

ORIGINAL ARTICLE

Generation of Insulin-Producing Cells from Human Adipose Stem Cells by Genetic Manipulation and Growth Factors Effects

Mostafa Bahrebar^{1*}, Masoud Soleimani², Mohammad Hossein Karimi^{3*}, Akbar Vahdati⁴, Ramin Yaghobi⁵

¹Department of Biology, Science and Research Branch, Islamic Azad University, Fars, Iran

²Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

³Organ Transplant Research Center, Nemazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

⁴Department of Biology, Science and Research Branch, Islamic Azad University, Fars, Iran

⁵ Organ Transplant Research Center, Nemazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding authors: ms_bahrebar@yahoo.com, Karimi70@yahoo.com

ABSTRACT

Replacement of the damaged cells with regulated insulin-producing cells (IPCs) considered the ultimate cure for type 1 diabetes. Human adipose tissue- derived mesenchymal stem cell (hAMSCs) is an optimal source for gene and cell-based therapy. This research was carried out to study the effects of growth factors and introduction of PDX-1 gene on the generation of IPCs from hAMSCs. After characterization of hAMSCs by flow-cytometric analysis and multilineage differentiation studies, PDX-1 gene was delivered into hAMSCs through lentiviral vector as PDX-1 transduced cells. Mock-transduced cells were infected with an empty lentivirus. The PDX-1 transduced cells were treated with growth factors such as activin A, fibroblast growth factor (FGF), and glucagon- like peptide-1 (GLP-1) in three stage of utilized protocol for 14 days. The morphologic and physiologic changes of cells were evaluated using dithizone-staining, Immunocytofluorescence, reverse transcription polymerase chain reaction (RT-PCR) and chemiluminescence immunoassay (CLIA). PDX-1 along with growth factors induced profound changes including formation of Islet- like cell aggregations (ICAs), the dithizone-stained cells, and insulin positive cells in the PDX-1 transduced cells related to Mock-transduced cells. Over expression of Pancreas-related genes like PDX-1, Ngn3, Nkx2-2, and Insulin (INS) and significant increase of insulin secretion were shown in the PDX-1 transduced cells compared with Mock transduced cells on the 10th and 14th days of differentiation ($P < 0.05$). The findings of this study demonstrated that hAMSCs could induce to differentiate into IPCs by effects of growth factors and PDX-1 introduction, which may provide a stem cell source for β -cell replacement in type 1 diabetes.

Key Words: Manipulation, PDX-1, Growth Factors, Mesenchymal Stem Cells, Insulin- Producing Cells, Adipose tissue.

Received 02/08/2014 Accepted 24/10/2014

©2014 Society of Education, India

How to cite this article:

Mostafa B, Masoud S, Mohammad H K, Akbar V, Ramin Y. Generation of Insulin-Producing Cells from Human Adipose Stem Cells by Genetic Manipulation and Growth Factors Effects. Adv. Biores., Vol 5 [4] December 2014: 11-19.

DOI: 10.15515 /abr.0976-4585.5.4.1119

INTRODUCTION

Type 1 diabetes mellitus is characterized with a progressive autoimmune destruction of the β -cells, leading to insufficient insulin production [1]. Replacement of the damaged cells with regulated insulin-producing cells (IPCs) is proposed as the ultimate cure for type 1 diabetes. Transplantation of intact human pancreas or isolated islets has been severely limited by the scarcity of human tissue donors, and requirement of life- long immunosuppressant. Recently many attempts have been made to generate insulin-producing cells from human mesenchymal stem cells [2]. The ability of MSCs for differentiating toward mature tissues depends on the surrounding microenvironment or genetic manipulation [3]. Chandra et al have shown that media containing extrinsic/growth factors such as activin A, sodium butyrate, 2-mercaptoethanol, insulin-transferrin-selenium (ITS), fibroblast growth factor (FGF), taurine, nicotinamide and glucagon- like peptide 1 (GLP-1) in the protocol of differentiation with three stages induced hAMSCs to differentiate into islet- like cell aggregates (ICAs). They promised that hAMSCs are

optimal source of stem cells for inducing and cell replacement therapy in diabetes [4]. The pancreatic and duodenal home box factor 1 (PDX-1) is proposed as a master gene for inducing stem cells to differentiate into IPCs [5]. PDX-1 has a critical role in pancreas development, β -cell differentiation, and maintenance of normal β -cell function through regulating several β -cell-related genes [6]. Signaling through the activin and Hedgehog pathways also controls pancreas size and shape [7]. Therefore, in this original article, the PDX-1 gene was initially delivered into hAMSCs by lentivirus, and then the PDX-1 transduced cells were treated with growth factors for generating of IPCs.

