Human Bone Marrow Mesenchymal Stem Cells Transfected with Human Insulin Genes Can Secrete Insulin Stably

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Abstract. Beta-cell replacement therapy by pancreatic islet transplantation has become a promising treatment for type 1 diabetes. However, the limited supply of human islet tissue prevents this therapy from being widely used to treat patients with type 1 diabetes. In order to obtain insulin-secreting cells, retrovirus vector pLNCX was used to transfer the human insulin gene into human bone marrow mesenchymal stem cells (hMSCs). The hMSCs were isolated from bone marrow of healthy volunteers and were expanded in vitro. The reverse transcriptase-polymerase chain reaction (RT-PCR) was used to amplify the insulin DNA fragment from a healthy pancreas sample. The recombinant vector pLNCX-Ins was constructed by cloning the insulin DNA fragment into retrovirus vector pLNCX. After being packaged by BD RetroPack PT67 packaging cells, the virus that contained the insulin gene was used to infect hMSCs. Transcription and expression of the insulin gene in transfected hMSCs were examined by RT-PCR and immunofluorescence. The transfected hMSCs stably secreted insulin into culture media for >3 weeks. Thus, insulin gene-transfected hMSCs can secrete insulin and provide a new way to cope with the shortage of beta cells for therapy of type 1 diabetes.

Keywords: mesenchymal stem cells, bone marrow, retroviral vector, gene therapy, insulin, type 1 diabetes

Introduction

Type 1 diabetes afflicts millions of people in the world and is responsible for untold morbidity. It is an autoimmune disorder in which the insulin-producing beta cells of the pancreatic islets of Langerhans are selectively destroyed [1]. Despite significant improvements in monitoring blood glucose levels and administration of insulin, insulin therapy cannot fully normalize glucose homeostasis at the present time. Therefore, interest has developed in finding mechanisms to increase beta cell mass by stimulating endogenous regeneration of islets [2-5] or by transplanting the donor beta cell mass [6]. During the past 35 years, whole organ pancreatic transplantation (WOP) has evolved gradually into a highly effective therapy for type 1 diabetic patients who are undergoing simultaneous renal transplantation [7-9]. But the risks of severe complications arising from this procedure and the necessity of lifelong immunosuppression have affected its development. The success and relative safety of the Edmonton protocol [10] has led some to suggest that isolated islet transplantation (IIT) might supersede WOP as the procedure of choice for replacement of beta cells in type 1 diabetic patients [11]. Recently, Huang [12] described a modified Euro-Ficoll sodium diatrizoate density gradient medium that improves the yield and viability of human islets suitable for islet transplantation. However, the success of clinical islet transplantation has been hampered by the fact that large masses of islets from cadaveric donors are needed to achieve complete graft function and insulin independence [6,13].

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Human bone marrow mesenchymal stem cells (hMSCs) are thought to be multipotent cells that are present in adult marrow, that can replicate as undifferentiated cells, and that have the potential to differentiate into adipocytes, osteoblasts, chondrocytes, tenocytes, myocytes, and hematopoietic-supporting stroma [14-16].

Pluripotent hMSCs in adult bone marrow can differentiate into mesenchymal lineage cells, endothelium [17,18], and endoderm tissue [19] in vitro and in vivo [20]. These cells display a stable phenotype and remain as a monolayer in vitro. MSCs have been explored as vehicles for both cell therapy and gene therapy. They have been used to perform transfection with viral vectors [21,22]. They have several characteristics that make them potentially useful for cell and gene therapy. The cells are relatively easy to isolate from the small aspirates of bone marrow that can be obtained under local anesthesia; they are also relatively easy to expand in culture and to transfect with exogenous genes [23]. A phase I clinical trial demonstrated that systemic infusion of autologous MSCs appears to be well tolerated [24]. Engraftment of whole bone marrow or of MSCs can be obtained in mice or dogs without the need for marrow ablation if large numbers of cells are infused or if they are infused at regularly spaced intervals [25]. Therefore, gene-engineered MSCs may be an effective vehicle for therapy of type 1 diabetes and other genetic diseases caused by deficiencies in circulating proteins.

