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PRELIMINARY REPORT

In vitro Generation of Functional Insulin-producing Cells from Lipoaspirated Human Adipose Tissue-derived Stem Cells

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Background and Aims. Tissue engineering strategy has been considered as an alternative treatment for diabetes mellitus due to lack of permanent pharmaceutical treatment and islet donors for transplantation. Various cell lines have been used to generate functional insulin-producing cells (IPCs) including progenitor pancreatic cell lines, embryonic stem cells (ESCs), umbilical cord blood stem cells (UCB-SCs), adult bone marrow stem cells (BMSCs), and adipose tissue-derived stem cells (ADSCs).

Methods. Human ADSCs from lipoaspirated abdominal fat tissue was differentiated into IPCs following a two-step induction protocol based on a combination of alternating high and low glucose, nicotinamide, activin A and glucagon-like peptide 1 (GLP-1) for a duration of 3 weeks. During differentiation, histomorphological changes of the stem cells towards pancreatic β-islet characteristics were observed via light microscope and transmission electron microscope (TEM). Dithizone (DTZ) staining, which is selective towards IPCs, was used to stain the new islet-like cells. Production of insulin hormone by the cells was analyzed via enzyme-linked immunosorbent assay (ELISA), whereas its hormonal regulation was tested via a glucose challenge test.

Results. Histomorphological changes of the differentiated cells were noted to resemble pancreatic β-cells, whereas DTZ staining positively stained the cells. The differentiated cells significantly produced human insulin as compared to the undifferentiated ADSCs, and its production was increased with an increase of glucose concentration in the culture medium.

Conclusions. These initial data indicate that human lipoaspirated ADSCs have the potential to differentiate into functional IPCs, and could be used as a therapy to treat diabetes mellitus in the future.

Key Words: Adipose tissue-derived stem cells, Diabetes mellitus, Glucose challenge test, Insulin-producing cells, Tissue engineering.

Introduction

The global incidence of diabetes mellitus has increased dramatically and currently >250 million people are living with diabetes. If no immediate action is taken, this figure is estimated to double within a generation (1). Both type 1 and type 2 diabetes are linked to a severely reduced islet cell mass, thus eventually leading to many devastating complications including retinopathy, nephropathy, stroke, and heart attack (2). The survival of diabetic patients relies on recurring insulin delivery but this does not cure the disease or prevent diabetic-associated maladies (2,3). Whole pancreas and isolated islet transplantation successfully demonstrated that diabetes can be cured by the replenishment of deficient β cells. This therapy, which was established by Shapiro et al., proved that a large population of islet recipients did not need further insulin therapy (3,4).

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However, this modality is restricted by the limited organ donors. Therefore, with an understanding of growth and developmental biology of β-pancreatic cells, regenerative medicine for renewable sources of IPCs has been explored.

Regenerative medicine focuses on harnessing the infinite self-renewal capacity of stem cells and their ability to differentiate into various lineages under appropriate conditions, to provide effective treatment for a range of diseases (5). This involves the repair or regeneration of cells, tissue or organs to restore impaired function, thus also offering new tools to tackle disorders for which there is currently no good therapeutic option (5,6). Ruszymah et al. successfully generated autologous tissue-engineered skin for the treatment of burns and ulcers, which is able to replace the conventional method of skin grafting or transplantation in replenishing severed skin loss (6). Tissue engineering strategy has been considered as an alternative treatment for diabetes mellitus due to lack of permanent pharmaceutical treatment and islet donors for transplantation. Despite pancreatic progenitor cells (7), other alternative cell sources of extra-pancreatic tissues such as embryonic stem cells (ESC) (8), bone marrow (BM) mesenchymal stem cells (9), human umbilical cord-blood (UCB)-derived mesenchymal stem cells (10) and human eyelid adipose tissue (11) have also been differentiated successfully into functional IPCs. Although these studies demonstrated that transplantation of differentiated cells could normalize hyperglycemia of diabetic animals, the sources of these cells are very limited and are not easily accessible (8–11).

