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Bone Marrow-Derived Mesenchymal Stem Cells Restored High-Fat-Fed Induced Hyperinsulinemia in Rats at Early Stage of Type 2 Diabetes Mellitus

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Abstract

Numerous studies have proposed the transplantation of mesenchymal stem cells (MSCs) in the treatment of typical type 2 diabetes mellitus (T2DM). We aimed to find a new strategy with MSC therapy at an early stage of T2DM to efficiently prevent the progressive deterioration of organic dysfunction. Using the high-fat-fed hyperinsulinemia rat model, we found that before the onset of typical T2DM, bone marrow-derived MSCs (BM-MSCs) significantly attenuated rising insulin with decline in glucose as well as restored lipometabolic disorder and liver dysfunction. BM-MSCs also favored the histological structure recovery and proliferative capacity of pancreatic islet cells. More importantly, BM-MSC administration successfully reversed the abnormal expression of insulin resistance-related proteins including GLUT4, phosphorylated insulin receptor substrate 1, and protein kinase Akt and proinflammatory cytokines IL-6 and TNFα in liver. These findings suggested that MSCs transplantation during hyperinsulinemia could prevent most potential risks of T2DM for patients.

Keywords

mesenchymal stem cells, insulin resistance, high fat diet, type 2 diabetes mellitus, lipometabolic disorder

Introduction

Diabetes mellitus (DM) is a major risk factor for many diseases such as ischemic heart disease and stroke, chronic kidney disease, and blindness among adults[1–3]. Long-term high-fat food is one of the causes leading to insulin resistance (IR) followed by a compensatory hyperinsulinemia[4–6]. Because of the high secretary activity, β cells are constantly exposed to various kinds of stresses, such as glucolipotoxicity and oxidative stress[7,8]. Eventually, this results in β-cell death, which is characterized as typical type 2 diabetes mellitus (T2DM) characterized by hyperinsulinemia and hyperglycemia[9,10].

IR might last for 10 years before the onset of β-cell dysfunction and diabetes[11]; therefore, alleviation of IR at the early stage could be the most efficient approach to prevent progressive and inexorable β-cell dysfunction. Clinical treatment of T2DM including oral antidiabetic drugs and exogenous supply of insulin could reverse neither IR nor β-cell dysfunction[12,13].

Mesenchymal stem cells (MSCs) can differentiate into different types of connective tissue cells, which have the

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capability to produce bone, adipose, and cartilage, modulate the local environment, activate endogenous progenitor cells, and secrete various factors[14,15]. Through clinical trials and mouse models, they have successfully restored insulin and stimulated glucose uptake in typical T2DM[16–20]. However, most effects are based on regenerating injured pancreatic tissues and anti-inflammatory or paracrine effects[16–20].

New therapeutic strategies to ameliorate IR have been tried with modulating intestinal microbiota[21], autophagy[22], and cell therapy with MSCs[23–25]. These reports present remarkable alleviation of IR by MSCs in a typical T2DM rats which has proceeded to a late state of disease. However, whether MSCs could prevent the deterioration of hyperinsulinemia at early stage of T2DM is not clear.

Therefore, we aimed to investigate the effect of bone marrow-derived MSCs (BM-MSCs) on hyperinsulinemia at the early stage of T2DM. The results suggested that early transplantation of MSCs holds a promising role in controlling the progress of T2DM at early stage.

Materials and Methods

Animals and Sample Collection

Male Sprague-Dawley rats (approximately 200 g, HFK bioscience, Beijing, China) were used for all studies. All experimental procedures were approved by Tongji Medical College, Huazhong University of Science and Technology Institutional Animal Care and Use Committee (2016IACUC number, 644). All efforts were made to reduce the number of animals tested and their suffering. Animals were fed either a normal chow diet or a high-fat diet (Animal Center, Huazhong University of Science and Technology)[26] for 4 wk. On experimental days, food was removed at 8 AM and blood was sampled 4 h later for analysis for insulin (Rat INS (Insulin) ELISA Kit, Elabscience, Wuhan, China), proinsulin (Rat PI (Proinsulin) ELISA Kit, Elabscience), triglyceride (TG) (glycerine phosphate oxidase peroxidase method, JCBio, Wuhan, China), low-density lipoprotein (LDL kit, JCBio), T-CHO (glucose oxidase-phenol amino phenazone method, JCBio), alanine aminotransferase (ALT) (microplate method, JCBio), and aspartate aminotransferase (AST) (microplate method, JCBio). Tail blood was directly subjected to glucose meter (ACCU-CHEK, Roche, Basel, Switzerland) for glucose concentration, or collected.

