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PDX1 transfection induces human adipose derived stem cells differentiation into islet-like cells: what is the benefit for diabetic rats?

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In this study, we observed the differentiation potential of human ADMSCs (hADMSCs) into functional islet-like cells and the therapeutic effect of hADMSCs transplantation in diabetic rats. Firstly, the PDX1 gene was transfected into hADMSCs by an adenovirus. Cell differentiation and insulin secretion were identified and detected by dithizone staining and ELISA, respectively. Twenty male Sprague-Dawley rats were randomly divided into control group (n=4), diabetes group (n=8) and transplantation group (n=8). Rats in the latter two groups were subjected to making diabetic models by 65 mg/kg streptozotocin injection. Afterwards, rats in the transplantation group were given PDX1 transfected ADMSCs via the tail vein. At 15 days after transfection, the number of insulin positive cells and insulin secretion were both increased significantly ($P < 0.05$). Fasting glucose levels in the transplantation group decreased significantly ($P < 0.05$), while the body weight increased significantly ($P < 0.05$). In the diabetic group, the fasting glucose level still maintained at a high level, and the body weight of rats was significantly decreased. These results implicated that the PDX-1 gene could induce hADMSCs differentiating into functional islet-like cells. PDX-1 transfected ADMSCs transplantation is effective in treating diabetic rats, but the mechanism needs further study.

1. Introduction

For the treatment of type 1 diabetes, islet transplantation is a relatively safe treatment method (Ramzy et al. 2018). Although recent advances in clinical islet transplantation have achieved enormous breakthroughs, there are still problems with the ability to avoid exogenous insulin use and with increasing the survival rate of transplanted islets. In recent years, the use of stem cell transplantation to overcome the problem of islets shortage has become a research focus, which will deliver cells with specific functions to the corresponding damaged sites of body, where they can partially restore damaged organ functions (Schmid et al. 2018).

Adipose-derived mesenchymal stem cells have a cell phenotype similar to bone mesenchymal stem cells as CD73 +, CD90 +, CD105 +, CD45-, CD34- (Luo et al. 2018). Adipose-derived mesenchymal stem cells have a multi-directional differentiation potential, and can differentiate into adipocytes, chondrocytes, osteoblasts and nerve cells under specific culture conditions, and they can even be turn into cardiomyocytes, corneal cells etc. (Siegel et al. 2017). Adipose tissue is easy to obtain in terms of extraction and ethical issues (Xu et al. 2017).

PDX1 (pancreatic and duodenal homeobox factor-1) acts as a specific transcription factor, which plays an important role in pancreatic development: early development of pancreas, late proliferative differentiation and maintaining the function of pancreatic β-cells. PDX1 regulates gene expression related to islet β-cell function. After transfection of PDX1 into certain non-islet β cells (such as bone marrow mesenchymal stem cells, umbilical cord mesenchymal stem cells, hepatocytes), these cells can produce insulin (Zhu et al. 2017). Studies on the transformation of human adipose-derived mesenchymal stem cells by PDX1 transfection have not been reported. Positive results of stem cells in reducing blood sugar level, improving clinical symptoms and complications have achieved. At present, adipose mesenchymal stem cells used in diabetes treatment is still in the exploratory stage, and there are many problems which still need to be further resolved. Our study aimed to observe the effect of PDX1 transfection into human adipose-derived mesenchymal stem cells, and differentiation ability after cell transfection. We also investigated the therapeutic effect on diabetic rats after cell transplantation, hoping to find an effective method for diabetes treatment.

2. Investigations and results

2.1. Identification of ADSCs

After 24 h of inoculation, the non-adherent cells were removed. Two or three days later, the morphological changes could be observed. The cells were homogeneous, fibroblast-like growth, cell growth was polar, and fusion was helical (Fig.1)

The expression of CD14, CD45, CD44 and CD105 in adipose mesenchymal stem cells were detected by flow cytometry (Fig. 2). Oil-red staining results were positive after 2 weeks of adipogenesis

