Cytotherapy, 2016; 18: 371–383

International Society for Cellular Therapy
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ADIPOCYTE-DERIVED CELLS

High glucose-induced reactive oxygen species generation promotes stemness in human adipose-derived stem cells

NAI-CHEN CHENG[1,2], TSUNG-YU HSIEH[3], HONG-SHIEE LAI[1] & TAI-HORNG YOUNG[3]

[1]*Department of Surgery, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan,* [2]*Research Center for Developmental Biology and Regenerative Medicine, National Taiwan University, Taipei, Taiwan, and* [3]*Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, Taipei, Taiwan*

Abstract

Background aims. Adipose-derived stem cells (ASCs) represent an important source of cell therapy to treat diabetic complications. However, hyperglycemia may alter several cellular functions, so the present study aimed to investigate the influence of a diabetic environment on the stemness and differentiation capabilities of ASCs. *Methods.* Human ASCs were obtained from subcutaneous adipose tissues of diabetic (dASCs) and nondiabetic donors (nASCs) and characterized. To reproduce an *in vitro* hyperglycemia environment, the nASCs were also cultured under prolonged high-glucose (HG; 4.5 g/L) or low-glucose (LG; 1.0 g/L) conditions. *Results.* The expression of cell surface markers in dASCs and nASC was similar and characteristic of mesenchymal stem cells. Although dASCs or HG-treated nASCs exhibited decreased proliferation, enhanced expression of the pluripotent markers Sox-2, Oct-4, and Nanog was observed. Moreover, HG-treated nASCs exhibited decreased cell migration, enhanced senescence, and significantly higher intracellular reactive oxygen species (ROS), whereas their adipogenic and osteogenic differentiation capacities remained comparable to LG-treated cells. With antioxidant treatment, HG-treated nASCs showed improved cell proliferative activity without stemness enhancement. This HG-induced biological response was associated with ROS-mediated AKT attenuation. When cultured in an appropriate induction medium, the HG-treated nASCs and dASCs exhibited enhanced potential of transdifferentiation into neuron-like cells. *Discussion.* Despite lower proliferative activity and higher senescence in a diabetic environment, ASCs also exhibit enhanced stemness and neurogenic transdifferentiation potential via a ROS-mediated mechanism. The information is important for future application of autologous ASCs in diabetic patients.

Key Words: *adipose-derived stem cell, diabetes, differentiation, reactive oxygen species, stemness*

Introduction

The increasing prevalence of diabetes mellitus worldwide necessitates the search for more effective therapies for this chronic debilitating disease [1]. Patients with diabetes invariably develop dyslipidemia, hypertension and atherosclerotic complications. Moreover, such patients are prone to delayed wound healing and development of nephropathy, retinopathy and neuropathy. These diabetes-associated complications have become crucial clinical concerns worldwide, and currently available therapies have limitations. Experimental evidence suggests that cell therapy might represent a novel and promising strategy for the management of various diabetes-associated complications [2]. Among the emerging cell therapies, stem cell therapy shows great potential for clinical application considering its unique biological properties. Particularly, mesenchymal stromal cells (MSCs) have gained increased attention because they can contribute to tissue repair and regeneration through differentiation and paracrine effects [3]. The use of MSCs circumvents the ethical or safety concerns regarding the use of the embryonic stem cells or induced pluripotent stem cells.

Adipose-derived stem cells (ASCs) represent an abundant source of multipotent adult stem cells that can be easily obtained from the subcutaneous adipose tissue through surgical excision or liposuction. In

Correspondence: **Tai-Horng Young**, PhD, Institute of Biomedical Engineering, National Taiwan University 1, Sec. 1, Jen-Ai Rd., Taipei, Taiwan. E-mail: thyoung@ntu.edu.tw

(Received 19 June 2015; accepted 16 November 2015)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2015.11.012

particular, their accessibility and low donor site morbidity has positioned ASCs as favorable candidates for various cell-based therapies [4,5]. The expression of pluripotency markers, including Sox-2, Oct-4, and Nanog, in ASCs has been demonstrated to increase the self-renewal potential and suppress the spontaneous differentiation of these cells [6]. In addition, ASCs exhibit the potential to differentiate into several cell lineages, including adipocytes, osteocytes, chondrocytes, myocytes and cells of neuronal lineages. The ability of ASCs to restore damaged tissues has been attributed to their multilineage differentiation potential, release of multiple cytokine and growth factors and immunomodulatory effects [1]. Previous studies have described the application of ASCs in treating diabetes-associated complications [2]. For instance, in a murine diabetic model, topically applied ASCs were able to engraft in the skin and contribute to wound repair [5].