Oral glucose tolerance test (OGTT) was assessed when animals were fasted overnight to determine their glucose response to the oral administration (by gavage) of a solution of 20% glucose (2 g/kg) before (time 0) and 30, 60, and 120 min after administration of glucose[26].

BM-MSC Preparation and Administration

BM-MSCs were isolated following a previously described method[27]. Briefly, rats were sacrificed and their hind limbs were harvested, bone marrow was flushed out and collected in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Grand Island, NY, USA) supplied with 10% fetal bovine serum (Gibco). Thereafter cells were cultured in a 25-cm^2 flask in 5% CO_2 incubator at 37 °C. Nonadherent cells were removed after 24 h and adherent cells were passaged every week using 0.05% trypsin. About 5×10^5 (for high concentration) or 10^5 (for low concentration) cells between passages 3 to 6 were intravenously injected via tail vein.

Histological and Immunohistochemical Staining

Pancreases were freshly removed from the rats, 6-μm sections were cut immediately, and fixed with acetone for hematoxylin-eosin (H&E) staining using standard techniques. Some sections were subjected to immunohistochemical staining (animals were previously intravenously injected with BrdU for 3 days). After the process consisting of 10 min of fixation with acetone, 2 h of permeabilization with 0.3% Triton X-100 (Sigma, St. Louis, MO, USA), and 1 h of blocking with 3% albumin from bovine serum (BSA) (Gibco) at room temperature, the sections were incubated with the primary antibody rabbit anti-rat BrdU (1:100, Proteintech Group, Chicago, IL, USA) overnight at 4 °C, followed by a further incubation with the secondary antibody goat anti-rabbit IgG-TRITC (1:50, Proteintech Group) for 60 min at room temperature to detect the cycling cells in the pancreas. 6-Diamidino-2-phenylindole (DAPI, Beyotime, Shanghai, China) was used to stain the nuclei for 10 min. The pictures were captured using Immuno Floure (Olympus, Tokyo, Japan).

Western Blot

Protein extracts of liver were prepared in radio immunoprecipitation assay (RIPA) buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, and 1 mM phenylmethylsulfonyl fluoride) according to the standard methods (Beyotime, Shanghai, China). Protein concentration was determined using the bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockland, ME, USA). About 30 μg of total protein per lane was resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane. Primary antibody rabbit anti-β-actin antibody (1:10,000, TDY Biotech Co., Ltd, Beijing, China), rabbit anti-GLUT4 antibody (1:1000, Abcam, Cambridge, MA, USA), rabbit anti-p-AKT (1:1000, Cell Signaling Technology, Inc., Danvers, MA, USA), rabbit anti-p-AKT (1:1000, Cell Signaling Technology, Inc.), rabbit anti-p-insulin receptor substrate (IRS)-1 (1:500, Abcam), rabbit anti-IRS-1 (1:1000, Cell Signaling Technology, Inc.), rabbit anti-IL-6 (1:1000, Affbiotech, Changzhou, China), and rabbit anti-TNFα (1:1000, Abcam) were diluted and detected using HRP-goat anti-rabbit IgG (ASPEN, Wuhan, China) and the enhanced chemiluminescent reagent (ECL; Pierce Biotechnology).

