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Research paper

The characteristics of human cranial bone marrow mesenchymal stem cells

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HIGHLIGHTS

- We established mesenchymal stem cells from human cranial bone marrow.
- We confirmed neural crest-associated gene expression of our stem cells.
- These cells should have a greater tendency to differentiate into neuronal cells.

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ABSTRACT

Recently, cell-based therapy has attracted attention for treatment of central nervous system (CNS) disorders. Bone marrow-derived mesenchymal stem cells (BMSCs) are considered to have good engraftment potential. Therefore, more efficient and less invasive methods to obtain donor cells are required. Here, we established human BMSCs from cranial bone waste (cBMSCs) obtained following routine neurosurgical procedures.

cBMSCs and cells obtained from the iliac crest (iBMSCs, standard BMSCs) showed expression of cell surface markers associated with mesenchymal stem cells and multipotency traits such as differentiation into osteogenic and adipogenic lineages. cBMSCs showed higher expression of the neural crest-associated mRNAs p75, Slug, and Snail than iBMSCs. Neurogenic induced cells from cBMSCs expressed the neural markers nestin, Pax6, neurofilament (NF)-L, and NF-M as seen with RT-PCR, and NF-M protein as seen with western blotting at higher levels than cells from iBMSCs. Immunostaining showed a significantly greater proportion of NF-M-positive cells in the population of induced cBMSCs compared with the population of iBMSCs. Thus, cBMSCs showed a greater tendency to differentiate into neuron-like cells than iBMSCs.

1. Introduction

Recovery after central nervous system (CNS) disorders such as brain stroke, traumatic brain injury, spinal cord injury, and degenerative disease is often restricted and poor because brain and nervous tissue have a limited ability for self-repair after injury [1,2]. After neuronal stem cells were identified in the adult mammalian brain [3], much attention has recently been focused on regenerative cell therapy for CNS disorders.

Currently, mesenchymal stem cells (MSCs) are expected to be candidate cells for grafting. Honmou et al. transplanted autologous

Abbreviations: BMSC, bone marrow-derived mesenchymal stem cell; cBMSC, cranial BMSC; CNS, central nervous system; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; DMEM, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; hBMSC, human BMSC; HLA-DR, human leukocyte antigen-DR; iBMSC, iliac BMSC; mAbs, monoclonal antibodies; MSC, mesenchymal stem cell; NF-L, neurofilament light chain; NF-M, neurofilament medium chain; Pax6, paired box 6; PBS, phosphate-buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; SDS, sodium dodecyl sulfate.

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human bone marrow-derived mesenchymal stem cells (hBMSCs) that were expanded in autologous human serum into stroke patients in a clinical study and showed reductions in neurological deficits and the lesion size. They described the feasibility and safety of this method [4].

Human MSCs secrete neurotrophins such as brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor that contribute to anatomical and functional motor recovery in the ischemic brain [5]. Therefore, selection of cells for grafting that have a greater tendency to differentiate into neuron-like or glial-like cells may provide a maximal therapeutic response in CNS regenerative medicine.

The bones of the face and part of the cranial vault are derived from the neural crest, and bones of the limbs and vertebrae are derived from the mesodermal germ layer [6]. Most previous studies that attempted to induce neuronal differentiation of hBMSCs used iliac bone marrow-derived cells, which are derived from the mesodermal germ layer. We have focused on hBMSCs derived from cranial bone marrow, which are obtained from bone waste in neurosurgical procedures. Previous reports suggest that neurogenic potential is an innate characteristic of MSCs, particularly those of dental origin that are derived from cranial neural crest cells, and MSCs derived from human teeth have high neurogenic potential [7]. Based on these findings, hBMSCs from cranial bone, which are derived from the neural crest, are likely to have high neurogenic potential and have been proposed to be a valuable source of stem cells for CNS cell therapy.

In this study, we isolated hBMSCs from cranial bone marrow (cBMSCs) and investigated their undifferentiated phenotype and differentiation potential into multi-lineage cell types. To investigate the neurogenic potential of cBMSCs, they were induced to differentiate into neural cells. Then, we analyzed the characteristics of differentiated cBMSCs by comparing them with hBMSCs from iliac crest bone marrow (iBMSCs), which are considered standard BMSCs.

2. Materials and methods

2.1. Tissue sampling

Cranial bone marrow samples were obtained from frontotemporal cranial bone waste following neurosurgical procedures after informed consent was obtained from the patient, according to the university hospital's guidelines. Iliac bone marrow was obtained from iliac bone waste following spinal surgery and served as a control.

