ORIGINAL RESEARCH

Allogeneic diabetic mesenchymal stem cells transplantation in streptozotocin-induced diabetic rat

Qing-Yu Dong^{a,b}

Li Chen^a

Guan-Qi Gao^b

Lei Wang^c

Jun Song^a

Bo Chen^c

Yu-Xin Xu^a

Lei Sun^a

^aDepartment of Endocrinology and Metabolism, Qilu Hospital of Shandong University, No. 107 Wenhua Road, Jinan, Shandong, 250012, P. R. China

^bPeople's hospital of Linyi city, 27 Jie Fang Road, Linyi 276003, P.R. China

^cDepartment of Hepatobiliary and Pancreatic Surgery, Qilu Hospital of Shandong University No. 107 Wenhua Road, Jinan, Shandong, 250012, P. R. China

Manuscript submitted 1st December, 2007

Manuscript accepted 15th September, 2008

Clin Invest Med 2008; 31 (6): E328-E337.

Abstract

Background: Bone marrow-derived mesenchymal stem cells (BM-MSCs) are multipotent stroma cells which can provide a potential therapy for diabetes mellitus. But the mechanism is still controversial. Also, the status of BM-MSCs under hyperglycemia is not known. In the present study, we investigated the status of BM-MSCs in experimental-diabetic rat and demonstrated the rescue of experimental diabetes by diabetic MSCs transplantation.

Methods: BM-MSCs were cultured and the potential of multiple-differentiation was identified through induction into osteoblasts. MSCs of passage 3 were used for the following experiment. The MSCs were labeled with 5-bromo-2'-deoxyuridine (BrdU). Diabetes in rats was induced by STZ injection. The rats were divided into three groups: normal control group (no DM, rats treated with saline through tail vein, n=10); DM control group (DM, no transplantation of MSCs, n=20); experimental group (DM and transplantation of MSCs, n=20). Body weight and blood glucose of the rats were monitored during the experiment after transplantation of MSCs. Paraffin sections of pancreas were obtained from rats of each group. Immunohistochemistry analysis and double immunofluorescence were used to detect the BM-MSCs in the pancreatic tissue and their differentiating state.

Results: MSCs were 89.5% labeled by BrdU and DAPI, which was green/blue double stained under fluorescent microscopy. Transplantation of diabetic MSCs resulted in a reduction of hyperglycemia on day 45 in experimental diabetic rats compared with control rats ($17.7 \text{ mM} \pm 3.9$ vs $27.8 \text{ mM} \pm 2.1$, $P < 0.05$). There was also a difference between MSC-treated experimental diabetic rats and control rats in body weight ($232.7 \text{ g} \pm 19.7$ vs $133.3 \text{ g} \pm 13.1$, $P < 0.05$). Histological and morphometric analysis of the pancreas of experimental diabetic rats showed the presence and differentiation of transplanted MSCs into insulin-producing cells which evidenced by double-staining of anti-BrdU and insulin. Also, there were many small islets throughout the sections. Their mean area and diameter analysis revealed that they were smaller than control islets ($1835.7 \pm 175.8 \mu\text{m}^2$ vs $13257.2 \pm 1457.6 \mu\text{m}^2$; $43.5 \pm 3.7 \mu\text{m}$ vs $119.9 \pm 5.8 \mu\text{m}$, respectively, $P < 0.05$).

Conclusion: Allogeneic MSCs transplantation can reduce blood glucose level in recipient rats. A relatively small quantity of transplanted diabetic MSCs survive and trans-differentiate into insulin-producing cells in the pancreas of recipient rats. Upon transplantation these cells initiate endogenous pancreatic regeneration by neogenesis of islet of recipient origin. The present study demonstrates that diabetic MSCs retains its stemness and potential to induce pancreatic regeneration on transplantation.

Reduction of β cell mass in the pancreas is the hallmark of the development of both type 1 and type 2 diabetes. Regeneration and maintenance of pancreatic endocrine tissue after the onset of islet destruction would have considerable therapeutic impact on diabetes mellitus (DM).