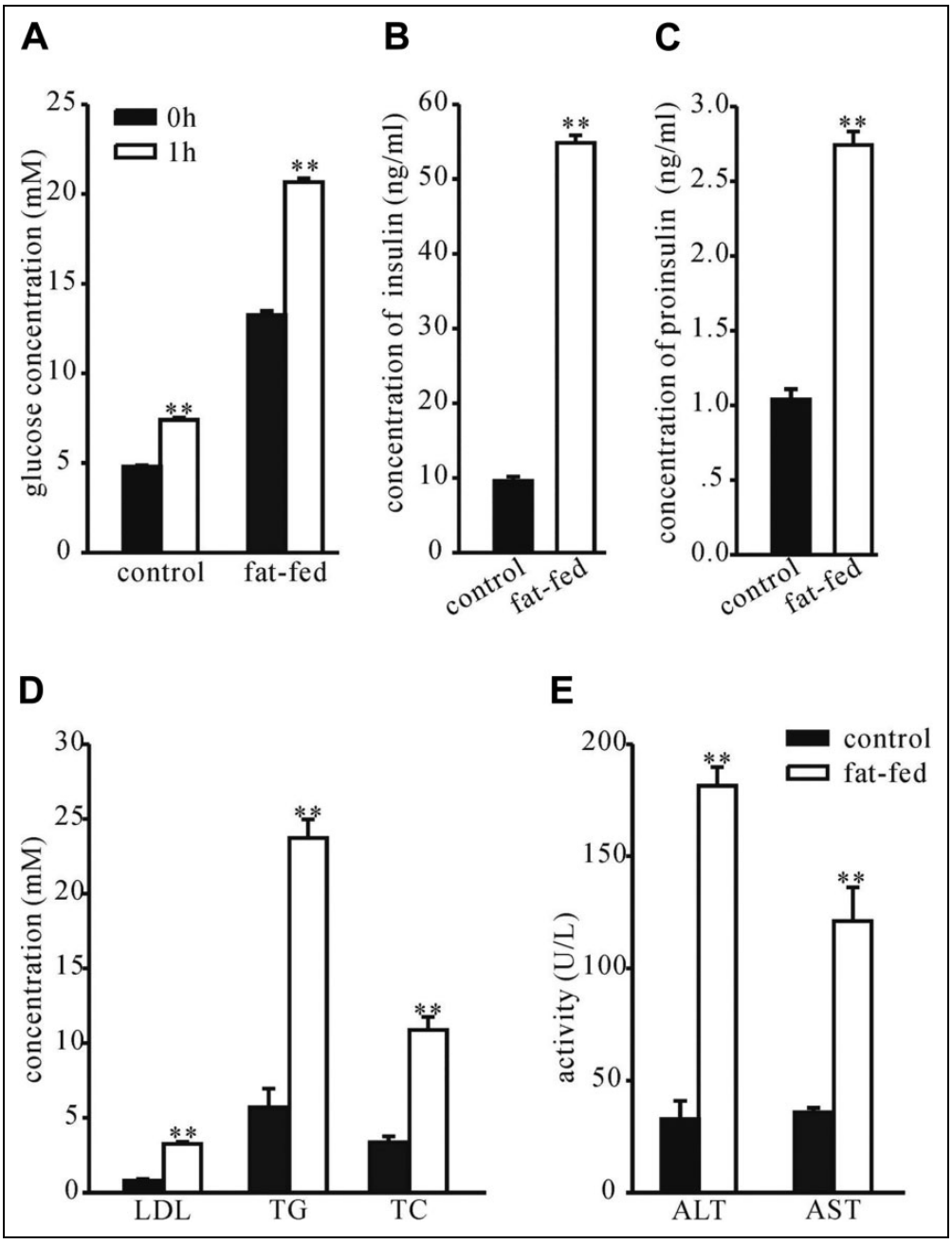

Fig. 1. Characteristics of the high-fat-fed hyperinsulinemia rat model. Glucose (A), insulin (B), and proinsulin (C) concentration increased in 4-week high-fat-fed rats with obvious deterioration in lipometabolism (D) and abnormal ALT/AST concentration (E). $^{**}P < 0.01$ vs control. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDL, low density lipoprotein; TG, triglyceride; TC, total cholesterol.

Immunoreactive bands were detected using Kodak BioMax ML film (Kodak, Rochester, New York, USA). The results were characteristic of at least three independent experiments.

Statistical Analysis

All results are presented as mean $\pm$ SEM. Treatments were compared using Student's unpaired t test or one-way ANOVA with least significant difference post hoc test. A $P < 0.05$ was considered to be statistically significant.

Results

Characteristics of the High-Fat-Fed Induced Hyperglycemia and Hyperinsulinemia Rat Models

Success of high-fat-fed hyperinsulinemia rat model was confirmed by checking blood glucose, insulin, and proinsulin, respectively (Fig. 1). Four weeks after high fat food, animals were subjected to OGTT. We measured basal blood glucose (normal diet: 4.80 $\pm$ 0.58 mM vs high fat diet: 13.23 $\pm$ 0.24 mM) as well as blood glucose levels 1 h after

