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A comparison of human cord blood- and embryonic stem cell-derived endothelial progenitor cells in the treatment of chronic wounds

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ABSTRACT

Endothelial progenitor cells (EPCs) promote new blood vessel formation and increase angiogenesis by secreting growth factors and cytokines in ischemic tissues. Therefore, EPCs have been highlighted as an alternative cell source for wound healing. EPCs can be isolated from various sources, including the bone marrow, cord blood, and adipose tissue. However, several recent studies have reported that isolating EPCs from these sources has limitations, such as the isolation of insufficient cell numbers and the difficulty of expanding these cells in culture. Thus, human embryonic stem cells (hESCs) have generated great interest as an alternative source of EPCs. Previously, we established an efficient preparation method to obtain EPCs from hESCs (hESC-EPCs). These hESC-EPCs secreted growth factors and cytokines, which are known to be important in angiogenesis and wound healing. In this study, we directly compared the capacity of hESC-EPCs and human cord blood-derived EPCs (hCB-EPCs) to benefit wound healing. The number of hESC-EPCs increased during culture and was always higher than the number of hCB-EPCs during the culture period. In addition, the levels of VEGF and Ang-1 secreted by hESC-EPCs were significantly higher than those produced by hCB-EPCs. After transplantation in a mouse dermal excisional wound model, all EPC-transplanted wounds exhibited better regeneration than in the control group. More importantly, we found that the wounds transplanted with hESC-EPCs showed significantly accelerated re-epithelialization. Thus, hESC-EPCs may be a promising cell source for the treatment of chronic wounds.

1. Introduction

Knowledge of the wound-healing process has significantly increased, which has resulted in the development of numerous therapeutic options, including cultured keratinocyte grafts [1–4], composite grafts [5–8], and preparations containing growth factors [9–11]. Despite the development of a vast array of therapeutics, the best available treatment for chronic wounds demonstrates only a 50% healing rate that is often temporary [12], and many people still suffer from chronic wounds. Thus, therapies must be sought for improving wound healing. Recently, endothelial progenitor cells (EPCs) have been suggested as a promising cell source that can both generate the epidermal component and improve the functionality of the dermal component [13–15].

The wound-healing process is a complex alignment of various biological processes and involves the collaboration of many different cells and tissues in various molecular and cellular events [16]. EPCs have the capacity to induce neovascularization, recruit other cells to a site of injury, and secrete growth factors and cytokines that have a paracrine effect on surrounding cells [17,18]. In addition, EPCs can be isolated from various sources, such as the peripheral blood, bone marrow, adipose tissue, and umbilical cord blood [19]. Indeed, transplantation of EPCs isolated from various sources has been attempted for wound healing in animal models

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[17,18,20–23], and animals receiving EPCs following experimental injury were shown to have accelerated wound closure, increased inflammatory cell recruitment, and improved neovascularization [17,18,20]. Although EPCs may have a beneficial effect on wound healing, EPCs isolated from the peripheral blood, bone marrow, adipose tissue, and umbilical cord blood have limitations, including the relatively low number of cells obtained from these sources and their difficult expansion [14,24,25]. Therefore, increasing focus is being placed on human embryonic stem cells (hESCs) as a potential source for EPCs.

hESCs could overcome the limitations of insufficient cell number and difficult expansion because these cells can be readily expanded *in vitro* [21,22]. Previously, we established an efficient preparation method to obtain EPCs from hESCs, and these hESC-derived EPCs (hESC-EPC) showed proper EPC characteristics. Furthermore, we demonstrated the therapeutic effect of hESC-EPCs in a mouse hind limb ischemia model [21]; unfortunately, this effect was not compared to the effect of EPCs isolated from other sources. Thus, questions remain regarding the functional significance of EPCs isolated from different sources, and a more in-depth comparison of EPCs from a variety of sources is required to discover useful applications for these cells in a clinical setting. The aim of the present study was to compare the benefit of hESC-EPCs and human cord blood-derived EPCs (hCB-EPCs) on wound healing. We selected hCB-EPCs as a control for this study because EPCs are present in significantly higher numbers in the umbilical cord blood than in other sources, such as the peripheral blood, bone marrow, and adipose tissue [26].

2. Material and methods

2.1. Derivation and culture of hESC-EPCs

Undifferentiated hESCs (the H9- and CHA3-hESC lines) [21,22,27,28] were grown on mitotically inactivated mouse embryonic fibroblasts in DMEM/F12 (50:50%; Gibco BRL, Gaithersburg, MD) supplemented with 20% (v/v) serum replacement (Gibco) and basic ES medium components, including 1 mM L-glutamine (Gibco), 1% nonessential amino acids (Gibco), 100 mM beta-mercaptoethanol (Gibco), and 4 ng/ml bFGF (Invitrogen, Grand Island, NY). The medium was changed every day, and the hESCs were transferred to new feeder cells every 7 days with dissecting pipettes. To induce the hESCs to differentiate into EPCs, the hESCs were allowed to form hEBs in suspension conditions with hESC culture medium containing BMP-4 (20 ng/ml) for 2 days. Subsequently, the hESCs were plated on a Matrigel-coated plate and cultured in DMEM supplemented with 10% FBS (Gibco) for 10 days (differentiation day 12). To isolate EPCs from the other differentiated cells, cell sorting was performed with a FACS Vantage flow cytometer (BD Biosciences, Bedford, MA) using a PE-conjugated mouse anti-human monoclonal PECAM antibody (BD Biosciences). To use the sorted cells for experiments, they were subsequently cultured on collagen-coated plates with EGM2MV (Clonetics, San Diego, CA) and expanded for 3 passages in culture.

