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Transplantation of insulin-secreting cells differentiated from human adipose tissue-derived stem cells into type 2 diabetes mice

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ABSTRACT

Currently, there are limited ways to preserve or recover insulin secretory capacity in human pancreas. We evaluated the efficacy of cell therapy using insulin-secreting cells differentiated from human eyelid adipose tissue-derived stem cells (hEAs) into type 2 diabetes mice. After differentiating hEAs into insulin-secreting cells (hEA-ISCs) in vitro, cells were transplanted into a type 2 diabetes mouse model. Serum levels of glucose, insulin and c-peptide were measured, and changes of metabolism and inflammation were assessed in mice that received undifferentiated hEAs (UDC group), differentiated hEA-ISCs (DC group), or sham operation (sham group). Human gene expression and immunohistochemical analysis were done. DC group mice showed improved glucose level, and survival up to 60 days compared to those of UDC and sham group. Significantly increased levels of human insulin and c-peptide were detected in sera of DC mice. RT-PCR and immunohistochemical analysis showed human gene expression and the presence of human cells in kidneys of DC mice. When compared to sham mice, DC mice exhibited lower levels of IL-6, triglyceride and free fatty acids as the control mice. Transplantation of hEA-ISCs lowered blood glucose level in type 2 diabetes mice by increasing circulating insulin level, and ameliorating metabolic parameters including IL-6.

1. Introduction

Recently, there has been a shift in paradigm about the pathogenesis of type 2 diabetes in which beta cell dysfunction is considered to be a primary defect or at least non-inferior to insulin resistance in the pathogenesis of type 2 diabetes [1–3]. Various drugs have been shown to ameliorate insulin resistance, yet there are limited ways to preserve or restore beta cell function. Stem cell therapy is an attractive treatment option for its abundant source and its potential to acquire glucose-dependent insulin secretory function [4]. Adult stem cell, in particular, is good candidate for its safety in terms of tumorigenicity and ethical concerns compared to embryonic or induced pluripotent stem cells [5,6], and depending on the source of the cells, some are easily acquired without an invasive procedure. Moreover, they allow autologous transplantation, thereby circumventing the adverse effects of immunosuppression [6].

Human adult stem cells derived from various sources including bone marrow mesenchymal stem cells [7], peripheral blood monocytes [8] umbilical cord mesenchymal stem cells [9] have been successfully differentiated into insulin-secreting cells and showed a glucose-lowering effect in diabetes murine models. Neural-crest derived cells are another good candidate source of stem cells in preparing insulin-secreting cells [10]. These adipose tissue-derived stem cells can be easily obtained from human facial adipose tissue without an invasive procedure, and they exhibit good proliferation and multi-differentiation potential with high levels of stem cell -related antigens [11]. Also, a lack of HLA-DR expression and

Abbreviations: DC, differentiated cell; hEAs, human eyelid adipose-derived stem cells; hEA-ISCs, insulin secreting cells differentiated from human eyelid adipose-derived stem cells; UDC, undifferentiated cell.

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immunosuppressive properties of these cells have been reported [12], and a successful engraftment was shown in immunocompetent mice without immunosuppressive agents [10].

The efficacy of stem cell therapy in type 2 diabetes has been suggested by several studies. Mononuclear cells from human umbilical cord blood were shown to improve blood glucose levels and survival when transplanted into type 2 diabetic mice [13]. Human pancreatic beta-like cells derived from induced-pluripotent stem cell (iPS) also exhibited a reversal of hyperglycemia in mouse diabetic models of type 2 as well as type 1 [14]. Moreover, clinical trials demonstrated the efficacy and safety of stem cell transplantation using autologous bone marrow cells [15] and placenta-derived mesenchymal stem cells [16] in patients with type 2 diabetes. However, all these studies on type 2 diabetes were conducted with undifferentiated cells or differentiated iPS cells, and there is no prior study that transplanted differentiated adult stem cells.

Mounting evidence points to the involvement of chronic inflammation and immune dysfunction in the pathogenesis of type 2 diabetes [17], and adult stem cells were shown to have anti-inflammatory, immune modulatory effects [18,19]. In the present study, we transplanted insulin-secreting cells derived from human eyelid-derived adipose stem cells (hEA-ISCs) into immunocompetent type 2 diabetes mouse model, and assessed their effects on glucose level, metabolic profiles as well as surrogate markers of insulin resistance, inflammatory marker, and survival.