Bone marrow (BM) is a complex tissue containing hematopoietic progenitor cells and a connective-tissue network of stromal cells. Marrow stroma includes a subpopulation of undifferentiated cells that are capable of becoming one of a number of phenotypes, including bone and cartilage, tendon, muscle, fat, and marrow stromal connective tissue that supports hematopoietic cell differentiation.^{1,2} These cells are referred to as mesenchymal stem cells (MSCs), since they have the capacity of proliferation and differentiation into the mesenchymal lineage. Due to their potential for differentiation into different tissues, MSCs have emerged as a promising tool for clinical applications such as tissue engineering and cell therapy.³⁻⁵

Some recent evidence suggests the possibility that BM cells differentiate into islet β cells.⁶⁻⁹ and it is proposed that the cell types in the BM responsible for pancreatic endocrine differentiation are MSCs.^{7,8} However, the role of BM-derived cells in β cell replacement is controversial. Other reports^{10,11} have contradictory findings that suggest that BM cells could be “feeder” cells for islet differentiation, proliferation, and vascularization¹² but they do not differentiate into β cells.¹³ Zorina et al. suggest that BM cells contribute to pancreatic β cell regeneration.¹⁴

Slack¹⁵ noted that the failure of regeneration following treatment with streptozotocin (STZ) or alloxan suggests that these drugs target the potential stem or transit cells as well as the differentiated β cells. On the contrary, Guz et al. have shown that STZ does not destroy the intra-islet stem cell reserve in experimental-diabetic subjects.¹⁶ However, the stem-cell activity of MSCs under the influence of sustained hyperglycemia is questionable. We investigated the status of MSCs in STZ-diabetic rats with respect to their capacity of transdifferentiation and inducing regeneration. We de-

signed this experiment to observe the outcome of labeled MSCs from STZ-diabetic rat in recipient diabetic rats after allogenic transplantation.

Materials and methods

STZ-induced diabetes in Wistar rats

Male Wistar rats (Laboratory Animal Center of Shandong University), weight c. 200g, were injected with 60 mg/kg STZ (Sigma) by tail vein. STZ was solubilized in sodium citrate buffer, pH 4.5, and injected within 15 min of preparation. Blood was drawn from the tail vein and blood glucose was measured between 8:00 and 10:00 AM, weekly from day 3 to day 45 with a blood glucose meter (Lifescan Inc.). The rats were maintained under sterile conditions, and all animal work was carried out under protocols approved by the Laboratory Animal Care and Use Regulations of Shandong University.

Isolation and culture of bone marrow mesenchymal stem cells

Bone marrow MSCs were isolated as described previously.¹⁷ Diabetic Wistar rats were anesthetized with intraperitoneal sodium pentobarbital (30mg/kg). Femurs and tibiae of rats were collected and adherent soft tissue was carefully removed. Bone marrow cells were flushed from the medullary cavities of femurs and tibiae and disaggregated into a single-cell suspension by sequential passage through a 25-gauge needle. Mononuclear cells were separated by density-gradient centrifugation over Ficoll-Paque ($\rho=1.077$) at 1100g for 30 min. Cells were rinsed twice with PBS to remove Ficoll-Paque and then plated in T-25 flask. The cells were cultured in Dulbecco's modified Eagle's medium-low glucose (DMEM-LG, Gibco) containing 10% fetal bovine serum (FBS, Gibco).

Three days later, non-adherent cells were removed by changing the medium. and every third or fourth day thereafter. After 10~14 days in culture, adherent cells formed homogenous fibroblast-like colonies.

Adherent cells were passed with 0.25% trypsin (Gibco) for ≈ 5 min, rinsed with serum-containing medium to remove residual trypsin, collected by centrifugation at 1200 rpm for 5 min, and seeded into fresh flasks 1-3 split. The culture medium was changed every 3-4 days and the passages 2-4 MSCs were used for the following studies.

Osteogenic differentiation of MSCs

Multipotentiality of the resulting cells was verified with the use of *in vitro* assays to differentiate into osteoblasts (alizarin staining). Cells were seeded following trypsinization into 6-well tissue culture-treated plates and grown to confluency. Differentiation was started by adding complete medium containing 0.1 μ M dexamethasone, 0.2 mM vitamin C and 50 mM β -glycerolphosphate. After 14 days, cells were fixed in 4% paraformaldehyde and stained with a solution of 1% alizarin red. Cells were photographed under an inverted microscope.

