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Bone marrow mesenchymal stem cells promote the repair of islets from diabetic mice through paracrine actions

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ABSTRACT

Transplantation of bone marrow mesenchymal stem cells (MSCs) has been shown to effectively lower blood glucose levels in diabetic individuals, but the mechanism has not been adequately explained. We hypothesized that MSCs exert beneficial paracrine actions on the injured islets by releasing biologically active factors. To prove our hypothesis, we tested the cytoprotective effect of conditioned medium from cultured MSCs on isolated islets exposed to STZ in vitro and on mice islets after the experimental induction of diabetes in vivo. We assessed islet regeneration in the presence of conditioned medium and explored the possible mechanisms involved. Transplantation of MSCs can ameliorate hyperglycemia in diabetic mice by promoting the regeneration of β cells. Both β cell replication and islet progenitors differentiation contribute to β cell regeneration. MSC transplantation resulted in increases in pAkt and pErk expression by islets in vivo. Treatment with MSC-CM promoted islet cell proliferation and resulted in increases in pAkt and pErk expression by islets in vitro. The MSC-CM-mediated induction of β cell proliferation was completely blocked by the PI3K/Akt inhibitor LY294002 but not by the MEK/Erk inhibitor PD98059. Together, these data suggest that the PI3K/Akt signal pathway plays a critical role in β cell proliferation after MSC transplantation.

1. Introduction

Type 1 and type 2 diabetes result from a lack of functional β cells. Pancreas and islet transplantation have proven successful in restoring functional β cells. However, donor organ shortage has limited the clinical application of these transplantations and incentivized efforts to generate insulin-producing cells from other sources. Two possible solutions exist: identifying insulin-producing cells to be used for cell therapy and promoting the regeneration of endogenous damaged β cells.

Considerable efforts have been directed toward the development of efficient protocols for the differentiation of stem cells of either embryonic or adult origin into insulin-expressing cells. Heretofore, robust glucose-induced insulin secretion, the main function of β cell, has only been possible in hESC-derived cells after in vitro transplantation for several months (Kroon et al., 2008; D'Amour et al., 2006). However, the application of hESCs has ethical issues and can cause teratoma formation and other problems (Furth and Atala, 2009). These issues remain to be addressed and require resolution before this approach can become a therapeutic option. In addition to identifying an exogenous source of β cells, another approach would be to increase the inherent β cells mass by promoting the regeneration of a patient's own β cells. If this method becomes possible, the limitations of in vivo regeneration could be overcome without requiring immunosuppressive therapy.

Recently, different types of bone marrow-derived cells have been proposed as potential sources of cells for diabetic cell therapy (Hess et al., 2003; Banerjee et al., 2005; Hasegawa et al., 2007; Lee et al., 2006; Zhao et al., 2008; Madec et al., 2009). One strategy uses mesenchymal stem cells (MSCs). Adult bone marrow-derived MSCs are multipotent stem cells that, given their ease of isolation, low immunogenicity, and amenability to ex vivo expansion, are optimal candidates for diabetic cell therapy (Pittenger and Martin, 2004). Indeed, transplantation of bone marrow MSCs has been demonstrated to be an effective strategy for the repair of diabetic islets in experimental models (Lee et al., 2006). Seven registered

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clinical trials on type 1 and/or type 2 diabetes in phase I/IIcan be found on the website http://www.clinicaltrial.gov (Si et al., 2011). In these clinical trials, MSCs exhibited exciting therapeutic effects in diabetic volunteers (Jiang et al., 2011). However, the mechanisms underlying these therapeutic effects have not been clearly defined. Bone marrow-derived MSCs have been shown to secrete various cytokines, including VEGF and FGF (Madec et al., 2009). Through these actions, the transplantation of MSCs has been experimentally reported to improve diabetic islets and promote the expansion of endogenous islet progenitors (Si et al., 2012; Park et al., 2010). However, the underlying mechanisms of this phenomenon are not yet fully understood. We hypothesized that MSCs exert beneficial paracrine actions on the injured islets by releasing biologically active factors. To prove our hypothesis, we tested the cytoprotective effect of conditioned medium from cultured MSCs on isolated islets exposed to STZ in vitro and on mice islets after the experimental induction of diabetes in vivo and assessed the regeneration of islets in the presence of conditioned medium.

2. Materials and methods

2.1. Animals

Six-week-old C57BL/6J mice were purchased from the Shanghai Laboratory Animal Center at the Chinese Academy of Sciences. Green fluorescent protein (GFP) transgenic mice with the C57BL/6J background were purchased from the Department of Cell Biology, Secondary Military Medical University, Shanghai. All animals were maintained in the animal facility of Zhongshan Hospital, Fudan University, Shanghai. The protocols of animal use complied with the principles of laboratory Animal Care (NIH Publication 85-23) and approved by the Ethics committee of Zhongshan Hospital, Fudan University.