In the present study, hMSCs were isolated from healthy volunteer donors and expanded in vitro, and the insulin gene was transferred to them by using a retrovirus vector pLNCX. The hMSCs transfected with insulin gene can steadily express and secrete insulin. Fundamental questions about MSCs need to be resolved before they can be safely used for effective clinical cell and gene therapy, but MSCs may provide a new way to deal with the shortage of beta cells for therapy of type 1 diabetes.

Materials and Methods

Isolation and expansion of hMSCs. The hMSCs were isolated as previously described [14]. Briefly, after a healthy volunteer donor signed an informed consent form, a bone marrow aspirate was collected from the posterior iliac crest into a syringe containing 6000 U of heparin. The marrow sample was washed with Dulbecco's phosphate-buffered saline (PBS, GIBCO Corp.) and cells were recovered after centrifugation at 900 x g. Nucleated cells were counted, and 1 x 10^8 nucleated cells were loaded onto 25 ml of 1.073 g/ml Percoll solution in a 50-ml conical tube. Cells were centrifuged at 1100 x g for 30 min at 20°C. The nucleated cells were collected from the upper layer and interface, diluted with 2 volumes of Dulbecco's PBS, and collected by centrifugation at 900 x g. The cells were cultured in low glucose Dulbecco's modified Eagle's medium (DMEM, Invitrogen Corp.) containing 10% fetal bovine serum (FBS, Hyclone). At 48 hr after plating, the supernatant containing non-adherent cells was removed and fresh medium was added. After growing nearly to confluence, cells were passaged 2 or 3 times by being detached with 0.25% trypsin-EDTA (Sigma Chemical Co.) and replated in expansion medium which consisted of 58% low-glucose DMEM and 40% MCDB-201 (Sigma Chemical Co.), supplemented with 1x insulin-transferrin-selenium, 1x linoleic acid–albumin, 10^-8 M dexamethasone, 10^-4 M ascorbic acid 2-phosphate (all from Sigma Chemical Co.), 1x penicillin-streptomycin-glutamine (GIBCO Corp.); 2% FBS, 10 ng/ml epidermal growth factor (EGF) (Sigma Chemical Co.), and 10 ng/ml platelet-derived growth factor (PDGF-BB) (R&D Systems, Inc.). Cells were fed every 4–6 days. When the cells were >80% confluent, adherent cells were detached and replated at 1:4 dilution in 25-cm² flasks coated with 5 ng/ml fibronectin, to maintain cell densities between 2 x 10^3 and 8 x 10^3 cells/cm². The cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂. In some instances, cells were subcultured after day 9 at a 1:4 dilution under the same culture conditions for more than 20 population doublings. Several aliquots of cells were frozen in liquid nitrogen for future use. Hematoxylin and eosin staining was used to show the morphological hMSCs.

Transmission and scanning electron microscopy. Cells were washed 3 x with PBS (pH 7.4), fixed with PBS containing 2.5% glutaraldehyde for 2 hr, and embedded in epoxy resin. Ultra-thin sections were cut horizontally parallel to the growing surface. The sections were double-stained in uranyl acetate and lead citrate prior to inspection in the transmission electron microscope. Similarly, hMSCs were washed 3 x with PBS (pH 7.4), fixed with PBS containing 2.5% glutaraldehyde for 1 hr, dried by the critical point drying technique, coated by ion sputtering, and inspected in the scanning electron microscope.