MATERIALES AND METHODS

Isolation and Culture of hAMSCs

Fat tissue samples were obtained by simple liposuction from abdominal subcutaneous fats of female cases (30-35years old). Human AMSCs were isolated from the resected fat tissue samples and cultured according to modifications of previous reported method [4]. Briefly, Fat tissues samples were washed and digested in phosphate-buffered saline (PBS) supplemented with 0.2% collagenase-type-II (1 mg/mL) (Sigma- Aldrich) under gentle agitation pre-warmed at 37 °C for 45 minutes. The collagenase was inactivated with an equal volume of Dulbecco's modified Eagle's medium (DMEM)/10% fetal bovine serum (FBS), and the digested tissues were filtered through a 100- μ m mesh filter to remove cellular debris. The cells were centrifuged (400 g for 4 minutes) at room temperature (RT) in 1–2 ml sterile PBS containing 2% (v/v) FBS (Sigma-Aldrich). Supernatant containing mature adipocytes were discarded. The pellet containing stromal vascular fraction was suspended and cultured in the expansion medium and seeded onto 25 cm² plastic tissue culture flasks (BD Biosciences) and incubated at 37 °C in a humidified atmosphere containing 5% CO₂ for 3 days. Expansion medium was contained: DMEM with 10% FBS (Sigma- Aldrich), L-glutamine (2Mm), penicillin (100units/ml), and streptomycin (100 μ g/ml). The Ethical Committee of Shiraz University of Medical Sciences (The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki) approved the study with code 90/171 registered in the Shiraz University of Medical science.

Flow cytometric analysis

The hAMSCs were detached from the tissue culture flasks at the third passage and counted. One- $\times 10^6$ cells were incubated on ice for 30 minutes with goat serum, suspended in phosphate buffered saline (PBS) and pelleted by centrifugation for 4 minutes at 2000 rpm. Subsequently, the cells were stained for 30 minutes at 4 °C with fluorescent isothiocyanate (FITC)-conjugated CD34, CD45 (BD Biosciences) antibodies and FITC- conjugated CD90 (BD Biosciences) and CD105 (R&D, USA) antibodies. Isotope control with FITC- and PE labeled was used in each experiment. Flow cytometry was performed on an Attune acoustic focusing cytometer (Applied Biosystems, USA) and the data were analyzed using Win Mdi software (Scripps, CA).

In Vitro Multilineage Differentiation Studies

Adipogenesis and osteogenesis for hAMSCs were evaluated in the appropriate induction media according to modifications of previously reported methods [3]. The differentiation of cells was documented using oil red O for adipocytes, and Alizarin staining for osteoblasts.

Packaging Plasmids for Virus Construction

HEK293T cell line was used for expressing the gag/pol packaging genes. To produce PDX-1 expressing lentiviral vectors, 3.5×10^6 HEK293T cell line were plated in 10 ml of DMEM with 10% FBS without antibiotic and incubated overnight at 37°C in a 5% CO₂. Three plasmids including psPAX2, pMD2.G and pEZ-Lv105-PDX-1 (Genecopoeia, USA) co-transfected into HEK293T cell line through calcium phosphate (CaPO₄) precipitation reaction using Express-In transfection reagent according to the manufacturer's instructions (Biosystems, USA). In addition, an empty lentiviral vector (LV null) was constructed by co-transfection of PCDH-CMV-MCS-EF1-cGFP-T2A-Puro (BioCat-GmbH, Germany) as an empty plasmid along with psPAX2 and pMD2.G into HEK293T cell line for producing of empty lentiviral vector (Lv-null) using same method. The culture supernatant containing lentiviral particles was harvested 48 h after transfection and clarified using a 0.45- μ m filter.

Transduction and Selection of Transduced Cells

Human AMSCs were incubated with human PDX-1 or empty lentivirus at a multiplicity of infection (MOI) of 100 in serum free medium (SFM). 1×10^6 cells per well were seeded in 6-well plates with 3 ml of unconcentrated lentiviral supernatant. Polybrene (Sigma-Aldrich, USA) with concentration of 4 μ g/ml was added to medium. Transduction was performed for 6 hours in serum free medium. After 6 hours, the medium was replaced with DMEM/F12 containing 10%FBS, 17.5mM glucose and 1% BSA. After 48 hours, the cells were collected and characterized by fluorescent microscopic method in order to determine

expression of GFP that it relatively proved the entrance of lentivirus into hAMSCs. Selection of transduced cells was performed with 1 µg/ml Puromycin.

In Vitro Differentiation of hAMSCs into IPCs

The transduced cells were counted and seeded with density of 1×10^6 cells per well of the ultra-low attachment 6- wells culture plates in SFM containing: DMEM/F12 (Gibco,USA), Penicillin/Streptomycin 1% (Pen/Strep), ITS-1X [insulin (5 mg/L), transferrin (5 mg/L), selenium (5mg/L)], glucose (17.5 mM) and 1%BSA (Sigma- Aldrich, USA).The PDX-1 transduced cells treated with growth factors in three stages. On the first stage, activin A (4 nM), sodium butyrate (1 mM), 2-mercaptoethanol (50 µM) and FGF (2 ng/ml) were added to SFM (SFM-B).The cells were cultured in this medium for 2 days. On the second stage taurine (0.3 mM) was added to SFM (SFM-B). On the third stage, taurine (3 mM), GLP-1 (100 nM) (Sigma- Aldrich, USA), nicotinamide (NIC) (1 mM), and 1X non-essential amino acids (NEAAs) were added to SFM (SFM-C). For the remaining 9 days, the culture medium of the experimental group was exchanged with fresh SFM-C every 2 days. The culture medium of Mock transduced cells was exchanged with fresh serum- free medium (SFM) every 2 days.

