AGGCAGAACTACTGCTGCT-3)\  and antisense primer (5:\textasciitilde GTCATAGATCCGGACTTC-3)\ were derived from the coding region of the human PECAM-1 cDNA. Sense primer (5:\textasciitilde CGACGACCCATTCGAACGTCT-3)\  and antisense primer (5:\textasciitilde GTATGGGACTGGAATTACCG-3)\ were used for the amplification of 18S rRNA. The PCR products were separated by electrophoresis on 2% agarose gels stained with ethidium bromide and the samples were then visualized by UV transillumination.

Flow Cytometric Analysis
MNCs from cord blood and MNCs (30-day-culture) from cord blood transplanted with VEGF gene at 12 days after isolation were subjected to flow cytometric analysis to examine the surface expression of Flk-1, VE-cadherin, PECAM-1, and von Willbrand factor (vWF). Cultured cells were detached from the culture plates by incubation with EDTA and trypsin. The cells were stained with primary antibodies specific for Flk-1 (Sigma, St. Louis, USA), VE-cadherin (Chemicon, Temecula, USA), PECAM-1, and vWF (Becton Dickinson, San Jose, USA) for 30 min at 37°C. The cells were then stained with fluorescein- or phycoerythrin-conjugated secondary antibody for 10 min at 37°C and were analyzed by flow cytometry (FACSCalibur, Becton Dickinson, Franklin Lakes, USA).

In Vivo Angiogenesis
Our investigation conformed to the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

MNCs collected from cord blood were cultured in EBM-2 in 6-well culture dishes coated with type I collagen. After 12 days of culture, the cells were transfected with HVJ-envelope vector containing pHVEGF165 for 3 h, and then they were washed with culture medium to remove the floating cells. Attached cells were detached with 0.05% trypsin (Gibco Life Technologies, Gaithersburg, USA) in Ca\(^{2+}\)- and Mg\(^{2+}\)-free Dulbecco’s PBS.

We used a rat model of surgically induced hindlimb ischemia to determine whether or not the transplantation of cord blood-derived cells transfected with the hVEGF gene would induce postnatal neovascularization in vivo. Because we used human cells for the transplantation, immunodeficient (F344/N nu/nu) nude rats were used to avoid graft-vs.-host disease. Rats were subjected to severe limb ischemia by resection of the left femoral artery under anesthesia with sodium pentobarbital (50 mg/kg, intraperitoneal delivery). Rats with chronic hindlimb ischemia were used for these experiments 4 weeks after surgery.

For the transplantation, 10\(^6\) cord blood-derived attaching cells transfected with or without the VEGF gene were injected into 10 points (10\(^7\) cells/body) in the chronic ischemic hindlimb area; transplantation was performed by local intra-muscular injection under anesthesia. Four weeks after transplantation (8 weeks after surgery), regional blood perfusion was evaluated by near-infrared spectroscopy (SciMedia, Irvine, USA). The hindlimb muscles of the rats were monitored for changes in the levels of oxyhemoglobin during a 10-s period of arterial occlusion and recovery. Upon initiation of occlusion, oxyhemoglobin levels fell progressively until they reached a plateau during the latter half of the occlusion period. After muscle oxygenation stabilized at the lowest value, the occlusion was released. At this point in the experiment, the oxyhemoglobin level increased rapidly and reached the maximal value within 1 min after release. The oxyhemoglobin level then returned to the control level within 7 min. The time required for recovery from ischemia was defined as the time elapsed from circulatory release to the maximal oxyhemoglobin value.

Histological Capillary Density
Nude rats with hindlimb ischemia, which had received a transplant of cord blood MNCs transfected with the VEGF gene, were sacrificed, and the hindlimb muscles were isolated and fixed in formalin. The muscles were then embedded in paraffin and 5-\(\mu\)m-thick sections were prepared. Anti-PECAM-1 antibody was used to identify endothelial cells, and the capillary density was assessed to determine the efficacy of treatment at the level of the microcirculation. Four microscopic fields from each section from each tissue were observed, and the capillary density was expressed as the number of capillaries per mm\(^2\).