Flow cytometry. The hMSCs were detached using 0.25% trypsin-EDTA and stained with antibodies to CD13, CD10, CD29, CD34, CD44, CD45, and HLA-DR (all from Becton Dickinson Immunocytochemistry Systems). Cells were washed and labeled with fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated secondary antibodies (Sigma Chemical Co.), and then washed and analyzed using a FACS Calibur flow cytometry system (Becton Dickinson Immunocytochemistry Systems).
Generating retroviral vector insulin-pLNCX. After written informed consent was obtained from a patient, a pancreas sample was procured by pancreas excision surgery. Total RNA was extracted from the pancreas sample using Nucleospin RNAIIKit (Clontech). DNase-treated total RNA was used for the first-strand cDNA and polymerase chain reaction (PCR) amplification. This reaction was performed using BD Titanium One-step RT-PCR kit (Clontech), according to the manufacturer’s protocol. The primers (produced by Shanghai Sangon Bio-Engineering Co.) used to amplify insulin gene were forward 5’-GAT AAG CTT TGC CAT GGC CCT GTG GAT G-3’ and reverse 5’-ATA TCG ATT GCG GGC TGC GTC TAG TTG-3’, which contained two suitable restriction sites HindIII and Clal. The PCR conditions were as follows: incubation at 50°C for 5 min and 94°C for 3 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 65°C for 30 sec, and elongation at 68°C for 1 min; with final incubation at 68°C for 2 min. PCR products were analyzed using GelWorks software after scanning the ethidium bromide-stained 2% agarose gel (TaKaRa). Insulin DNA fragments were extracted from the PCR products using Nucleospin extract kit (Clontech) for future use. The retroviral vector pLNCX and insulin DNA fragments were digested respectively with the appropriate restriction enzymes HindIII and Clal (TaKaRa) and incubated at 37°C for 1 hr. After that, they were purified by using the Nucleospin extract kit. The digested vector pLNCX and the target gene fragment were ligated using DNA ligation kit Ver 2.1 (TaKaRa), following the manufacturer’s protocol. The ligation mixture products were transformed into E. coli (TaKaRa) to expand. The recombinant plasmid and the pLNCX plasmid control were purified using Nucleospin plasmid kit (Clontech). The desired recombinant plasmid was identified by restriction analysis, and analyzed using a 377 ABI automated sequencer (Sangon, Shanghai).

Virus production. Packaging cells (BD RetroPack PT67, Clontech) were plated on a 60-mm diameter plate at 60–80% confluency (1–2 x 10^6 cells/plate) 24 hr before transfection. A 25 mM stock solution of chloroquine (Sigma) was prepared in distilled water and sterilized by filtration. At 1 hr before transfection, the medium of packaging cells was replaced with medium containing chloroquine. Each 60-mm plate was transfected with 10 µg of recombinant plasmid DNA insulin-pLNCX (or the pLNCX plasmid control) using BD CLONfectin transfection reagent (Clontech). At 24 hr post-transfection, the culture medium of RetroPack PT67 cells was aspirated; the packaging cells were washed twice with PBS, and 3 ml of complete medium was added. The transfected packaging cells were plated in selection medium containing 400 µg/ml antibiotic G418 (Clontech) 36 hr post-transfection and cultured for 1 week with the appropriate antibiotic. Large, healthy colonies of the packaging cells were isolated and transferred to individual plates to expand. The colonies were cultured until cell culture reached the desired culture volume. One plate was retained for the continuation of the culture; the remaining cells were plated at 60–80% confluency in the desired number of culture vessels. Viral supernatants were harvested at 24 hr intervals until the cells were no longer viable. All cells were discarded once the virus had been harvested. The viral titer MOI (number of viral particles per target cell) was determined by use of NIH 3T3 cells (ATCC).

Transfection of hMSCs. At 18 hr before infection, hMSCs were plated at a cell density of 1–2 x 10^5 per 60-mm plate in two groups (group 1, experiment group, treated with virus-carrying insulin gene; group 2, control group, treated with an insert-free virus). Medium was collected from packaging cells and filtered through a 0.45-µm cellulose acetate (low protein binding) filter (Clontech) and then were added to hMSCs. At the same time, polybrene (Sigma) was added to a final concentration of 6 µg/ml. After 24 hr of incubation, the medium were replaced with fresh medium. Then the infected hMSCs were used for selection as soon as possible by plating in selection medium containing 300 µg/ml G418. Large, healthy colonies of the packaging cells were isolated and transferred to individual plates to expand and be used for future experiments.

Reverse transcription polymerase chain reaction. In the experiment group, transfected hMSCs were harvested 1, 7, 14, or 21 days after transfection. In the control group, cells were also harvested. Total RNA was extracted from each group of cells (5 x 10^6 cells) using Nucleospin RNA II kit (Clontech). DNase-treated total RNA was used for the first-strand cDNA and PCR amplification. RT–PCR was undertaken in a volume of 50 µl of buffer containing 5 µl 10X One-Step buffer, 1 µl 50X dNTP mix, 0.5 µl recombinant RNase inhibitor (40 units/µl), 25 µl thermostabilizing reagent, 10 µl GC-Melt, 1 µl oligo(dT) primer, 1µl 50X RT-TITANIUM Taq enzyme mix, 3 µl total RNA sample, 2 µl PCR primer mix (45 mM each), and 1.5 µl RNase-free water. The PCR conditions were the same as described above. PCR products were analyzed using GelWorks software after scanning the ethidium bromide-stained 2% agarose gel.

