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Characterisation of insulin-producing cells differentiated from tonsil derived mesenchymal stem cells

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ABSTRACT

Tonsil-derived (T-) mesenchymal stem cells (MSCs) display mutilineage differentiation potential and self-renewal capacity and have potential as a banking source. Diabetes mellitus is a prevalent disease in modern society, and the transplantation of pancreatic progenitor cells or various stem cell-derived insulin-secreting cells has been suggested as a novel therapy for diabetes. The potential of T-MSCs to trans-differentiate into pancreatic progenitor cells or insulin-secreting cells has not yet been investigated. We examined the potential of human T-MSCs to trans-differentiate into pancreatic islet cells using two different methods based on β-mercaptoethanol and insulin–transferin–selenium, respectively. First, we compared the efficacy of the two methods for inducing differentiation into insulin-producing cells. We demonstrated that the insulin–transferin–selenium method is more efficient for inducing differentiation into insulin-secreting cells regardless of the source of the MSCs. Second, we compared the differentiation potential of two different MSC types: T-MSCs and adipose-derived MSCs (A-MSCs). T-MSCs had a differentiation capacity similar to that of A-MSCs and were capable of secreting insulin in response to glucose concentration. Islet-like clusters differentiated from T-MSCs had lower synaptotagmin-3, -5, -7, and -8 levels, and consequently lower secreted insulin levels than cells differentiated from A-MSCs. These results imply that T-MSCs can differentiate into functional pancreatic islet-like cells and could provide a novel, alternative cell therapy for diabetes mellitus.

1. Introduction

Diabetes mellitus, the most prevalent endocrine and metabolic disease in the world, is characterised by hyperglycaemia caused by either impaired insulin secretion in the pancreas (type 1 diabetes) or insulin resistance in peripheral tissues such as liver, muscle, and adipose tissues (type 2 diabetes). Chronic insulin resistance in type 2 diabetes can also combine with a progressive decrease in the

Abbreviations: ABCC8, ATP-binding cassette, sub-family C, member 8; A-MSC, dipose-derived mesenchymal stem cell; BSA, bovine serum albumin; CFSE, carboxyfluorescein succinimidyl ester; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; FBS, foetal bovine serum; FGF, fibroblast growth factors; GLP-1, glucagon-like peptide; GLUT2, glucose transporter 2; GSIS, glucose-stimulated-insulin-secretion; HKRB, HEPES-added Krebs-Ringer bicarbonate buffer; HGF, hepatocyte growth factor; IPC, insulin producing cells; ITS, insulin–transferrin–selenium; K-ATP, ATP-sensitive K^+; KCNJ11, potassium channel, inwardly rectifying subfamily J, member 11; LDHA, lactate dehydrogenase A; MSC, mesenchymal stem cell; NGN3, neurogenin 3; PAX4, paired box 4; PBS, phosphate buffered saline; PBST, BSA in PBS containing 0.1% Tween-20; PAX4, paired box 4; PC, pyruvate carboxylase; PDX1, pancreatic and duodenal homeobox 1; SYT, synaptotagmin; T-MSC, tonsil-derived mesenchymal stem cell

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insulin secretory capacity of the pancreas over time (Guillausseau et al., 2008). The current therapy for diabetes includes blood glucose-lowering medications and exogenous insulin administration, but patient noncompliance and difficulties with insulin dosing techniques challenge proper glycaemic control. Islet cell transplantation is expected to overcome the current therapeutic limitations, and recent studies reported significantly improved therapeutic outcomes of islet cell transplantation compared with those of conventional treatments (Barton et al., 2012). The clinical usage of islet cell transplantation is limited, however, by a shortage of organ donations, difficulties in human pancreatic islet isolation, and chronic immune suppression (Rezania et al., 2014).

Recently, stem cell therapy has been suggested as a potential therapeutic tool for diabetes. Stem cells help damaged endogenous pancreatic β-islet cells to regenerate (Milanesi et al., 2012). The transplantation of insulin-secreting cells produced from various stem cells such as embryonic stem cells (Rezania et al., 2014), induced pluripotent cells (Saito et al., 2011), and mesenchymal stem cells (MSCs[1]) (Karaoz et al., 2013; Gabr et al., 2013; Kim et al., 2012b) have shown therapeutic effects in diabetic animals (Villani et al., 2014; Karaoz et al., 2013; Rezania et al., 2012). MSCs are plastic-adherent cells, phenotypically characterized by the expression of CD73, CD90, CD105 and lack of CD11b, CD31 and CD45, and can differentiate into mesodermal lineages (Horwitz et al., 2005; Uccelli et al., 2008; Dominici et al., 2006). Recently, the differentiating capacity of MSCs into other lineages including endodermal and ectodermal lineages have also been reported (Kuroda and Dezawa, 2014). MSCs have immunosuppressive capability, and allogenic transplantation of MSCs without the use of immunesuppressive drugs is possible. Therefore, MSCs provide an attractive source of banking for clinical transplantation. MSCs can be isolated from several tissues such as bone marrow, adipose tissue, placenta, and umbilical cord blood. Previous reports suggested that tonsil-derived MSCs (T-MSCs) isolated from waste tissue obtained during tonsillectomy have multilineage differentiation abilities and immunosuppressive properties comparable to those of bone marrow-derived MSCs (Ryu et al., 2014, 2012; Janjanin et al., 2008). In addition, T-MSCs maintain their stemness during the freezing and thawing process (Ryu et al., 2012) and until passage 10 during in vitro expansion (Yu et al., 2014). In addition, considering the young age at which tonsillectomy is usually performed, T-MSCs can be a more suitable source for MSCs compared with other sources.

