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Transplantation of differentiated umbilical cord mesenchymal cells under kidney capsule for control of type I diabetes in rat

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ABSTRACT

Nowadays, stem cells have been introduced as an appropriate source of regenerative medicine for treatment of type I diabetes. Human umbilical cord matrix-derived mesenchymal cells (hUCMC) have successfully been differentiated into insulin producing cells. The isolated hUCM cells were characterized by the expression of stem cell surface markers and by differentiation into adipocytes and osteocytes. The hUCMCs were cultured with different concentrations of neural conditional medium (NCM) and were induced to differentiate into insulin producing cells (IPCs). As 60% NCM concentration resulted in higher nestin and PDX1 expression, the cells were first exposed to 60% NCM and were then induced for IPCs differentiation. PDX1 and insulin gene expression was evaluated in the treated cells. Also, the secretion capacity of the IPCs was assessed by glucose challenge test. IPCs were transferred under the rat kidney capsule. Blood glucose level, weight gain and immunohistochemistry assessments were done in the treated animals. hUCMC expressed mesenchymal cell surface markers and successfully differentiated into adipocytes and osteocytes. Higher NCM concentration resulted in higher PDX1 and nestin expression. The IPCs expressed insulin and PDX1. IPCs were detectable under the kidney capsule 2 months after injection. IPCs transplantation resulted in a sharp decline of blood sugar level and less weight loss. Differentiated hUCM cells could alleviate the insulin deprivation in the rat model of type I diabetes. In addition, higher NCM concentration leads to more differentiation into IPCs and more nestin and PDX1 expression. Kidney capsule can serve as a suitable nominee for IPCs transplantation.

1. Introduction

The autoimmune destruction of insulin producing cells (IPCs) of pancreas eventuates in the ablation of pancreatic β cells. The outcome is insufficient insulin secretion and type I diabetes; a complex metabolic disorder which is increasing worldwide (Wild et al., 2004). Insulin is administered for the control of hyperglycemia but it cannot prevent long term complications such as vascular disorders, kidney failure, neuropathy, cardiovascular disease, blindness and stroke (Froud et al., 2005). A combination of treatments including drug administration and islet transplantation are applied to minimize the risk of further complications (Drucker et al., 2010). A new form of treatment known as cell transplantation which is based on pancreatic islet cells injection, has recently been innovated. However, the number of accessible donated pancreatic islets is very limited (Hussain and Theise, 2004). To unravel this problem, some studies have focused on finding a suitable cell sources, an appropriate differentiation protocol and on the route of cell transplantation with minimal complications (Soria et al., 2008). Till now, many types of stem cells have been introduced for the treatment of diabetes, which are classified into embryonic stem cells (ESC), adult stem cells and extra embryonic or infant stem cells which are isolated from cord blood and placenta (Van, 2011). Umbilical cord matrix-derived cells (Wharton's jelly cells) have the properties of

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mesenchymal cells (Anzalone et al., 2011). These multipotent cells (Fong et al., 2007) have an embryonic origin and they do not face any ethical precautions and teratoma formation following transplantation. Also transplantation of differentiated hUCMCs, has resulted in no immuno rejection complications (Weiss et al., 2006, 2008).

Although stem cell therapy in diabetes is still in its infancy period, by considering the hUCM cells accessibility and unique immunological characteristics, establishing an appropriate protocol for in vitro differentiation of these cells into IPCs and transplantation of induced cells into a suitable animal model would be a long step toward the type I diabetes treatment.

In the present study we assessed the effect of different concentrations of neural conditional medium (NCM) on nestin and PDX1 expression followed by a three steps protocol to induce hUCMCs to IPCs. The insulin and C-peptide secretion levels were evaluated by glucose challenge test. Also the insulin and PDX1 expressions were detected by semi quantitative RT-PCR. In the next step, we transplanted the induced cells under the kidney capsule of the rat models of type I diabetes and the blood glucose level and body weight were assessed in the treated animals within 8 weeks.

2. Materials and methods

All the materials were purchased from Sigma Company (Sigma–Aldrich, MO, USA) unless those otherwise stated. The institutional ethical review board (approval number 69-1780) of Kerman University of Medical Sciences, Kerman, Iran, issued ethical approval.

2.1. Isolation of human umbilical cord Wharton jelly mesenchymal cells

We used a previously reported protocol for harvesting hUCM cells (Salehinejad et al., 2012) with few modifications. Briefly, the umbilical cords (UC) were obtained from cesarean section of full-term pregnancy with no complications, after a written consent from the mothers. The UCs (Fig. 1A) were washed with PBS, the amnion and vessels were removed, the matrix was cut into small pieces and transferred into culture dishes (Becton Dickinson & Company Franklin Lakes) (Fig. 1B) with DMEM, 20% FBS (Gibco), 200 IU/ml penicillin, streptomycin and 2 μg/ml amphotericin B. The culture dishes were incubated at 37 °C with 5% CO_2 in the humidified air. The culture media was refreshed every 72 h. After the hUCM cells appeared at the margin of fragments, the matrix fragments were removed and the culture continued to reach 80% confluence. The cells were dissociated by trypsin and EDTA in PBS. Cells were either cultured or cryopreserved for further use.

