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Characterization and hepatogenic differentiation of mesenchymal stem cells from human amniotic fluid and human bone marrow: A comparative study

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Abstract

Since stem cells can differentiate into hepatocyte, stem cell-based therapy becomes a potential alternative treatment for terminal liver diseases. However, an appropriate source of human mesenchymal stem cells (hMSCs) for hepatocytes has not yet been clearly elucidated. The aim of the present study was to investigate the *in vitro* biological characterization and hepatic differentiation potential of human amniotic fluid-derived mesenchymal stem cells (AF-hMSCs) and human bone marrow-derived mesenchymal stem cells (BM-hMSCs). Our results show that AF-hMSCs possess higher proliferation and self-renewal capacity than BM-hMSCs. Cytogenetic studies indicate that AF-hMSCs are as genetically stabile as BM-hMSCs. Following incubation with specific hepatogenic agents, AF-hMSCs showed a higher hepatic differentiation potential than BM-hMSCs. Expression of several liver-specific markers was significantly greater in AF-hMSCs than in BM-hMSCs, as shown by real time RT-PCR and immunofluorescence (IF). In conclusion, AF-hMSCs possess superior potential for hepatic differentiation, making them more suitable for diverse terminal liver diseases.

Keywords: Human amniotic fluid; Human bone marrow; Mesenchymal stem cells; Hepatogenic differentiation; Hepatocytes

1. Introduction

Stem cells with self-renewal and multipotential can be induced to differentiate into hepatocytes (Schwartz et al., 2002; Ishii et al., 2005) and show promise as a new therapy to treat terminal hepatic diseases. The two alternative stem cells with potential for hepatic differentiation derived from extra-hepatic tissue are embryonic stem cells (ESCs) and adult stem cells (ASCs), referring mainly to BM-hMSCs. However, ESCs derived from blastocysts grow as teratocarcinomas when implanted *in vivo* (Cowan et al., 2004), which raises significant ethical concerns. Accordingly, many scientists have recently focused on the derivating alternative sources of stem cells from various mesenchymal tissues, such as bone marrow (Horwitz et al., 2002), adipose tissue (Seo et al., 2005), and umbilical cord (Romanov et al., 2003; Hong et al., 2005). The difficulties with bone marrow is obtaining tissue samples from donors, and finding a significant decrease in both quantity and differentiation potential of BM-hMSCs with age (Stenderup et al., 2003). Meanwhile, the derivation of autologous hMSCs from appropriate tissue cannot be readily obtained or expanded in culture from patients with recent acute liver disease (Atala, 2006). Recent observations also indicate that the hepatic differentiation capability of BM-hMSC can be impaired to some extent as a result of exposure to environmental pollution such as insecticides, heavy metals and anti-putrefactives (Ghen et al., 2006). These disadvantages of ESCs

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and BM-hMSCs have restricted their use in tissue engineering and organogenesis, which has accelerated the search for alternative sources of stem cells.

Stem cells derived from second-trimester amniocentesis are pluripotent stem cells capable of differentiating into multiple lineages, including representatives of all three embryonic germ layers (De Coppi et al., 2007). Therefore, amniotic fluid may be a suitable alternative source of cells. However, there has been no specific report regarding hepatic differentiation of AF-hMSCs, and therefore appropriate source of hMSCs has not yet been found.

We have investigated and compared various biological characteristics, including the hepatic differentiation potential, of hMSCs from amniotic fluid and bone marrow. AF-hMSCs exhibit a higher capacity for proliferation, self-renewal and hepatic differentiation compared with BM-hMSCs, and may provide an ethically uncontroversial and easily accessible source of human hepatocytes for future clinical applications and experimentation. Specifically, our study was aimed at verifying AF-hMSCs.

2. Materials and methods

2.1. Isolation and culture of human mesenchymal stem cells

All biological samples were obtained after informed consent, according to the policy approved by the local Ethical Committee (The Third Affiliated Hospital of Sun Yat-Sen University).