Results
Culture of MNCs from Peripheral Blood or Cord Blood in EBM
MNCs isolated from peripheral blood and cord blood on day 4 during cell separation were floating in dishes coated with type I collagen. The number of floating cells gradually decreased, and the cells attached to the dishes on days 8 and 16. By day 30, only attached, spindle-shaped cells were observed. The number of attached cells from cord blood was significantly greater than that from peripheral blood on days 16.

Histological Capillary Density
Nude rats with hindlimb ischemia, which had received a transplant of cord blood MNCs transfected with the VEGF gene, were sacrificed, and the hindlimb muscles were isolated and fixed in formalin. The muscles were then embedded in paraffin and 5-\(\mu\)m-thick sections were prepared. Anti-PECAM-1 antibody was used to identify endothelial cells, and the capillary density was assessed to determine the efficacy of treatment at the level of the microcirculation. Four microscopic fields from each section from each tissue were observed, and the capillary density was expressed as the number of capillaries per mm\(^2\).

Results
Culture of MNCs from Peripheral Blood or Cord Blood in EBM
Fig. 1. Morphological changes in mononuclear cells (MNCs) from adult peripheral blood and cord blood. MNCs were isolated by using lymphoprep, and the cells were cultured in endothelium basal medium-2 (EBM-2) supplemented with 2% fetal bovine serum, human fibroblast growth factor-B, human VEGF, insulin-like growth factor-1, ascorbic acid, and human epidermal growth factor. Cell morphology was assessed every 4 days by conventional light microscopy (Day 4 and 8, ×40; Day 16 and 30, ×200). The white column represents peripheral blood, and the black column represents cord blood. * p<0.05, ** p<0.01 between the indicated columns.

Fig. 2. Immunostaining of cultured mononuclear cells (MNCs) for Flk-1, VE-cadherin, PECAM-1, CD34, and Tie-2 (×200). Isolated MNCs were cultured in 8-chamber slides. Thirty days after separation, the cells were stained with primary antibodies (Flk-1, VE-cadherin, PECAM-1, CD34, and Tie-2) and biotinylated secondary antibodies.
16 (p<0.05) and 30 (p<0.01) (Fig. 1).

To characterize the attached cells at day 30, they were immunostained with endothelial cell-specific surface markers (Flk-1, VE-cadherin, PECAM-1, CD34, and Tie-2). Cells from cord blood were stained with endothelial markers, indicating that the cord blood MNCs had changed into endothelial cells in the culture with EBM (Fig. 2).

**Effects of VEGF Gene Transfer into Cord Blood MNCs on Endothelialization**

To enhance endothelialization, phVEGF165 was transfected into cord blood MNCs. The transfection efficiency for pGFP with HVJ-envelope was 40% (Fig. 3A).

Cord blood MNCs were incubated with either 100, 250, or 500ng/well phVEGF165 or with 1, 10, or 100ng/ml recombinant hVEGF protein. Five hundred nanograms/well of phVEGF165 was found to be most effective for inducing endothelialization. Equal efficiency was observed with 10ng/ml of recombinant hVEGF protein (Fig. 3B).

Changes in the number of attached cells in culture from peripheral blood MNCs and cord blood MNCs transfected with phVEGF165 are shown in Figs. 4 and 5, respectively. The number of attached cells increased rapidly, with a peak observed at day 8. This number gradually decreased after transfection with phVEGF165, whereas it increased and remained elevated after transfection with pGFP (Fig. 4). These results were also obtained with cultures of cord blood MNCs (Fig. 5).