Labelling of the MSCs

The MSCs were labeled *in vitro* for later identification by adding 10 μ g/mL 5-bromo-2'-deoxyuridine (BrdU, Sigma) containing media to $\approx 70\%$ confluent cultures for 48 h.¹⁸ At the completion of the incubation period, the cells were harvested from culture flask using 0.25% trypsin solution, and rinsed twice with phosphate-buffered saline (PBS), then resuspended to a concentration of $2-4 \times 10^6$ bone marrow MSCs in 1 mL of culture medium.

Total body irradiation and MSCs transplantation

In order to destroy the stem cell niche of bone marrow of the recipient STZ-diabetic rat, they were subjected to total body irradiation with a dose of 900 rads from a ^{60}Co source. Recipient rats (diabetic, irradiated) were transplanted intravenously through tail vein approximately $2-4 \times 10^6$ labelled bone marrow MSCs per

rat. And the same volume of saline was injected into diabetic control rats.

Histological and morphometry analyses

At 45 days, the number of survived rats without administration of insulin in normal control group, DM control group and experimental group was 9, 8, and 12 respectively. For tissue section analysis, animals were anesthetized and perfused intracardially with 4% paraformaldehyde/PBS. The distal portions of pancreases were dissected and further fixed in 4% paraformaldehyde at 4°C for 24 h. Fixed tissues were washed once in PBS, then processed for paraffin embedding. Three sections per animal were stained with hematoxylin/eosin to allow the assessment of pancreatic islet morphology after STZ treatment and subsequent transplantation.

Immunohistochemistry staining for insulin was performed as follows: The sections were deparaffinized, immersed in 3% H_2O_2 to quench endogenous peroxidase activity, and microwaved in 10 mM sodium citrate (pH 6.0) for 15 min for antigen retrieval. Then, avidin and biotin were applied to eliminate endogenous biotin-related background staining. The sections were incubated with primary antibodies (1:100, Cell Signaling) at 4°C overnight and incubated with rabbit anti-rat secondary antibody (1:100, Santa Cruz) for 1 hour at room temperature. The slides were washed and visualized using the Vectastain Elite ABC Kit (Vector Labs) with 3'3' diaminobenzidine tetra-chloride (DAB, Boehringer-Mannheim Inc.).

Immunofluorescence for labeled MSCs and pancreatic tissue: Three sections per animal were selected for the measurement of insulin-BrdU-positive cells in the pancreas. The sections were immunostained with rabbit anti-insulin (1:100, Cell Signaling), mouse anti-BrdU (1:100, Neomarkers), goat anti-rabbit IgG-TRITC (1:100, Kpl) and goat anti-mouse IgG-FITC (1:100, Kpl). For BrdU staining, cryosections were pretreated with 1N HCL for 1 hour at 37°C to denature DNA. The nuclear regions of MSCs were

FIGURE 1. Photomicrographs of bone marrow MSCs in culture (A); osteogenic differentiation evidenced by the alizarin red staining (B); labelling of MSCs by double-staining with anti-BrdU and DAPI (C), ($\times 200$ magnification).

stained by DAPI (Roche) counterstaining. The labelling rate for MSCs was analyzed by the ratio of cells double-staining with BrdU-DAPI and cells single-staining DAPI. In pancreatic tissue, cells visualized as that coexpressed insulin and BrdU were considered transplanted cells which have transdifferentiated into insulin-producing cells.

Islet image analysis: Islets labeled for rat insulin per section were counted manually. And islets size and area were scored with an Olympus light microscope and a computer-assisted image analysis program (Image Pro Plus 5.1.0.20).

Statistical Analyses

For statistical analysis, SPSS (Version 10.0, SPSS Inc) was used. Differences among groups were determined with one-way ANOVA analysis. Differences between specific groups was performed with Student's *t* test. Differences were considered significant at $P < 0.05$.

Results

Isolation, differentiation and labeling of MSCs

The isolated MSCs had a fibroblastlike shape, attached to the culture dish tightly, and proliferated in

the culture medium (Fig 1A). For the differentiation assay, osteogenic differentiation, evidenced by the formation of mineralized matrix, is shown as alizarin red staining (Fig. 1B). Before transplantation, the nuclei of MSCs were labeled with BrdU for 48 h, and 89.5% ($n=3$) of the cultured MSCs were stained positively with BrdU and DAPI (Fig. 1C).