2. Materials and methods

2.1. Isolation and differentiation into hEA-ISCs

The human eyelid adipose tissue was obtained from nine subjects undergoing cosmetic surgery with informed consent. All experiments were approved by Institutional Review Boards of Seoul Women's University and Yonsei University. Human eyelid adipose-derived stem cells (hEAs) were obtained as described elsewhere [10]. Briefly, minced adipose tissue was reacted with 0.075% type I collagenase for 30 min at 37 °C with gentle stirring. Cell suspensions were cultured in DMEM-LG supplemented with 10% FBS at 5% CO_2, 37 °C. Medium was changed twice a week. Then, hEAs at passage 3–4 were induced into insulin-secreting cells using differentiation medium (IS1 kit, Bcellbio, Korea), for 21 days according to the manufacturer's instructions.

2.2. Immunocytochemistry

The cells cultivated in chamber slides were incubated with antigen specific antibodies followed by biotinylated 2nd antibodies, and horseradish peroxidase-conjugated streptavidin. Immunoreactivity was visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB) and counterstained with haematoxylin. Negative control staining was performed by omitting primary antibody.

2.3. Flow cytometric analysis

Cells were fixed with 10% formaldehyde for 10 min, then permeabilized with Phosphate-buffered Saline with Tween (PBST) for 15 min. After washing, cells were incubated with rabbit anti-human insulin antibody for 20 min at 4 °C, then labeled with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit secondary antibody. Flow cytometry analyzes were performed using a FACS Caliber apparatus (Beckman Coulter).

2.4. Measurement of insulin and C-peptide

Cells were incubated with DMEM-LG containing 0.5% BSA for 12 h, washed with PBS, and then stimulated by DMEM-HG for 2 h at 37 °C. The amount of insulin and c-peptide in media was measured using the ultrasensitive human insulin and c-peptide ELISA kit (Mercodia). For the measurement of insulin and c-peptide levels in blood, mice fasted overnight were intraperitoneally injected with glucose (1.5 g/kg body weight), then blood sample collected by cardiac puncture at 30 min. Mouse insulin and c-peptide in serum were determined using mouse insulin ELISA kit (Mercodia) and c-peptide ELISA kit (Yanaihara).

2.5. Reverse-transcription polymerase chain reaction (RT-PCR)

RNA was isolated using Tri-reagent (Invitrogen) according to the manufacturer's instructions. Subsequent PCR reactions were performed using cDNA, primer pairs (Supplementary Table 1) and PCR mixture (Fermentas) according to the manufacturer's instructions. RNAs from human embryonic stem cells were kindly donated from Prof. Kim (Korea University, Korea), and human total RNAs from pancreas (hPAN) was purchased from Ambion.

2.6. Establishment of type 2 diabetes mouse model

Eight-week-old female C57BL/6 mice (Samtako Bio, Korea) were induced by a single intraperitoneal low dose streptozotocin (STZ, 120 mg/kg) followed by high fat diet (HFD) [20]. Three weeks after STZ injection, mice were fed on high-fat diet consisting (as a percentage of total kcal) of 45% fat, 35% carbohydrate and 20% protein (D12451; Research Diets). After 5 weeks of HFD feeding, mice were intraperitoneally injected with 0.5 units/kg of rapid-acting insulin (Humalog®; Lilly) under fasted condition for 4 h. Blood glucose levels were measured at 0, 15, 30, 60, and 90 min post-injection [21]. The rate constant for plasma glucose disappearance (K_{itt}) was calculated from following formula [22].

2.7. Transplantation into type 2 diabetes mice

Diabetes mice with glucose level of higher than 300 mg/dl and K_{itt} value of less than 5% were selected and randomly allocated to sham, hEAs, and hEA-ISCs group. Total 1.5×10^6 cells loaded into a PE50 tube were transplanted underneath the kidney capsule using a Hamilton syringe. Blood glucose level was measured under feeding condition weekly thereafter until 60 days, and 1 mouse that received hEA-ISCs was observed for 210 days.

2.8. Immunohistochemistry

The methods have been described previously [10]. Briefly, graft-bearing kidneys were embedded in paraffin and cut into 4-μm section. The sections were incubated with antigen specific antibodies followed by fluorescence conjugated 2nd antibodies and visualized under confocal microscopy (Nikon). Cell nuclei were visualized by DAPI. Pancreata sections were incubated with anti-mouse insulin monoclonal antibody. Then, cells were incubated with biotinylated 2nd antibody followed by horseradish peroxidase-conjugated streptavidin. Immunoreactivity was visualized using DAB and counterstained with haematoxylin.

2.9. Metabolic parameters and interleukin-6 (IL-6)

Plasma free fatty acids, triglyceride, total cholesterol, adiponectin, and IL-6 levels were measured using quantification kits (Triglyceride, free fatty acids and cholesterol: BioVision; adiponectin and IL-6: AbFrontier) according to the manufacturer's instructions.

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