Immunofluorescence. The transfected hMSCs were plated on fibronectin-coated chamber slides, fixed with 4% paraformaldehyde (Sigma Chemical Co.) for 10 min at room temperature, and washed twice in 0.01M phosphate-buffered saline (PBS, GIBCO Corp.). For nuclear ligands, cells were further permeated with 0.1% Triton X-100 at room temperature, and washed twice in 0.01M phosphate-buffered saline. Primary antibody against human insulin was used at a 1:75 dilution and incubated for 2 hr with mouse anti-human insulin antibody (Ab) at 4°C, then incubated with FITC-coupled goat anti-mouse IgG antibody for 24 hr at 4°C. After each incubation step, slides were washed with PBS. Primary antibody against human insulin was used at a 1:75 dilution and secondary antibody at 1:25 dilution. Between each step, slides were washed with PBS. Cells were examined by fluorescence microscopy using a Zeiss Axiovert microscope.

Measurement of insulin secretion. Transfected cells were plated at a density of 10^6 cells per well in a 6-well plate and were divided into 3 groups (group 1, experiment group, cells treated with virus carrying insulin gene; group 2, control group, cells treated with an insert-free virus; group 3, no-
treatment control group). When the cells reached 80% confluence, they were washed with serum-free medium containing 5 mM glucose and exposed to fresh serum-free medium for 10 hr in the presence of different concentrations of glucose (5mM, 25mM) for 1, 3, 5, 7, 14, or 21 days after transfection. Insulin released into the medium was measured by a human insulin radioimmunoassay kit (Linco) and the insulin concentrations were expressed as IU/L (mean ± SE). Statistical analysis was performed using SAS v6.12 software (SAS Institute Inc., Cary, NC).

Results

Growth of hMSCs. Nucleated cells were collected from bone marrow of healthy volunteers and counted. The cells (2 x 10⁵/cm²) were cultured in low glucose DMEM (Fig. 1A). Non-adherent hematopoietic cells in the culture were removed during changes of medium. Colonies of fibroblastic cells began to appear in the culture flasks 5 to 7 days after plating of bone marrow nucleated cells (Fig. 1B). Initially, fibroblastic cells in a single colony were often separated from each other; however, after continuous culture for 1 to 2 weeks, the number and density of cells were greater in the colonies (Fig. 1C). H&E staining was used in some experiments to show the morphology of hMSCs. There were many nucleoli in the nuclei of the cells (Fig. 1D). The hMSCs were inspected by transmission electron microscopy (Fig. 1E) and scanning electron microscopy (Fig. 1F). Microvilli were present on the surface of the cells and abundant mitochondria and rough endoplasmic reticulum (RER) were in the cytoplasm near the nucleus.

Flow cytometric analysis. The hMSCs were harvested and labeled with antibodies against CD10, CD13, CD29, CD34, CD44, CD45, HLA-DR, or control IgGs and analyzed by FACS. Fig. 2 shows phenotypic characterization of the culture-expanded cells by flow cytometry of their surface antigens. The analyses revealed that hMSCs were positive for CD29 (β1-integrin), CD44 (vascular cell adhesion molecule, VCAM), and CD13; they were negative for CD34, CD45, and HLA-DR. The hMSCs also expressed a low level of CD10.

Construction of retrovirus vector systems for transgenic delivery. RT-PCR was used to amplify the insulin DNA fragment from the healthy pancreas sample that was procured by pancreas excision surgery. The insulin DNA fragment was analyzed using GelWorks software after scanning the ethidium bromide-stained 2% agarose gel (Fig. 3A). Then the HindIII/ClaI fragment of insulin DNA was inserted into the pLNCX vector digested with HindIII and ClaI. The resulting recombinant pLNCX-Ins plasmid was analyzed by restriction enzyme digestion (HindIII and ClaI). Then, 2 DNA fragments (ie, the insulin DNA fragment and the linear pLNCX (6.6 kb)) were evident in the ethidium bromide-stained 1% agarose gel (Fig. 3B, Lane 2). The recombinant pLNCX-Ins plasmid also was analyzed by using a 377 ABI automated sequencer. The sequence of the inserted insulin DNA fragment (shown in Fig. 3C) was consistent with the human insulin mRNA sequence as recorded in Gene Bank. The pLNCX plasmid control and recombinant pLNCX-Ins plasmid were prepared and purified with the Nucleoprin plasmid kit. They were packaged by means of the packaging cells (BD RetroPack PT67). After antibiotic selection, the transfected packaging cells PT67 produced virus, which could infect hMSCs. The viral titer MOI, determined by NIH 3T3 cells, was 2 to 4 x 10⁶ cfu/ml.