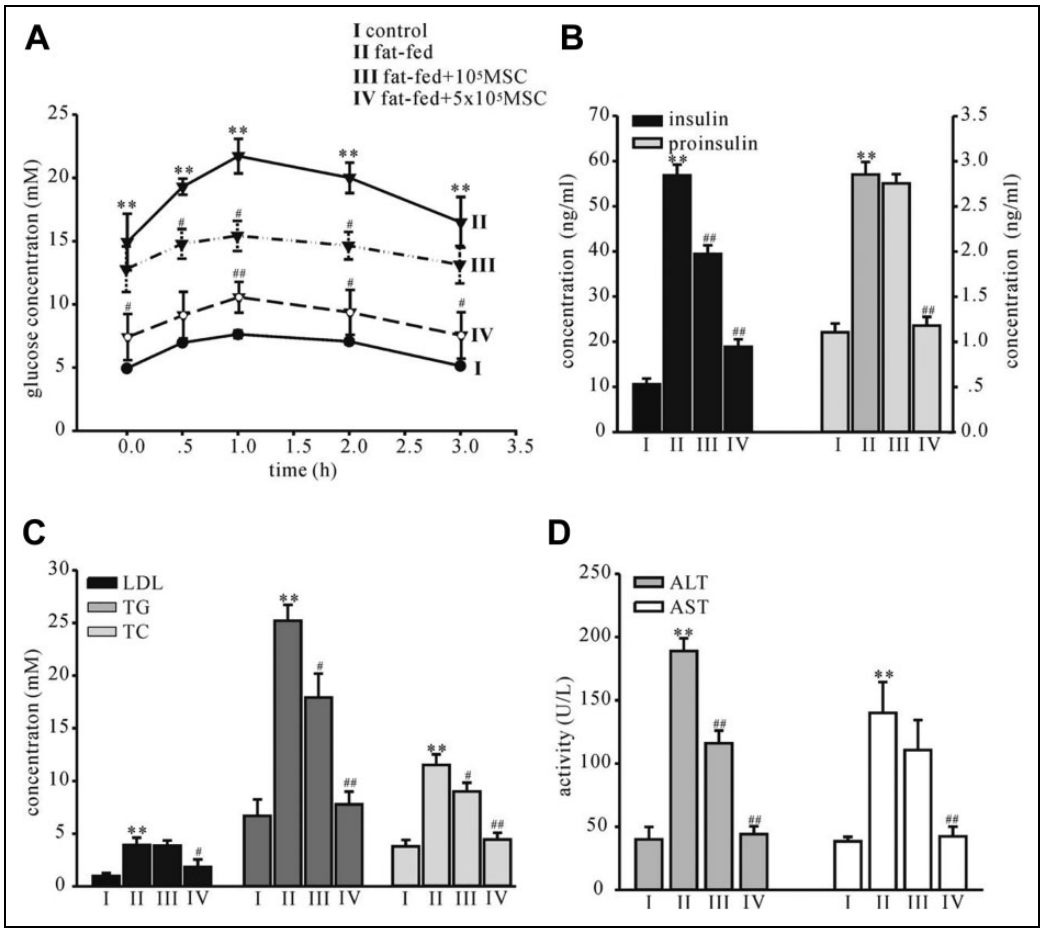

Fig. 2. BM-MSCs attenuated changes in glucose/insulin/proinsulin concentration and restored lipometabolism disorder in high-fat-fed rats. MSCs induced a significant decrease in both glucose (A) and insulin/proinsulin concentration (B). Lipometabolic disorders (C) and concentration of AST/ALT (D) were successfully restored and almost to the normal level by 5×10^5 MSCs. $^{**}P < 0.01$ vs control (I), $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs fat-fed (II). ALT, XXX; AST, XXX; BM-MSCs, bone marrow-derived mesenchymal stem cells; LDL, XXX; MSCs, mesenchymal stem cells; TG, XXX; TC, XXX.

oral administration of glucose (normal diet: 7.40 $\pm$ 0.12 mM vs high-fat diet: 20.66 $\pm$ 0.20 mM) and found that in high-fat diet rats levels were increased by 150% more than normal diet rats (Fig. 1A). Significant insulin (normal diet: 9.57 $\pm$ 0.62 ng/ml vs high fat diet: 54.84 $\pm$ 0.99 ng/ml) and proinsulin (normal diet: 1.04 $\pm$ 0.07 ng/ml vs high fat diet: 2.74 $\pm$ 0.09 ng/ml) elevations were also detected (Fig. 1B, C). These data validated that the high-fat diet rats were clinically in early stage of T2DM. Moreover, in these high-fat diet rats, significant deterioration in lipometabolic disorders and abnormal ALT/AST concentration were observed (Fig. 1D, E).