RNA Isolation, cDNA Synthesis and RT-PCR

Total RNA was extracted from the Mock and PDX-1 transduced cells using RNX-Plus according to the manufacturer's instructions (High Pure RNA isolation kit, CinnaGen Co, Iran). Synthesis of cDNA was carried out with M-MuLV reverse transcriptase (RT) and random hexamer according to the manufacturer's instructions (Fermentas, Lithuania).The PCR product was run using electrophoresis in 2% agarose gel in 1X TBE buffer at 80V for 30min. The PCR products were identified by 50 bp size marker DNA (Biolabs, England). All RT-PCR results were normalized against B2M as an internal control, and compared with Mock transduced cells and human pancreas tissue. The primer sequences and the length of the amplified products used for RT-PCR are summarized in table 1.

Table 1: The list of the primers for detection of the human pancreas-related genes.

Name	Sequence	Tm (°C)	products' Length (bp)
PDX-1	F: ATGGATGAAGTCTACCAAAGC	57.4	159
	R: CGTGAGATGTACTTGTGAATAG	59.3	
Ngn3	F: AGAGAGCGTGACAGAGGC	58.4	182
	R: GCGTCATCCTTTCTACCG	56.1	
Nkx2-2	F: AGTACTCCCTGCACGGTC	58.4	103
	R: GTCTCCTTGTCATTGTCCG	57.3	
INS	F: GAACGAGGCTTCTTCTACAC	58.4	143
	R: ACAATGCCACGCTTCTG	52.4	
B2M	F: ATGCCTGCCGTGTGAAC	55	91
	R: ATCTTCAAACCTCCATGATG	54	

Immunocytofluorescence staining

The Mock and PDX-1 transduced cells were washed with PBS and fixed with 4% cold para-formaldehyde for 20 minutes at 4 °C. The cells were incubated for 5 minutes at room temperature (RT) and washed twice with cold PBS for 5-10 minutes. The cells were permeabilized with 0.1% Triton X-100/PBS (Sigma-Aldrich, USA) for 10 minutes at RT. The fixed cells were blocked with goat serum 5% (Sigma-Aldrich, USA) for 45 minutes at RT, and then it was removed. The cells were stained with first antibody, Rat anti-human insulin antibody (1:200) (R&D systems, MAB1417, USA) and diluted in BSA/PBS 0.2% at 4 °C for overnight. The cells were washed 3 times with PBS-Tween 0.1% (Merck, Germany) for 5 minutes. For detection of primary antibody, a fluorescent- labeled secondary antibody, mouse anti rat IgG-FITC (eBioscience11-4811-85), was utilized for 1h at RT then the cells were washed 3 times with PBS-Tween 0.1% for 5 minutes. PBS was added to the wells and kept in dark. DAPI (4, 6- diamidino-2- phenylindole) (Invitrogen, USA) was used for staining of cell nuclei. Images were captured using a fluorescent microscope (Nikon, Japan).

Dithizone (DTZ) Staining

A DTZ (Merck, Germany) stock solution was prepared with 50 mg of DTZ in 5 ml of dimethyl sulfoxide (DMSO) and stored briefly at -15°C. In vitro DTZ-staining was performed by adding 10 µl of the stock solution to 3 ml of culture medium of the Mock- and PDX-1 transduced cells on the day 14. The cells were incubated at 37°C for 15 minutes in the DTZ solution, rinsed three times with HBSS and were examined with a stereomicroscope [8].

Measurement of Spontaneous Insulin Secretion

10^6 cells of the Mock - and PDX-1 transduced cells were plated in 6- well plates. At the days 10 and 14 of differentiation, the cells were washed with PBS and incubated for 3 hours in low glucose DMEM (5.5 mM) (Gibco, USA). The medium was collected and stored at -20°C until being assayed. Measurement of spontaneous secreted insulin was performed with chemiluminescence enzyme immunoassay (CLIA) system according to the manufacturer's instructions [9].

Measurement of Glucose- Stimulated Insulin Secretion (Glucose Challenge Test)

To test whether the insulin release of induced endocrine cells was glucose dependent, two glucose concentrations (5.5 mM and 25 mM) were used. After 14th day of differentiation, Mock- and PDX-1 transduced cells were cultured under low- or high-glucose. The cells were incubated for 1 hours in low glucose DMEM (5.5mM), and the medium was collected and stored at -20°C . Next, the cells were washed with PBS and incubated for 1 hour in high glucose DMEM (25 mM) and the medium was collected and stored at -20°C . Measurement of glucose- stimulated insulin was performed with chemiluminescence enzyme immunoassay (CLIA) system according to the manufacturer's instructions [9].

Statistical Analysis

Data were reported as mean \pm SE and the differences between groups were analyzed with one way ANOVA and Student's t-test. All statistical analysis was performed using SPSS 15.0 Software. Significance was set at $P < 0.05$. Band scoring of the RT-PCR analysis was performed using Lab Image V.3.3.3 Software.

RESULTS

In-vitro growth and phenotype analysis of hAMSCs

Among the early stage of this protocol, the hAMSCs were spindle- shaped with a fibroblast- like morphology and were attached to the plate during cell culture with typical mesenchymal morphology. The hAMSCs were significantly positive for CD90 (86.6%) and CD105 (99.8%), but negative for CD34 (0.411%) and CD45 (0.65%) (Fig.1A). Upon specific induction, hAMSCs exhibited in-vitro competence to differentiate into adipogenic (Fig.1B) and osteogenic (Fig.1C) lineages as confirmed by Oil red O-staining and Alizarin- red staining respectively.