Effect of STZ and MSCs transplantation on blood glucose of rat

STZ was used to produce diabetes in Wistar rat. About 60mg/kg STZ were administered to the rats (Scheme). At the 3rd day after STZ administration, blood glucose levels increased from normal levels ($4.3 \text{ mM} \pm 0.8$) to severe hyperglycemic levels ($24.8 \text{ mM} \pm 5.7$). STZ-induced diabetic rats, which had non-fasting glucose levels of $>13.9 \text{ mM/L}$ for 14 consecutive days, were selected for further experiment. Some STZ-treated rats were randomly selected for sublethal irradiation and used as recipients. The effect of injections of MSCs is described in Fig. 2. The diabetic control rats weighed less on days 45 than normal controls ($133.3\text{g} \pm 13.1$ vs. $358.83\text{g} \pm 21.6$, $P < 0.05$). There was a difference in body weight between diabetic control rats and MSCs-treated experimental diabetic rats

SCHEME 1. Experimental design.

on day 45 ($133.3\text{g} \pm 13.1$ vs $232.7\text{g} \pm 19.7$, $P < 0.05$), Fig 2A. Reduction in hyperglycemia was found among the experimental group of rats whereas the diabetic control group of rats remained hyperglycemic throughout the experiment ($17.7\text{mM} \pm 3.9$ vs $27.8\text{mM} \pm 2.1$, $P < 0.05$), Fig 2B.

Effect of STZ and MSCs transplantation on the pancreas of rat

Pancreases from the STZ-diabetic rats showed fewer islets, altered islet morphology (Fig. 3A), less rat insulin immunoreactivity (Fig. 3B and C), and a decreased number of islets per section (Fig. 3D). In pancreases from MSC-treated diabetic rat, many islets appeared small compared with islets from untreated diabetic rat (Fig. 3A). Also, the islets had an increase in insulin immunoreactivity (Fig. 3 B and C), and there was an increase in number of islets per section (Fig. 3D). Many of the islets in the MSC-treated diabetic rats appeared to bud off the pancreatic ducts, suggestive of new islet formation. (Fig. 3B).

Image analysis of normal control as well as newly generated islets has been performed with paraffin sections of pancreas. The mean area of control islets was $13257.2 \pm 1457.6\text{ }\mu\text{m}^2$ whereas the mean area of newly generated islets was $1835.7 \pm 175.8\text{ }\mu\text{m}^2$. Mean

diameter of control islets was $119.9 \pm 5.8\text{ }\mu\text{m}$ whereas, for newly generated islets, it was $43.5 \pm 3.7\text{ }\mu\text{m}$. Thus, the values for islet area as well as diameter of control islets were higher than those of newly generated islets.

Transdifferentiation of transplanted MSCs in recipient pancreas

A small number of transplanted MSCs were detected around and inside the islets of the MSC-treated diabetic rat by labeling sections with antibodies to BrdU (Fig. 4B). A few of the cells labeled for BrdU colabeled with insulin antibodies which means that a few of the transplanted MSCs have transdifferentiated into insulin-producing cells (Fig. 4C). Without transplantation of MSCs, no transplanted MSCs were detected in islets of diabetic control rats that had received saline (Fig. 4).

Discussion

This study demonstrated that a small amount of allogeneic MSCs from STZ-diabetic rats homed to the injured pancreas and survived after transplantation. Two aspects of the observations are remarkable: the ability of MSCs homing to the pancreas; and the ability of transplanted MSCs to repair the injured pan-

FIGURE 2.(A) Effects of MSCs on blood glucose : Blood glucose levels in normal control group, diabetic control group and experimental MSCs-treated diabetic group. Values are mean $\pm$ SD from three groups. (B) Body weight in normal control group, diabetic control group and experimental MSCs-treated diabetic group. Values are mean $\pm$ SD. *, Values that differ from each other with $P < 0.05$.

creas by transdifferentiation into islet β cells and inducing islet regeneration.

