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Comparative characterization of bone marrow-derived mesenchymal stromal cells from four different rat strains

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Background aims

Bone marrow (BM) multipotent mesenchymal stromal cells (MSC) hold great potential for cell-based regenerative medicine. Because of the growing use of autologous rat MSC transplantation in various rat models, there is a need to establish minimal criteria for rat MSC characterization independent of the specific strain employed in each study. We aimed to compare the phenotypic and functional traits of BM MSC from the four strains of rats commonly used in research: Fisher, Lewis, Sprague–Dawley and Wistar.

Methods

Rat MSC were isolated from the BM of the four different rat strains in an identical fashion. Cells were characterized for their cell-surface phenotype in early and late passage. Functional mesenchymal differentiation capacities were examined following adipogenic and osteogenic inductions. Population doubling times were determined across the four strains throughout 10 passages. In vitro *proliferation assays of immune cells were conducted following co-culture of spleen cells and MSC of the four different strains.*

Results

We found that rat MSC from different strains exhibited similar cell-surface phenotype. Expansion rates and differentiation capacities of the MSC were also similar across the different strains. Co-culture of rat MSC with spleen cells obtained from rats of a different strain did not induce proliferation of immune cells.

Conclusions

Our findings suggest that BM-derived MSC from different strains share similar characteristics, in contrast to the variations previously described in the characterization of mice MSC from different strains.

Keywords

Mesenchymal stromal cells, differentiation, Fisher, Lewis, splenocytes, Sprague–Dawley, Wistar.

Introduction

Bone marrow (BM) multipotent mesenchymal stromal cells (MSC) have been reported for their multilineage differentiation potential [1,2], and have been shown to be clinically effective when transplanted into animal models of various diseases [3–6]. One of the prominent advantages of MSC for future cell-based regenerative medicine is the prospect of enabling autologous transplantation. Hopefully, the future of MSC-based therapy will include autologous human to human transplantations [7]. In the mean time, because of the convenience and availability of rat animal models for various diseases, rat MSC have been used widely over the last few years as an autologous cell source for the treatment of diseases affecting the bones, cartilage, nervous system, heart, liver, blood vessels, gastric system and kidneys [8–24].

Human MSC have been studied extensively and are characterized in accordance with clear criteria agreed between researchers [25]. Mice BM-derived MSC have been described previously to differ in their cell-surface phenotype, proliferation rates and differentiation potential between different inbred strains [26]. Rat MSC from

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different strains have been characterized in some detail as a part of studies performed with Fisher [27], Lewis [15,24], Sprague-Dawley [12,13,20,21,28] and Wistar strains [19,29]. However, no report has been made regarding the comparative characterization of rat MSC derived from different strains under the same experimental conditions.

This study describes the functional and phenotypic comparison of BM MSC derived from four different rat strains commonly employed in medical research. The characteristics evaluated in this study were the ones most often used to fulfill the minimal criteria for defining cells as MSC [25], namely cell-surface epitope expression, mesenchymal differentiation capacity and expansion rate. The results indicate that rat MSC isolated from all four strains share similar characteristics and do not differ significantly from one another in the examined traits. Moreover, *in vitro* analysis demonstrates that the MSC do not induce immune cell proliferation when co-cultured with spleen cells of a rat from a different strain.

Methods

Animals

Male Fisher, Lewis, Sprague–Dawley and Wistar rats (Harlan, Jerusalem, Israel) at 8–12 weeks of age were used. Each primary culture was pooled from two to four different animals from the same stain. All experimental protocols were approved by the University Committee of Animal Use for Research and Education. Every effort was taken to reduce the number of animals used and minimize their suffering.

Isolation of rat MSC

Following killing of the rats with CO_2, tibiae and femora were dissected and the bones placed in Hanks' balanced salt solution (HBSS; Biological Industries, Beit HaEmek, Israel). The epiphyses of the bones were removed, and the marrow was flushed out using a syringe filled with HBSS. Low-density BM mononuclear cells were separated, based on density gradient, using UNISEP maxi tubes (NovaMed, Jerusalem, Israel). Next, the mononuclear cells (at a density of approximately 250,000/cm^2) were plated in a polystyrene plastic flask (75 cm^2; Corning, NY, USA) in a growth medium consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal calf serum (FCS), 2 mM L-glutamine, 100 μg/mL streptomycin, 100 U/mL penicillin, 12.5 U/mL nystatin (SPN) (all from Biological Industries) and 0.001% 2-mercaptoethanol (Sigma-Aldrich, St Louis, MO, USA). The next day, non-adherent cells were removed with medium replacement. Cells were then cultured for 2 weeks; the medium was changed twice a week and cells maintained at 37°C in a humidified 5% CO_2 incubator. Confluent cultures (passage 0) were subcultured according to experimental requirements.

Growth rate analysis

Passage 0 cells were trypsinized and reseeded at a density of 5000 cells/cm^2. Following 7 days, cells (passage 1) were trypsinized, counted and subcultured onwards in the same manner up to passage 10. The medium was changed twice a week throughout all experiments.

Mesenchymal differentiation

For adipogenic induction, cells (passage 5) were grown to confluence and treated with adipogenic differentiation medium for 21 days. The medium contained DMEM, L-glutamine (12 mM), SPN (as in the growth medium), FCS (10%) (all from Biological Industries), insulin (5 μg/mL), indomethacine (50 μm), dexamethasone (1 μm) and 3-isobutyl-1-methylxanthine (IBMX; 0.5 μm; all from Sigma-Aldrich). Oil Red O staining was conducted at the end of the differentiation protocol to assess lipid accumulation, as a marker for adipogenic differentiation. For osteogenic induction, cells (passage 5) were plated on wells coated with vitronectin (12.5 μg/mL; Millipore, Billerica, MA, USA) and collagen 1 (12.5 μg/mL; Sigma), grown to confluence and treated with osteogenic differentiation medium for 21 days. The medium contained DMEM, L-glutamine (12 mM), SPN (as in the growth medium), FCS (10%) (all from Biological Industries), dexamethasone (0.1 μm), ascorbic acid (0.2 mM) and glycerol 2-phosphate (10 mM) (all from Sigma-Aldrich). Alizarin red staining was conducted at the end of the differentiation protocol to detect calcium deposits as a marker for osteogenic differentiation.

Flow cytometry analysis of cell-surface markers

Rat MSC were harvested at the second or seventh passage. Cells were trypsinized, washed, suspended in phosphate-buffered saline (PBS) and distributed into duplicate samples containing $2–5 \times 10^5$ cells in 50 μL. Cells were incubated with appropriate antibodies for 45 min on ice, washed twice in flow-buffer consisting of 5% FCS and 0.1% sodium azide in PBS, and centrifuged for 10 min. The cells were resuspended in 0.2 mL PBS and studied with a

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