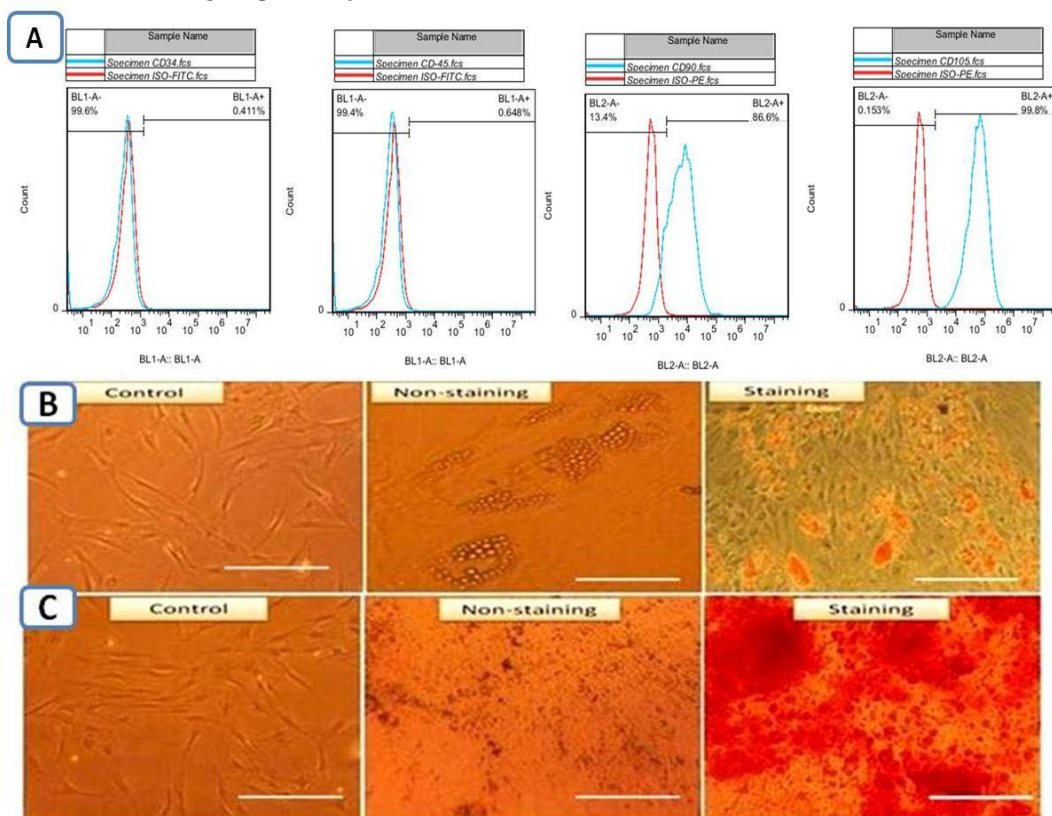


Figure1.Characterization of hAMSCs. (A) Flow cytometric analysis. The cells were positive for CD90 (86.6%) and CD105 (99.8%), but negative for CD34 and CD45 antigens. (B) Adipogenic differentiation including Control, Non-staining and Oil red O- staining (original magnification 100 \times). (C) Osteogenic differentiation including Control, Non-staining and Alizarin red staining (Scale bars A-C: 100 μm).

Morphologic Changes of the PDX-1 Transduced Cells

To investigate the effects of extrinsic/growth factors and introduction of PDX-1 gene into hAMSCs, the changes in cell morphology of the Mock- and PDX-1 transduced cells were studied on days 0, 3, 5, 10 and 14. Phenotypic changes in the Mock transduced cells were not observed from day 0 to 14 (Fig.2A). While, the phenotypic changes in the PDX-1 transduced cells were observed from day 3 to 14 (Fig.2B). After the third day of differentiation protocol (SFM-A), the PDX-1 transduced cells showed a remarkable transition from bipolar fibroblast- like morphology to a round epithelial- like shape. During further culturing, the rate of cell proliferation become slower and spindle- like cells become short and changed into round epithelial- like cells. Meanwhile, some new islet- like clusters started to appear (Fig.2B, day3). After the 5th day of differentiation protocol (SFM-B), cellular aggregation occurred as a gradual process (Fig.2B, day5). After the 10th day of differentiation protocol (SFM-C), complete aggregates were formed (Fig.2B, days 10&14).