2.2. Antibodies

Guinea pig (GP) anti-mouse insulin (Invitrogen, Carlsbad, CA, USA), rabbit anti-GFP (Millipore, Billerica, MA, USA), goat anti-mouse insulin, rabbit anti-mouse glucagon, rabbit anti-mouse insulin, goat anti-mouse nestin, goat anti-mouse PDX-1, goat anti-Pax4, goat anti-Pax6, goat anti-Nkx6.1, goat anti-Nkx2.2, goat anti-Ngn3, and goat anti-NeuroD antibodies were all purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA. Mouse monoclonal anti-BrdU antibody was purchased from Sigma–Aldrich, St. Louis, MO, USA. FITC conjugated chicken anti-mouse secondary antibody was purchased from Santa Cruz. Rhodamine-conjugated donkey anti-GP secondary antibody was purchased from Millipore. Cy2-conjugated donkey anti-rabbit and Cy3-conjugated donkey anti-goat secondary antibodies were purchased from Jackson ImmunoResearch, West Grove, PA, USA. PE anti-mouse CD117, PE anti-mouse CD29, FITC anti-mouse CD44, PE anti-mouse SCA-1, and PE anti-mouse CD34 antibodies were purchased from Biolegend, San Diego, CA, USA. 4′-6-diamidino-2-phenylindole (DAPI) was purchased from Sigma–Aldrich. Rabbit antiphosphorylated Akt (pAkt, Ser473), rabbit anti-Akt, rabbit antiphosphorylated Erk1/2 (pErk, thr202.tyr204) and rabbit anti-Erk antibodies were purchased from Cell Signaling, Beverly, MA.

2.3. Induction of diabetes

Mice were injected intraperitoneally (i.p.) with 50 mg/kg of streptozotocin (STZ; Sigma–Aldrich) daily for 7 consecutive days. STZ was solubilized in sodium citrate buffer, pH 4.5, and injected within 15 min of preparation. Non-fasting blood glucose was measured using a One Touch Sure Step meter (Johnson & Johnson, Shanghai, China) between 9:00 and 11:00 a.m. every 3 days after STZ injection and then weekly after bone marrow MSC transplantation.

2.4. Mesenchymal stem cell purification and transplantation

Bone marrow was collected from the femurs of mice or GFP-transgenic mice. The mononuclear fraction of bone marrow was isolated from a Ficoll density gradient. The nucleated cells were plated in plastic culture dishes (Corning, NY, USA) and incubated in Dulbecco's modified Eagle's medium (DMEM; Sigma–Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA) at 37 °C in a 5% humidified CO_2 atmosphere. MSCs were selected based on their adherent property of preferentially attaching to culture dishes. The cells were continuously cultured as MSCs until passage 3. The cells were then incubated with PE anti-mouse CD117, PE anti-mouse CD29, FITC anti-mouse CD44, PE-Cy anti-mouse SCA-1, or PE anti-mouse CD34 antibodies (Biolegend, San Diego, CA, USA). Isotype-identical antibodies served as controls. Passage 3 MSCs were incubated to differentiate into adipocytes, osteoblasts in corresponding induction medium for 3 weeks. MSCs adipogenic differentiation medium kit (Cyagen, Santa Clara, CA, USA) includes medium A and B. Medium A includes basal medium A 175 ml, FBS 20 ml, Glutamine 2 ml, Insulin 400ul, Indomethacin 200ul, Dexamethasone 200ul. Medium B contains Basal medium 175 ml, FBS 20 ml, Glutamine 2 ml and Insulin 400ul. For adipogenesis, we followed the instruction of adipogenesis protocol. MSC osteogenic differentiation kit (Cyagen, Santa Clara, CA, USA) contains basal medium 175 ml, FBS 20 ml, Glutamine 2 ml, Ascorbate 400ul, β-Glycerophosphate 2 ml and Dexamethasone 20ul. For osteogenic differentiation, we followed the instruction of osteogenesis protocol. Eight hours before transplantation, recipient mice were irradiated (500 cGy), and MSCs (6.5×10^6) were transplanted into the diabetic mice through their tail veins.

2.5. Conditioned medium

Conditioned medium was generated as follows: 80% confluent, passage-3 MSCs in 10-cm tissue culture dishes (Corning) were fed with 5 mL of serum-free DMEM per dish for 12 h in a chamber. The conditioned medium was further concentrated (50 times) by ultra-filtration using 5-kDa cut-off centrifugal filter units (Millipore) following the manufacturer's instructions.

2.6. Isolation of islets

After the mice were killed, their pancreata were inflated via the pancreatic duct with type V collagenase (0.7 mg/mL in Hank's balanced salt solution, Sigma), excised, and digested at 37 °C for 7–10 min. The resulting digest was washed twice with cold Hank's balanced salt solution containing 5% bovine serum albumin, and the islets were separated using a Histopaque density gradient. The interface-containing islets were removed and washed with Hank's balanced salt solution containing bovine serum albumin, and the islets were resuspended in RPMI 1640 medium (Gibco, Grand Island, NY, USA) containing 5% bovine serum albumin, 100 U/mL penicillin, and 100 ug/mL streptomycin. Following 2 h of incubation at 37 °C, the islets were handpicked into fresh media and washed twice in PBS. The islets were fed with RPMI 1640 medium containing 10% fetal bovine serum albumin on plastic dishes at 37 °C in a 5% humidified CO_2 atmosphere. Each dish (30 mm) contained approximately 40 islets. For islet injury, we prepared the solution of STZ (10^{-2} mmol/L). And each dishes added about 100 μl solution of STZ. After an hour later, we changed the medium and stop the injury of STZ. For MSC-CM treatment, we changed the