The differentiation of various MSCs, including bone marrow-derived MSCs and adipose-derived MSCs (A-MSCs), into insulin-producing cells has been reported previously (Moshtagh et al., 2013; Xie et al., 2009) using various methods (Karaoz et al., 2013; Xie et al., 2009; Kim et al., 2012b, 2012a; Moshtagh et al., 2013; Jafarian et al., 2014) that can be summarised into two classes: (1) the β-mercaptoethanol method (Chen et al., 2004; Moshtagh et al., 2013) and (2) the insulin-transferin-selenium (ITS) method (Kanafi et al., 2013; Kim et al., 2012a; Jafarian et al., 2014). A direct comparison between the two reprogramming methods has not been conducted, however, and the potential of T-MSCs to differentiate into insulin-producing cells (IPCs) has not yet been investigated. We directly compared the efficacy of the two differentiation methods to generate IPCs from T-MSCs and A-MSCs, respectively, and confirmed the ability of T-MSCs to be reprogrammed into IPCs.

2. Materials and methods

2.1. Isolation and culture of MSCs

Human tonsils were extracted during tonsillectomies performed in the Department of Otorhinolaryngology, Head and Neck Surgery at Ewha Womans University Mok-Dong Hospital. The experimental protocol including informed consent templates was approved by the Ewha Womans University Medical Center institutional review board (ECT-11-53-02). This study used cryopreserved-thawed T-MSCs that were isolated, frozen, and thawed according to a protocol described previously (Ryu et al., 2014, 2012). Briefly, extracted tonsils were minced and digested in medium containing 210 U/ml collagenase type I (Invitrogen, Carlsbad, CA, USA) and 10 μg/ml DNase (Sigma-Aldrich, St. Louis, MO, USA). Cells were collected by passing the digested tissues through a cell strainer (BD Labware, Bedford, MA, USA). Mononuclear cells were obtained from among the collected cells by Ficoll-Paque (GE Healthcare, Little Chalfont, UK) density gradient centrifugation and cultured for 48 h in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal bovine serum (FBS; Invitrogen) and 1% penicillin/streptomycin. Then, non-adherent cells were removed, and adherent T-MSCs were replenished with fresh medium. The T-MSCs were then frozen in 90% FBS and 10% dimethyl sulfoxide (DMSO) solution. The T-MSCs used for the experiments were thawed once and expanded through 5–7 passages.

Human A-MSCs purchased from Invitrogen were cultured in MesenPRO RS medium (Invitrogen) for five passages according to manufacturer's protocol. After passage five, the A-MSCs were cultured in DMEM containing 10% FBS (Invitrogen) and 1% penicillin/streptomycin. We used A-MSCs obtained between passages five and seven for the experiments.

2.2. In vitro differentiation of MSCs into insulin-secreting cells

MSCs were differentiated into insulin-secreting cells using two different methods, as described previously (Karaoz et al., 2013; Xie et al., 2009; Kim et al., 2012b, 2012a; Moshtagh et al., 2013; Jafarian et al., 2014) with some modifications. The two different methods can be summarised into: 1) the β-mercaptoethanol method (Chen et al., 2004; Moshtagh et al., 2013) and (2) the ITS method (Kanafi et al., 2013; Kim et al., 2012a; Jafarian et al., 2014).

The first step of the β-mercaptoethanol method was to incubate T-MSCs and A-MSCs in high-glucose DMEM containing 5% FBS (Invitrogen), 1 mM β-mercaptoethanol, and 10 mM nicotinamide (Sigma) for 2 days. Then, the medium was replaced by high-glucose DMEM containing 1 mM β-mercaptoethanol, 10 mM nicotinamide, 20 ng/ml basic fibroblast growth factors (FGF; Peprotech, Rocky Hill, New Jersey, USA), and 2 mM L-glutamine (Sigma), and the cells were cultured in the new medium for 8 days. As the last step, the cells were cultured for 14 days in high-glucose serum-free DMEM/F12 (Hyclone, GE Healthcare, Logan, Utah, USA) containing 10 mM nicotinamide, 1 mM β-mercaptoethanol, 1% penicillin/streptomycin, 10 ng/ml β-cellulin (Peprotech), 2 nM activin A (Peprotech), 10 nM exendin-4 (Sigma), and 100 pM hepatocyte growth factor (HGF; R&D Systems, Minneapolis, MN, USA; Table 1). Fresh medium was supplied every 3 days during steps 2 and 3. Standard tissue culture-treated plates (BD Biosciences, San Jose, CA, USA) were used for the β-mercaptoethanol method.

The first step of the ITS method was seeding T-MSCs and A-MSCs into a non-adherent 100 mm dish (2×10^6 cells/dish) containing high-glucose α-MEM (Hyclone) with 1% fatty acid-free bovine serum albumin (BSA) and 1 × ITS (Sigma). The cells were then incubated for 2 days. In the second step, the cells were cultured for 4 days in high-glucose α-MEM containing 1% fatty acid-free BSA, ITS, 0.3 mM taurine (Sigma), and 10 mM nicotinamide. In the last step, the cells were incubated for 4 days in high-glucose α-MEM with 1% fatty acid-free BSA, ITS, 3 mM taurine, 10 mM nicotinamide, 100 nM glucagon-like peptide (GLP-1; Sigma), and 10 nM exendin-4 (Table 2). Fresh medium was supplied every 2 days during steps 3. Non-treated sterile plates (BD Biosciences) were used for the ITS method.

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