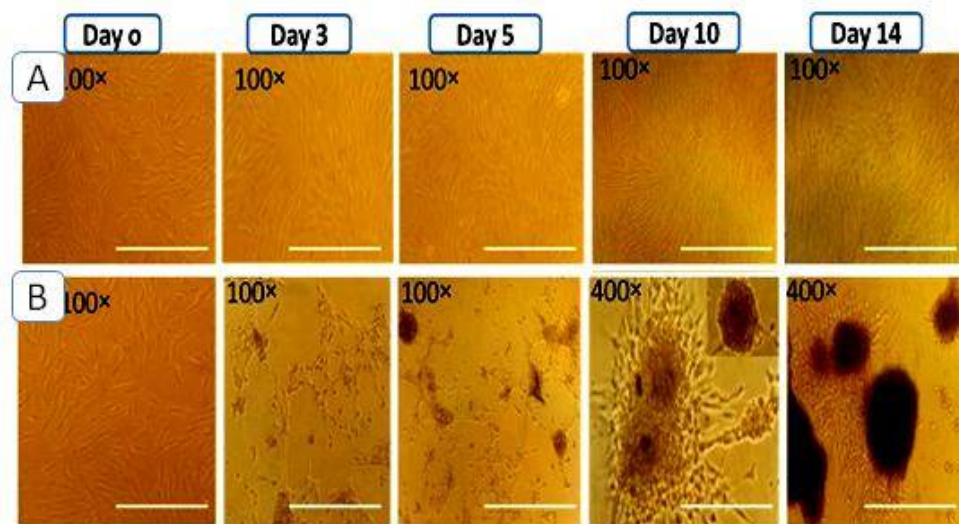


Figure 2. Morphologic changes of hAMSCs during 14 days of differentiation. (A) Mock transduced cells (original magnification $\times 100$). (B) PDX-1 transduced cells (original magnification $\times 100$).

Expression of Pancreas -Related Genes

The expression of genes involved in pancreatic β -cell related genes like PDX-1, Ngn3, Nkx2-2 and insulin were detected by RT-PCR. As illustrated in figure 3, a significant over-expression of pancreas- related genes like PDX-1, Ngn3, Nkx2-2, and insulin were observed in the PDX-1 transduced cells in comparison to Mock-transduced cells after day 10 and 14 of differentiation.

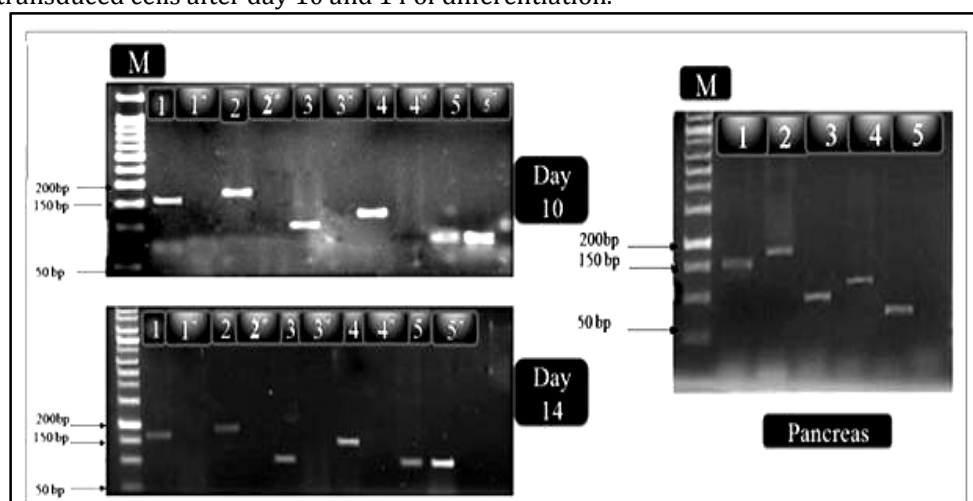


Figure 3. Gel electrophoresis of RT-PCR products for detection of pancreatic marker genes on the 10th and 14th days of differentiation. Human pancreas tissue was used as control that observed in the right part in this figure. M: Marker (Ladder 50bp), 1: PDX-1, 2: Ngn3, 3: Nkx2-2, 4: Insulin (INS), 5: B2M refer to PDX-1 transduced cells and pancreas tissue, while 1*: PDX-1, 2*: Ngn3, 3*: Nkx2-2, 4*: Insulin (INS), 5*: B2M refer to Mock- transduced cells.

***In vitro* hAMSCs differentiation and DTZ Staining**

To determine whether the hAMSCs differentiated into IPCs, the cells were stained with DTZ, a zinc-chelating agent known to selectively stain β cells due to their high zinc content [8]. Results are shown in the figure 4. The Mock-transduced cells were not stained (Fig.4 A), while most of the PDX-1 transduced cells could be seen stained with DTZ and it was found they were mostly crimson red (Fig. 4B).

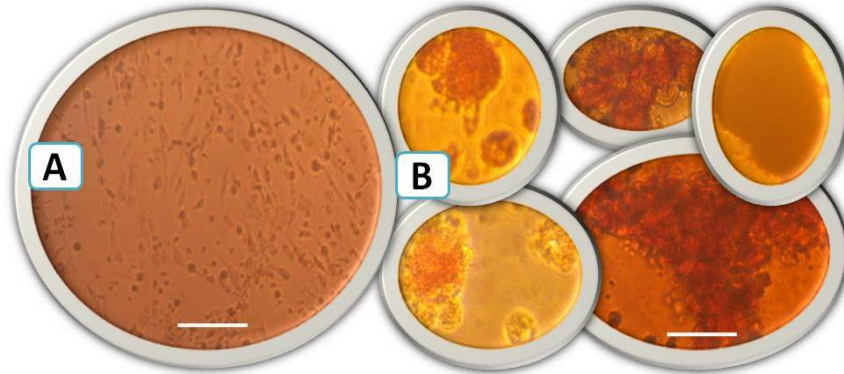


Figure 4. Characterization of IPCs derived from hAMSCs by DTZ staining at day 14 of differentiation. (A) Negative DTZ-stained cells of Mock-transduced cells (original magnification 100 \times). (B) Positive DTZ-stained cells of PDX-1 transduced cells (original magnification 100 \times).

