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Multipotent Adult Progenitor Cells from Bone Marrow Differentiate into Chondrocyte-Like Cells

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ABSTRACT

Cartilage tissue engineering has great potential for treating chondral and osteochondral injuries. Efficient seed cells are the key to cartilage tissue engineering. Multipotent adult progenitor cells (MAPCs) have greater differentiation ability than other bone-marrow stem cells, and thus may be candidate seed cells. We attempted to differentiate MAPCs into chondrocyte-like cells to evaluate their suitability as seed cells for cartilage tissue engineering. Toluidine blue and Alcian blue staining suggested that glycosaminoglycan was expressed in differentiated cells. Immunofluorostaining indicated that differentiated human MAPCs (hMAPCs) expressed collagen II. Based on these results, we concluded that bone-marrow-derived hMAPCs could differentiate into chondrocyte-like cells in vitro.

Chondral and osteochondral injuries are currently very frequent in clinical practice. A study of 993 arthroscopies showed 66% articular cartilage pathologies, with 11% full-thickness chondral lesions suitable for cartilage-regeneration treatment [1]. A further retrospective study of 31,000 arthroscopic procedures reported a 63% incidence of cartilage defects [2]. Although routine clinical treatments for chondral and osteochondral injuries can reduce clinical symptoms, they fail to regenerate injured cartilage tissue. Cartilage tissue engineering that could solve a series of shortcomings associated with routine clinical treatments would have great potential for treating chondral and osteochondral injuries. One of the major components of cartilage tissue engineering is seed cells.

In 2002, the group of Catherine Verfaillie found a new subpopulation of stem cells in bone-marrow stromal cells, termed multipotent adult progenitor cells (MAPCs) [3]. These cells have greater differentiation potential than other bone-marrow stromal stem cells and are able to differentiate into mesoderm, ectoderm and endoderm cells in vitro [3]. In terms of their differentiation potential, human MAPCs (hMAPCs) could be expanded in vitro for >70 population doublings. Furthermore, these cells had a high amplification potential for multi-directional differentiation without losing differentiation ability. Recent studies demonstrated that hMAPCs did not express significant levels of Oct4 and they had low levels of major histocompatibility complex (MHC) class I and CD146, but were negative for CD56, alkaline phosphatase, CD140a, CD140b and MHC class II [4]. Several groups showed that MAPCs could differentiate towards adipocytes, osteoblasts, chondrocytes, smooth-muscle cells [5] and hepatocyte-like cells [6]. In summary, MAPCs have the following properties to be ideal candidates as seed cells for cartilage tissue engineering: avoid ethical constraints [7]; are obtained easily; have a stable morphology; have a strong differentiation capability.

Because of the strong differentiation ability of MAPCs, we hypothesized that these cells could be induced to differentiate into chondrocytes, to evaluate their suitability to be seed cells for cartilage tissue engineering. Unlike mesenchymal stem cells (MSCs), which have been investigated extensively, the study of the differentiation of bone-marrow MAPCs into chondrocytes has been rarely reported. The main disadvantage of MSCs is their biological instability, thus readily differentiating, which became a major difficulty for researchers. Compared to MSCs, MAPCs have a greater and more stable differentiation potential. We envisage MAPCs providing a new direction in stem-cell differentiation. We demonstrated herein that hMAPCs could differentiate in vitro into chondrocyte-like cells with some of the functional characteristics of chondrocytes. Based on these experimental results, we believe that the present study provides a possible strategy for future cartilage tissue engineering.

Materials and Methods

Isolation and Culture of MAPCs

hMAPCs were isolated from iliac bone marrow obtained from 30 Asian patients who underwent pelvic-fracture operations. Informed consent was obtained in accordance with the guidelines of the Medical Ethics Committee. Cell isolation and culture were performed as described previously [8,9]. Briefly, the marrow was incubated in phosphate-buffered saline (PBS, Hyclone, USA) containing 0.2% collagenase (Sigma, USA) and 0.02% DNase (Invitrogen, USA) at 37 °C. Cell suspensions were filtered using a 40-μm cell strainer and centrifuged at 800 ×g for 5 min. The

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cells were plated in 2-ml Dulbecco's modified Eagle's medium (DMEM)-LG (Gibco, USA) supplemented with 2% fetal bovine serum (Hyclone). The cells were inoculated at a concentration of $2 \times l0^6$/ml in 50-ml culture bottles and incubated under 5% CO_2 at 37 °C. The medium was changed after 24 h and subsequently every 3 days. When the cells covered approximately 80% of the bottom of the bottle, they were digested using 0.25% trypsin (Hyclone) and subcultured 1:2 with the same culture medium. Nucleated cells were counted using a hemacytometer to allow adjustments in cell concentrations for later experiments. Clonal populations were obtained by plating five cells of passages 3–10 per well in a 96-well plate. The expansion medium consisted of 60% DMEM-LG (Gibco), 40% MCDB-201 (Sigma, USA), 1× insulin–transferrin–selenium, 1× linoleic acid–bovine serum albumin (Gibco), 10.9 M dexamethasone (Sigma), 10.4 M ascorbic acid 2-phosphate (Sigma), 100 U of penicillin, 1000 U of streptomycin, 2% fetal calf serum (both Gibco), 10 ng/ml human platelet-derived growth factor-BB (hPDGF-BB, R&D Systems,USA), 10 ng/ml epidermal growth factor (EGF, Sigma), and 1000 U/ml leukemia inhibitory factor (LIF, Chemicon, Temecula, CA, USA).