BM-MSCs Attenuated the Increased Insulin and Glucose as Well as Restored Lipometabolic Disorder in High-Fat-Fed Rats

Notably tail vein injection of MSCs induced a significant decrease in both insulin and glucose levels in high-fat-fed rats (Fig. 2A, B). Both 10^5 MSCs and 5×10^5 MSCs significantly attenuated fat-fed induced hyperinsulinemia and hyperglycemia (Fig. 2B, C); 5×10^5 MSCs exerted a greater effect, while incompletely reversed the abnormal OGTT and insulin concentration. Lipometabolic disorders and concentration of AST/ALT were successfully restored to the normal level by 5×10^5 MSCs.

BM-MSCs Favored the Histological Structure Recovery in High-Fat-Fed Hyperinsulinemia Rat Model

Fat-fed diet rats had bigger islets (Fig. 3B) than normal diet rats (Fig. 3A) but with irregular morphology and disruption of basement membrane. Lipid accumulation was observed in some islet cells (Fig. 3B). Treatments with 10^5 MSCs remarkably favored the recovery of the islets and the islet cells. Although abnormal morphology of islets was only partially rescued, lipid in the cells decreased significantly (Fig. 3C). Treatment with 5×10^5 MSCs exhibited better therapeutic

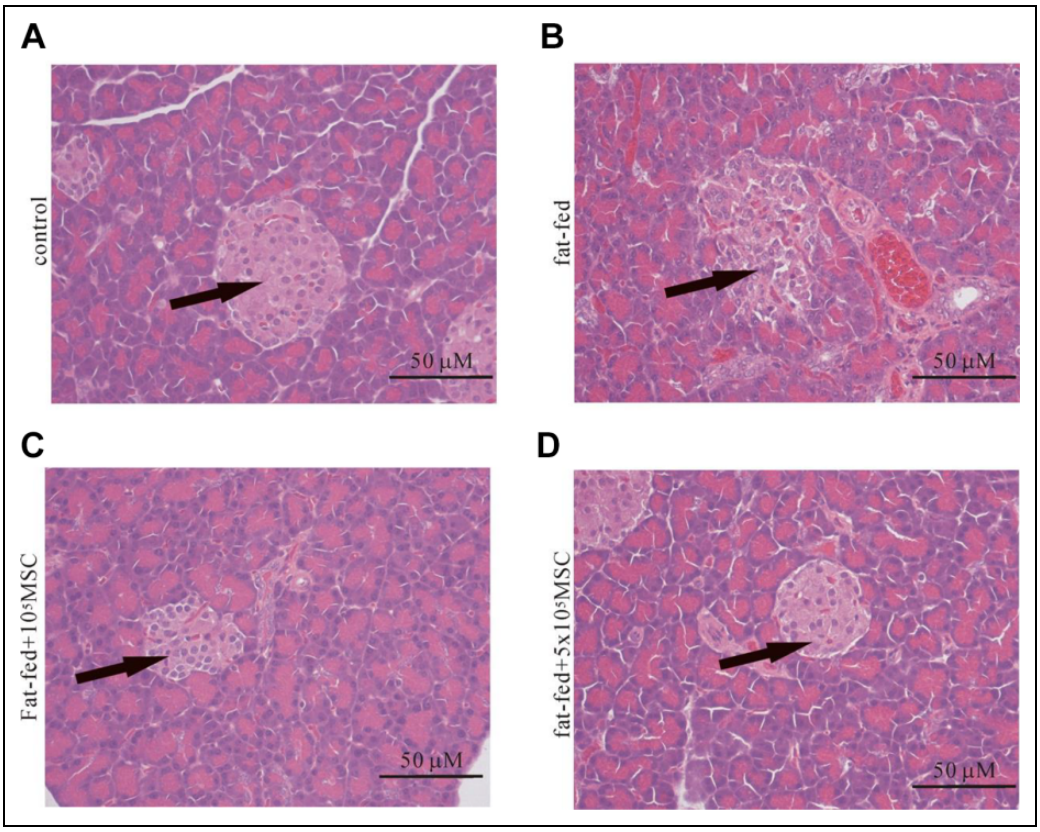

Fig. 3. BM-MSCs favored the histological structure recovery in high-fat-fed rat model. With comparison to control (A), normal diet rats with hyperinsulinemia had irregular islets with lipid accumulation in some islet cells (B). Treatment with 10^5 MSCs remarkably favored the recovery of the islet and the cells. Although islets remained partially irregular, lipid in the cells decreased significantly (C). Treatment with 5×10^5 MSCs exhibited better therapeutic effects. The islets had normal regular morphology and lipid was almost undetectable in the islet cells (D). BM-MSCs, bone marrow-derived mesenchymal stem cells; MSCs, mesenchymal stem cells.

effects, where the islets had normal morphology and lipid was almost undetectable in the islet cells (Fig. 3D). Further immunohistochemical staining of $BrdU^+$ cells in the pancreas revealed that BM-MSCs countervailed high-fat-fed induced damage to proliferation ability (Fig. 4).