Insulin immunostaining

The production of insulin was evaluated by immunofluorescent staining. Insulin immunoreactivity at the 14 day of differentiation within Mock-transduced cells revealed negative cells for insulin (Fig.5A), whereas the PDX-1transduced cells were positive for insulin (Fig.5 B).

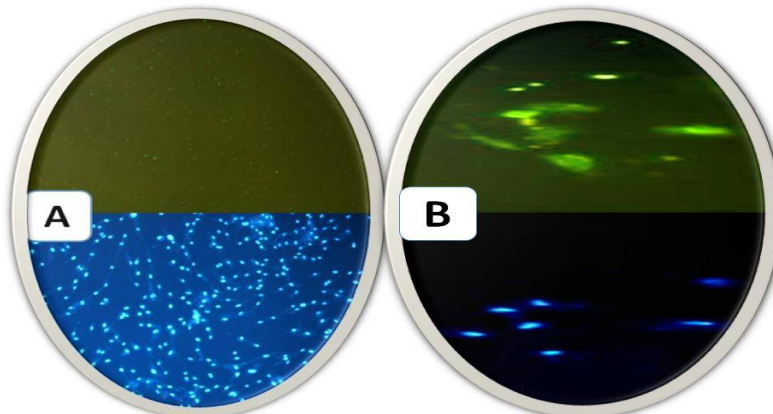


Figure 5. Immunocytofluorescence staining has been performed to detect insulin- positive cells. (A) Insulin-negative cells of the Mock-transduced cells (B) Insulin-positive cells of the PDX-1 transduced cells. The cellular nuclei were stained by DAPI (labeled blue staining) in lower parts of the figure for each group (Scale bars A-C: 100 μ m).

Spontaneous and Glucose- stimulated insulin secretion

As illustrated in figure 6A, the amount of spontaneous insulin secretion in the PDX-1 transduced cells at 10th day was 18.03 μ g/L, while it was 0.82 μ g/L in the Mock-transduced cells ($P < 0.05$). At 14th day, it was 21.05 μ g/L in the PDX- transduced cells, while it was 1.50 μ g/L in the Mock-transduced cells. Data analysis indicated that spontaneous insulin secretion was significantly increased in the PDX-1 transduced cells related to Mock-transduced cells ($P < 0.05$). To determine whether these insulin- producing cells were responsive to glucose challenge, the amount of insulin secretion in response to different glucose concentrations of medium was measured at the day 14 of differentiation. As illustrated in figure 6B, data analysis showed that the mean of amounts of insulin secretion at high glucose (20.85 μ g/L) was significantly increased related to low glucose (12.38 μ g/L) ($P < 0.05$) in PDX-1 transduced cells. The mean of amounts of secreted insulin in the medium of Mock-transduced cells was 0.10 μ g/L at low, and 0.3 μ g/L at high glucose; that they had not significantly different ($P > 0.05$).

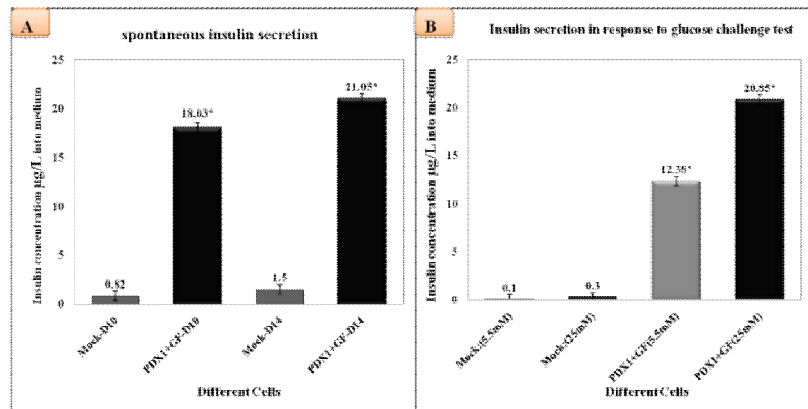


Fig.7 Insulin secretion into medium. (A) Spontaneous insulin secretion. (B) Insulin secretion in response to glucose challenge test. Abbreviations: Mock-D10, Mock-transduced cells at day 10 of differentiation; PDX-1 +GF-D10, PDX-1 transduced cells at day 10 of differentiation; Mock-D14, Mock-transduced cells at day 14 of differentiation; PDX-1 +GF-D14, and PDX-1 transduced cells at day 14 of differentiation.

