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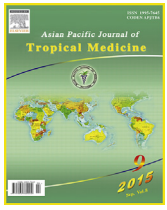

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Comparative analysis of biological characteristics of adult mesenchymal stem cells with different tissue origins

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ABSTRACT

Objective: To invest the differences among mesenchymal stem cells (MSCs) derived from different tissues and their impacts on clinical applications.
Methods: In this study, MSCs were isolated from adipose tissue (AD), umbilical cord tissue (UC), and menstrual blood (Men) and compared their biological characteristics in terms of proliferation capacity, passage capacity, colony formation, and surface markers were compared.
Results: The stem cells (SCs) obtained from different sources were all characterized as MSCs, but demonstrated some differences. Umbilical cord-derived MSCs (UCMSCs) were able to overcome density inhibition. The proliferation rate decreased in the order UCMSCs > MenSCs > ADSCs, while the colony-forming ability decreased in the order MenSCs > ADSCs > UCMSCs. Based on gene-expression data for MSCs from different sources within the same donor, 768 MenSC genes were found that were specifically upregulated or downregulated compared with bone marrow-derived MSCs and UCMSCs, most of which were involved in cell function-related pathways. In addition, MenSCs appeared to be superior in terms of immune inflammation, stress response, and neural differentiation potentials, but weaker in terms of osteogenic and chondrogenic differentiation capacities, compared with UCMSCs and bone marrow-derived MSCs.
Conclusions: MenSCs have higher extraction efficiency, colony-forming ability, and long time passage capacity. Although the proliferation capacity is inferior to UCMSCs.

1. Introduction

Mesenchymal stem cells (MSCs) are a class of adult stem cells with characteristics of self-renewal and pluripotency. MSCs can not only differentiate into mesenchymal cell lineages, but also into non-mesenchymal cell lineages, including astrocytes, oligodendrocytes, and neurons. Studies on the pluripotency of MSCs have laid a solid foundation for their clinical application in the field of regenerative medicine [1–3].

Current research on MSCs is mainly focused on their self-renewal capacity, multi-lineage differentiation potential, surface markers, and immune regulation. Several studies have shown that MSCs derived from different tissues demonstrate a certain extent differences in terms of some of the above-mentioned aspects.

Although the immunophenotypes of MSCs comply with the minimum standards of the International Society for Cellular Therapy, there is no agreement on the expression of other molecular markers. For example, amniotic fluid-derived MSCs sustainably expressed embryonic stem cell-specific markers such as Nanog, SSEA-4 and OCT-4. OCT-4 expression levels were 9.4 times higher than in bone marrow-derived MSCs (BMSCs), while SSEA-4 expression was low in BMSCs but high in amniotic fluid-derived MSCs [4]. Menstrual blood-derived stem cells (MenSCs) are novel stem cells with the basic characteristics of MSCs, but

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expressing different molecular markers. Meng *et al*. found that MenSCs expressed OCT-4, but not NANOG or SSEA-4 [5–7], while Rossignoli *et al*. [8] reported that 19.4% of MenSCs were SSEA-4-positive by flow cytometry. Zemel'ko *et al*. [9] showed high expression of SSEA-4 by immunofluorescence.

MSCs from different tissues also display significant differences in proliferative potential. Barlow *et al*. [10] suggested that the proliferation rate of adipose-derived mesenchymal stem cells (ADSCs) was much higher than that of BMSCs or placenta-derived MSCs. However, placenta-derived MSCs were able to maintain a longer proliferative phase and be continuously cultured for up to 160 d, with up to 64 population doublings. In contrast, BMSCs can only be continuously cultured for 60 d, with up to 12 population doublings [10]. Another study reported that the population-doubling time in umbilical cord-derived mesenchymal stem cells (UCMSCs) was much lower than that of ADSCs, but UCMSCs showed earlier morphological changes and a more rapid decline in amplification ability [9–11]. Some studies found that amniotic fluid-derived MSCs and ADSCs exhibited different levels of telomerase activity [12], suggesting different amplification characteristics.