BM-MSCs Reversed the Abnormal Insulin Signaling Transduction and Inflammation in High-Fat-Fed Rat Liver

Protein extracts of liver were used to investigate the influence of BM-MSCs on insulin signaling transduction. High fat-fed rats had less GLUT4 and downregulation of phosphorylated insulin receptor substrate 1 (p-IRS-1) and protein kinase Akt (p-AKT). Administration of BM-MSCs resulted in an increase of GLUT4 expression and enhanced p-IRS-1 and Akt (Fig. 5A–D). Additionally, a high fat-fed induced upregulation of proinflammatory cytokines TNFα and IL-6, which was reversed by BM-MSCs (Fig. 5E–G). These data suggest that BM-MSCs can effectively potentiate the transduction of insulin signaling and inhibit the inflammation in insulin target tissues.

Discussion

A high-caloric diet has been broadly characterized as the trigger of T2DM[28–30], and T2DM accounts for 90%–95% of all DM cases, IR being the typical symptom and mechanism at the early stage of dietary-induced T2DM[4–6,31]. High-fat-induced IR could generally associate with alterations in lipid cellular intake and accumulation, followed by disorders of the metabolism of β-cells, stroke, and other diseases[32,33]. Here in our model, the high fat diet for 4 wk successfully induced hyperinsulinemia and elevation of blood glucose concentration, which was associated with lipometabolic disorders and rising ALT/AST. With this model, we showed that the intervention of MSCs at early stage of T2DM could significantly lead to decline of insulin/glucose as well as rescue lipometabolic disorders and liver dysfunction. A much lower cell dose (5×10^5; vs 2×10^6 cells in typical T2DM model)[22,24] induced remarkable effects, which suggested an economic time window for application of MSCs in T2DM.

MSCs harbor great potential to become a routine therapeutic measure for T2DM, partially due to reversing IR.

