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Ectopic vascularized bone formation by human umbilical cord-derived mesenchymal stromal cells expressing bone morphogenetic factor-2 and endothelial cells

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ABSTRACT

Mesenchymal stromal cells (MSCs) isolated from numerous tissues including human fetal tissue are currently used in cell therapy and regenerative medicine. Among fetal tissues, the umbilical cord (UC) is one of the sources for both MSCs and endothelial cells (ECs). To establish ectopic vascularized bone tissue formation, UC-derived MSCs and ECs were isolated. UC-MSCs expressing human BMP-2 (hBMP-2-MSCs) were generated using an adenoviral system to promote bone formation. These cells were then transplanted with Matrigel into the subcutaneous tissue of an immune deficient NSG mouse, and bone tissue was analyzed after several weeks. The osteogenic differentiation ability of MSCs was elevated by transduction of the hBMP-2 expressing adenoviral system, and vascularization of bone tissue was enhanced by human umbilical vein endothelial cells (HUVEC). In this study, our results provide evidence that MSCs and HUVECs from human umbilical cord are suitable cells to investigate bone tissue engineering. The results also suggest that the co-transplantation of hBMP2-MSCs and HUVECs may be a simple and efficient strategy for improving tissue generation and angiogenesis in bone tissue engineering using stem cells.

1. Introduction

Mesenchymal stromal cells (MSCs), also referred to as mesenchymal stem cells, are widely used and reported in cell therapy and regenerative medicine because they are self-renewable, multipotent, easily accessible and culturally expandable in vitro [1]. Since the first report of MSCs from bone marrow in 1960s, MSCs have been isolated from almost all adult and fetal tissues. Among the fetal tissues, the human umbilical cord is a special source of MSCs. Anatomically, the umbilical cord (UC) is roughly divided into several regions, such as the artery, vein, cord lining, and Wharton's jelly (WJ). Each of these regions has been described previously as giving rise to numerous MSCs [2,3]. In particular WJ is a general source for MSCs because it is thought that the cells in the WJ are actually primitive MSCs originating from the mesenchyme that were already present within the UC matrix [4].

Bone formation using MSCs harboring osteogenic differentiation

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potential is thought to be a valuable tool in tissue regeneration. Moreover, during the process of bone formation, MSCs acquire specific phenotypes under the control of respective regulatory factors [5]. Bone morphogenetic proteins (BMPs), which are members of the transforming growth factor (TGF)-β superfamily, are one of the regulatory factors. BMPs influence osteoblast differentiation and rapid bone formation when implanted into mouse tissue [6]. It has been reported that local administration of human BMP-2 (hBMP-2) protein improved not only ectopic bone formation [7], but also healing of bone defects in vivo [8]. In ectopic bone formation, BMP-2 plays an important role in the rapid induction of bone matrix by remodeling high-grade mineralized mature bone similar to that observed in normal bone development [9].

Vascularization is also important in tissue engineering because it supplies oxygen and nutrients to cells in tissues. The classical bone engineering strategies have a weakness due to the lack of vascularization within engineered bone tissues. Among various strategies to enhance the vascular networks in bone tissues, co-transplantation of MSCs with human umbilical vein endothelial cells (HUVECs) may represent an efficient method to establish vascularized tissue engineering because endothelial cells promote vascularization through release of angiogenesis factors [10]. Therefore, vascularized bone formation is a multipurpose regenerative process using MSCs.

In this study, to generate vascularized bone tissue in vivo, MSCs and HUVECs were isolated from human UC, and hBMP-2 expressing MSCs were generated using an adenoviral system. Generated hBMP-2-MSCs were subcutaneously transplanted with HUVECs into mice, and we observed enhanced function of hBMP-2 and HUVECs in bone tissue generation and vascularized structure, respectively. Our results provide evidence that hBMP-2 plays an important role in bone formation and suggests that co-transplantation of hBMP2-MSCs and HUVECs may be a simple and efficient strategy for improving the tissue generation and angiogenesis for bone tissue engineering using stem cells.

