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Comparison of cell proliferation, apoptosis, cellular morphology and ultrastructure between human umbilical cord and placenta-derived mesenchymal stem cells

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HIGHLIGHTS

- We showed that HUCMSCs had higher self-renewal capacity than HPDMSCs.
- The ability of secretion of VEGF, IGF-1, HGF from HPDMSCs was greater than HUCMSCs.
- HPDMSCs had a greater number of large cuboidal or flat cells than HUCMSCs.
- Myofilaments and pseudopods of HPDMSCs were more intensively organized than HUCMSCs.
- Cellular characteristics should be considered when selecting an appropriate source of MSC.

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ABSTRACT

Research in mesenchymal stem cells (MSCs) is mainly focused on applications for treatments of brain and spinal cord injury as well as mechanisms underlying effects of MSCs. However, due to numerous limitations, there is little information on selection of appropriate sources of MSCs for transplantation in clinical applications. Therefore, in this study we compared various properties of human umbilical cord-derived MSCs (HUCMSCs) with human placenta-derived MSCs (HPDMSCs), including cell proliferation, apoptosis, cellular morphology, ultrastructure, and their ability to secrete various growth factors (*i.e.* vascular endothelial growth factor, insulin-like growth factors-1, and hepatocyte growth factor), which will allow us to select appropriate MSC sources for cellular therapy. Cell culture, flow cytometry, transmission electron microscope (TEM) and atomic force microscope (AFM) were used for assessment of HUCMSCs and HPDMSCs. Results showed that the two types of cells appeared slightly different when they were observed under AFM. HUCMSCs appeared more fibroblast-like, whereas HPDMSCs appeared as large flat cells. HUCMSCs had higher proliferative rate and lower rate of apoptosis than HPDMSCs ($p < 0.05$). However, HPDMSCs secreted more of the three growth factors than HUCMSCs ($p < 0.05$). Results of TEM revealed that the two types of MSCs underwent active metabolism and had low degree of differentiation, especially HUCMSCs. Results of AFM showed that HUCMSCs had stronger ability of mass transport and cell migration than HPDMSCs. However, HPDMSCs displayed stronger adhesive properties than HUCMSCs. Our findings indicate that different sources of MSCs have different properties, and that care should be taken when choosing the appropriate sources of MSCs for stem cell transplantation.

1. Introduction

In recent years, increasing evidence has shown that transplantation of mesenchymal stem cells (MSCs) is a promising therapeutic strategy for acute brain or spinal cord injury and progressive degenerative diseases of the central nervous system [12], such as stroke [8], Parkinson's disease (PD) [11], spinal cord

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injury [16], amyotrophic lateral sclerosis (ALS) [10], autoimmune encephalomyelitis (EAE) [1], and multiple system atrophy (MSA) [5]. The therapeutic effects rely not only the differentiation of MSCs into cells of neural lineage to repair damaged tissues, but also on their ability to create a favorable environment for regeneration, such as expression of growth factors or cytokines.

However, MSCs derived from adult tissues, such as the bone marrow, fat, liver, skin, have many disadvantages: for example, insufficient supply of stem cells, decreased proliferation and differentiation capacity with age, and associated risk of infection and development of tumors after stem cell transplantation [14]. It has been shown that bone marrow-derived MSCs (BMSCs) can cause secondary damage in patients after transplantation [15]. By contrast, MSCs derived from fetal tissues have advantages over proliferation and differentiation, however, these tissues are difficult to obtain. Moreover, embryo-derived MSCs are ethically controversial and they are difficult to be cultured *in vitro*, implying that this type of cells is not suitable for clinical research for large-scale applications. Therefore, many researchers have tried to isolate MSCs from fetal appendages, such as the umbilical cord blood, amniotic fluid, umbilical cord and placenta, which can be extensively used in clinical studies because of their availability, the absence of ethical concerns, low oncogenicity and resistance to bacterial and viral contamination. However, the MSCs derived from the umbilical cord blood have unavoidable immunogenicity, and the amount of the amniotic fluid are often limited. Therefore, human umbilical cord-derived MSCs (HUCMSCs) and human placenta-derived MSCs (HPDMSCs) are the most preferable choice when compared with the above-mentioned sources. Based on these advantages, HUCMSCs and HPDMSCs have been widely used in research for treatment of many diseases, such as cirrhosis [19], myocardial infarction [6], spinal cord injury [2], diabetes [7] and tissue engineering [9]. Researchers have therefore proposed HUCMSCs and HPDMSCs to be promising sources of cells for future clinical applications [17]. In this study, we focused on comparing the biological characteristics and ultrastructures of HUCMSCs and HPDMSCs, in order to select suitable sources of MSCs for future clinical application according to their connatural characteristics.