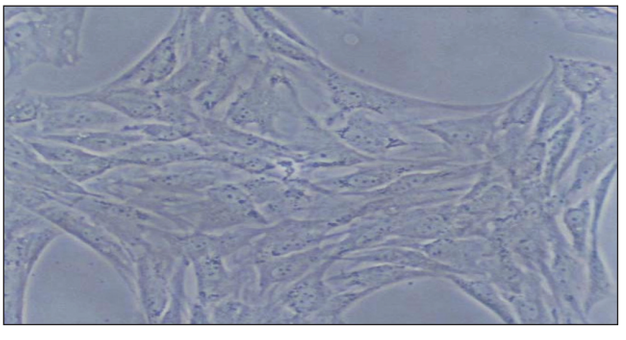

Fig. 1: Third passage of human adipose-derived mesenchymal stem cells (×200)

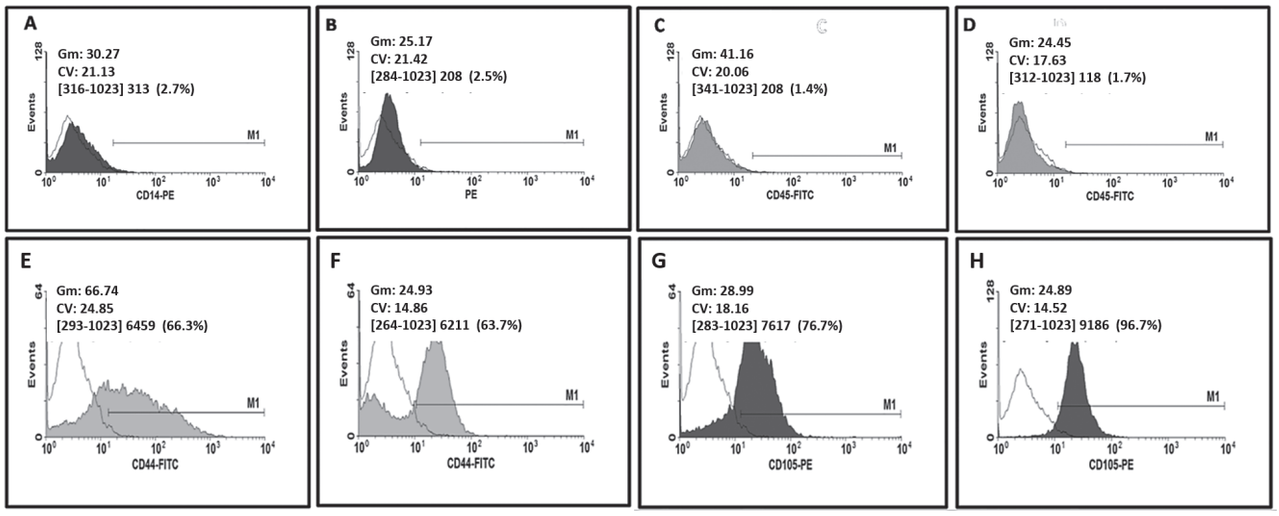

Fig. 2: Flow cytometry for human adipose-derived mesenchymal stem cells detection

