Biochemical and Biophysical Research Communications 500 (2018) 682–690

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Aging impairs beige adipocyte differentiation of mesenchymal stem cells via the reduced expression of Sirtuin 1

Vuong Cat Khanh [a, 1], Amin Firman Zulkifli [a, 1], Chiho Tokunaga [b], Toshiharu Yamashita [a], Yuji Hiramatsu [b], Osamu Ohneda [a, *]

[a] Graduate School of Comprehensive Human Science, Laboratory of Regenerative Medicine and Stem Cell Biology, University of Tsukuba, Tsukuba 305-8575, Japan
[b] Department of Cardiovascular Surgery, University of Tsukuba, Japan

ARTICLE INFO

Article history:
Received 14 April 2018
Accepted 17 April 2018

Keywords:
Beige adipocyte
Aging
Mesenchymal stem cells
Sirtuin
Senescence

ABSTRACT

In the body, different types of adipose tissue perform different functions, with brown and beige adipose tissues playing unique roles in dissipating energy. Throughout life, adipocytes are regenerated from progenitors, and this process is impaired by aging. One of the progenitors of adipocytes are mesenchymal stem cells (MSCs), which have recently become a promising tool for stem cell therapy. However, whether or not aging impairs the brown/beige adipocyte differentiation of adipose tissue-derived MSCs (AT-MSCs) remains unclear. In the present study, we isolated AT-MSCs from two different age groups of donors (infants and elderly subjects) and examined the effects of aging on the AT-MSC brown/beige adipocyte differentiation ability. We found that none of the AT-MSCs expressed Myf5, which indicated the beige (not brown) differentiation ability of cells. Of note, an inverse correlation was noted between the beige adipocyte differentiation ability and age, with AT-MSCs derived from elderly donors showed the most severely reduced function due to induced cellular senescence. The impaired expression of Sirtuin 1 (Sirt1) and Sirt3 proved to be responsible for the induction of senescence in elderly AT-MSCs; however, only Sirt1 was directly involved in the regulation of beige adipocyte differentiation. The overexpression of Sirt1 impaired the p53/p21 pathway, thereby preventing elderly AT-MSCs from entering senescence and restoring the beige differentiation ability. Thus, our study represents the important role of Sirt1 and senescence in the regulation of beige adipocyte differentiation during aging.

1. Introduction

Brown adipose tissue (BAT) possesses the unique ability to produce energy during thermogenesis based on the role of uncoupling protein (UCP)-1 in the inner mitochondrial membrane, where it dissolves the mitochondrial proton gradient, thereby uncoupling respiration from ATP production [1]. BAT is known to be abundant in newborn mammals and gradually decreases with age [1]. The existence of brown-like adipocytes known as beige adipocytes (Beige adipocytes), which come from distinct cell lineages but possess the same ability of thermogenesis as BAT, was recently reported [2]. Beige adipocytes can be formed inside white adipose tissues and appear in the adult body [2]. Both BAT and Beige adipocytes control energy homeostasis, thereby regulating the fat accumulation in the body [2].

Obesity is defined as the abnormal fat accumulation that may impair health [3]. In 2014, 39% of adults were overweight and 13% of the world adult population was obese [3]. The body fat content peaks between 40 and 70 years old which reveals an association with aging [3]. Thus, obesity in the elderly has become a serious concern and studying the mechanisms related to aging and obesity has become necessary. The incidence and activity of brown/beige adipose tissue was reported to decrease with age, being >50% in younger donors (20–29 years old) but <10% in the older donors (over 40 years old) [3]. Aging causes cell-autonomous defects, including a reduced mitochondrial function and changes in the endocrine balance of the microenvironment [4]. In addition, aging impairs the regeneration of brown and Beige adipocytess through a defect in progenitor proliferation and differentiation [4]. However,

* Corresponding author. Laboratory of Regenerative Medicine and Stem Cell Biology, University of Tsukuba, 1-1-1 Tsukuba 305-8575, Japan.
E-mail address: oohneda@md.tsukuba.ac.jp (O. Ohneda).
[1] These authors contributed equally to the work.

https://doi.org/10.1016/j.bbrc.2018.04.136
0006-291X/

whether or not the Beige adipocytes differentiation ability of adipose tissue-derived mesenchymal stem cells (AT-MSCs) derived from elderly donors is similar to that in younger donors has been unclear.

MSCs are the progenitors of all three types of adipocytes in the body (white, brown, and Beige adipocytess) in response to small molecules, including insulin, dexamethasone, and rosiglitazone [5]. The expression of the myogenic lineage protein Myf5 in MSCs has been reported to determine the fate of adipocytes [5]. Brown adipocytes are derived from myogenic lineages that express Myf5, while white and beige adipocytes come from different lineages with no history of Myf5 expression [5]. Given the unique functions of brown and Beige adipocytes, the regeneration of brown/Beige adipocytes from MSCs has been considered a promising therapy for treating obesity in elderly population [5]. However, whether or not aging impairs the beige adipocyte differentiation ability of human MSCs has been unclear.

In the present study, we compared the beige adipocyte differentiation ability of AT-MSCs from donors of different age groups (infant and elderly donors) and assessed the negative effects of aging on the beige adipocyte differentiation ability of MSCs. Furthermore, our findings showed that the low expression of Sirt1 in elderly MSCs impairs the beige adipocyte differentiation ability by promoting cellular senescence.