Transcription and expression of insulin gene in hMSCs. At 1, 7, 14, or 21 days after transfection, transfected hMSCs were harvested to extract total RNA that was used for RT-PCR amplification. The hMSCs in the experiment group were transfected with the insulin gene, while the control cell groups received the empty vector or no vector. (Fig. 4A). The transfected hMSCs that had been selected by G418 were plated on fibronectin-coated chamber slides for immunofluorescence microscopy. Expression of the insulin gene was evident in hMSCs of the experiment group (Fig. 4C), but not in the control groups.

Insulin secretion. Three groups of hMSCs were exposed to fresh serum-free medium for 10 hr in the presence of two different concentrations of glucose (5 mM, 25 mM) for 1, 3, 5, 7, 14, or 21 days after transfection, respectively. In group 2 (control group, cells treated with an insert-free virus) and group 3 (no-treatment control group,
cells treated with nothing), no insulin was detected in the media with either concentration of glucose at any day. In group 1 (experiment group, cells treated with virus carrying insulin gene), insulin concentrations (mean ± SE) in culture media with 5 mM glucose at 1, 3, 5, 7, 14, or 21 days after transfection were $8.14 \pm 0.12$, $8.28 \pm 0.14$, $10.57 \pm 0.36$, $16.53 \pm 0.27$, $16.51 \pm 0.26$, and $16.65 \pm 0.35$ IU/L and in the media with 25 mM glucose were $8.12 \pm 0.15$, $8.28 \pm 0.13$, $10.62 \pm 0.28$, $16.60 \pm 0.27$, $16.43 \pm 0.30$, and $16.59 \pm 0.30$ IU/L, respectively (Fig. 5). Data were analyzed by two way ANOVA; $p < 0.05$ was considered statistically significant. There were no significant differences in insulin secretion between the cells grown in media with 5 or 25 mM glucose concentrations.
Fig. 2. Phenotype of hMSCs. The hMSCs were plated in expansion medium and passaged for 10 cell doublings. Cells were harvested and labeled with antibodies to CD10, CD13, CD29, CD34, CD44, CD45, HLA-DR, or control IgGs as indicated and analyzed by FACS. (A) Control IgG-staining profile. (B) hMSCs expressed low level of CD10. The analyses revealed that (C) hMSCs were positive for CD13, (D) β1-integrin (CD29), and (F) vascular cell adhesion molecule (VCAM, CD44); the hMSCs were negative for (E) CD34, (G) CD45, and (H) HLA-DR.

Fig. 3. (A) The insulin DNA fragment was amplified from a healthy pancreas sample that was procured by pancreas excision surgery. Lane 1, DNA marker DL2000; lane 2, insulin DNA fragment (333 bp). (B) The recombinant pLNCX-Ins plasmid was analyzed by restriction enzyme digestion (HindIII and Clal) and shown in the ethidium bromide-stained 1% agarose gel. Lane 1, DNA marker (λ-EcoT14 I, Takara); lane 2, two DNA fragments: insulin DNA fragment (333 bp) and the linear pLNCX (6.6 kb); lane 3, recombinant super spiral pLNCX-Ins plasmid (6.9 kb); lane 4, super spiral pLNCX plasmid control (6.6 kb); lane 5, insulin DNA fragment (333 bp). (C) The sequence of the insulin DNA fragment inserted in the recombinant pLNCX-Ins plasmid was analyzed by using a 377 ABI automated sequencer and was consistent with the human insulin mRNA sequence in Gene Bank.
Fig. 4. (A) RT-PCR analysis of the transcription of insulin gene. Lane 1, DNA marker DL2000; lane 2-5, strong transcription of insulin gene in the hMSCs of the experiment group at 1, 7, 14, and 21 days after transfection, respectively; lane 6, no transcription of insulin gene in the control group. Transfected hMSCs treated with FITC-coupled antibody were examined by microscopy under normal light (panel B) and by fluorescence (panel C) (enlargement = 40x).