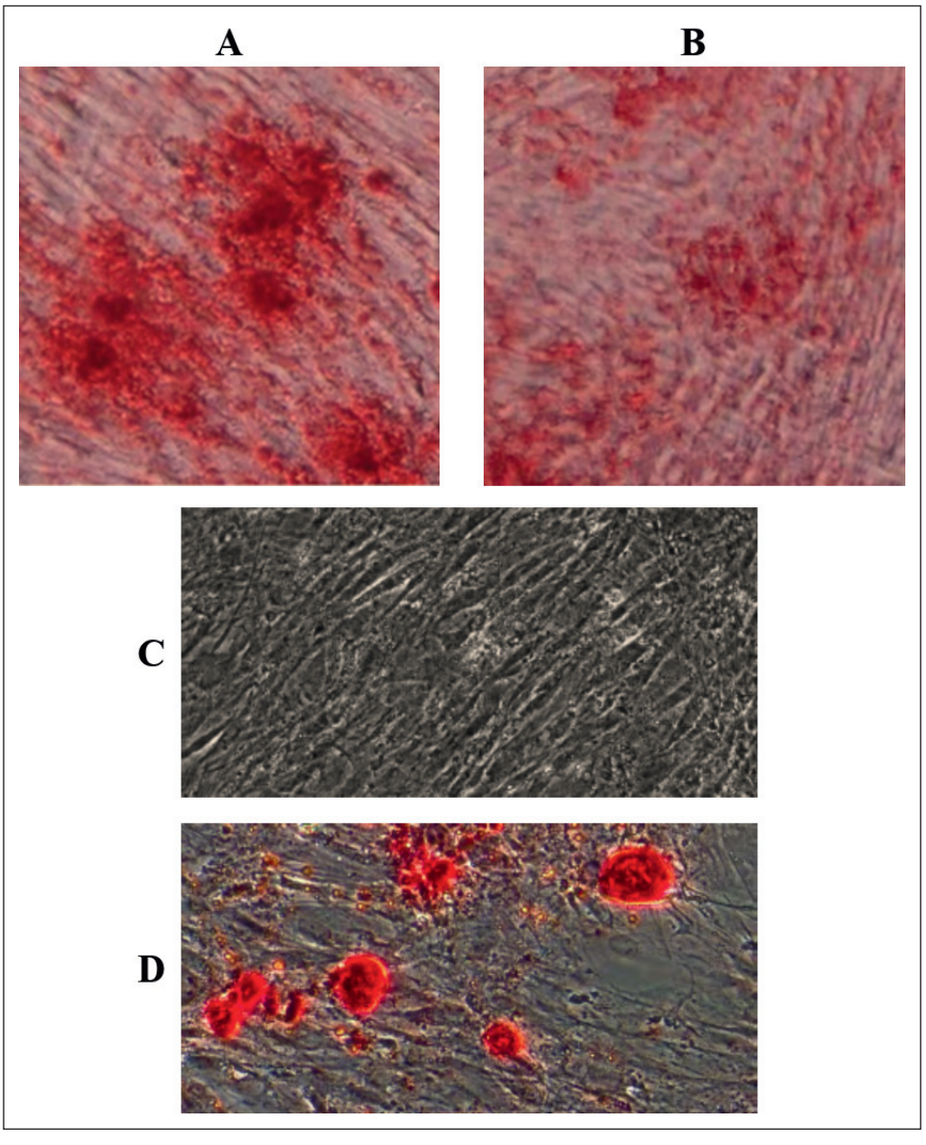

Fig. 3: Adipogenic and osteogenic differentiation potentials of human adipose-derived mesenchymal stem cells (Oil-red staining on ADSCs after adipogenic induction (A) and before adipogenic induction (B); alkaline phosphatase staining on ADSCs before osteogenic induction (C) and after osteogenic induction (D))

induction of ADSCs, demonstrating their ability to differentiate into adipocytes (Fig. 3AB); Alkaline phosphatase staining results showed strong positive reaction after 2 weeks of osteogenesis induction of ADSCs, demonstration their ability to differentiate into osteoblast (Fig. 3CD).

2.2. PDX1 expression after cell transfection

The expression of PDX1 was detected by Western blot 48h after PDX1 transfection into ADSCs, while PDX1 expression was not observed in the control group and PDX1 expression could still be detected 15 days after virus infection (Fig. 4).

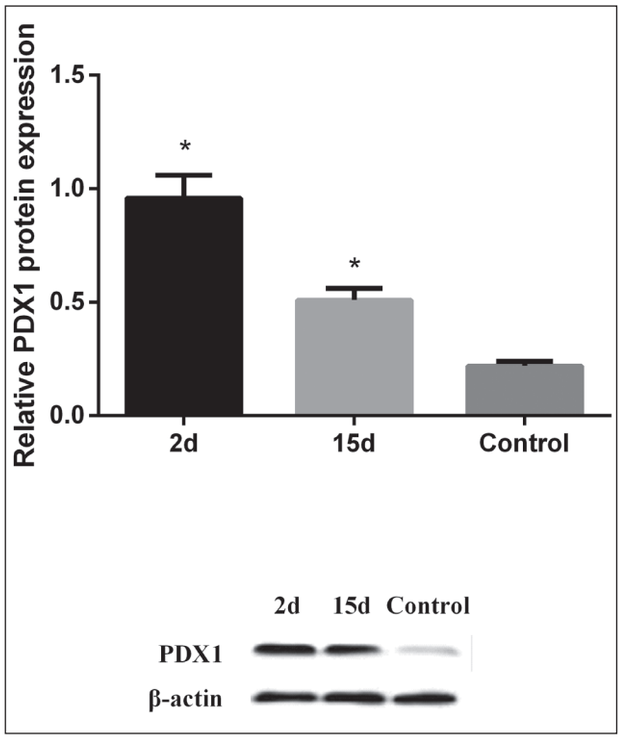

Fig. 4: Western blot for detection of PDX1 expression in transfected adipose-derived mesenchymal stem cells

2.3. Dithizone staining results

The cells were stained with dithizone and no insulin-positive cells appeared in the control group. Dithizone-positive cells were stained in the experimental group (Fig. 5).