In patients with diabetes, prolonged hyperglycemia damages several organs, including heart, kidneys and eyes, and also affects blood vessels and nerves [7]. At the cellular level, hyperglycemia can cause damage in several cell types. For example, endothelial progenitor cells from diabetic patients exhibit impaired endothelial adhesion, proliferation, and tube formation [8]. Diabetic hyperglycemic microenvironment also plays a major role in inducing the differentiation of human bone marrow–derived MSCs [9]. Although diabetic ASCs are not considered ideal for autologous cell therapy because of their impaired neovascular potential [10,11], the stemness and differentiation capabilities of human ASCs in a hyperglycemic milieu has not been thoroughly investigated. To elucidate the possible clinical applications of autologous ASCs in patients with diabetes, the present study aimed to investigate the influence of a diabetic environment on the stemness and differentiation potentials of ASCs.

Methods

Isolation and culture of ASCs

The study protocol was approved by the Internal Ethics Committee of National Taiwan University Hospital. Informed consent was obtained from all patients involved in this study. The ASCs derived from four patients with type 2 diabetes with an average age of 59 years (range 48–71 years) and an average body mass index of 28.5 (range 25.4–32.8) were cultured, yielding four independent diabetic ASCs (dASCs). These patients exhibited average HbA1c levels of 8.8% (range 6.9–11.8%). The control nondiabetic ASCs (nASCs) were obtained from a combination of four nonsmoking, nondiabetic donors with an average age of 45 years (range 32–57 years) and an average body mass index of 24.6 (range 21.0–26.6). The nASCs from different donors were pooled as a single population, and they have been well characterized in our previous studies [12,13].

The ASCs were isolated as described previously [6]. In brief, small sections of subcutaneous adipose tissue were collected in a physiological solution (0.9% NaCl), washed twice with phosphate-buffered saline (PBS; Omics Biotechnology) and finely minced. The scraped adipose tissue was then placed in a digestion solution: 1 mg/mL collagenase type I (Gibco) dissolved in PBS at 37°C in agitation for 60 min. After digestion, the cell suspension was filtered through 40-μm cell strainers (BD Falcon). The cells were cultured in an expansion medium comprising Dulbecco's Modified Eagle's Medium (DMEM)/F-12 (Hyclone), 10% fetal bovine serum (FBS; Biological Industries), 1% penicillin-streptomycin (Biological Industries) and 1 ng/mL of basic fibroblast growth factor (bFGF; R&D Systems). The cells were cultured at 37°C in 5% CO_2, and the medium was changed every 2–3 d. On reaching 90% confluence, the cells were lifted with 0.05% trypsin-ethylenediaminetetraacetic acid (Biological Industries) and replated until the third passage for different experiments.

To simulate a diabetic or nondiabetic environment *in vitro*, the third passage nASCs were further cultured for 49 days in DMEM-high glucose (HG; 4.5 g/L) or DMEM-low glucose (LG; 1 g/L), containing 10% FBS and 1% penicillin-streptomycin. The cells were passaged every 7 days, and a previous study observed no significant DNA damage in ASCs up to passage 10 [14]. The LG- or HG-treated cells were analyzed using reverse transcription polymerase chain reaction (RT-PCR), immunofluorescence and Western blotting. The cells were cultured at 37°C in 5% CO2, and the medium was changed every 2 to 3 d.

Flow cytometry analysis

ASCs derived from various sources and experimental conditions were subjected to flow cytometry to determine their cell surface antigen expression. The cells were incubated with the following antibodies: human monoclonal antibodies against CD31 (BD Pharmingen), CD34 (BioLegend), CD44 (BD Pharmingen), CD73 (BioLegend), CD90 (BioLegend), CD105 (eBioscience) and CD166 (BioLegend). The samples were analyzed using a flow cytometer (FACScan; Becton Dickinson), which counts 10,000 cells per sample. The positive cells were defined as those with fluorescence >95% of that of the isotype controls.

Proliferation and senescence assay

The nASCs maintained in LG or HG culturing conditions were seeded at a density of 9000 cells/cm^2. Every 7 days, the cells were lifted, counted using a Scepter 2.0 Cell Counter (Millipore), and then