2.2. Tissue culture

The bone marrow samples were seeded in culture dishes containing low-glucose Dulbecco's modified Eagle medium (DMEM-L; Sigma–Aldrich Co., St. Louis, MO, USA) with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific HyClone, South Logan, UT, USA), penicillin (100 U/ml), and streptomycin (100 μg/ml; both from Sigma–Aldrich). The dishes were maintained at 37 °C in 5% CO_2 in a humidified chamber. The medium was changed twice a week to eliminate floating bone powder and non-adherent cells, and adherent cells were incubated until 90% confluent. The cells that adhered to the bottom of the culture dish were used as BMSCs [8] and were passaged several times.

2.3. Flow cytometry analysis for specific cell markers

To examine specific cell markers of stem cells derived from cranial bone marrow, flow cytometry analysis was performed. Alexa Fluor-conjugated primary monoclonal antibodies (mAbs) against CD105, APC-conjugated primary mAbs against CD73, and PE-conjugated primary mAbs against CD44 were used as MSC markers, and FITC-conjugated primary mAbs against CD11b, CD34, CD45, and human leukocyte antigen-DR (HLA-DR) (each from BioLegend Inc., San Diego, CA, USA) were used as endothelial/hematopoietic markers.

Data acquisition and analyses were performed with BD FACSAria (BD Biosciences, San Jose, CA, USA).

2.4. Multi-lineage cell differentiation

BMSCs were differentiated using osteogenic and adipogenic induction conditions. To induce osteogenic or adipogenic differentiation, cells were seeded at 2.0×10^4 cells/10-cm culture dish and maintained in growth medium until confluent. For osteogenic induction, cells were cultured in DMEM-L containing 10% FBS, 2 mM L-glutamine (Sigma–Aldrich Co.), 100 nM dexamethasone (Sigma–Aldrich), 10 mM β-glycerophosphate disodium salt hydrate (Sigma–Aldrich), 50 μg/ml L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate (Sigma–Aldrich), penicillin (100 U/ml), and streptomycin (100 μg/ml) for 7 days. To visualize the mineralized deposits, the cultures were fixed in 95% ethyl alcohol and stained with 1% alizarin red S followed by microscopic examination using a multifunctional microscope (BZ-9000; KEYENCE Co., Osaka, Japan). For adipogenic induction, the AdvanceSTEM Adipogenic Differentiation Kit (Thermo Fisher Scientific HyClone) was used. Cells were cultured in AdvanceSTEM Adipogenic Differentiation Medium (89%) containing AdvanceSTEM Growth Supplement (5%), penicillin (100 U/ml), and streptomycin (100 μg/ml) for 3 weeks. Lipid-laden fat cells were washed twice with phosphate-buffered saline (PBS) and stained with Oil red-O for 10 min at room temperature followed by microscopic examination. Growth and differentiation medium was changed every 3–4 days.

2.5. Neural differentiation

After at least four passages, the BMSCs were induced to differentiate into neural cells with neurotropic factors. We used modified neural differentiation conditions including neural conditioning medium and neural differentiation medium according to previously reported methods [9]. Specifically, the BMSCs were seeded at a density of 2.0×10^4 cells/10-cm dish and maintained in growth medium until 80% confluent. Then the medium was changed to the neural conditioning medium containing Dulbecco's modified Eagle's/F12 (Invitrogen Co., Carlsbad, CA, USA) with 1% FBS (Thermo Fisher Scientific HyClone), basic fibroblast growth factor (100 ng/ml; PeproTech, Rocky Hill, NJ, USA), penicillin (100 U/ml), and streptomycin (100 μg/ml; both from Sigma–Aldrich). After incubating in this neural conditioning medium for 3 days, the cells were cultured in neural differentiation medium composed of neural conditioning medium with forskolin (10 μM; Sigma–Aldrich) added for 7 days. The differentiation medium was changed every 3 or 4 days.

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

Cultured cells were collected in ISOGEN (Nippon Gene Co., Ltd., Toyama, Japan), and RNA was isolated according to the manufacturer's protocol. Reverse transcription was performed with ReverTra Ace-α (Toyobo Co., Ltd., Osaka, Japan). Using cDNA as the template, PCR was performed with BD Advantage 2 PCR Kits (BD Biosciences Clontech, Palo Alto, CA, USA). We used nestin as a neural progenitor cell marker, paired box 6 (Pax6) as a neural precursor cell marker, and neurofilament-L (NF-L) and neurofilament-M (NF-M) as neuronal markers. Glyceraldehyde-3-phosphate dehydrogenase