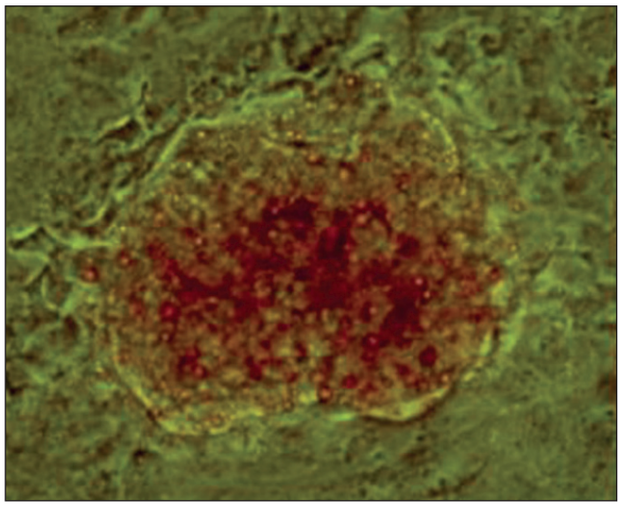

Fig. 5: 15 days after transfection, Dithizone staining showed positive result in floating islet-like cells (×200)

2.4. Insulin secretion level

There was no significant difference in insulin level secreted between the transfection group and the control group ($P > 0.05$) within low glucose medium, while there was a significant difference between the two groups ($P < 0.05$) within high glucose medium. Insulin secretions showed no significant difference before and after glucose stimulation (Fig. 6).

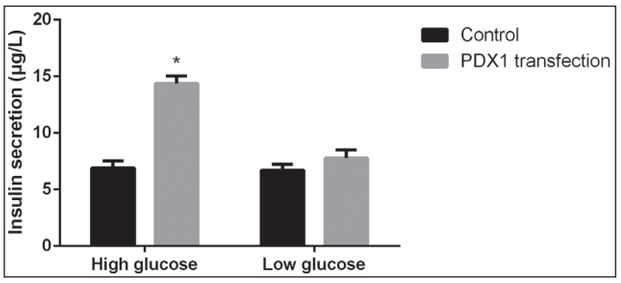

Fig. 6: Insulin secretion in each group after glucose stimulation (* indicated that $P < 0.05$ compared to control group)

2.5. Effect of PDX1 transfection on fasting blood glucose in diabetic rats

Compared with the control group, the fasting blood glucose of the model group increased significantly ($P < 0.05$), and significantly higher than that of normal group. At 2 weeks after administration, fasting blood glucose remained at a high level and higher than that in the control group ($P < 0.05$, Fig. 7). The fasting blood glucose level of transplanted rats was significantly lower than that of the

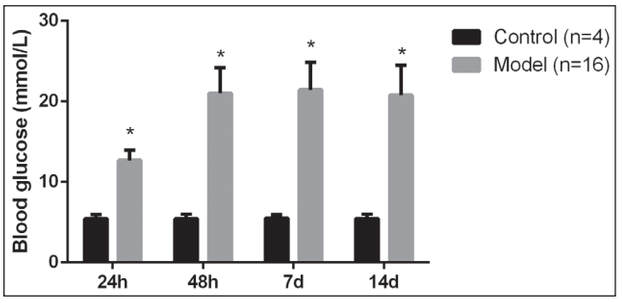

Fig. 7: Blood glucose changes in rats at different time after streptozotocin injection (* indicated that $P < 0.05$ compared to control group)

diabetic group at 14 days ($P < 0.05$). The fasting blood glucose of diabetic rats maintained a high level, without significant change (Fig. 8).