2. Materials and methods

2.1. Materials

Fresh human umbilical cords and placentae were obtained from healthy women ranging from 25 to 30 years old ($n = 8$) who delivered healthy full-term infants by cesarean section at the Department of Obstetrics and Gynecology of the Zhujiang Hospital (Southern Medical University, Guangzhou, Guangdong, PR China) (03/05/2010 to 05/21/2010). These women were healthy with no history of infectious diseases or pregnancy complications, and were confirmed to be negative for HBV, HIV, and syphilis. Informed consent was obtained, and all procedures were approved by the Ethics Committee of our hospital.

2.2. Primary culture of MSCs

2.2.1. Primary culture of HUCMSCs

Fresh human umbilical cords were obtained after birth and washed in phosphate-buffered solution (PBS) (Hyclone, Logan, USA) several times. After removal of blood vessels, the umbilical cords were minced into 1 cm^3 fragments, and subsequently homogenized into 1–2 mm^3 pieces. Tissue cultures were maintained in DMEM medium (Hyclone) supplemented with 10% FBS (Hyclone), and were incubated in a humidified atmosphere with 5% CO_2 at 37 °C.

2.2.2. Primary culture of HPDMSCs

The tissues were collected from the chorionic plate under sterile condition and rinsed with PBS to remove the remaining blood. The tissues were cut into fragments (approximately 1 mm^3) and digested with type-II collagenase solution (Sigma) in a 37 °C water bath for 30 min. The resulting fluid was collected and centrifuged at 1500 rpm for 10 min. The precipitated cells were collected, resuspended with DMEM-LG medium (Hyclone), and transferred into a centrifugation tube containing Ficoll–Hypaque solution, and centrifuged at 2000 rpm for 15 min. The white layer was aspirated, rinsed twice with PBS, and centrifuged again to separate HPDMSCs. The cells were plated in DMEM-F12 medium (Hyclone) containing 10% FBS and incubated in an atmosphere of 5% CO2 at 37 °C with saturated humidity for 7 days (d). The incubation medium was refreshed every 3–4 d to remove non-adherent cells. Cell growth and morphology were observed daily under an inverted microscope. The cells were trypsinized and passaged after reaching a confluence of 80–90%.

2.3. Flow cytometric analysis

Secondary passages of HUCMSCs and HPDMSCs were trypsinized and dissociated into different single cell suspensions. Monoclonal antibodies (Pharmingen, San Diego, USA) that recognize CD29, CD44, CD73, CD105, CD90, CD14, CD34, CD45, CD106, CD133 (at a concentration of 20 μl/10^6 cells) were used. HLA-DR (5 μL) were added into suspension of each antibody solution. Mouse IgG1 (5 μL) was used as a negative control. All of the resulting suspension samples were incubated at 4 °C for 30 min and then analyzed by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA).

2.4. Analyses of cell cycle distributions

Appropriate volumes of above single cell suspensions were dispensed into tubes (1×10^6 cells/tube) and centrifuged. The supernatants were discarded, and the precipitated cells were fixed with ice-cold (−20 °C) 75% ethanol. The fixed cells were resuspended and centrifuged. The cell pellet was treated with RNase (Sigma) to remove RNA, followed by staining with propidium iodide in the dark (4 °C, 30 min). Cell cycle distributions were then analyzed by flow cytometry.

2.5. Detection of cellular apoptosis

Secondary passages of HUCMSCs and HPDMSCs were rinsed twice with 4 °C PBS and resuspended in binding buffers; the cell concentrations were adjusted to 1×10^6 cells/mL. A small volume (100 μL) of these cell suspensions was dispensed into a 5 mL FACS tube, and 5 μL of Annexin V/FITC (Pharmingen, San Diego, USA) and 10 μL of propidium iodide (20 μg/mL) were also added to the tube. The resulting cell suspension was thoroughly mixed and incubated at room temperature in the dark for 15 min. Then, 400 μL of PBS (Hyclone) was added to the tube, and the levels of cellular apoptosis were analyzed by flow cytometry.

2.6. Analysis of cell proliferation

Secondary passages HUCMSCs and HPDMSCs (1000 cells) were plated onto two 96-well plates with 100 μL per well. Over the following 7 days, 10 μL of CCK-8 (Beyotime Institute of Biotechnology, Haimen, PR China) was added into 5 wells, and incubated for 1 h in CO_2 incubator once daily. The absorbance module at 450 nm was analyzed by an auto-microplate reader, and mean value was calculated.

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