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Effect of mild heat stress on the proliferative and differentiative ability of human mesenchymal stromal cells

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) are an attractive candidate for autologous cell therapy, but regenerative potential can be compromised with extensive *in vitro* cell passaging. Development of viable cell therapies must address the effect of *in vitro* passaging to maintain overall functionality of expanded MSCs. *Methods.* We examined the effect of repeated mild heat shock on the proliferation and differentiation capability of human adipose-derived MSCs. Adipose tissue MSCs were characterized by means of fluorescence activated cell sorting analysis for expression of CD3, CD14, CD19, CD34, CD44, CD45, CD73, CD90 and CD105. Similarly, the expression of *SIRT-1*, $p16^{INK4a}$ and p21 was determined by means of polymerase chain reaction. Measurements of population doubling, doubling time and superoxide dismutase activity were also determined. Differentiation of expanded MSCs into bone and adipose were analyzed qualitatively and quantitatively. *Results.* The strategy led to an increase in expression of *SIRT-1* concomitant with enhanced viability, proliferation and delayed senescence. The stressed MSCs showed better differentiation into osteoblasts and adipocytes. *Conclusions.* The results indicate that mild heat shock could be used to maintain MSC proliferative and differentiation potential.

Key Words: *heat shock, mesenchymal stromal cells, senescence*

Introduction

Mesenchymal stromal cells (MSCs) show therapeutic potential for tissue engineering and regenerative medicine applications. Adipose tissue represents a rich source of MSCs, with more abundant cells than other sources. MSCs from adipose tissue are easier to harvest, isolate, culture and expand than the bone marrow counterpart. However, several studies have suggested that age-related changes may make MSCs less effective in the treatment of certain diseases and disorders [1]. Similarly, *in vitro* passaging of cells has a negative impact on the regenerative potential of MSCs [2]. It has been shown that the adipogenic [3], osteogenic [4], chondrogenic [2] and myogenic [5] differentiation potential of MSCs declines with *in vitro* passaging. Because many promising tissue engineering applications require cell expansion after harvest, increased senescence would be a severe limitation for use.

The term "hormesis" is derived from a Greek word meaning "to excite" and refers to a cascade of beneficial biological effects in response to low doses of harmful stressors that are otherwise lethal in higher quantities. It has recently been shown that the hormetic effects of temperature can limit age-related dysfunction in cells and have recently been used with success to enhance stem cell functionality [6,7]. Culture temperatures below (32°C) [6] and above (41°C) [8] standard culture temperature have been shown to prevent stem cell aging and age-related impairments. Similarly, repeated heat stress has been shown to avert various characteristics associated with age [7,9]. However, the effects of heat stress on the growth characteristics and differentiation potential of MSCs largely remains unexplored.

The present study was designed to study the effect of repeated mild heat shock on the proliferation and

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differentiation potential of human adipose-derived MSCs (AT-MSCs). AT-MSCs were analyzed for osteogenic and adipogenic potential along with various growth characteristics. The older "stressed" cells showed increased proliferative potential and better differentiation into osteoblasts and adipocytes as compared with older control cells. The results indicated that mild heat shock could be applied to AT-MSCs to maintain both proliferative and differentiation potential during *in vitro* expansion.

Methods

MSC isolation and expansion

Adipose tissue was harvested during a liposuction procedure ($n = 8$, 51.50 ± 5.17 years of age) with a 2.4-mm cannula or with a hand-held 10-mL syringe. All samples were obtained with written consent from the donors. MSCs from adipose tissue were isolated as described [10]. The tissue was washed with phosphate-buffered saline (PBS) and treated with 0.2% collagenase type IV in PBS for 20 min at 37°C. Collagenase activity was neutralized with 20 mL of fetal bovine serum (FBS) containing media and filtered through a sieve. The cell suspension was centrifuged for 10 min at 150*g* and the supernatant was discarded. The cells were seeded in 25-cm^2 culture flasks and incubated at 37°C with 5% CO_2 in humidity. The non-adherent cells were removed after 72 h to obtain a pure population of plastic adherent cells (MSCs).

MSCs were expanded in minimum essential medium (MEM, ThermoScientific) supplemented with 10% FBS and 1% each of non-essential amino acids, sodium pyruvate, L-glutamine and streptomycin/penicillin solution. The adipogenic AdvanceSTEM adipogenic differentiation medium (catalogue No. SH30886.02, ThermoScientific) supplemented with 10% AdvanceSTEM stem cell growth supplement (catalogue No. SH30b78.02) and osteogenic AdvanceSTEM osteogenic differentiation medium (catalogue No. SH30881.02, ThermoScientific) supplemented with 10% AdvanceSTEM stem cell growth supplement (catalogue No. SH30b78.02) media were used to induce MSCs into adipose and bone, respectively.

Flow cytometry

Cultured cells were examined for surface markers by means of flow cytometry. The cells were stained with the following primary antibodies: AF-700–conjugated CD3 (BD BioSciences), phycoerythrin (PE)-conjugated CD14 (BD, Immunocytometry), Allophycocyanin (APC)-conjugated CD19 (BD BioSciences), PE-conjugated CD34 (BD, BioSciences), APC-conjugated CD44 (BD, Pharmingen), fluorescein isothyocyanate–conjugated CD45 (BD Pharmingen), PE-conjugated CD73 (BD Pharmingen), AF-700–conjugated CD90 (Biolegend) and APC-conjugated CD105 (Biolegend). Samples were analyzed on an LSR II flow cytometer (BD Biosciences), and at least 10,000 events were acquired for each population. Data acquisition and analysis were performed with the use of FACS DIVA software (BD Biosciences). Unstained cells were used to establish flow cytometer settings. Debris and auto-fluorescence were removed by means of forward scatter.

Mild heat shock

Confluent primary cultures of MSCs (at passage 1) after trypsinization were divided into 2 groups: group 1 (control) and group 2 (subjected to mild heat shock). MSCs in group 2 were subjected to heat shock for 60 min in a water bath set at 41°C once in a week. No heat shock was given for at least 24 h after sub-culturing, and no sub-culturing was done within 24 h of heat shock. These conditions were maintained throughout the study. MSCs in group 1 were kept in the 37°C incubator. MSCs were cultured through 8 cell passages (approximately 7 weeks) before differentiation was induced.

Senescence-associated β-galactosidase staining

Cellular senescence was detected by means of senescence-associated β-galactosidase (SA–β-gal) staining (Cell Signaling). Briefly, 5×10^3 cells were seeded in 12-well plates at passages 5 and 8. After 24 to 48 h, cells were incubated with freshly prepared β-gal staining solution for 60 min at 37°C in the absence of CO_2. MSCs were washed with water, and blue-colored senescent cells were observed under microscopy. Phase-contrast images were taken, and the percentages SA–β-gal–positive cells were calculated by dividing blue-stained cells by the total number of cells, multiplied by 100.

Superoxide dismutase assay

A calorimetric assay (Abcam) was used to evaluate the activity of superoxide dismutase (SOD) produced as a result of heat shock. Briefly, at passages 5 and 8, protein was extracted from both groups of MSCs and the total protein extract (10 μg) was used to determine SOD activity. Absorbance values were measured with the use of Spectra max PLUS 384 (Molecular Devices) at 450 nm.

Viability assay

MSCs of both types (at passages 5 and 8) were treated with H_2O_2 (100 μmol/L) for 90 min (for