Human adipose-derived stem cells (ADSCs) that can be easily isolated from the fat tissue of the body through surgical procedures such as abdominoplasty or liposuction (12,13) have been shown to be multipotent and are able to be differentiated into osteogenic, adipogenic, myogenic, and chondrogenic lineages, making them suitable for cell therapy (13–15). Recently, as the demand for cosmetics surgery increases and with the new development of high technology devices, surgery has become safer and less invasive; thus, more patients are undergoing liposuction procedures. The lipoaspirated waste tissue is finely minced and forms an excellent starting material for ADSC cell isolation as the shear forces exerted during the suction process do not significantly alter cell viability (16–18). Because the sources of these stem cells are abundant and easily accessible, it is able to supply the large number of cells required for stem cell therapy (11–18).

Timper et al. (19) demonstrated that human ADSCs are able to adopt a pancreatic endocrine phenotype *ex vivo* in response to defined culture medium; however, no functional evaluations of the induced cells were emphasized. Meanwhile, Kang, et al. (11) described the generation of functional IPCs from the ADSCs isolated from human eyelid adipose tissue that possesses neural crest characteristics. Our study was designed to generate IPCs using human ADSCs harvested from lipoaspirated abdominal fat tissue. The differentiation was performed using a simple two-step protocol based on a combination of alternating high-to-low glucose, nicotinamide, activin A and glucagon-like peptide 1 (GLP-1) for 3 weeks as described by Kang et al.[11]

Materials and Methods

Collection of Adipose Tissue from Human Subjects

This study was approved by the Research Ethics Committee, Faculty of Medicine, Universiti Kebangsaan Malaysia with the fund approval no. FF-249-2010. Adipose tissue was obtained from six patients aged between 20 and 40 years undergoing abdominal liposuction cosmetic surgery. Informed consent was obtained from all participants. All patients fasted for at least 10 h prior to surgery. None of the patients had a history of chronic illnesses or were taking medications. Fat tissue was surgically lipoaspirated from the abdominal subcutaneous zone (external to the fascia superficialis) and a total of 100–200 mL of fat tissue was obtained from each patient.

Isolation and Culture of Human ADSCs

Lipoaspirated adipose tissue was washed in phosphate-buffered saline (PBS) (Cellgro, Manassas, VA) and then digested with 0.075% type 1 collagenase (Gibco, Grand Island, NY) for 45 min at 37°C. The digests were then filtered with a cell strainer (BD Biosciences, San Jose, CA) and centrifuged at 800 g for 10 min. The pellets were washed twice with PBS and resuspended in Dulbecco Modified Eagle's medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT). Cells were cultured in a 6-well culture dish with the same medium at 5% CO_2 at 37°C. The medium was changed twice a week and, at 80% confluence, cells were detached by trypsinization to perform serial culture. Cells were collected at P3, P4 and P5 for subsequent experiments.

In Vitro *Differentiation Procedure*

ADSCs at P3, P4 and P5 were plated on 24-well culture dishes at 1 x 10^4 cells/well. For IPC differentiation, cells were induced by a two-step protocol in which they were cultured in DMEM of two different glucose concentration with a combination of 10 mmol nicotinamide (Sigma, St. Louis, MO), 4 nmol activin A (Sigma) and 10 nmol glucagon-like peptide 1 (GLP-1) (Sigma). During the first week of culture, cells were grown in high-glucose DMEM (25 mmol of glucose) (Gibco) containing 10% FBS and after 2 weeks were cultured in low-glucose DMEM (5.5 mmol of glucose) (Gibco) containing 10% FBS. The waste medium was changed every 3 days. Throughout the differentiation, cell histomorphological changes were observed and monitored via light microscope (Olympus, Center Valley, PA).

Transmission Electron Microscopy

Differentiated and undifferentiated pancreatic endocrine cells were fixed in 5% glutaraldehyde for 2 h at 4°C,