Flow Cytometry

The phenotype of the hMAPCs was analyzed by flow cytometry. Cells were trypsinized and incubated for 5 min with 4% paraformaldehyde. They were then washed with PBS twice and blocked using 5% normal goat serum for 30 min at room temperature. The cells were washed twice, incubated with the secondary antibody for 30 min at 4 °C and washed. The primary antibodies included stage-specific embryonic antigen-1 (SSEA-1), CD44, CD45, human major histocompatibility complex (hMHC) class II (all Santa Cruz, USA) and isotype control. The immunolabeled cells were analyzed by fluorescence-activated cell sorting (FACS, Becton, Dickinson and Company, USA).

Differentiation of MAPCs

To induce differentiation of MAPCs into chondrocytes, the cells were trypsinized, plated into 24-well plates at a density of $1 \times 10^5/cm^2$ and incubated in expansion medium. After 48 h, the expansion medium was removed and differentiation medium was added. The differentiation medium was similar to the expansion medium but without hPDGF-BB, EGF and LIF, while including 10 ng/ml of transforming growth factor-β1 (TGF-β1, R&D Systems, USA) to induce differentiation. The differentiation medium was changed every 3 days.

Identification of Chondrocyte-like Cells

Toluidine blue staining, Alcian blue staining and immunofluorostaining for collagen II were performed on days 7 and 14 after induction.

Toluidine Blue Staining

Cell-climbing films were washed using distilled water and stained using toluidine blue for 4 h. Excess toluidine blue dye was washed off using 95% alcohol. The cell-climbing films were dehydrated in 100% acetone for 5 min. Cell morphology and the expression of glycosaminoglycans (GAG) were assessed.

Alcian Blue Staining

Cell-climbing films were washed using distilled water and stained using Alcian blue for 30 min. The cells were washed again with distilled water for 15 min and dehydrated in 100% alcohol. Cell morphology and the expression of GAG were assessed.

Immunofluorostaining for Collagen II

Immunofluorostaining was performed on methanol-fixed cells using Cy3-conjugated rabbit monoclonal anti-collagen II (Sigma). Alexa 488-labeled goat anti-rabbit secondary antibody (Invitrogen) was used to detect anti-calponin and anti-collagen II antibody localization. Collagen-II expression was assessed.

Results

Morphology of hMAPCs

Primary hMAPCs were normally triangular or long-spindle shaped, and of 8–10 μm (Fig. 1). The morphology of the hMAPCs was similar to that of murine MAPCs, as reported previously [10].

FACS Analysis

The phenotype of hMAPCs cultured on fibronectin for 15–40 population doublings was SSEA-1^+, $CD44^-$, $CD45^-$, hMHC-II^- (Fig. 2). The result was identical to the phenotype described for murine and human bone-marrow-derived MAPCs [3,9,11].

Identification of Chondrocyte-like Cells

Toluidine blue staining and Alcian blue staining of differentiated hMAPCs were positive, indicating that GAG were expressed in differentiated cells (Fig. 3). Immunofluorostaining demonstrated that the

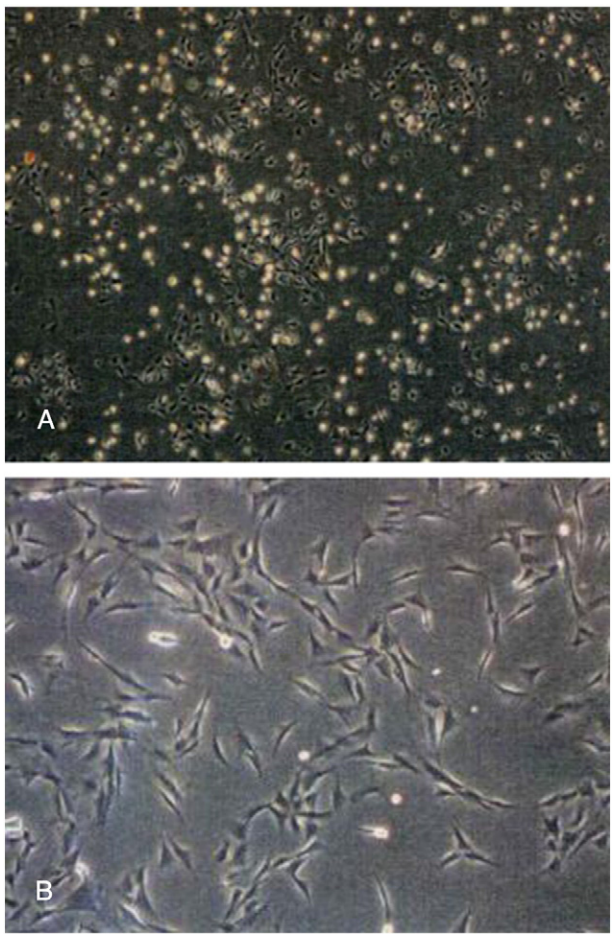

Fig. 1. (A) Morphology of hMAPCs—after primary passage (×40). (B) Morphology of hMAPCs—after passage 5 (×100).