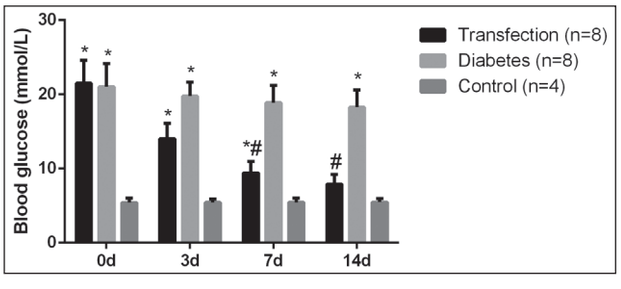

Fig. 8: Effect of PDX-1 transfected adipose-derived mesenchymal stem cells on blood glucose level in diabetic rats (* indicated that $P < 0.05$ compared to control group, # indicated that $P < 0.05$ compared to diabetes group)

2.6. Effect of PDX1 transfection and ADSCs transplantation on diabetic rats' body weight changes

After transplantation, the body weight of the transplanted rats increased gradually, which was significantly higher than that of diabetic rats ($P < 0.05$), while the body weight of diabetic rats continued to decline (Fig. 9).

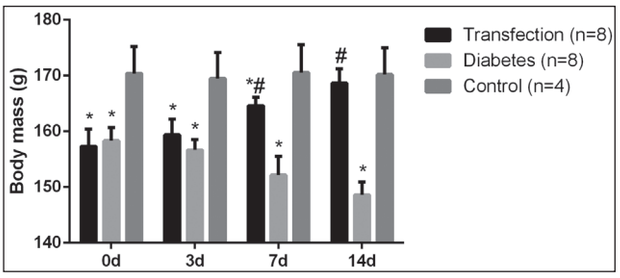

Fig. 9: Effect of PDX-1 transfected adipose-derived mesenchymal stem cells on body mass in diabetic rats (* indicated that $P < 0.05$ compared to control group, # indicated that **$P < 0.05$ compared to diabetes group)**

3. Discussion

Type 1 diabetes is mainly caused by a destruction of islet β cells which leads to an absolute deficiency of insulin secretion (Kyvsgaard et al. 2017). Studies have shown that CD4 + and CD8 + T lymphocytes which are involved in autoantigen tolerance damage, cause selective islet cell destruction and functional failure, leading to inadequate insulin secretion, causing diabetes symptoms. Insulin injection has become the main way to treat current type 1 diabetes, although it can stabilize patient's blood glucose level to improve life quality, but it cannot totally prevent the occurrence of diabetes complications. Therefore, cell therapy for type 1 diabetes attracts more and more attention.

ADSCs are widely known because of their richness and ease of separation (Li et al. 2018). In recent years, it has been found that ADSCs not only have pluripotent differentiation potential, but also play an important role in balancing blood glucose and hormone production (Sun et al. 2017). It has been reported that adipose-derived mesenchymal stem cells are better able to induce islet-like cells than bone marrow mesenchymal stem cells (Karaoz et al. 2013), making them an ideal insulin-secreting cell source. Our study used the type I collagenase digestion method, and high purity ADSCs were obtained from adipose tissues. PDX1 gene is one of the main regulators of pancreatic development which can also promote pancreatic progenitor cell differentiation into β cells. At early stages of embryonic development, PDX1 is expressed in both endocrine and exocrine cells, which can induce endodermal development and maturation to pancreas, activation of β cells can promote expression of important genes and maintain normal function of β cells. During development of the pancreas, PDX1 expression is limited to β cells (Thurner et al. 2017). The principle of differentiation from stem cell into insulin cells is to initiate the expression of PDX-1 in stem cells (Vinogradova and Sverdlov 2017), thereby expressing more islet cell-specific genes and altering function and morphology of stem cells (Kimura et al. 2017). Studies have shown that PDX1 overexpressing in mouse adipose-derived stem cells can induce expression of pancreatic specific transcription factors like Ngn3, Nkx2-2, and insulin 1 in stem cells (Zhao et al. 2017).

