Am J Transl Res 2018;10(8):2445-2456
www.ajtr.org /ISSN:1943-8141/AJTR0075977

Original Article

Islet transplantation attenuates cardiac fibrosis in diabetic rats through inhibition of TGF-β_1/Smad3 pathway

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Received March 13, 2018; Accepted July 2, 2018; Epub August 15, 2018; Published August 30, 2018

Abstract: Although islet transplantation has been identified as a promising endocrine replacement treatment for patient with diabetes mellitus (DM), it still remains unclear whether islet transplantation can inhibit the diabetic-induced myocardial injury and subsequent adverse ventricular remodeling. Here, we sought to explore the molecular mechanism underlying the cardioprotective effect of islet transplantation. We established the diabetic rat model by intraperitoneal injection of STZ, which was followed by either islet transplantation or conventional insulin treatment. Compared with insulin treatment, islet transplantation further reduced the elevated blood glucose which was nearly restored to normoglycaemia. In addition, islet transplantation attenuated the increased levels of cTn-I and CK-MB, cleaved-caspase-3 in response to DM, and ameliorated diabetic-induced cardiac hypertrophy and interstitial fibrosis, along with improved extracellular matrix (ECM) deposition. Moreover, diabetic rats that underwent islet transplantation had lower expression of TGF-β1 and lower phosphorylation levels of Smad3. Therefore, islet transplantation exerted protective effect against diabetic-induced myocardial injury and fibrotic remodeling through deactivation of TGF-β_1/Smad3 signaling pathway.

Keywords: Diabetes mellitus, islet transplantation, cardiac fibrosis, TGF-β_1/Smad3 signaling

Introduction

Diabetes mellitus (DM), a chronic metabolic disease, is characterized by either impaired insulin secretion or the loss of insulin producing pancreatic β cells, which results in hyperglycaemia and is closely related with cardiovascular events, stroke, kidney failure or peripheral neuropathy [1]. Myocardial dysfunction secondary to direct cardiomyocytes loss or atherosclerotic complications is the leading cause of morbidity and mortality in DM patients, indicating the heart as a primary target of DM pathological process [2]. Previous studies have demonstrated that oxidative stress, inflammation, apoptosis, cardiac hypertrophy and interstitial fibrosis are the major determinants of diabetic-induced myocardial injury [3-5]. Notably, excessive reactive interstitial fibrosis and dysregulated extracellular matrix (ECM) cross-link initially decrease the compliance of mycardium, subsequent perivascular fibrosis hinders the diffusion of oxygen and disrupts the supply-consumption balance, contributing to both diastolic and systolic dysfunction [6]. Therefore, fibrotic response may have a pivotal role in the diabetic remodeling of myocardium and heart failure, thus providing a rational for targeting the diabetic-induced cardiac fibrosis.

Although the insulin is seen as the well-accepted therapeutic intervention in clinical practice, it is limited by unstable glucose control, severe hypoglycaemia and insulin resistance, thus failing to reduce the long-term complication rate. Currently, pancreatic transplantation or islet transplantation is recognized as the most effective endocrine replacement treatment for type 1 or type 2 diabetes, with restored endogenous insulin secretion by the provision of suf-

ficient β cells [7-9]. In contrast to pancreatic transplantation, islet transplantation has been developed as a minimal invasive approach, which was eligible for patients (older than 50 years) that were at higher risk of postoperative complications [10]. Evidence from previous studies has demonstrated that islet transplantation or pancreatic transplantation could retarded the progression of hyperglycemia-induced nephropathy, neuropathy and retinopathy, and even reversed it in terms of excellent metabolic control [11, 12]. Additionally, pancreas transplantation has been associated with improved histological changes of left ventricular and cardiac function in diabetic patients [13]. However, thus far the beneficial effect of islet transplantation on diabetic-induced myocardial injury have not yet been reported and the underlying molecular mechanism remained unclear.

The present study was designed to explore the potential cardioprotective roles of islet transplantation in diabetic hearts. Our results confirmed that islet transplantation protected against diabetic-induced myocardial dysfunction by reducing diabetic-induced interstitial fibrosis through inhibition of transforming growth factor-β_1 (TGF-β_1)/Smad3 signaling pathway.