DISCUSSION

The aim of this study was to examine the potential of PDX-1 transduced hAMSCs to differentiate into insulin expressing cells following a differentiation protocol previously optimized for differentiating into IPCs [4]. Adipose tissue has been offered as a novel and rich source of MSCs that can be isolated and generated in a short time [10]. Our protocol resulted in isolation of hAMSCs that expressed CD90 and CD 105 surface antigen profile (Fig.1A) and these cells showed high potential to differentiate into adipocyte (Fig.1B) and osteoblast cells (Fig. 1C). These characteristics proved that they were MSCs. To achieve differentiation into pancreatic endocrine cells, specific stepwise conditions were formulated like earlier reported protocol [4, 12]. In the first stage of protocol, SFM-A induced profound phenotypic changes. The hAMSCs that proliferated as an adherent monolayer, after SFM-A effects, they were aggregated into spherical cell clusters (Fig.2 B). After approximately 36-48h, islet-like cell aggregates were formed and the number of islets increased at the day 10 of differentiation of the utilized protocol (Fig.2 B). According to work of previous researcher [13], FGF stimulated the initial process of cell aggregation and cluster formation in the PDX-1 transduced cells, that it is an important step in the development and differentiation of pancreas via FGF-receptors. They were showed that human pancreatic precursor cells secrete FGF-2 to stimulate clustering into hormone-expressing islet-like cell aggregates [13]. The very low attachment plates and primary high plating density of the cells provided the optimum conditions for increasing efficiency of the formation of the 3D aggregates.

Evaluation of mRNA expression of pancreas-related genes like PDX-1, Ngn3 and Nkx2-2 showed a significant up regulation in the expression of these genes in the PDX-1 transduced cells related to Mock-transduced cells. In addition, introduced PDX-1 along with growth factors significantly enhanced expression of insulin gene in these transgenic cells compared with non-transgenic cells, which is in accordance with the recent report [12]. Previous studies demonstrated that PDX-1 is critically required for organogenesis in mice and human. In addition, it is upstream of Ngn3 during embryonic development that Ngn3- expressing progenitors selectively populate the endocrine compartment [14]. Ngn3 is a bHLH transcription factor that binds to the E-box and is involved in endocrine pancreas development. Role of Ngn3 in giving rise to hormone- expressing cells during the secondary transition is dependent upon PDX-1[15]. The findings of the present study in concordance with above reports indicate that PDX-1 contributes to the specification of endocrine progenitors by up regulating Ngn3 directly or in concert with other transcription factors that they participate in a cross- regulatory transcription factor network during Tran's differentiation. Nkx2-2, aNk-homeodomain protein, has an equally profound effects on islet cell differentiation. As illustrated in figure 3, expression of Nkx2-2 is up regulated in the PDX-1 transduced cells in comparison to Mock transduced cells (days 10 and 14). The Nkx2-2 persists with many of the mature endocrine cells especially all β - cells. Mice lacking Nkx2-2 have complete absence of insulin-producing cells [16]. DTZ- stained clusters demonstrated that PDX-1 transduced cells synthesized insulin, because pancreatic islets from human are known to be stained crimson red by DTZ treatment [8] Fluorescent staining also revealed the insulin positive cells in the PDX-1 transduced cells based this result it could suggest these cells were insulin- producing cells. Spontaneous insulin secretion and response to high glucose medium in accordance with previous research demonstrated that PDX-1 functions as mediator of glucose-regulated gene expression in the IPCs [17]. PDX-1 protein identified as an islet-

specific glucose- sensitive factor whose DNA binding to an AT- rich motif in the insulin promoter is modulated by extracellular glucose levels. In addition to being the major regulator of the insulin gene, PDX-1 also regulates the expression of glucose transporter-2 (GLUT2) and glucokinase in the β -cells that control glucose utilization and sensing. Glucose enters the beta cell through GLUT2 and is phosphorylated by glucokinase, which catalyzes the rate- limiting reaction in glucose metabolism [17]. In according to the study of Chandra et al. that the taurine supplemented with nicotinamide and GLP-1 in the differentiation cocktail resulted in the up regulation of a number of pancreatic related genes like PDX-1, Ngn3, NeuroD, Pax-4, Nkx2-2, Nkx6.1, pax-6, Isl-1 and pancreas specific glucose transporter (Glut-2) involved in pancreatic beta cells development cascade [4], the results of this study have shown that taurine supplemented with nicotinamide and GLP-1 in the stage 3 of differentiation (SFM-C) resulted in the up regulation of a number of pancreatic related genes like PDX-1, and Ngn3 in the PDX-1 transduced cells in comparison to mock transduced cells and human pancreas sample. Because of this, it is concluded that these pancreas marker genes were activated and involved in conversion of hAMSCs to insulin- secreting cells. Previous study have shown that supplementation of taurine, a non-essential amino acid modulate glucose homeostasis and islet function and regulates insulin release from pancreatic beta cell line. Based on their findings, taurine can alter the electronic response in β -cell lines, leading to a change in calcium homeostasis and a subsequent decrease in intracellular insulin levels and initiate the calcium-dependent release of insulin [18]. They suggested that taurine enters β -cell via taurine transporter (TauT) and binds to the sulfonylurea receptor protein of the K_{ATP} channel, inactivating the channel resulting in Ca^{2+} flux via voltage sensitive Ca^{2+} channel (VSCC). Calcium-dependent exocytosis of large dense-core vesicles release insulin and GABA [18]. On the other hand, Hoorens et al. demonstrated that nicotinamide protects human beta cells against radical- induced necrosis and increases the total cell number and the frequency of beta cells. This could be caused either by formation of new beta cells through differentiation or by increased survival of beta cells [19].