We found some transplanted labeled MSCs in the pancreas of recipient rats which suggests selective homing of MSCs to the pancreas. MSCs have the ability to migrate.¹⁹⁻²³ Of particular interest for tissue remodeling, intravenous delivery of MSCs resulted in their specific migration to the site of injury.¹⁹⁻²¹ This ability of implanted MSCs to seek out the site of tis-

sue damage has been demonstrated in bone or cartilage fractures²⁴, myocardial infarction²¹, and ischemic cerebral injury.¹⁹ Sordi V et al.²⁵ provided evidence that BM-MSCs are attracted by pancreatic islets *in vitro* and *in vivo*, and confirmed that CXCL12 and its ligand CXCR4 play an important role in homing. In all, the ability of pancreatic islets to attract BM-MSCs suggests a potential role for these cells in β cell replacement.

In the present study transplantation of MSCs from diabetic rats reduced blood glucose and prevented further rise in blood glucose in experimental-diabetic rats. This suggests that the diabetic BM-MSCs retain their stem-cell activity. Histological examination of rat pancreas suggested that BM-MSCs from STZ-diabetic rats initiates pancreatic regeneration upon transplantation into recipient experimental-diabetic rats. Pancreatic regeneration was evidenced by the appearance of many small islets. Several of these small islets were found near the pancreatic ducts that are the source of islets during early development of the pancreas.²⁶ On the other hand, A few of the transplanted MSCs could transdifferentiate into insulin-producing cells evidenced by costaining with anti-BrdU and insulin. We believe that the major effect of the MSCs treatment was to increase the number of islets and insulin-producing cells, but we cannot exclude the effect of MSCs transdifferentiation. These two aspects may contribute together to the cellular mechanism and physiological relevance of BM-MSCs for the restoration of tissue function after pancreatic injury. Thus, the effects of the MSCs may be similar to the recent observations that MSCs implanted into the dentate gyrus of the hippocampus of immunodeficient mice enhanced proliferation, migration, and neural differentiation of the nearby endogenous mouse neural stem cells.²⁷

We found that transplantation of MSCs, although helping to reduce blood glucose and preventing further blood glucose increase in experimental-diabetic rats, is insufficient to restore normoglycemia. The main reason is that only a very small percentage of the

FIGURE 3. Histology of pancreas from control rat (Normal), diabetic control (STZ-treated) and experimental (MSCs-treated diabetic rat) at day 45, sections (6 μ m) are magnified $\times 400$. (A) Morphology of islets stained with hematoxylin and eosin; (B) Islets labeled antibodies for rat insulin with immunohistology; (C) Islet labeled antibodies for rat insulin with immunofluorescence; (D) Islets per section. Values are mean $\pm$ SD. *, Values that differ from each other $P < 0.05$.

FIGURE 4. Fluorescent immunohistology of pancreas from diabetic control and experimental (MSCs-treated diabetic rat) on day 45. Sections were costained with antibodies for BrdU and insulin. Sections (6 μ m) are magnified $\times 400$. arrows, transplanted MSCs; arrowheads, transplanted MSCs colabeled for insulin.

infused cells are seen in the injured pancreas²¹ Although several reports underline the ability of MSCs to migrate,^{19,20} suggesting that CXCR4 and related receptors were important in the trafficking of MSCs. However, this type of trafficking action is not strong enough to attract more MSCs to reside in the injured pancreas. The present study was not designed to analyze the ability of the transfused cells homing to injured tissue. In future studies harnessing the migratory potential of MSCs by modulating their chemokine-chemokine receptor interaction may be a powerful way to enhance the effect of tissue repair after MSCs transplantation.

The therapeutic utility of bone marrow transplantation in diabetic patients to overcome deficient β -cell population is an attractive proposal. The data provided in our experiment may allow treatment of diabetes via a stem cell therapy approach. MSCs are readily obtained from a diabetic patient and rapidly multiply in culture so that it is feasible to administer very large numbers of autologous cells. A further attractive feature of MSCs is that they were tested in clinical trials and provided promising results without any apparent toxicity in patients.²⁸⁻³¹ BM-MSCs from the DM patient himself may serve as a constant potential autologous source for cell therapy for DM.

Acknowledgments

We thank the Key Laboratory of Cardiovascular Remodeling and Function Research Center, Qilu hospital of Shandong university.