Material and methods

Animal

A total of 42 male Wistar rats weighing 200-220 g were provided by the Experimental Animal Center of Wenzhou Medical University. All the animals were housed in an environment with temperature of 24 ± 1°C, relative humidity of 50 ± 1% and a light/dark cycle of 12/12 hr. In addition, all animal studies (including the mice euthanasia procedure) were done in compliance with the regulations and guidelines of Wenzhou Medical University institutional animal care and conducted according to the AAALAC and the IACUC guidelines.

Establishment of diabetic models

Diabetes mellitus was induced over a period of 8 weeks using a single intraperitoneal (I.P.) injection of streptozotocin (STZ) (50 mg/kg) in 0.1 ml of citrate buffer (pH 4.5). Subsequently, glucose levels in tail vein blood were measured using an Accu-Check Active glucometer (Roche, Basel, Switzerland); rats with nonfasted glucose concentrations > 16.67 mmol/l that was maintained for 3 days were used as experimental diabetic model. Twelve weeks later, rats were randomly divided into three different groups and were treated with saline, insulin (3U at 9 a.m. and 9 p.m./day, I.P.) or islet transplantation. After 4 weeks, rats were anaesthetized and sacrificed for further study. Normal rats were used as control.

Islet transplantation and assessment

Islets transplantations were performed according to our protocols as previously described [14]. Briefly, rats were anesthetized with chloral hydrate; pancreas was then exposed, separated from the surrounding tissues, and digested at 37°C. After washing, the islets were centrifuged, purified and cultured in RPMI-1640 (Gibco, Carlsbad, CA, USA) containing 10% FBS, 2 mM L-glutamate, 100 U/ml penicillin and streptomycin. The activity of purified islets was determined by FDA-PI staining with a fluorescence microscope. Subsequently, the kidney of recipient rat was exposed, the islet was gently transferred into the capsule after capsulotomy, and the incision was sutured layer-by-layer. 4 weeks after transplantation, hematoxylin-eosin (HE) staining and immunofluorescence were applied for assessing the function of insulin secretion.

Histopathology

The hearts were rapidly excised, fixed in 4% formalin, dehydrated, and embedded in paraffin. To determine the cross-sectional area or the extent of fibrosis, 5 μm sections were deparaffinized and stained with HE and Masson's trichrome according to the manufacturers' instruction, respectively. Interstitial fbrosis was quantified as the percentage of fibrotic area over the total LV area. Images was obtained using a Leica DMI3000B microscope and analyzed by Image-Pro Plus software.

Immunohistochemistry

Tissue sections were deparaffinized and rehydrated. Subsequently, the sections were incubated with 3% hydrogen peroxide and followed by 5% goat serum. Immunohistochemical α-SMA, collagen Ia1 and collagen IIIa1 staining of LV sections were performed using anti-α-SMA (Abcam), anti-collagen Ia1 (Abcam), anti-collagen IIIa1 (Abcam) and cleaved-caspase-3