**Changes in the Expression of Endothelial Cell Marker mRNAs**

Changes in the expression of mRNAs encoding endothelial cell markers (VEGF, Flt-1, and PECAM-1) in cultured cord blood MNCs, with or without transfection of phVEGF165, are shown in Fig. 6. Expression of VEGF mRNA increased gradually in untransfected cord blood MNCs until day 24. The levels of VEGF mRNA were considerably higher from days 1 to 24 in transfected MNCs, in comparison with the levels observed in the untransfected cells. The mRNA for Flt-1, a receptor for VEGF, was not detected in cord blood MNCs on day 1, but it was detected on days 16 and 24 in the untransfected cells. Flt-1 mRNA was not expressed in the cord blood MNCs transfected with phVEGF165. Moreover, PECAM-1, a platelet/endothelial cell adhesion molecule, was expressed from days 1 to 24 in the control cord blood MNCs. The levels of PECAM-1 mRNA were higher on day 1, but were lower on days 16 and 24 in the cord blood MNCs transfected with phVEGF165, in comparison with the levels obtained in the untransfected cells (Fig. 6).
Fig. 4. (A) Differentiation and growth of mononuclear cells (MNCs) from peripheral blood transfected with GFP plasmid (pGFP, 40) or human VEGF165 plasmid (phVEGF165). MNCs from cord blood were incubated with an HVJ-envelope vector containing 500 ng of phVEGF165 or pGFP for 4, 8, 16, and 30 days. (B) Changes in the number of attached cells from peripheral blood MNCs transfected with phVEGF165 or pGFP. The white column represents MNCs transfected with pGFP, and the black column represents MNCs transfected with phVEGF165. * p<0.05, ** p<0.01 between the indicated columns.

Fig. 5. (A) Differentiation and growth of mononuclear cells (MNCs) from cord blood transfected with GFP plasmid (40) or human VEGF165 plasmid (phVEGF165). MNCs from cord blood were incubated with an HVJ-envelope vector containing 500 ng of phVEGF165 or pGFP for 4, 8, 16, and 30 days. (B) Changes in the number of attached cells from cord blood MNCs transfected with phVEGF165 or pGFP. The white column represents MNCs transfected with pGFP, and the black column represents MNCs transfected with phVEGF165. * p<0.05, ** p<0.01 between the indicated columns.
Endothelialization of Cord Blood MNCs by VEGF Gene Transfer

We investigated the efficiency of VEGF gene transfer after different culture periods using cord blood MNCs for the endothelialization. MNCs were transfected with phVEGF165 on days 1, 8, 12, and 16 of culture, and the number of attached cells was counted on day 30 of the culture period. The number of attached cells increased beginning on day 1 of the culture period, and the number peaked on day 12 (Fig. 7A and B). We then analyzed the expression of surface markers in order to determine whether or not the attached cells were endothelial cells. Flk-1, VE-cadherin, PECAM-1, and vWF were positive in these cells (Fig. 7C). These results indicate that the transfection of hVEGF gene on day 12 of culture most effectively enhanced the endothelialization of cord blood MNCs. Cells transfected on day 12 were used for later experiments.

Effects of Transplantation of Cord Blood MNCs Transfected with VEGF Gene on Blood Flow and Capillary Density in Ischemic Hindlimbs

We examined the effects of the transplantation of cord blood MNCs transfected with or without the VEGF gene on nude rats with hindlimb ischemia. The blood flow recovery time was immediately prolonged after resection of the left femoral artery and at 4 weeks after resection. Recovery time was significantly lower in rats that had received a transplant of control MNCs. VEGF gene transfer into the cord blood MNCs further shortened the recovery time, suggesting that blood flow to the ischemic area had improved (Fig. 8A). Hindlimb muscle tissues were stained with anti-PECAM-1 antibody. The capillary density was increased in rats trans-
planted with MNCs without the VEGF gene, in comparison with the results obtained for the control rats. VEGF gene transfer into cord blood MNCs was thus found to significantly increase capillary density (Fig. 8B). These findings indicate that neovascularization is enhanced by transplantation with either MNCs or VEGF-transfected MNCs.