Recombinant adenovirus is a tool for gene transfer and expression as, first of all, it can infect a variety of cells, including stem cells. Secondly, the virus titer is high; thirdly, a high titer of the virus can achieve high infection rates and high expression; fourthly, the virus genome is not integrated into cell chromosomes. Because of these advantageous characteristics, adenovirus gain many researchers' preferences, however, preventive measures are needed during whole experiments to ensure safety and transfection efficiency. Our study used the human serotype 5 adenovirus, which is the most widely used replication-defective adenovirus. ADSCs were infected with adenovirus carrying PDX1 gene for 48 h. Western blot and immunofluorescence showed that the adenovirus had a high infection ability to ADSCs. Since zinc ions are rich in islet cells, and dithizone is a zinc chelating agent, this is a specific staining method to identify islet cells. Fifteen days after transfection, the islet-like cell mass was observed under inverted microscope, and dithizone staining showed a brownish red coulour. Results of ELISA showed that PDX1 transfection significantly increased the differentiation percentage from ADSCs to insulin-positive cells, suggesting that PDX1 could significantly promote differentiation of ADSCs into islet-like cells, which is similar to the studies of Grapin-Botton et al. (2001) and Feber et al. (2000), which used PDX1 to induce hepatocytes, small intestinal epithelial cells and adipose-derived mesenchymal stem cells to differentiate them into islet-like cells.

Studies have shown that stem cell transplantation can effectively improve renal function in patients with advanced diabetes and improve patient survival rate (Song et al. 2017). In our study, compared with the control group, the rats in the model group were injected with streptozotocin, and 48 h after injection, the fasting blood glucose of the rats in model group increased significantly, the rats in the transplanted group were treated with PDX1 transfected adipose-derived mesenchymal stem cells, and their fasting blood glucose decreased gradually, and remained lower than that of the model group 2 weeks later. While the fasting blood glucose of diabetic rats maintained at a high level without significant changes during whole study. The body weight of rats in the ADSCs transplanted group increased gradually, then two weeks later, the body weight of rats was significantly higher than that of the diabetes group, while the body weight of diabetic rats decreased continuously. The results suggested that PDX1 transfection in adipose-derived mesenchymal stem cells can effectively reduce blood glucose in diabetic rats, which is important for the clinical application of ADSCs in diabetes treatment. However, the localization of ADSCs transfected by adenovirus carrying PDX1 in rat, the toxic and side effects to rats and its molecular mechanism of diabetes treatment need further investigations.

In summary, human ADSCs are readily available and can be induced to differentiate into islet-like cells by transfection of adenovirus carrying PDX1 gene into ADSCs. The use of insulin cells derived from ADSCs as cell therapy has a broad research prospects and application value in the future for type 1 diabetes treatments.

4. Experimental

4.1. Animals

Male SD rats weighing 180-200 g were obtained from the Wenzhou Medical University Animal Center. Animals were housed at constant room temperature with a 12:12 hour light-dark cycle, and fed with a standard rodent diet and water. The animals were acclimatized to the laboratory for at least 7 days before use in experiments. Protocols involving the use of the animals were approved by the Wenzhou Medical University Animal Policy and Welfare Committee (Approval documents: wydw2014- 0001). All animal care and experiments were performed in accordance with the approved protocols and the 'The Detailed Rules and Regulations of Medical Animal Experiments Administration and Implementation' (Document No. 1998-55, Ministry of Public Health, China).

4.2. Reagents and instruments

Type I collagenase, dithizone, streptozotocin (Sigma, USA); DMEM/F12, DMEM-Low glucose and DMEM-High Glucose Medium (HyClone, USA); Fetal Bovine Serum (Gibco, USA); 40 g/L paraformaldehyde, Triton X-100, trypsin inhibitor (Solarbio, Beijing); Trypsin, 4', 6-diamidino-2-phenylindole (Beyotime Biotechnology, Jiangsu); human insulin ELISA kit (Boster Biological, Wuhan); Rabbit anti-human CD29, CD73, CD49d, CD90, CD14, CD45, CD34, HLA-DR, PDX-1 antibody (Bioss, Beijing); carbon dioxide incubator (Thermo, USA); inverted phase contrast microscope (Nikon, Japan); flow cytometry (Becton Dickinson, USA); fluorescence microscopy and color photography system (Yuguang Instrument, Shanghai); microplate reader (Thermo, USA).