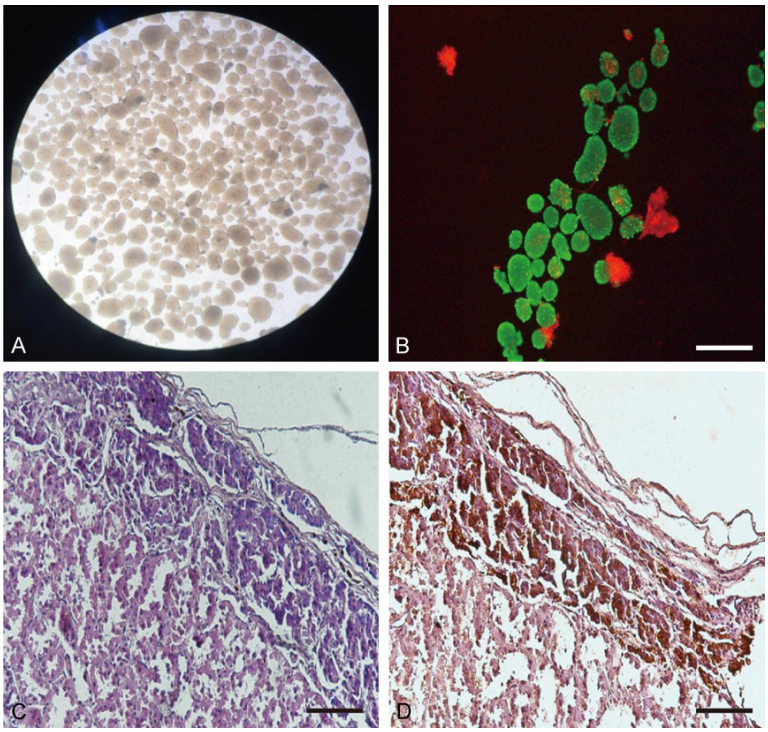


Figure 1. The morphology and activity of isolated islets before transplantation. A. Representative images of isolated islets from rat pancreas. The morphology of islets were observed and assessed under microscope. B. Immunofluorescence staining for FDA-PI to determine the activity of isolated islets. The contrast in relative fluorescence intensity reflected a high level activity (> 99%) of isolated islets. Bar = 25 μm. C. Representative HE staining of transplanted islets under kidney capsule. Islets were injected and subsequent located under the kidney capsule revealed by HE staining. Bar = 25 μm. D. Immunohistochemistry staining of insulin after islets transplantation. Bar = 25 μm.

(Abcam) antibodies with a dilution factor of 1:200. The sectioned tissue was then incubated with goat anti-rabbit secondary antibody and visualized with DAB. The extent of immunostaining was assessed using Image-Pro Plus software.

Western blot analysis

Western blotting was performed as described previously [15]. Briefly, hearts were extracted, homogenized in lysis buffer and centrifuged. 50 μg proteins were fractionated by SDS-PAGE and transferred to PVDF membranes. After blocking with 5% non-fat milk, the membranes were incubated with following primary antibodies at 4°C overnight: TGF-β_1 (CST, 1:1000), α-SMA (Abcam, 1:1000), cleaved-caspase-3 (Abcam, 1:500), p-Smad3 (Abcam, 1:500) and CTGF (CST, 1:1000), Bcl-2 (Abcam, 1:1000), Bax (Abcam, 1:500), cleaved-caspase-3 (Abcam, 1:500). After washing, the membranes were incubated with secondary antibodies, and the bands were visualized with ECL and quantified using Image-Pro Plus software.

Quantitative real-time PCR

Total RNAs were prepared using Trizol reagent (Invitrogen, USA) as described previously. Quantitative real-time RT-PCR was then performed in an ABI 7500 Fast (Applied Biosystems) with the following primers: ANP F: 5'-CTGCTAGACCACCTGGAGGAGAAG and R: 5'-TCATCGGTCTGCTCGCTCAGG-3'; β-MHC F: 5'-GTGCCAATGACGACCTGAAGGAG-3' and R: CTGGTTGATGAGGCTGGTGTTCTG-3'; collagen-IA1 F: 5'-TGTTGGTCCTGCTGGCAAGAATG-3' and R: 5'-GTCACCTTGTTCGCCTGTCTCAC-3'; collagen-IIIA1 F: 5'-GACACGCTGGTGCTCAAGGAC-3' and R: 5'-GTTCGCCTGAAGGACCTCGTTG-3'; α-SMA F: 5'-GCGTGGCTATTCCTTCGTGACTAC-3' and R: 5'-CCATCAGGCAGTTCGTAGCTCTTC-3'; CTGF F: 5'-CACCGCACAGAACCACCACAC-3' and R: 5'-GGCAGGCACAGGTCTTGATGAAC-3'; GAPDH F: 5'-ACAAGGCTGCCCCGACTAC-3' and R: 5'-CTCCTGGTATGAAATGGCAAATC-3'. For each sample, the transcript levels was normalized to that of GAPDH.

Measurement of blood glucose, cTn-I, and CK-MB

Blood samples were collected and centrifuged. Plasma was than isolated and the extent of myocardial injury was assessed by measuring cTn-I, and CK-MB levels according to the manufacturers' instruction.