2. Materials and methods

2.1. Isolation of UC-MSCs

Human UCs were obtained after birth from healthy donors according to a protocol approved by the Institutional Review Board (IRB) of the Samsung Medical Center. UCs were manually chopped into small fragments 1–2 mm^3; in size. These fragments were incubated with 0.1% type I collagenase (Sigma-Aldrich, St Louis, MO) for 1 h with gentle agitation at 37 °C. After sieving with a 70 μm strainer, the cells were plated with Dulbecco's modified Eagle's medium (DMEM) (low glucose), 10% fetal bovine serum (FBS; HyClone, Logan, UT) and 1% penicillin/streptomycin (Gibco, Carlsbad, CA). The cell cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C and the medium was changed twice weekly thereafter.

2.2. Isolation of HUVECs

The isolation of HUVECs was performed according to the process outlined in a previous report [11] with some modifications. Briefly, the umbilical vein was perfused with Hank's Balanced Salt Solution (HBSS; Sigma-Aldrich) to wash out the blood. The vein was then infused with 10 ml of 0.05% collagenase I and incubated in a water bath at 37 °C for up to 20 min. After incubation, the collagenase solution containing HUVEC was flushed from the cord by infusing the vein with HBSS. The suspension was collected and centrifuged at 250 × g for 10 min, resuspended in endothelial growth media-2 (EGM-2; Lonza, Walkersville, MD) with 1% penicillin/streptomycin (Gibco), and cultured at 37 °C in a 5% CO_2 incubator. The medium was changed twice weekly thereafter.

2.3. Flow cytometry

To determine the expression of the positive and negative markers on the UC-MSCs and HUVECs, human anti-CD29 (clone TS2/16), CD34 (4H11), CD44 (IM7), CD45 (HI30), CD44 (IM7), CD45 (HI30), CD105 (SN6), HLA-DR (LN3; eBioscience, SanDiego, CA), CD31 (M89D3), CD73 (AD2), CD90 (5E10), CD144 (55-7H1), CD146 (P1H12), and VEGFR1 (89106; BD Biosciences, San Jose, CA) antibodies were used. Flow cytometry analysis was performed on UC-MSCs and HUVEC at passage 4 using a FACSVerse flow cytometer and Cell Quest Pro software (BD Biosciences).

2.4. In vitro differentiation

2.4.1. Adipogenic differentiation

To induce adipogenic differentiation, UC-MSCs (3×10^3 cells/cm^2) were cultured in DMEM supplemented with 10% FBS, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 1 μM hydrocortisone and 0.1 mM indomethacin (Sigma-Aldrich) for 14 days. The cells were stained with oil red O to observe the fat droplets.

2.4.2. Osteogenic differentiation

To induce osteogenic differentiation, the UC-MSCs (3×10^3 cells/cm^2) were cultured in DMEM with low glucose supplemented with 10% FBS (HyClone), 0.1 μM dexamethasone, 10 mM β-glycerophosphate and 0.25 mM ascorbic acid (Sigma-Aldrich). After 21 days, during which time the medium was changed every 3–4 days, mineralized deposits were observed by Alizarin Red S staining.

2.5. Tubule formation assay

For the tubule formation, 50 μl of Matrigel (Corning, Lowell, MA) was poured into 96-well plates and incubated for 30 min at 37 °C. The HUVECs (1–2 × 10^4 cells) were then re-suspended with EGM-2 and loaded onto the Matrigel. Following incubation at 37 °C overnight, each well was analyzed directly under a microscope.

2.6. In vitro adenovirus transduction in UC-MSCs

To facilitate BMP-2 expression in UC-MSCs, the pGA30-hBMP-2 vector was constructed by cloning a PCR-amplified fragment of hBMP-2 into the pGA30 adenoviral backbone vector (Ad-BMP-2) as described in our previous report [12]. The empty pGA30 vector without hBMP-2 cDNA (Ad-Empty) was used as the 'mock' control. UC-MSCs were plated at a density of 2 × 10^5 cells in 100 mm tissue culture plate. After 24 h, the UC-MSCs were incubated in serum free medium containing Ad-Empty and Ad-BMP-2 virus at a multiplicity of infection (MOI) of 25–175 with 0.01 μM of 4HP4. After 1 h, adenoviruses were removed and replaced with normal MSC culture medium.

2.7. hBMP-2 ELISA analysis

Two days after Ad-BMP-2 transduction, the cell culture supernatants were collected. The concentrations of soluble BMP-2 protein in the supernatants were determined using the Quantikine BMP-2 Immunoassay ELISA kit (R&D system, Minneapolis, MN) according to the manufacturer's instructions.

2.8. Immunoblot analysis

Immunoblot assays were performed using standard methods.