4.3. Adipose derived stem cell culture

Adipose tissues were obtained from patients with cholelithiasis aged 35-50 years (without other co-morbidity) in the hepatobiliary department of our hospital. Patients and their families gave their informed consent, and research was approved by the hospital ethics committee. Abdominal adipose tissue was obtained under sterile conditions. After cutting the tissue, samples were centrifuged at 1500 r/min for 10 min. The upper indigested adipose tissue was removed, the same volume of DMEM/F12 medium (containing the volume fraction of 10% fetal bovine serum) was added to terminate the digestion, then it war filtered with sterile 200 mesh cell screen. The filtrate was centrifuged again at room temperature (1500 r/min 10 min). The supernatant was discarded and the bottom sediment was resuspended in DMEM/F12 medium (containing 10% fetal bovine serum). The cells were inoculated into 25cm2 cell culture flask, and cultured in a cell incubator (37 °C, volume fraction 15% CO2). After 24-36h, cell growth was observed under inverted microscope. When the cell

growth reached 80% -90%, the cells were passaged by trypsin digestion in 1:2 ratio. In the process of cell passage, cell growth was observed under inverted microscope and recorded.

4.4. Identification of ADSCs

CD14, CD45, CD44, CD105 were used as positive markers for adipose-derived stem cells, while CD14, CD45 were negative markers, their purity was detected by flow cytometry. The adipose-derived mesenchymal stem cells were labeled with oil red O two weeks after adipogenesis induction, and were stained with alkaline phosphatase 2 weeks after osteogenesis induction.

4.5. Transfection of ADSCs with recombinant adenovirus

The adenovirus carrying PDX1 gene was synthesized by Vigenebio Co., Ltd (Shandong, China). The pdx-1 gene was cloned from the NM00029 gene sequence into the pAD-Amp vector. Adenovirus is packaged with the His, Flag at the C-terminus of the vector. Human serotype 5 adenovirus was selected as the adenovirus vector in our study. ADSCs of third generation (cell confluence was between 50 % and 70 %) were inoculated into 6-well plates. Before the infection, the original culture medium was abandoned, and complete culture medium was added, the virus solution were added with MOI = 50 into the cells. The recombinant medium was incubated for 48 h at 37 °C. The expression of PDX-1 gene was detected by Western blot and immunofluorescence.

4.6. Western blot

Cell lysates were prepared by suspending 1×106 cells in 150 µl of RIPA lysis buffer (50 mM Tris, 150 mM NaCl, 1% Triton X-100,1% sodium deoxycholate,0.1% SDS, 100 mM phenylmethylsulfonyl fluoride) followed by centrifugation at 12000 rpm for 5 min at 4 °C to collect the supernatant. Then, 20 mg of cell lysates were separated by 12 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto nitrocellulose membranes. The membrane was probed with relevant primary antibodies and corresponding HRP-conjugated secondary antibodies. West Pico chemiluminescent (Thermo Fisher, USA) was used to visualize protein bands. The intensity of bands was quantified using ImageJ software. Normalization of specific proteins was performed based on GAPDH expression.

4.7. Dithizone staining

Cells were washed twice with D-Hank's buffer solution at 15 days after infection. Add 1 mL of D-Hank's buffer and 10 µL of dithizone stock solution, 37 °C incubation for 30 min, samples were observed under inverted microscope.

4.8. Enzyme linked immunosorbent assay

15 days after the end of cell transfection, samples were rinsed twice with 1 × PBS, 37 °C incubated for 1 h; 1 × PBS slowly washed 2 times, then add 25.0 mmol / L high glucose DMEM medium. 37 °C incubation for 1 h, the insulin content in the culture medium was determined by ELISA.

4.9. Animals grouping

Four among twenty SD rats were used as control group, and the other 16 were used to establish the diabetes model. After the model was successfully established, the rats were randomly divided into transplantation group and diabetes mellitus group. The rats in transplantation group were transplanted with ADSCs. Diabetes models were established by intraperitoneal injection of streptozotocin (65 mg/kg) dissolved in citrate buffer (pH 4.0), blood glucose concentration after 48 h of injection > 16.8 mmol/L indicated the success of diabetic model. The rats in transplanted group were injected with 1 mL of transfected adipose mesenchymal stem cells (cell concentration 1.5×10^9 L^{-1}). The fasting blood glucose and body mass were measured at regular time after transplantation.

4.10. Statistical analysis

Data were presented as the mean±standard deviation (SD) and analyzed using SPSS 13.0 statistical software (SPSS Inc., USA). Statistical analysis between two sets of data was performed using Student's t-test. $P < 0.05$ was considered statistically significant.

Conflicts of interest: None declared.

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