Statistical analysis

Statistical analysis was performed utilizing SPSS 20.0. Data were presented as the means ± SD. Statistical significance among the four groups (control group, DM group, DM + insulin group and DM + islet transplantation group) was initially determined by one-way ANOVA

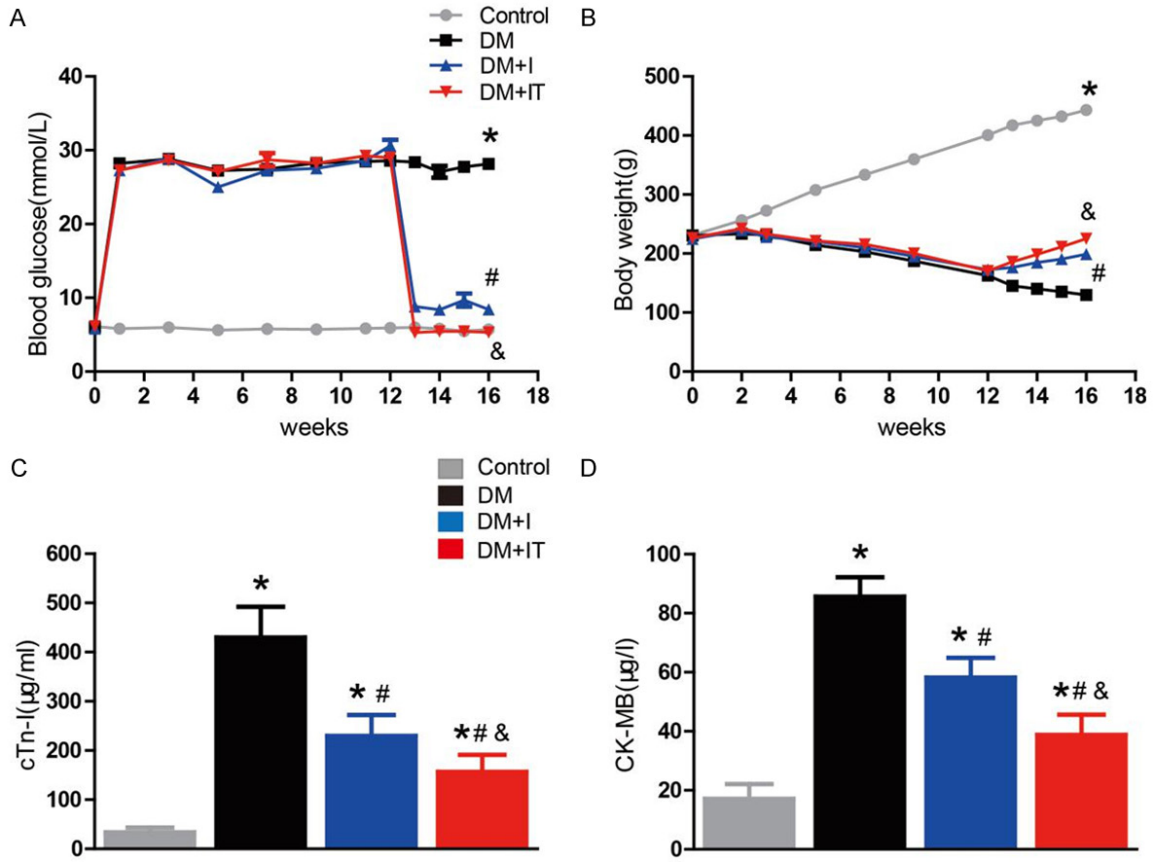


Figure 2. Effect of islets transplantation on blood glucose, body weight and biomarker of cardiac injury. A and B. The blood glucose levels and body weight in each group (n = 6-8) over the 16 weeks. The blood glucose of diabetic rats was significantly increased and maintained at the high level during the 16 weeks, which was significantly reduced by insulin treatment and nearly restored to normal level by islet transplantation. Meanwhile, diabetic rats treated with islet transplantation significantly gained weight in comparision with insulin treatment. C and D. Quantitative analysis of of cTn-I and CK-MB (n = 6 for each). Diabetic induced myocardial damage was associated with a higher levels of cTn-I and CK-MB, which was significanly alleviated by insulin treatment. However, islet transplantation further decreased the release of cTn-I and CK-MB from the myocardium. DM, diabetes mellitus. I = insulin, IT = islets transplantation. $^{*}P < 0.05$ vs. Control. $^{\#}P < 0.05$ vs. DM. $^{\#}P < 0.05$ vs. DM + I.

test, and further evaluated with Turkey post hoc analysis for data homogeneous variance. When the data were heterogeneous variance, Dunnett's T3 test was performed for post hoc analysis. And $P < 0.05$ was considered statistically significant.