AF-hMSCs were prepared as previously described Cipriani et al. (2007). Briefly, amniotic fluid samples were obtained from surplus amniotic fluid cell (AFC) cultures taken by amniocentesis performed between 16 and 20 weeks of gestation for routine karyotype analysis. Each amniotic fluid sample (10 ml) was centrifuged for 10 min at 1600 rpm. Pellets were resuspended in Amniomax II complete (Invitrogen, USA), plated in 25 cm^2 culture flasks (Corning, USA), and incubated in 95% humidified air plus 5% CO_2 at 37 °C. After 6 days, AF-hMSCs were obtained by detaching amniotic fluid cells with 0.25% trypsin 0.02% EDTA (Sigma-Aldrich, China) and plated at 1000 cells/cm^2 in low-glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) supplemented with 20% fetal bovine serum (FBS, HyClone, USA), 100 U/ml penicillin, 0.1 mg/ml streptomycin (Sigma-Aldrich, China), 10 ng/ml basic-fibroblast growth factor (bFGF; R&D systems, USA), and 2 mM L-glutamine (Sigma-Aldrich, China). The medium was changed every 3 days. When the cell cultures reached ~70% confluence, they were detached and replated at 1:3 under identical culture conditions.

Preparation of BM-hMSCs was done as previously described (Miao et al., 2006). Isolation of human adipose tissue-derived mesenchymal stem cells (AD-hMSCs) was accomplished as previously described Banas et al. (2007). Culturing of human amniotic membrane-derived mesenchymal stem cells (AM-hMSCs) was done as before (Tsai et al., 2007).

2.2. Growth kinetics

Following the third passage, AF-hMSCs and BM-hMSCs were seeded initially at 1000 cells/cm^2 in a six-well plate (Corning, USA). Cells were detached by treatment with 0.25% Trypsin-0.02% EDTA and counted with a hemocytometer at days 3, 6, 9, 12, 15, 18, and 21. Dead cells were excluded by Trypan blue staining (Sigma-Aldrich, China). Both of these experiments were performed in triplicate for each point described in Fig. 2A.

2.3. Flow cytometric analysis

Passage 3 AF-hMSCs and BM-hMSCs were stained with various combinations of saturating amounts of monoclonal antibodies conjugated with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE) as follows: CD19-FITC, CD29-FITC, CD34-PE, CD44-FITC, CD45-PE-cy_5, CD80-PE, CD86-PE-cy_5, CD117-PE, HLA-ABC-PE, HLA-DR-FITC (BD PharMingen, USA), CD106-PE (eBiosciences, USA). Approximately 5×10^5 cells were analyzed by flow cytometry (FACScan, BD Biosciences, USA) using Cellquest software.

2.4. Hepatic differentiation

Hepatic differentiation was induced as previously described Snykers et al. (2007). Briefly, passage 3 AF-hMSCs and BM-hMSCs were plated at 2×10^4 cells/cm^2 in 25 cm^2 flasks and 12-well plates coated with 5 μg/cm^2 collagen gel type I (Sigma, USA) in basal medium consisting of 60% (v/v) DMEM and 40% (v/v) MCDB-201 (Sigma, USA) supplemented with 2% (v/v) fetal bovine serum, 100 IE/ml penicillin, 100 μg/ml streptomycin, 1 mg/ml linoleic-acid, 0.1 mM L-ascorbic acid, 0.03 mM nicotinamide, 0.25 mM sodium pyruvate, and 1.623 mM glutamine. At confluence, hepatogenic cytokines and growth factors were added sequentially according to the following schedule. Days 0–2: basal medium +10 ng/ml FGF-4; days 3–5: basal medium +20 ng/ml hepatocyte growth factor (HGF); days 6–18: basal medium +20 ng/ml HGF +1 × insulin-transferrin-sodium-selenite (ITS) and 20 μg/l dexamethasone +1 μM trichostatin A (TSA) (all from Sigma, USA). Differentiation media were changed every 3 days. To evaluate the inducibility of CYP_1A_1, cells from 18 days after differentiation were treated with 1 mM phenobarbital (PB, Sigma, USA). Differentiation media, supplemented with PB, were daily renewed.

2.5. Total RNA isolation and real time quantitative RT-PCR

RNA was extracted from 5×10^5 cells, including undifferentiated hMSCs from passages 1, 5 and 10, and differentiated cells from stated time-points (days 0, 7, 14, 21, 28), as well as from HeLa cells, which were used as a positive control for Oct-4 mRNA (Octamer-4 mRNA) during the course of differentiation, using RNAprep cell kit according to the manufacturer's protocol. Complementary DNA was synthesized