References

- Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*; 1997;276:71-4.
- Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*; 1999;284:143-7.
- Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*; 2002;418:41-9.
- Asahara T, Kalka C, Isner JM. Stem cell therapy and gene transfer for regeneration. *Gene Ther*; 2000;7:451-7.
- Bianco P, Robey PG. Stem cells in tissue engineering. *Nature*; 2001;414:118-21.
- Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest* 2003;111:843-50.
- Tang DQ, Cao LZ, Burkhardt BR, et al. In vivo and in vitro characterization of insulin-producing cells obtained from murine bone marrow. *Diabetes* 2004;53:1721-32.
- Kodama S, Kuhtreiber W, Fujimura S, Dale EA, Faustman DL. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science*; 2003;302:1223-7.
- Oh SH, Muzzonigro TM, Bae SH, LaPlante JM, Hatch HM, Petersen BE. Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type I diabetes. *Lab Invest* 2004;84:607-17.
- Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 2004;429:41-6.
- Lechner A, Yang YG, Blacken RA, Wang L, Nolan AL, Habener JF. No evidence for significant transdifferentiation of bone marrow into pancreatic beta-cells in vivo. *Diabetes* 2004;53:616-23.
- Mathews V, Hanson PT, Ford E, Fujita J, Polonsky KS, Graubert TA. Recruitment of bone marrow-derived endothelial cells to sites of pancreatic beta-cell injury. *Diabetes* 2004;53:91-8.
- Hess D, Li L, Martin M, et al. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol* 2003;21:763-70.
- Zorina TD, Subbotin VM, Bertera S, et al. Recovery of the endogenous beta cell function in the NOD model of autoimmune diabetes *Stem Cells* 2003;21:377-88.
- Slack JM. Developmental biology of the pancreas, *Development* 1995;121:1569-80.
- Guz Y, Nasir I, Teitelman G. Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes, *Endocrinology* 2001;142:4956-68.
- Tomita S, Li RK, Weisel RD, et al. Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* 1999;100(suppl):II247-56.
- Krupnick AS, Kreisel D, Engels FH, et al. A novel small animal model of left ventricular tissue engineering. *J Heart Lung Transplant* 2002;21:233-43.

19. Ji JF, He BP, Dheen ST, Tay SS. Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. *Stem Cells* 2004;22:415-27.
20. Munoz-Elias G, Marcus AJ, Coyne TM, Woodbury D, Black IB. Adult bone marrow stromal cells in the embryonic brain: engraftment, migration, differentiation, and long-term survival. *J Neurosci* 2004;24:4585-95.
21. Barbash IM, Chouraqui P, Baron J, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation* 2003;108:863-8.
22. Annabi B, Lee YT, Turcotte S, et al. Hypoxia promotes murine bone-marrow-derived stromal cell migration and tube formation. *Stem Cells* 2003;21:337-47.
23. Lu D, Li Y, Wang L, Chen J, Mahmood A, Chopp M. Intraarterial administration of marrow stromal cells in a rat model of traumatic brain injury. *J Neurotrauma* 2001;18:813-9.
24. Murphy JM, Fink DJ, Hunziker EB, Barry FP. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum* 2003;48:3464-74.
25. Sordi V, Malosio ML, Marchesi F, et al. Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood* 2005;106:419-27.
26. Hardikar AA. Generating new pancreas from old. *Trends Endocrinol Metab* 2004;15:198-203.
27. Munoz JR, Stoutenger BR, Robinson AP, Spees JL, Prockop DJ. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. *Proc Natl Acad Sci USA* 2005;102:18171-6.
28. Horwitz EM, Prockop DJ, Gordon PL, et al. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood* 2001;97:1227-31.
29. Koc ON, Day J, Nieder M, Gerson SL, Lazarus HM, Krivit W. Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). *Bone Marrow Transplant* 2002;30:215-22.
30. Le Blanc K, Rasmusson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004;363:1439-41.
31. Lazarus HM, Koc ON, Devine SM, et al. Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol Blood Marrow Transplant* 2005;11:389-98.

Correspondence to:

Dr. Li Chen,
Department of Endocrinology,
Qilu Hospital,
Shandong University,
Wenhua Xilu 107,
Jinan, Shandong 250012, China
E-mail: nfmcl@163.com