Results

Therapeutic effects of islet transplantation on diabetic-induced myocardial damage

The islets were separated from the rat pancreas, purified and cultured for transplantation. The morphology of islets was evaluated under microscope (**Figure 1A**). Immunofluorescence staining for FDA-PI identified a high-level activity (> 99%) of isolated islets (**Figure 1B**). After transplantation, HE staining revealed that the islets were located under the kidney capsule with normal function for insulin secretion (**Figure 1C**, **1D**). After STZ injection, glucose level in the blood was markedly elevated, however rats treated with insulin or islet transplantation significantly reduced the blood glucose (**Figure 2A**). Compared with normal control, the body weight in diabetic rats gradually decreased during the study, whereas rats partially gained weight after insulin treatment or islet transplantation (**Figure 2B**). Diabetic rats presented increased cTn-I and CK-MB release, however, insulin treatment markedly decreased all biochemical markers of myocardial damage (**Figure 2C**, **2D**). Surprisingly, islet transplantation showed lower levels of cTn-I and CK-MB when compared with insulin treatment.

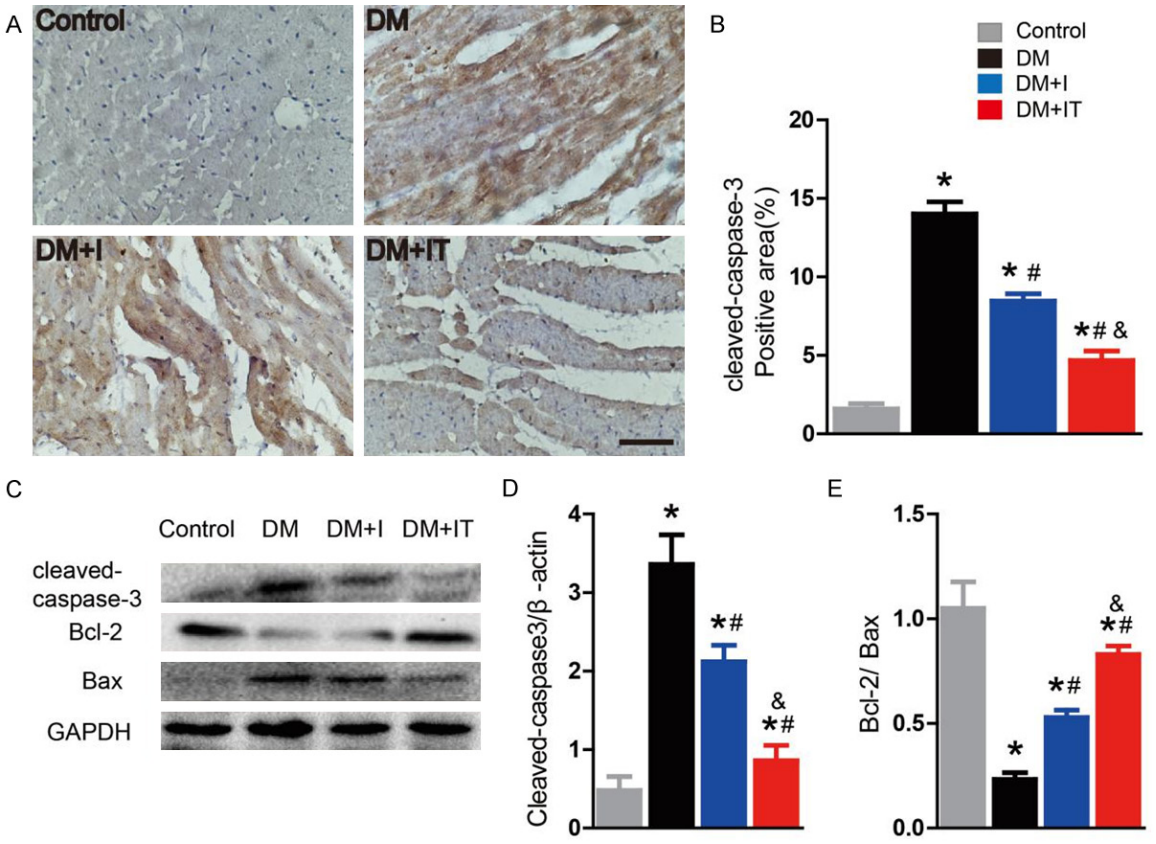


Figure 3. Islets transplantation reduces cardiomyocytes apoptosis in diabetic rats. A and B. Representative immunohistochemistry images and quantitative analysis of cleaved-caspase-3 in myocardium (n = 6 for each). Immunohistochemistry staining showed a larger area of cleaved-caspase-3 in the myocardium of diabetic rats, however, insulin treatment was associated with a smaller area of cleaved-caspase-3 and further decreased by islet transplantation. Bar = 25 μm. C. Representative WB images of cleaved-caspase-3, Bcl-2 and Bax. D and E. Quantitative analysis of the expression of cleaved-caspase-3 and the ratio of Bcl-2/Bax (n = 6 for each). DM induced the protein expression of cleaved-caspase-3 and upregulated the ratio of Bcl-2/Bax, nonetheless, islet transplantation significantly inhibited the activation of caspase-3 and attenuated the dysregulation of Bcl-2 and Bax. DM, diabetes mellitus. I = insulin, IT = islets transplantation. *P < 0.05 vs. Control. #P < 0.05 vs. DM. #P < 0.05 vs. DM + I.

Islet transplantation attenuated diabetic-induced myocardium apoptosis

Apoptosis was deeply implicated in the pathogenesis of diabetic-induced myocardial damage; thus we determined the activity of caspase-3 in the heart of diabetic rats. Immunohistochemistry staining presented an increased expression of cleaved-caspase-3, which had an important role in triggering the subsequent progression of apoptosis (**Figure 3A**, **3B**). Compared with insulin treatment, islet transplantation showed a more profound protection against myocardial apoptosis by decreasing the expression of cleaved-caspase-3, which was further convinced by western blotting analysis (**Figure 3C**, **3D**). In addition, the expression levels of Bcl-2 and Bax were respectively, decreased and increased, thus contributing to reduced Bcl-2/Bax ratio (**Figure 3C**, **3E**). Insulin treatment restored the Bcl-2/Bax ratio; however, islet transplantation further reduced the dysregulation of both Bcl-2 and Bax.

Islet transplantation improved myocardial remodeling in diabetic rats

Diabetic-induced myocardial damage was followed by adverse myocardial remodeling; therefore, we investigated the cardiac hypertrophy and interstitial fibrosis in diabetic rats. As shown in **Figure 4C**, insulin treatment significantly reduced the diabetic-induced cardiac hypertrophy as evidenced by the heart weight (HW)/body weight (BW) ratio, nonetheless, islet transplantation further decreased the HW/BW ratio. Subsequently, HE staining demonstrated