Discussion

In the present series of experiments, a greater number of ECs differentiated from cord blood MNCs than from peripheral blood MNCs, indicating that cord blood is a useful source of EPCs. Kalka et al. (11) recently reported that EPCs derived from human umbilical cord blood show faster differentiation than EPCs derived from adult peripheral blood. These data support findings that EPCs derived from cord blood have greater proliferative activity than those derived from adult peripheral blood. In addition, Mayani and Lansdorp (12) and Vaziri et al. (13) showed that cord blood has an accelerated cell cycle duration, as well as longer telomere activity. Thus, cord blood-derived EPCs may have greater endothelialization capabilities, which may prove to be useful for future application in cases requiring therapeutic angiogenesis.

The transplantation of cells with genes encoding growth factors such as VEGF, HGF, and FGF is expected to facilitate the differentiation of EPCs into ECs. Cord blood contains hematopoietic stem cells and EPCs. These progenitor cells are thought to be derived from a common precursor known as the hemangioblast (14, 15). EPCs differentiate into ECs in order to induce vasculogenesis and angiogenesis. Badorff et al. (16) recently reported that EPCs can also differentiate into cardiomyocytes. Because cord blood MNCs include multifunctional progenitor cells, phenotypic modulation by factors that induce and potentiate differentiation into ECs could be useful for the endothelialization of cord blood MNCs in cases requiring therapeutic angiogenesis. Young et al. (17) recently reported that both the incorporation of donor ECs into the newborn neovascularature and tissue vascularity were increased by the coadministration of VEGF with bone marrow cells, suggesting that bone marrow-derived EPCs are responsive to VEGF. Thus, transfection of the VEGF gene into cord blood MNCs is expected to en-

Fig. 8. (A) Effect of transplantation of mononuclear cells (MNCs) transsected with hVEGF165 plasmid (phVEGF165) on blood flow in nude rats with chronic hindlimb ischemia. MNCs were incubated for 3 h with an HVJ-envelope vector containing phVEGF165. MNCs were transplanted at 10^7 cells/animal in the chronic ischemic hindlimb area. Four weeks after inoculation (8 weeks after the surgery), regional blood perfusion was evaluated by near-infrared spectroscopy. The hindlimb muscles of the rats were monitored for changes in oxyhemoglobin during a 10-s period of arterial occlusion and recovery. The recovery time from ischemia was defined as the time elapsed from circulatory release to the maximal oxyhemoglobin value. *p < 0.05, **p < 0.01 vs. Before binding. *p < 0.05, **p < 0.01 between the indicated columns. (B) The capillary density in the hindlimb muscle tissues transplanted with cord blood MNCs carrying the hVEGF gene. Anti-PECAM-1 antibody was used to identify the endothelial cells ( immunostaining), and the capillary density was evaluated histologically. Four microscopic fields from each section from each tissue sample were analyzed, and the capillary density was expressed as the number of capillaries per square millimeter. *p < 0.05, **p < 0.01 between the indicated columns.
enhance endothelialization, which would be useful for angiogenesis to treat arterial obstructive diseases.

VEGF acts via the Flt-1 and Flk-1 receptors to induce EC proliferation (18–20). Therefore, in order to optimize this system, we first investigated the efficiency and timing of VEGF gene transfer into cord blood MNCs. The expression of Flt-1 was not detected in the cord blood MNCs after a short period of culture, and was detected in untransfected cells 16 days after separation. However, the expression of Flt-1 mRNA was not detected in cord blood MNCs transfected with the VEGF gene. The expression of mRNA for PECAM-1 was detected between days 1 to 24, inclusively, in the cord blood MNCs, and this expression increased transiently early on in the experimental period. PECAM-1 mRNA expression was lower later in the experimental period in the case of the cord blood MNCs transfected with the VEGF gene. These results indicate that the control MNCs from cord blood differentiated into ECs, whereas VEGF gene transfer transiently enhanced endothelialization, but suppressed endothelialization after a prolonged culture period.