2.2. Cells characterization

2.2.1. Immunocytochemistry of actin filament

To assess the myofibroblastic nature of the harvested cells, passages two of hUCMCs were immunostained for α-SMA (mouse monoclonal Clone 1A4; Sigma, A2547) to clarify the presence of actin filaments in isolated cells. The cells were cultured on a glass slide for 48 h. The cells were then rinsed with PBS and fixed in 4% paraformaldehyde for 5 min at 4 °C. The slides were then blocked with 10% normal goat serum for 30 min at room temperature and washed with PBS. The slides were incubated with primary antibody for 60 min, washed with PBS and incubated with secondary antibody (human anti-mouse IgG) for 60 min at room temperature. Finally, the cells were stained with 3,3′-diaminobenzidine and observed by a phase contrast inverted microscope (Olympus, IX71, Tokyo, Japan).

2.2.2. Alkaline phosphatase assay

Passage two cells were cultured in 35 mm culture dishes until colony formation by refreshing the medium every 72 h. Alkaline phosphatase activity was detected using an AP Kit (Sigma–Aldrich Chemie GmbH, Germany, Catalog No. 86-1) according to the manufacture's instruction. A red reaction product after exposure to alkaline dye mixture confirmed AP activity.

2.2.3. Osteogenic and adipogenic differentiation

For osteogenic and adipogenic differentiation of hUCM cells, 5×10^3 viable passage three cells were cultured in 35 mm culture plates for 24 h. The osteogenic medium; DMEM, supplemented with 10% FBS, 10 nM dexamethasone, 10 mM β-glycerophosphate and 82 μg/ml ascorbic acid, was added to the culture plates for 21 days. The adipogenic medium consisted of DMEM-F12 supplemented with 15% equine serum and 100 nM dexamethasone was added to the culture dishes for 14 days. The same procedure was carried out for negative control group, except the inducing agents were removed. To visualize osteogenic and adipogenic differentiation, the induced cells were respectively exposed to Von Cossa to detect black calcium phosphate deposits and Oil-red-O to detect red lipid droplets in adipocytes.

2.2.4. Cell surface analysis by flowcytometry

1×10^4 viable cells at passages two were harvested by trypsinization. Suspended cell were fixed by 4% paraformaldehyde for 15 min and permeabilized with triton ×100 for 15 min at 4 °C. After washing in PBS, the cells were incubated with 10% goat serum in PBS at 4 °C for 15 min to block non-specific binding sites. The cells were then incubated for 1 h with FITC conjugated human anti mouse primary antibodies; CD34, CD44, CD45 and PE conjugated anti CD73, CD90 and CD105. At least 10,000 events were recorded with FACS Canto flow cytometer (BD Biosciences, San Jose, CA) machine. The data were analyzed on FACS Diva software (BD Biosciences). The cells of control group were stained with matched isotype antibodies (FITC- and PE-conjugated mouse IgG monoclonal isotype standards), which were confirmed by positive fluorescence of the limbal samples.

2.3. Differentiation of hUCM into insulin-producing cells

For differentiation to IPCs, 3rd passage cells were cultured for a week with basal medium containing DMEMF12 supplemented with 10% FBS, as pre treatment stage. This was for cells expanding and reaching confluence. Then the hUCMs were induced for IPC differentiation by a three steps protocol as follow:

Step 1: The cells were cultured in 20%, 40% and 60% NCM to determine nestin expression ratio according to the different concentrations of NCM. Then the cells of each concentration were immuno-stained for nestin.
Step 2: NCM pre-treated cells of each NCM concentration were cultured in DMEMF12 (5 mM glucose) supplemented with 2% FBS, 10 mM nicotinamide and 1/100 ITS (Insulin-transferrin-selenium, Gibco) for one week, and the cells' morphology was assessed daily. Then RT-PCR was done on cells of different concentrations of NCM to detect the PDX1 expression. After observing higher PDX1 expression with 60% NCM concentration, the other analyses of the cells of 2nd and 3rd steps were done with the cells, cultured in 60% of NCM.
Step 3: The cells, cultured with 60% NCM, were cultured for 2 weeks in DMEMF12 (25 mM glucose) plus 2% FBS, 10 mM nicotinamide, 1/100 ITS and 20% stem cell conditioned medium (SCM) to produce islet-like cell clusters.