Studies on the differentiation potentials of MSCs from various sources have confirmed the differentiation potential of mesoderm three-line, but their capacity to differentiate into cells of other lineages remains controversial. Panepucci *et al*. [13] suggested that the functional difference between MSCs from different sources may be related to the origin of the cells. BMSCs are prone to differentiate into osteoblasts, while UCMSCs are prone to angiogenesis, confirming the 'imprint' differentiation potential theory of MSC lineage proposed by Satomura *et al*. [14]. However, Liu *et al*. [15] believed that the strong potential of BMSCs to differentiate into osteoblasts was due to the presence of more osteogenic and chondrogenic progenitor cells in BMSC cultures, rather than in the inherent characteristics of BMSCs. Numerous studies have investigated the differentiation potential of MSCs. Some researchers reported that sex differences affected the osteoblast-cytogenesis efficiency of MSCs, with ADSCs from male donors being more likely to differentiate than those from female donors [16]. Researchers also found that the osteogenic, but not adipogenic potential of ADSCs declined with age [17].

Low immunogenicity and immune regulation are important features of MSCs, making them suitable for allotransplantation. Melief *et al*. showed that, although both BMSCs and ADSCs had immunomodulatory functions, differences in cytokine secretion led to ADSCs having a stronger immunomodulatory function, equal to that of BMSCs [18]. Another similar study suggested that the immunomodulatory ability of placenta-derived MSCs was superior to that of BMSCs and ADSCs [19].

Biological differences among MSCs derived from different tissues determine their different clinical applications. In the current study, we isolated MSCs from adipose tissues, umbilical cord tissues and menstrual blood using different separation methods, and compared their biological characteristics, genetic stabilities, and gene expression patterns, to provide reference information to aid future clinical cell therapies.

2. Materials and methods

2.1. Sample sources

Adipose tissues (n = 6), umbilical cord tissues (n = 6), bone marrow samples (n = 1) and menstrual blood samples (n = 6) were obtained from 17 healthy donors recruited from the First Affiliated Hospital of Zhejiang University, Hangzhou, China. The donors were informed about the preparation and application of the specimens before collection, and signed informed consent was obtained. The study was approved by the ethical committee of the First Affiliated Hospital of Zhejiang University.

2.2. Cell isolation and culture

2.2.1. ADSC isolation and culture

Liposuction was performed in healthy donors to aspirate lipid, as described by Zuk *et al*. [20]. The lipid was cut into pieces, digested with 0.1% type I collagenase (Roche, Penzberg, Germany) at 37 °C for 30 min, neutralized with medium, and washed. The cells were then resuspended in DMEM containing 10% fetal bovine serum (Hyclone, Logan, UT), seeded into a culture flask, and placed in a 5% CO_2 incubator with saturated humidity at 37 °C. When the cells reached 80%–90% confluence, they were digested with 0.25% trypsin-EDTA (Gibco, Carlsbad, CA), seeded in a flask at a density of 5 × 10^3/cm^2, and successively passaged until cell senescence (cells were unable to be amplified).

2.2.2. UCMSC isolation and culture

Umbilical cord tissue (5–10 cm) was stripped from healthy women during labor, treated with Watertown's glue, and cut into pieces about 1 mm^3. DMEM medium (Hyclone) containing 10% fetal bovine serum was added and the cells were seeded into a culture flask, and placed in a 5% CO_2 incubator with saturated humidity at 37 °C for primary adherent culturing. Passaging was carried out as above.

2.2.3. MenSC isolation and culture

Menstrual blood was collected from healthy women on the second day of menstruation using a menstruation cup (E-vans Biotech, Hangzhou, China), mixed thoroughly, and filtered through 150–μm mesh. Mononuclear cells were isolated by density-gradient centrifugation. Menstrual stem cell culture medium (E-vans Biotech) was then added, the cells were seeded in a culture flask and cultured in a 5% CO_2 incubator with saturated humidity at 37 °C. Cells were passaged as described above.

2.2.4. BMSC isolation and culture

Human bone marrow was collected from healthy adult donors. BMSCs were harvested according to the methods described by Barlow *et al*. [10]. Mononuclear cells were isolated and plated in culture flasks.

2.3. Analysis of cell proliferation characteristics

2.3.1. Growth curve

The three kinds of cells of the same generation (P5) were selected by MTT assay to determine the growth curve. The cells were seeded in 96-well culture plates at a density of 1 000 cells per well. The cells were measured every 24 h, at six parallel points. Another group served as a blank control. The culture was maintained for 7 d, after which 20 μL of MTT solution (Sigma–Aldrich, St. Louis, MO) was added into each test well. The cells were further cultured in an incubator at 37 °C for 4 h, and the cultures were then terminated. The supernatant was carefully aspirated and discarded