Therefore, we transfected the VEGF gene into cord blood MNCs at a later stage of culture, i.e., when the attached cells expressed Flt-1 mRNA, and we found that the transfer of the VEGF gene at a later stage enhanced endothelialization. These findings indicate that delayed transfer of the VEGF gene enhances endothelialization of cord blood MNCs. In our in vivo study, we injected cultured cord blood MNCs transfected with the VEGF gene at a later stage into the ischemic areas in the hindlimbs of nude rats. The injection of cord blood MNCs with the VEGF gene significantly increased blood flow in the ischemic hindlimb in comparison with the effects of control cord blood MNCs. Iwaguro et al. (21) also transfected the VEGF gene into peripheral blood MNCs and transplanted these cells into the ischemic hindlimb of rats and found that VEGF-transfected MNCs induced angiogenesis. Their findings are therefore consistent with our results. However, we used cord blood instead of peripheral blood, and we transfected the VEGF gene into cord blood MNCs at a specific culture time to enhance angiogenesis. The increased blood flow observed after the transplantation of cord blood MNCs with the VEGF gene was likely due to angiogenesis resulting from the expansion of EPCs.

The transfer of expression plasmids with viral vectors, including adenoviruses and retroviruses, has been recognized as potentially useful in the field of gene therapy. However, viral vectors are problematic due to the potentially compromised safety associated with their use, especially with respect to the replication of the viral vector. The HVJ-envelope vector used in the present study is composed of inactivated HVJ, which cannot replicate (22), suggesting that the HVJ-envelope vector is safer than adenov- or retroviral vectors.

Stem cells and progenitor cells may circulate in the body and act to repair tissues when an organ is damaged and begins to fail (23). Stem cells, including embryonic stem cells, have the potential to differentiate into several types of cells. In addition, one mechanism of plasticity was reported to be relevant to stem cell fusion with other cells, by which the stem cells transdifferentiate into the fusion cell type (24). Therefore, stem cells appear to have potential for use as a vector for the transfer of a target gene into a host. In the present experiments, the transplantation of cord blood MNCs carrying the VEGF gene efficiently increased blood flow in the ischemic hindlimbs of rats, suggesting that EPCs acted as a vector, transferring the VEGF gene to the ischemic area and inducing angiogenesis.

The transplantation of cord blood MNCs with the VEGF gene is a promising therapy for arterial obstructive diseases and ischemic heart diseases. However, as a source of EPCs, cord blood may induce graft-vs.-host disease because it is allogenic; on the other hand, self-derived peripheral blood is difficult to use in the treatment of acute ischemic diseases. Umbilical cord blood transplantation has been used safely to treat the bone marrow suppression that occurs with chemotherapy for leukemia. Thus, transplantation of umbilical cord blood is applicable to cases requiring therapeutic angiogenesis. Therefore, allograft transplantation of banked cord blood transfected with the VEGF gene, in conjunction with immunosuppressive therapy, may be a new standard therapy for patients with severe ischemic diseases.

EPCs appear to be important for vasculogenesis and angiogenesis in ischemic tissues and for the maintenance of healthy arteries. Hill et al. (25) investigated EPC formation in the peripheral blood of patients with cardiovascular diseases; they found that impaired EPC function is correlated with cardiovascular risk factors such as hypertension, atherosclerosis, and diabetes mellitus. These findings suggest that endothelial injury in the absence of a sufficient number of circulating EPCs may influence the progression of cardiovascular diseases.

In conclusion, hVEGF gene transfer is effective at enhancing the endothelialization of EPCs. Furthermore, the transplantation of cord blood MNCs expressing VEGF may be feasible as an angiogenic cell and gene therapy for the treatment of ischemic diseases.

References