2.2. Derivation and culture of hCB-EPCs

The derivation and culture of hCB-EPCs were performed as described previously [14]. Briefly, hCB-EPCs were isolated using Ficoll reagent (GE Healthcare) from a fresh cord blood sample donated from a healthy volunteer at the CHA General Hospital (Seoul, Korea) and cultured on a fibronectin-coated culture dish in EGM-2/MV medium (Lonza).

2.3. Matrigel assay and DiI-labeled ac-LDL uptake

For the Matrigel assay, the sorted hESC-EPC and hCB-EPC suspensions (2.0×10^5 cells) were seeded on Matrigel (BD Biosciences)-coated 6-well plates, incubated at 37 °C for 12 h, and incubated with 10 mg/ml DiI-labeled ac-LDL (Biomedical Technologies, Stoughton, MA) for 4 h at 37 °C. After the incubation, the cells were washed three times with PBS and fixed with 4% (w/v) paraformaldehyde for 20 min. The images were analyzed using an LSM 510 META confocal microscope (Carl Zeiss Inc.).

2.4. Cell proliferation analysis

To test the proliferative ability of EPCs, human fibroblasts (BJ, ATCC, Manassas, VA) served as a control. The EPCs were cultured in EGM2MV medium and human fibroblasts were cultured in DMEM containing 10% FBS for optimal proliferation condition. The cell number and viability were determined with a hemocytometer and trypan blue staining, respectively (Sigma).

2.5. Enzyme-linked immunosorbent assay (ELISA) for FGF-2, VEGF, and Ang-1

The hESC-EPCs, hCB-EPCs, and human fibroblasts were cultured for 48 h in growth factor-free endothelial cell basal medium with 1% (v/v) FBS (EBM, Clonetics). The supernatants were collected and centrifuged to harvest a cell-free solution. EBM containing 1% (v/v) FBS without supplements served as a control. An ELISA for FGF-2, VEGF, and Ang-1 was performed using a Quantikine immunoassay kit (R&D Systems Inc., Minneapolis, USA), according to the manufacturer's instructions. All measurements were performed in duplicate from 3 different experiments.

2.6. Co-culture of human epidermal keratinocytes and EPCs

To assess the effect of EPCs on human epidermal keratinocytes (ATCC), a co-culture system was used. When keratinocytes were co-cultured with hCB-EPCs and hESC-EPCs, the droplet size and proliferation rate of the keratinocytes were measured. A modified co-culture system was applied as shown in Fig. 4A. Briefly, a total of 2.0×10^5 hCB-EPCs and hESC-EPCs were seeded in 35 mm plates and cultured with 2% FBS in DMEM medium. After 24 h, keratinocyte droplets (1.6×10^4 cells; 10 μl) were carefully placed in each well. The keratinocyte droplets were

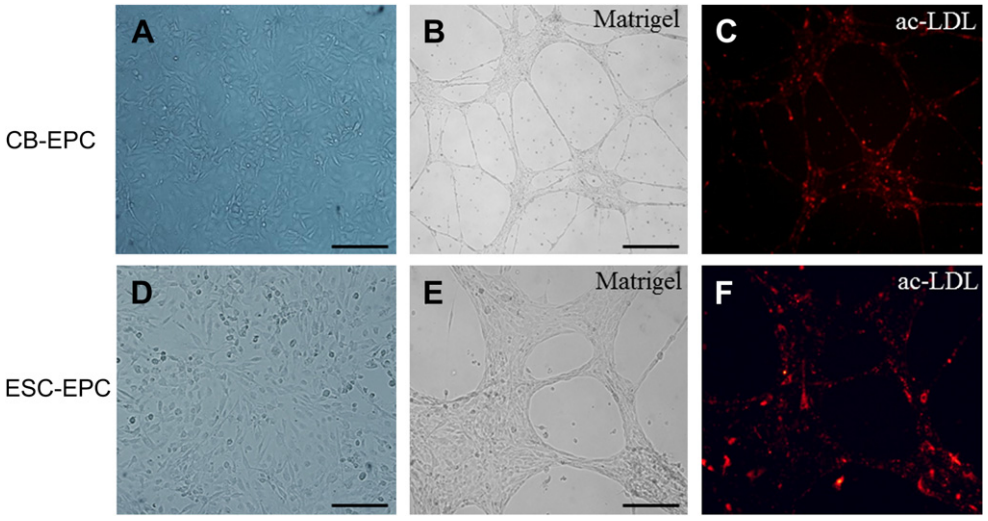

Fig. 1. (A and D) The morphology, (B and E) capillary formation in Matrigel and (C and F) ac-LDL uptake properties of hCB-EPCs and hESC-EPCs for EC-specific functions at passage 7 *in vitro*. Red in cytoplasm represents DiI-labeled acetylated LDL. The hCB-EPC and hESC-EPC were positive. The scale bars indicate 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)