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Immunophenotype characterization of rat mesenchymal stromal cells

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Background

Mesenchymal stromal cells (MSC) have shown diverse therapeutic potential. While characterization of human and mouse MSC has seen significant advances, rat bone marrow-derived MSC (rBM-MSC) remain under-characterized. We detail the isolation, expansion, differentiation, and detailed immunocharacterization of rBM-MSC.

Methods

Rat MSC were isolated and expanded in multipotent adult progenitor cell (MAPC) media, and cell-surface marker expression through 10 passages was used to characterize the population and multipotency was confirmed via differentation.

Results

By passage 3, rBM-MSC were found to be CD11b$^-$, CD45$^-$, CD29$^+$, CD49e$^+$, CD73$^+$, CD90$^+$, CD105$^+$ and Stro-1$^+$, without the use of cell sorting. Media selection was responsible for the isolation of a nearly homogeneous population of rBM-MSC. The rBM-MSC immunophenotype changed by passage 10, showing decreases in CD73, CD105 and Stro-1 expression.

Discussion

Detailed characterization of cell populations facilitates accurate and reproducible cell therapy investigation. Given the expanding body of research involving rBM-MSC, these results advance our ability to compare rBM-MSC populations.

Keywords

cell therapy, immunocharacterization, marker expression, mesenchymal stromal cell, rodent, stem cell.

Introduction

Mesenchymal stromal cells (MSC) have shown great promise as therapeutic agents in various fields of study [1–5]. However, varied isolation and expansion techniques, along with diverse methods of cell characterization, have yielded ill-defined and incomparable cellular populations. While characterization of human and mouse MSC has seen significant advances in the last few years [6–10], characterization of rat bone marrow-derived MSC (rBM-MSC) has lagged behind. Rats serve as critical models in numerous diseases where cellular therapy is being studied. As these results are translated to clinical trials, ensuring consistent cellular populations is vital. Therefore, we sought to isolate, expand and characterize the immunophenotype of rBM-MSC.

Since the early 1970s MSC have been defined by their ability to adhere to plastic and proliferate [11]. Nearly 40 years later, this property remains the key step in the isolation of MSC. More recently, MSC have been defined further by their ability to differentiate down multiple lineages (most commonly adipocytes, chondrocytes and osteocytes) [6,12], including reports of differentiation into neural [13,14] and hepatic [15], among other, cell types. Cells similar to MSC, known as multipotent adult progenitor cells (MAPC), have been reported to differentiate down all three germ cell lines [16]. Others have

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defined MSC based on cell surface marker expression or lack thereof [7,8].

Most of the defining properties, markers and actions that characterize rBM-MSC were originally identified while using human and mouse cell lines [6,10,16]. Many of the same characteristics have been shown to transcend species, however some may not. We detail our methods for isolating rBM-MSC. We do not require magnetic-activated cell sorting (MACS) or fluorescent-activated cell sorting (FACS) to isolate our population of rBM-MSC. We follow three cell lines through to passage 10, characterizing the following cell-surface markers over time via flow cytometry: CD45, CD11b, CD29, CD49e, CD73, CD90, CD105 and Stro-1. A near homogeneous population of rBM-MSC is seen by passage 3 (14 days). By passage 8 or 9 (*c.* 1 month), cells develop suboptimal growth patterns and altered cell-surface marker expression. The rBM-MSC were differentiated to adipocytes, chondrocytes and osteocytes, confirming their multipotency.

Methods

Cell isolation from BM

Sprague–Dawley rats (200–225 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN, USA) for use in this study. The animals were housed on a 12-h light/dark cycle with *ad libitum* access to food and water. All protocols involving the use of animals were in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee (protocol HSC-AWC-06–038).

Immediately after killing, the muscle was dissected from the femurs and tibiae, leaving isolated bone. The tips of the bones were shaved off with a Rongeur. Each bone was flushed with *c.* 10 ml of Medium 199 (Gibco, Carlsbad, CA, USA), leaving a cell suspension. After repetitive flushing (10–15 times) through an 18-gauge needle, the cell suspension was filtered through a 40-μm nylon cell strainer (BD Falcon, Bedford, MA, USA). The cell suspension was then centrifuged for 6 min at 800 ***g***. Cells were counted and plated in 6-well plates (Nunc, Rochester, NY, USA) coated with fibronectin (Sigma, St. Louis, MO, USA; 100 ng/mL in 1 × PBS) at *c.* 10^6 cells/cm^2 (*c.* 10^7 cells/well) in 2 mL media/well. Warm media (1 mL) were added after 48 h. After 72 h, all media were removed, the wells gently washed with 1 × PBS, and refed with fresh, warm media. Media were replaced every 48 h for another 4–6 days. All cells were then separated from the plastic (passage), using 0.05% trypsin EDTA (Cellgro, Manassas, VA, USA), every 48–72 h, through to passage 10. See Figure 1 for an overview of cell isolation and expansion.

Cells were maintained in a rat media mixture as described previously [17]. The rat media contains 60% low-glucose Dulbecco's modified Eagle media (DMEM; Gibco), 40% MCDB-201 (Sigma), 1 × insulin-transferrin-selenium (ITS; Sigma), 1 × linoleic acid bovine serum albumin (LA-BSA; Sigma), 10^{-9} m dexamethasone (Sigma), 10^{-4} m ascorbic acid 3-phosphate (Sigma), 100 U penicillin, 1000 U streptomycin (Gibco), 2% fetal bovine serum (FBS; HyClone, Logan, UT, USA), 10 ng/mL human platelet-derived growth factor (R&D Systems, Minneapolis, MN, USA), 10 ng/mL mouse epidermal growth factor (Sigma) and 1000 U/mL mouse leukemia inhibitory factor (Chemicon, Temecula, CA, USA). Cells isolated and expanded in the above media (MAPC media) were compared with cells isolated and expanded in basic media consisting of DMEM (low glucose; Gibco),supplemented with 10% FBS (HyClone) and penicillin/streptomycin (Gibco).

Flow cytometric characterization

Flow cytometry analysis was performed using a BD LSR II (BD Biosciences, San Jose, CA, USA). Cells were collected from three Sprague–Dawley rats and flow cytometric analysis performed at passage zero (P = 0 or BM) and P = 1, 2, 3, 4, 5 and 10. For each passage, cells were detached with trypsin (as above) and labeled with a fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated primary antibody (Ab) or purified primary Ab, followed by a conjugated secondary Ab. The following cell-surface markers were characterized at each passage: CD11b, CD45, CD49e, CD73, CD90 (all from BD Biosciences), CD105 (Santa Cruz, Santa Cruz, CA, USA), CD29 (BioLegend, San Diego, CA, USA) and Stro-1

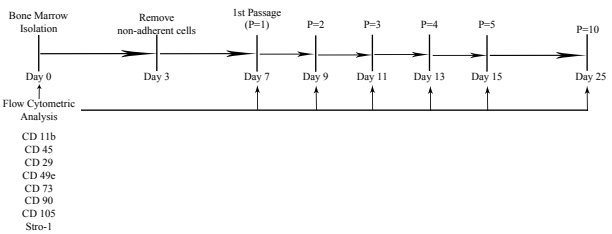

Figure 1*. Overview of cell isolation, expansion and immunophenotyping.*