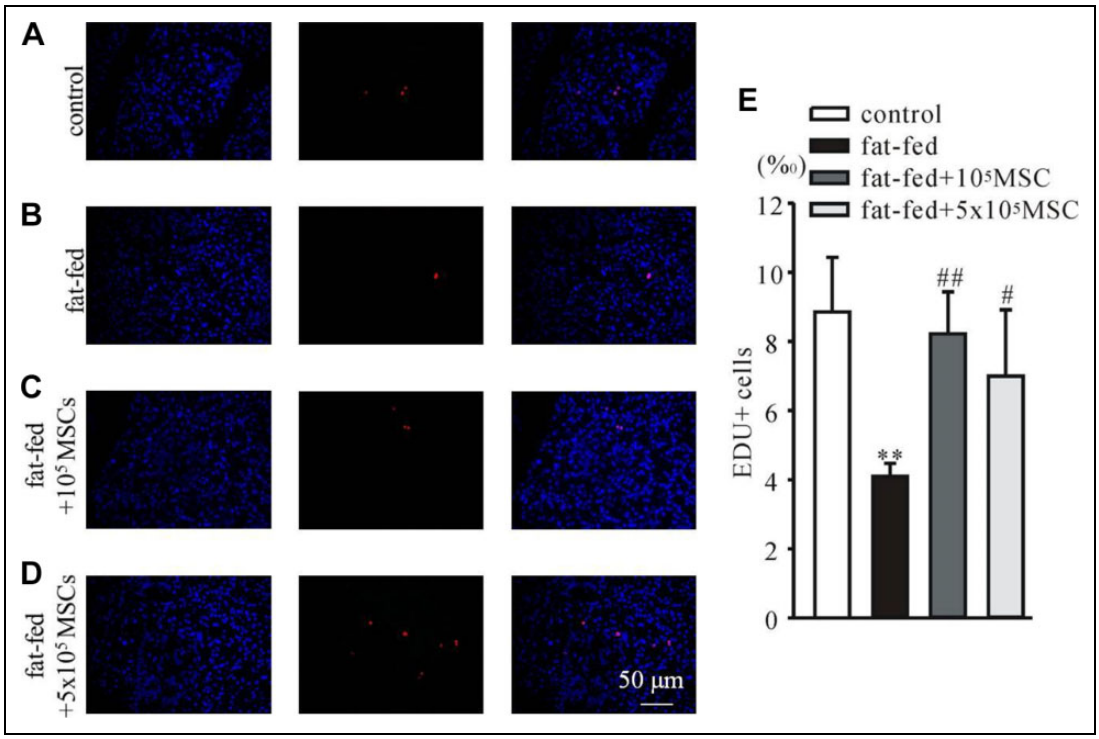

Fig. 4. BM-MSCs countervailed high-fat-fed-induced damage to proliferation ability in the pancreas. Immunohistochemical staining of pancreatic sections of control (A), fat-fed (B), fat-fed rat with treatments of 10^5 MSCs (C) and 5×10^5 MSCs (D) was performed to evaluate the cellular proliferation by detecting $BrdU^+$ (red) cells; nuclei were labeled by DAPI (blue). Statistical data (E) showed that treatments with MSCs significantly countervailed the high-fat-fed induced damage. $^{**}P < 0.01$ vs control, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs fat-fed. BM-MSCs, bone marrow-derived mesenchymal stem cells; DAPI, 4,6-diamino-2-phenyl indole; EDU, 5-ethynyl-2-deoxyuridine; MSCs, mesenchymal stem cells.

Previous studies have shown that infusion of MSCs ameliorates hyperglycemia by alleviating IR in T2DM rats[23–25]. Consistently, we found that in vivo transplantation of BM-MSCs attenuated increase in insulin and glucose resulting from a high-fat diet. It was notable that such therapeutic potential was not observed in a typical T2DM model induced by both high-fat diet and injection of streptozotocin[26], but in a high-fat diet triggered model. This strongly suggests early transplantation of MSCs could serve as a better strategy than have been proposed by previous studies to restore pancreatic or multiple organ dysfunction at later stage of T2DM[33,34].

IR could produce hyperinsulinemia, this in turn induces multiple organic dysfunction due to an abnormal intake of lipid and lipid accumulation in cells[28,32]. We found that MSCs restored lipometabolic disorders and liver dysfunction, as evidenced by the concentration of LDL/TG/TC and AST/ALT. More importantly, high-fat diet caused lipid accumulation in islet cells and disruption of the islet basement membrane; BM-MSCs favored the histological structure recovery, and obviously improved the proliferation potential of islet cells. These findings indicated that the potential risk of T2DM for other organs as well as the pancreas could be prevented if MSC transplantation is exerted at early stage of T2DM.

GLUT4 and phosphorylation of IRS-1 (p-IRS-1) and AKT (p-AK) are crucial for conferring insulin-signaling transduction, and glucose uptake therefore related intensively to IR[35,36]. Here in our high fat-fed induced IR model, BM-MSCs successfully enhanced GLUT4, p-IRS-1, and p-AKT, which is similar to the findings in typical T2DM model with MSC treatment[24]. A lot of evidence has shown that chronic activation of proinflammatory pathways within insulin target cells could lead to IR[37]. We found that BM-MSC remarkably reversed the upregulation of IL-6 and TNFα in the liver. These observations suggested that both the insulin-signaling pathway and proinflammatory pathways are involved in the favorable function of BM-MSCs in high fat-fed induced IR.

Due to the increase in associated risk factors, such as being overweight or obese, the global prevalence of (age-standardized) DM has been rising dramatically and it might become reality that 1 adult in every 10 will have diabetes in 2040[29,30]. Our study proposed a new strategy with MSC-based cell therapy for T2DM, i.e., MSC transplantation during hyperinsulinemia before onset of diabetes could prevent most possible risks of T2DM for patients.

It is the limitation of our study that we did not go further to investigate the detailed mechanisms for MSCs to reverse

