Characterization of a Growth-elevated Cell Line of Human Bone Marrow-derived Mesenchymal Stem Cells by SV40 T-Antigen

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Abstract Human bone marrow-derived mesenchymal stem cells (hMSCs) are capable of self-renewal and differentiation into various tissue lineages, attracting attention as tools for use in cell therapy. However, hMSCs have very poor proliferative capacity and a short life span in culture. To overcome this problem, we expressed the T antigen of SV40 in hMSCs because it is known to have the ability to elevate the growth rate of various primary animal cells. We obtained several hMSC lines (hMSCs-T) known for stable expression of T antigen. Cells expressing T antigen proliferated on the monolayer of hMSCs, forming high density foci. hMSCs-T showed changed morphology and improved growth rate and life span, and demonstrated preservation of the potential for differentiation into osteoblasts. In addition, hMSCs-T did not proliferate in soft agar culture, indicating that the cells did not transform into tumor cells. In order to evaluate metabolic change of amino acids in hMSCs-T compared to primary hMSCs, we investigated altered amino acids (AA) with gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring (SIM) mode (GC-SIM-MS). A total of 14 AAs were positively measured. Results from the Student’s t-test on the hMSCs group mean of the hMSCs-T group showed significantly elevated levels of glycine, proline, pipecolic acid, aspartic acid, lysine and tryptophan, whereas valine, leucine and isoleucine as branched-chain amino acids (BCAAs), and phenylalanine showed a significant decrease. Altered AAs metabolic pattern in the hMSCs-T may explain the disturbance of AA metabolism related to the expression of SV40 T antigen in hMSCs.

Keywords: hMSCs, SV40 T antigen, amino acids, gas chromatography-mass spectrometry, stable cell line

1. Introduction

Stem cells are characterized by their ability to generate progenitors capable of differentiation into various and distinct cell lineages. It is this ability of stem cells that makes them potentially ideal candidates for use in stem cell therapy [1,2]. Mesenchymal stem cells (MSCs), which exist in various tissues including bone marrow and adipose-tissue, can differentiate into osteoblasts, chondrocytes, adipocytes, and hepatocytes. The possibility that they might be able to transdifferentiate into other phenotypes as well, including neuronal cells, has been suggested [3,4]. In particular, human bone marrow-derived mesenchymal stem cells (hMSCs) have been...
used as tools for cell therapy due to their ability of self-renewal and differentiation into various tissue lineages [5-8]. However, hMSCs have very poor proliferative capacity and a short life span, which is an impediment in biological analysis and application to therapy. Therefore, in order to obtain consistent results in biological investigation using hMSCs, it is necessary to isolate single cell-derived clones of hMSCs that have a capacity for proliferation in long term culture.

For immortalization of primary cells, v-myc, telomerase and SV 40 T antigen have been used [9-11]. Among them, v-myc was used for immortalization of human neuronal stem cells using a retroviral vector encoding v-myc, and the immortalized cells expressed NSC specific markers including nestin [12]. Although v-myc is a good candidate gene for immortalization of stem cells, it has been known as a typical oncogene and its transformed cells showed that a heavy percentage of cells underwent apoptosis, which was dependent on the tumor suppressor P53 pathway [9]. On the other hand, the T antigen of Simian virus 40 (SV40) is known to have the ability to immortalize and transform various animal cells [11]. From studies conducted over several decades after SV40 was found as a contaminant of vaccines prepared from cultures of monkey cells in 1960, the proposition that the T antigen might be involved in human carcinogenesis was virtually dismissed, despite evidence showing that it was highly tumorigenic in rodents [13]. Therefore, SV40 T antigen might be a novel candidate as a tool for establishment of hMSCs lines maintaining the non-tumoral and normal phenotype.

To investigate metabolic changes following stable expression of T antigen in hMSCs, amino acids (AAs) were analyzed, because AAs occurring in metabolic pathways are important indicators for cellular physiological and pathological conditions including cell signal, oxidative stress, synthesis of ATP and utilization of nutrients [13-15]. In our previous report, we demonstrated that a two-phase aqueous ethoxycarbonyl (EOC) derivatization combined with tert-butyldimethylsilyl (TBDMS) reaction and subsequent gas chromatography–mass spectrometry in selected ion monitoring mode (GC-SIM-MS) method was useful for rapid screening and monitoring of biologically important AAs in biological samples in the presence of co-extracted endogenous metabolites [16].

In this study, we have established a growth-elevated cell line of hMSCs by stably expressing SV40 T antigen in hMSCs and characterized the growth properties, capacity of differentiation and AA metabolites.

2. Materials and Methods

2.1. Cell culture and transfection

Human bone marrow-derived hMSCs were purchased from Promo Cell (Germany).

The hMSCs and rat fibroblast 3Y1-T cells stably expressing SV40 T antigen were cultured in high glucose-DMEM (Invitrogen) containing 10% FBS (Invitrogen), and maintained in 5% CO₂, 37°C. Subconfluent cultures of hMSCs were transfected with pEF321-T plasmid DNA (20 µg/10 cm plate) using Fugene 6 (Roche Molecular Biochemicals) [17]. Plasmid DNA was mixed with Fugene 6 in serum-free DMEM for 15 min at rt. and applied to culture plates as directed by the manufacturer.

2.2. Preparation of cell extracts and western blotting

Total cell extract was prepared from cultures in 10 cm plates by suspending cells in lysis buffer (pH 7.9 20 mM Heps, 25% glycerol, 450 mM NaCl, 0.4 mM EDTA, 0.5 mM dithiothreitol, 1% NP-40) and incubating on ice for one hour. Cell extracts were run on 10% SDS-polyacrylamide gel and subjected to Western blotting with anti-SV40 T monoclonal antibody (1:1,000) (Santa Cruz Biotechnology) and chemiluminescence reagents (Amersham Pharmacia Biotech.).

2.3. Soft agar culture assay

Cells were suspended in DMEM containing 0.28% low-melting point agarose (FMC Bioproducts) at a density of 4 × 10^5 cells per 60 mm plate and cultured on the bottom layer of DMEM containing 1% agarose and 20% FBS for 20 days [11].

2.4. Immunocytochemical analysis

The antibodies used for immunocytochemistry were α-smoothmuscle (α-SM) monoclonal (1:50, R&D systems), CD105 monoclonal (1:100, R&D systems), fibronectin monoclonal (1:100, Santa Cruz), CD34 monoclonal (1:100) (Santa Cruz), Alexa Fluor 488 secondary antibody (1:200) (Molecular Probes). After being washed with PBS, the cells were fixed in 3.7% formaldehyde for 10 min at room temperature, permeabilized with 0.3% Triton X-100 for 15 min, and blocked with 3% bovine serum albumin for 30 min. Cells were then incubated at 4°C for 24 h with the appropriate primary antibody diluted in PBS containing 1% bovine serum albumin. After the cells were washed three times with PBS, the secondary antibody diluted in PBS containing 1% bovine serum albumin was added for 1 h at room temperature. Cell nuclei were counterstained with Hoechst 33342 (1:1,000) (Molecular Probes). Samples were visualized with a fluorescence confocal laser scanning microscope (LSM 510, Zeiss).

2.5. Osteogenic differentiation

Cells were plated at a density of 2,500 cells/cm² in two wells of 12-well plates 24 h before induction of differentiation.