Fig. 5. Insulin secretion of transfected hMSCs. The chart shows the insulin concentrations in culture media with 5 mM glucose or 25 mM glucose at 1, 3, 5, 7, 14, and 21 days after transfection. At 1 week after transfection, the insulin secreted by the transfected hMSCs attained a high level that was maintained for >3 weeks.
Discussion

Although studies indicate that hMSCs are a rare population of cells in bone marrow, representing perhaps 0.001% to 0.01% of the nucleated cells, almost 10-fold less abundant than hematopoietic stem cells (HSCs), hMSCs can be readily grown in culture [26]. In this study, we used a density gradient method in the isolation procedure to eliminate unwanted cell types that were present in the marrow aspirate. HSCs and non-adherent cells were removed with changes of the medium. We analyzed the surface antigens of hMSCs by flow cytometry. These expanded attached mesenchymal cells were positive for CD10, CD13, CD29, and CD44. Some of these antibodies have been used to characterize the expanded mesenchymal stem cell population [14,27]. In comparison, the hMSCs were negative for other markers of the hematopoietic lineage, including CD34 and CD45. CD45 was identified on some hMSCs from bone marrow at low levels and was quickly lost in culture [28].

Although understanding of hMSCs will further evolve, use of these multi-potential cells is already helping to answer critical questions that could not previously be addressed. For example, recently, cultured hMSCs were infused in humans for safety and early clinical testing for support of bone marrow transplantation, and treatment for osteogenesis imperfecta and glycogen storage disease, which are diseases with few therapeutic options [29-31].

The hMSCs do not differentiate spontaneously during culture expansion and they express exogenous DNA in an efficient manner. In this study, retrovirus vector pLNCX was used to transfer insulin gene to hMSCs. No good universal vector exists. The specific advantages and disadvantages of each available viral vector system have been reviewed [32]. The retrovirus vector pLNCX contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV) [33]. After having been transfected into packaging cell PT67, the recombinant vector pLNCX-Ins can stably express a transcript containing ψ+(the extended viral packaging signal), a selectable marker, and the insulin gene [34]. The 5’ viral LTR in this vector contains promoter/enhancer sequences that control expression of the neomycin resistance gene for antibiotic selection in packaging cells and hMSCs. RNA from the vector was packaged in infectious, replication-incompetent retroviral particles. These retroviral particles could infect hMSCs and transmit the insulin gene, but could not replicate within hMSCs since the cells lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimized the chances of producing replication-competent virus during hMSCs proliferation [34,35].

Diabetes is caused by an absolute insulin deficiency due to destruction of insulin-secreting pancreatic beta cells (type 1 diabetes) or by a relative insulin deficiency due to decreased insulin sensitivity (type 2 diabetes). In both types of diabetes, an inadequate mass of functional beta cells is the major determinant for the onset of hyperglycemia and the development of the overt disease. Maintenance of beta cell mass results from a dynamic balance of neogenesis, proliferation, and apoptosis [36]. These processes are adaptive since beta cells can proliferate physiologically in postnatal life (during growth or pregnancy), in response to injury, in disease states such as obesity, or in other genetic forms of insulin resistance [37]. The capacity of beta cells to replicate may be more limited in type 1 diabetes or at later stages of life. Beta cell replacement by islet transplantation has recently been shown to restore normoglycemia in type 1 diabetics [6,10,11]. However, a limited supply of human islet tissue prevents this therapy from being used to treat the myriad patients with type 1 diabetes [6,13].

The insulin gene-transfected hMSCs, as a new approach to beta cell replacement therapy, may provide a new way to deal with the limited supply of human islet tissue. Insulin gene-transfected hMSCs have the potential for ex vivo expansion and autologous transplantation. Thus, immunosuppression to prevent rejection could be avoided. However, before insulin gene-transfected hMSCs can be used for treatment of type 1 diabetes, it will be necessary to elucidate the mechanisms of insulin secretion in these cells and to identify environmental signals, such as different concentrations of glucose, that may trigger insulin secretion.
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