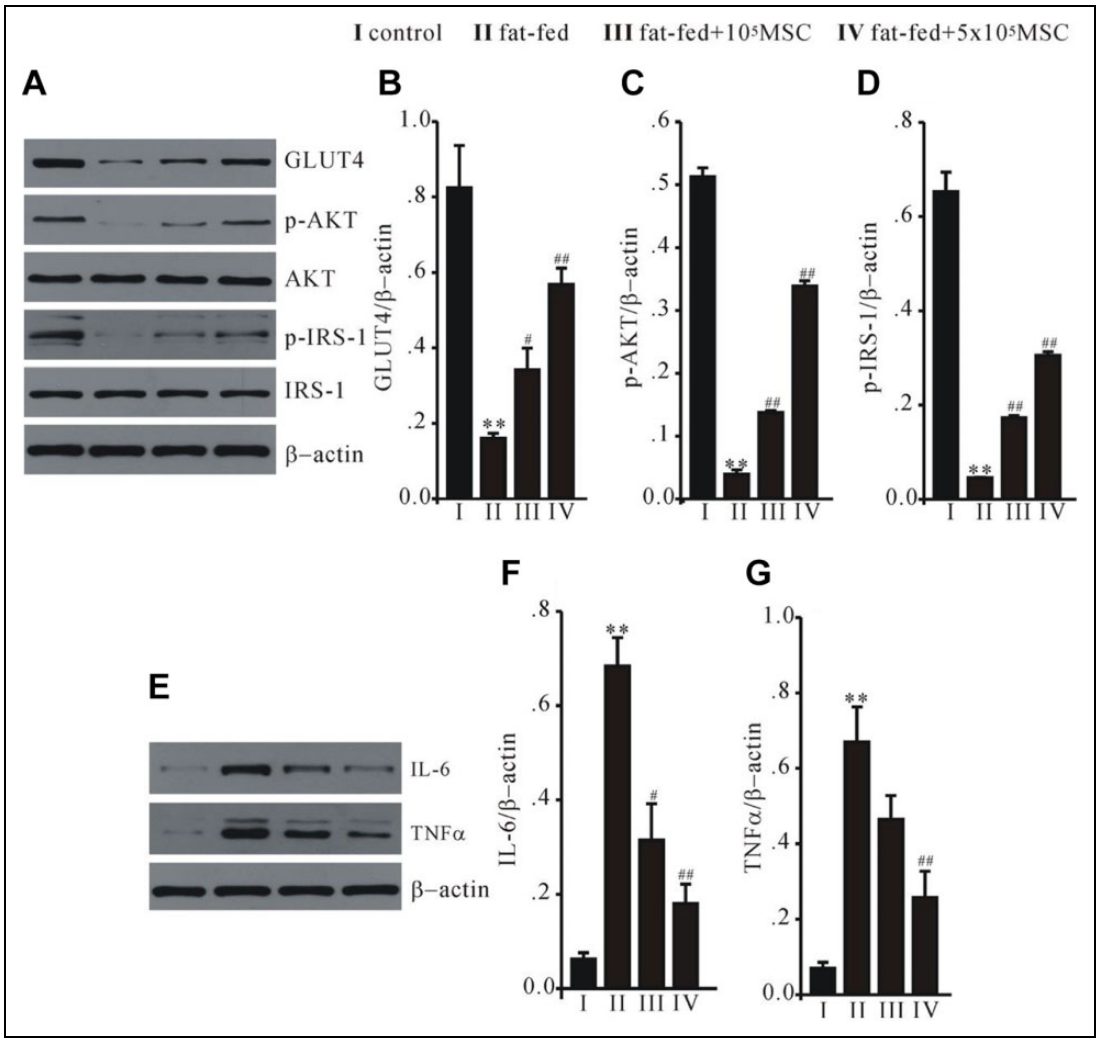

Fig. 5. BM-MSCs reversed insulin signaling transduction and inflammation in a high fat-fed rat liver. Western blot analysis on GLUT4, p-IRS-1, and p-AKT (A), and proinflammatory cytokines IL-6 and TNFα (E). Statistical data (B–D, F–G) revealed that administration of MSCs successfully reversed the abnormal expression of these proteins in fat-fed rat model. $^{**}P < 0.01$ vs control, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs fat-fed. BM-MSCs, bone marrow-derived mesenchymal stem cells; MSCs, mesenchymal stem cells.

IR in the early stage of T2DM. The widely accepted idea is that MSC infusion with host cells is one of the possibilities[24]. This could also be the underlying phenomenon in our study. Future work is necessary to validate it and to explore other possible mechanisms.

Data Availability

All the data used to support the findings of this study are included within the article files.

Ethical Approval

Ethical approval to report this case was obtained from Tongji Medical College, Huazhong University of Science and Technology Institutional Animal Care and Use Committee (Approval: 2016IACUC number, 644).

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Tongji Medical College, Huazhong University of Science and Technology Institutional Animal Care and Use Committee (Approval: 2016IACUC number, 644) approved protocols.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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