CONCLUSION

The findings of this study demonstrated that hAMSCs could be induced to differentiate into insulin-producing cells, which may provide a stem cell source for β -cell replacement.

ACKNOWLEDGEMENTS

The authors would like to thank the members of the Stem Cell Technology Research center, Tehran, Iran, and also Transplant Research Center Shiraz of Medical Science, Shiraz, Iran for their helpful guidance, financial support and providing suitable conditions in this work and no conflict of interest.

REFERENCES

1. Zulewski, H. (2006). Stem cells with potential to generate insulin-producing cells in man. *Swiss. Med. Wkly*, 136(41/42):647-653.
2. Karnieli, O., Izhar-Prato, Y., Bulvik, S., & Efrat, S. (2007). Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem Cells*, 25:2837-2844.
3. Ra, J.C., Shin, I.S., Kim, S.H., Kang, S.K., Kang, B.C., Lee, H.Y., Kim, Y.J., Jo, J.Y., Yoon, E.J., Choi, H.J., & Euna-Kwon, E. (2011). Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. *Stem Cells. Dev.*, 20(8):1297-1308.
4. Chandra, V.G.S., Muthyala, S., Jaiswal, A.K., Bellare, J.R., Nair, P.D., & Bhonde, R.R. (2011). Islet- like cell aggregates generated from human adipose tissue derived stem cells ameliorate test diabetes in mice. *PLoS One*, 6(6):e20615.
5. Ohlsson, H., Karlsson, K., & Edlund, T. (1993). IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO. J.*, 12(11):4251-4259.
6. Holland, A.M., Hale MA, Kagami H, Hammer RE, MacDonald RJ. 2002. Experimental control of pancreatic development and maintenance. *Proc. Natl. Acad. Sci. USA*, 99(19):12236-41.
7. Kim, S.K., & Hebrok, M. (2001). Intercellular signals regulating pancreas development and function. *Gene. Dev.*, 15(2):111-27.
8. Shiroy, A., Yoshikawa, M., Yokota, H., Fukui, H., Ishizaka, S., Tatsumi, K., & Takahashi Y. (2002). Identification of Insulin-Producing Cells Derived from Embryonic Stem Cells by Zinc- Chelating Dithizone. *Stem Cells*, 20:284-292.
9. Manley, S.E., Stratton, I.M., Clark, P.M., & Luzio, S.D. (2007). Comparison of 11 human insulin assays: implication for clinical investigation and research. *Clin. Chem.*, 53(5):922-932.
10. Reinders, M.E. & Rabelink, T.J. (2010). Adipose tissue- derived stem cells: can impure cell preparations give pure results? *Nephrol. Dial. Transplant.*, 25: 3805-3807.
11. Bobis, S., Jarocho, D., & Majka, M. (2006). Mesenchymal stem cells: characteristic and clinical applications. *Folia. Histochemica. E.T. Cytobiologica*, 44(4):215-230

12. Li, Y., Zhang, R., Qiao, H., Zhang, H., Wang, Y., Yuan, H., Liu, Q., Liu, D., Chen, L., & Pei, X. (2007). Generation of Insulin-Producing Cells from PDX1 Gene-Modified Human Mesenchymal Stem Cells. *J. Cell. Physiol.*, 211:36-44
13. Hardikar, A.A., Marcus-Samuels, B., Geras-Raaka, E., Raaka, B.M., & Gershengorn, M.C. (2003). Human pancreatic precursor cells secrete FGF2 to stimulate clustering into hormone-expressing islet-like cell aggregates. *Proc. Natl. Acad. Sci. USA*, 100(12):7117-22.
14. Oliver-Kransinski, J.M., Ksner, M.T., Yang, J., Crutchlow, M.F., Rustgi, A.K., Kaestner, K.H., & Stoffers, D.A. (2009). The diabetes gene PDX1 regulates the transcriptional network of pancreatic endocrine progenitor cells in mice. *J. Clin. Invest.*, 119:1888-1898.
15. Grapin-Botton, A., Majithia, A.R., & Melton, D.A. (2001). Key events of pancreas formation are triggered in gut endoderm by ectopic expression of pancreatic regulatory genes. *Gene. Dev.*, 15(4):444-54.
16. Sussel, L., Kalamaras, J., Hartigan-O'Connor, D.J., Meneses, J.J., Pedersen, R.A., Rubenstein, J.L., & German, M.S. (1998). Mice lacking the homeodomain transcription factor Nkx2.2 have diabetes due to arrested differentiation of pancreatic beta cells. *Development*, 125:2213-2221.
17. Melloul, D. (2004). Transcription Factors in Islet Development and Physiology: Role of PDX-1 in Beta-Cell Function. *Ann. N. Y. Acad. Sci.*, 1014(1):28-37.
18. L'Amoreaux, W.J., Cuttitta, C., Santora, A., Blaize, J.F., Tachjadi, J., & Idrissi, A.E. (2010). Taurine regulates insulin release from pancreatic beta cell lines. *J. Bio. Med. Sci.*, 17(Suppl 1):S1-S11.
19. Hoorens, A., & Pipeleers, D. (1999). Nicotinamide protects human beta cells against chemically-induced necrosis, but not against cytokine-induced apoptosis. *Diabetologia*. 42:55-59.