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Differentiation potential of bone marrow mesenchymal stem cells in duck

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Abstract

The bone marrow mesenchymal stem cells (MSCs) are multipotent stem cells which can differentiate into mesenchymal cells *in vitro*. In this study, MSCs in duck were isolated from bone marrow by density gradient centrifuge separation, purified and expanded in the medium. The primary MSCs were expanded for 11 passages. The different-passage MSCs were induced to differentiate into osteoblasts and neuron-like cells. Karyotype analysis indicated that MSCs kept diploid condition and the hereditary feature was stable. The different-passage MSCs expressed CD44, ICAM-1 and SSEA-4, but not CD34, CD45 and SSEA-1 when detected by immunofluorescence staining. There was no significant difference among the positive rates of passages 2, 6 and 8 ($P > 0.05$), but a significant difference existed among those of passages 2, 6, 8 and 11 ($P < 0.05$). After the osteogenic inducement was added, the induced different-passage MSCs expressed high-level alkaline phosphatase (ALP), and are positive for tetracycline staining, Alizarin Red staining and Von Kossa staining. After the neural inducement was added, about 70% cells exhibited typical neuron-like phenotype, the induced different-passage MSCs expressed Nestin, neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP) when detected by immunofluorescence staining. There was no significant difference among the positive rates of passages 3, 4 and 6 ($P>0.05$), but a significant difference existed among those of passages 3, 4, 6 and 8 ($P<0.05$). These results suggest that MSCs in duck were capable of differentiating into osteoblasts and neuron-like cells *in vitro*.

Keywords: duck; mesenchymal stem cells; induction; differentiation

Introduction

Bone marrow mesenchymal stem cells (MSCs) are generally known as ancestors of bone marrow stromal cells, which can assist hematopoiesis. Recently, MSCs were found to be more effective in their differentiation potential in that they were able to give rise to many kinds of mesenchymal cells, such as osteoblast, chondrocyte, tenocyte, adipocyte and cardiac myocyte, etc (Prockop et al., 1997; Pittenger et al., 1999). Furthermore, they were even able to differentiate into neurocyte which derived from the ectoderm (Woodbury et al., 2000). MSCs expressed numerous surface markers such as SH2, SH3, CD29, CD90, CD106 and CD166, but not hematopietic markers such as CD14, CD34 and CD45 (Deans and Moseley, 2000). MSCs could be expanded in culture and cryopreserved without loss of phenotype or multilineage differentiation potential (Majumdar et al., 1998). It was indicated in recent studies that MSCs were capable of homing to the bone marrow of non-human primates following systemic infusion (Devine

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et al., 2001). All the results mentioned above suggest that MSCs might be useful in cell therapy, gene therapy and tissue engineering.

MSCs are useful model system for better understanding of cell behavior and differential gene expression in the context of a focused and easily manipulated biological system. The continued understanding of cellular origin and MSCs' behavior will enable further identification of diagnostic markers and therapeutic targets. MSCs assist gene transfer, so that they can be used to produce medical antibodies. In addition, it is easy to obtain MSCs of duck, and MSCs are suitable for large scale preparation and are an ideal medicine screening model for the treatment of cell diseases. Therefore, MSCs of duck were chosen as our experimental material.

In this study, we examined the biological characteristics of MSCs and further evaluated the feasibility of inducing the differentiation of MSCs into osteoblasts and neuron-like cells *in vitro*. To characterize the differentiated MSCs, we examined their morphology and analyzed several markers at different passages after induction by means of histochemistry and immunofluorescence methods, which provides a theoretical foundation and a technological method for the utilization of MSCs.

Materials and methods

Isolation, purification and expansion of MSCs

Bone marrow samples were drained from the tibia of one-day-old ducks. Firstly, cells were pelleted by centrifugation, then resuspended with PBS and fractionated on a density gradient (Ficoll-Hypaque, 1.077 g/mL, Amersharm Biosciences, Sweden) for 20 min at 400 g. The interface was collected and seeded at 1.0×10^6 cells/cm^2 in T-25 flasks with 5 mL L-DMEM (Stem Cell Co., Canada). Cultures were maintained at 37.5°C in a humidified atmosphere containing 95% air and 5% CO_2. The non-adherent cells were removed after three days and the culture medium was changed every 3 days. When cultures became 80% confluence, cells were detached with 0.25% trypsin and 0.02% EDTA (1:1) for 3–5 min at 37.5°C, and reseeded at 8.0×10^3 cells/cm^2 in T-25 flasks with 5 mL L-DMEM for serial passaging.

Growth dynamical analysis of MSCs

MSCs of passages 2, 6 and 8 were harvested and made into cell suspensions, incubated in 24-well plates at the same concentration, and then put into an incubator containing CO_2 of 0.05 volume fraction at 37.5°C. The culture medium was changed every 3 days. MSCs in 3 culture foramens were randomized every day for the cell growth dynamical analysis and the growth curve of different-passage MSCs was drawn.

Chromosome analysis

Cells were harvested at 80%–90% confluence during their exponential growth phase, and microslides were prepared and the chromosome staining was performed according to the approach described (Hirofumi et al., 2006). The number of chromosomes in 50 to 100 spreads was counted. The three important chromosomal parameters, i.e., relative length, arm ratio and centromere index, were calculated by the method proposed by Kawarai et al. (2006).

Surface antigen characteristics of MSCs

Surface antigens of the different passages of MSCs were detected by immunofluorescence staining. MSCs of passages 2, 6, 8 and 11 were fixed in 4% paraformaldehyde/PBS for 15–20 min, and then blocked for 10 min with methanol containing 0.1% Triton X-100 and 0.3% peroxid (H_2O_2) to eliminate endogenetic peroxides, incubated in goat serum working solution for 15 min to block nonspecific binding, then the MSCs were incubated with primary antibodies at 4°C overnight. The MSCs were incubated with secondary antibodies which were conjugated with FITC (goat anti-mouse IgG). For negative control, 0.01 mol/L PBS was used to replace primary antibodies. For blank control, MSCs were stained directly. Fluorescence images were observed by fluorescence microscope. Ten non-overlapped visions (×100) were randomized from different-passage MSCs, then the percentage of positive cells relative to total count of MSCs was calculated and the results were expressed as mean ± SD using SPSS 10.0 software for variance analysis.

Differentiation of MSCs into osteoblasts

MSCs of passages 3, 4, 6 and 8 were vaccinated in a 24-well plate at 3×10^3 cells/cm^2. These MSCs were divided into two groups when covering 80% surface of the culture plate. MSCs in the induction group were incubated in the medium of inducers (10 mmol/L β-sodium glyc-