2. Materials and methods

2.1. The isolation and culture of AT-MSCs

Human adipose tissues were obtained from the Department of Cardiovascular Surgery, University of Tsukuba Hospital, Tsukuba, Japan. All cell experiments were approved by the ethics committee of the University of Tsukuba. Human adipose tissues were divided into two groups of ages: infants (n = 4, 1–11 months old) and elderly donors (n = 4, 70–80 years old). AT-MSCs were isolated from adipose tissues. In brief, adipose tissues were cut into small pieces and digested with 0.1% collagenase (Invitrogen, Carlsbad, CA, USA) in phosphate-buffered saline (PBS) at 37 °C for 1 h. The digested tissues were then separated by centrifugation to harvest the cells and the cell pellets were re-suspended in culture medium, Iscove's Modified Dulbecco Medium (IMDM; Invitrogen), supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen), 2 mg/ml L-glutamine (Invitrogen), 100 units/ml penicillin (Invitrogen), and 5 ng/ml bFGF (Peprotech, Rocky Hill, NJ, USA). Cells were maintained and cultured at 37 °C in a 5% CO_2 atmosphere. All AT-MSCs used in this study were from passages 3 to 8.

2.2. A fluorescent-activated cell sorting (FACS) analysis of AT-MSCs

After the cells become confluent, AT-MSCS were purified using cell sorting (MoFlo XDP); Beckman Coulter (Pasadena, CA, USA). In brief, $CD45^+$ and $CD31^+$ cells were eliminated from adherent cells in tissue culture dishes. AT-MSCs were characterized with the AT-MSCs markers: Fluorescein isothiocyanate (FITC)-labeled anti-CD90 (BioLegend, San Diego, CA, USA), phycoerythrin (PE)-labeled anti-CD13 (BioLegend), PE-labeled anti-CD73 (BD Biosciences, [please describe the company location]), PE-labeled anti-CD31 (BioLegend), and allophycocyanine (APC)-labeled anti-CD45 (BioLegend). FITC-labeled anti-IgG1 (BioLegend), APC-labeled anti-IgG1 (BioLegend), and PE-labeled anti-IgG1 (BioLegend) were used as the isotype controls.

2.3. Multi-differentiation of AT-MSCs

AT-MSCs were differentiated into specific cell lineages of adipocytes, chondrocytes, and osteoblasts. For adipogenic differentiation, cells were cultured in culture medium until 80% confluency and then switched to adipogenic differentiation medium containing 0.1×10^{-6} M dexamethasone (Invitrogen), 0.1 mM indomethacin (Invitrogen), and 2 μg/ml insulin (Invitrogen). After 20 days, adipocyte differentiation was confirmed by Oil Red O staining (Wako, Tokyo, Japan).

For osteogenic differentiation, adherent cells were grown in culture medium until 80% confluency and then switched to osteogenic differentiation medium containing 0.1×10^{-6} M dexamethasone (Invitrogen), 0.2 mM ascorbic acid (Invitrogen), and 10 mM β-glycerophosphate (Invitrogen). After 20 days of culture, calcium deposits were detected by Alizarin Red staining (Wako).

For chondrogenic differentiation, the cells were incubated at 5×10^4 cells/well in culture medium for 1 h to achieve micromass formation and then switched to the medium supplemented with 0.1×10^{-6} M dexamethasone (Invitrogen), 0.25 mM ascorbic acid (Invitrogen), 40 ng/ml proline (Invitrogen), and 10 ng/ml of transforming growth factor β3 (TGFβ3; Invitrogen) for 7 days. Chondrogenic differentiation was confirmed by hematoxylin and eosin staining (Muto Pure Chemicals, Tokyo, Japan) and Toluidine blue staining (Muto Pure Chemicals) and visualized under a microscope (Olympus, Tokyo, Japan).

2.4. Cell proliferation assay

AT-MSCs were seeded in 35-mm tissue culture dishes (Sumitomo Bakelite, Tokyo, Japan) at a density of 2×10^4 cells/dish in culture medium and cultured for 10 days. The cell culture medium was changed after three days. The cells were washed with sterile PBS and treated with 0.05% trypsin/EDTA (Invitrogen) at 24-h intervals for 10 days to separate single cells. Dead cells were excluded using trypan-blue staining solution (Invitrogen), and the numbers of live cells in triplicate dishes were counted using a hemocytometer. The doubling time were calculated as described previously.

2.5. Beige adipocyte differentiation of AT-MSCs

Cells were cultured in culture medium until 80% confluency and then switched to culture in IMDM medium supplemented with 10% FBS, 2 mg/ml L-glutamine, and 100 units/ml penicillin for 24 h. The medium was then replaced with the induction medium containing dexamethasone (Invitrogen), 2 μg/ml insulin (Invitrogen), IBMX, transferrin, and T3 to form pre-adipocytes. After 72 h, the induction medium was changed to beige adipocyte differentiation medium containing insulin, transferrin, T3, and Rosiglitazone. After 7 days, the formation of adipocytes was confirmed by Oil Red O staining, and the number of mitochondria was measured. After 21 days, the expression of UCP-1 was confirmed.

2.6. Cell senescence assay

Senescence was assessed using the β-Galactosidase Enzyme Assay System (ThermoFisher Scientific, Carlsbad, CA, USA). ATßß-MSCs were seeded in 12-well plates (Sumitomo, Japan) at a density of 5×10^3 cells/well in culture medium for 24 h β-galactosidase was added to each well and incubated at 37 °C for 30 min in the absence of CO_2. Measured the absorbance at 405 nm.

2.7. Mitotracker

To label the mitochondria in beige adipocytes derived from AT-MSCs, cells were incubated with MitoTracker® probes (MitoTracker Green FM; ThermoFisher Scientific, Carlsbad, CA, USA) according to the instructions from the manufacturer. In brief, after