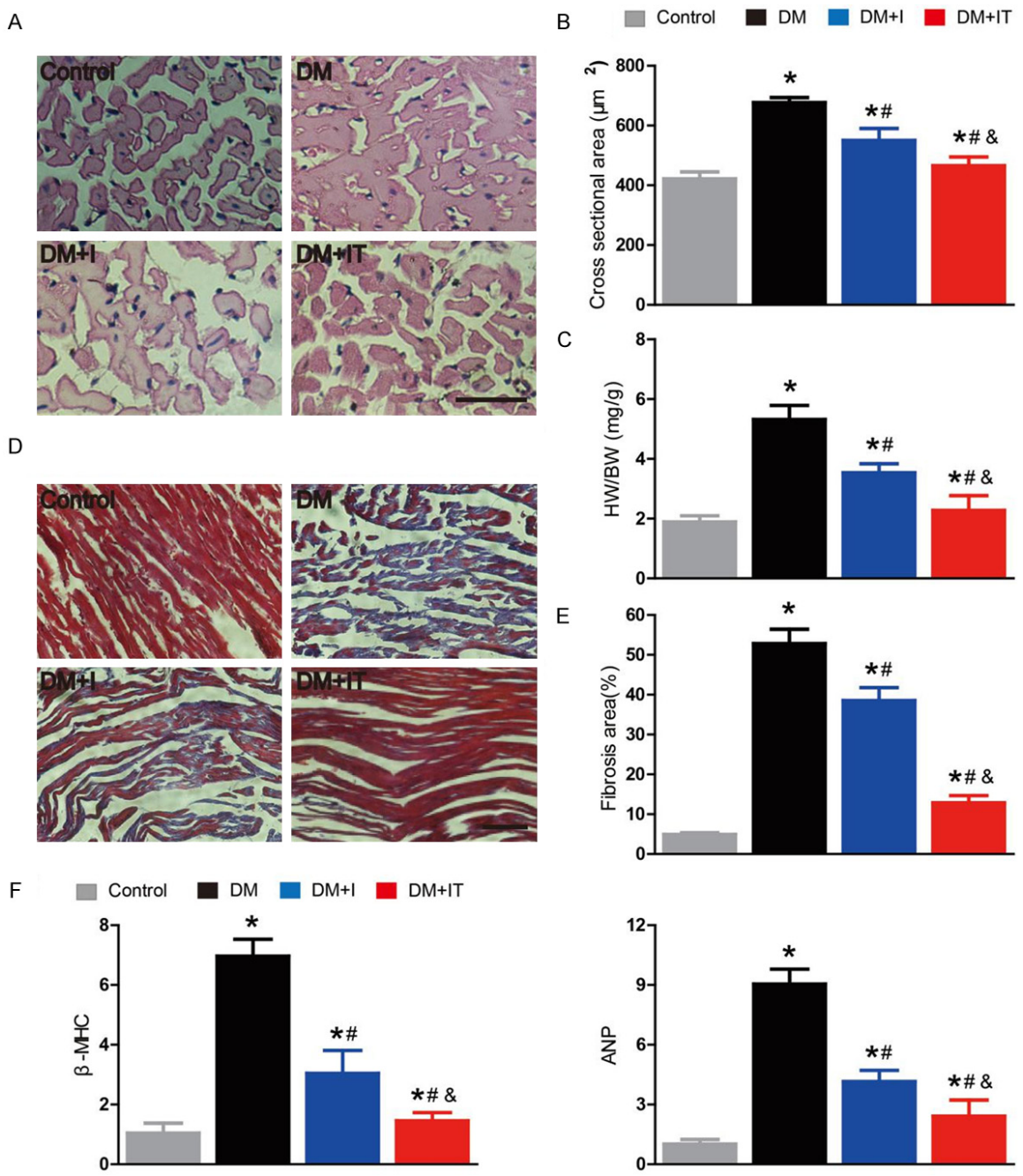


Figure 4. Islet transplantation attentuates myocardial remodeling in diabetic rats. A. Representative HE staining of myocardium. Bar = 25 μm. B. Quantitative analysis of cross-sectional area of cardiomyocytes (n = 6 for each). The cross-sectional area of cardiomyocytes was significantly larger than that of control, and decreased by insulin treatment. Intriguingly, islet transplantation further decreased the cross-sectional area of cardiomyocytes in comparision with insulin treatment. C. Measurement of H/W ratio (heart weight/body weight) in each group (n = 6-8 for each). The increased H/W ratio of diabetic rats was significantly reduced by insulin treatment and further reduced by islet transplantation. D and E. Representative Masson's trichrome staining and quantitative analysis of fibrosis area in myocardium (n = 6-8 for each). Diabetic induced myocardial damage was associated with a severe cardiac fibrosis, islet transplantation showed a more significant protection against fibrotic remodeling than insulin treatment. Bar = 25 μm. F. Transcription levels of ANP and β-MHC in the myocardium (n = 4-6 for each). DM induced the mRNA expression of ANP and β-MHC in the myocardium, islet transplantation significantly decreased the transcription levels of them when compared with insulin treatment. DM, diabetes mellitus. I = insulin, IT = islets transplantation. $^{*}P < 0.05$ vs. Control. $^{\#}P < 0.05$ vs. DM. $^{\#}P < 0.05$ vs. DM + I.

that administration of insulin attenuated the increased cross-sectional area of cardiomyocytes in diabetic rats (**Figure 4A**, **4B**). In accordance with the HW/BW ratio, islet transplantation presented smaller cardiomyocytes size than insulin treatment. Unsurprisingly, the transcript levels of ANP and β-MHC, two markers of cardiac hypertrophy were lower in islet transplantation group than that in insulin treatment group (**Figure 4F**). Additionally, severe interstitial fibrosis, a hallmark of cardiac remodeling, was detected in myocardium of diabetic-rats (**Figure 4D**, **4E**). As expected, fibrosis was consistently decreased by insulin treatment and was further alleviated in diabetic rats that underwent islet transplantation.

Islet transplantation inhibits diabetic-induced ECM accumulation

Cardiac fibrosis was characterized by myofibroblasts transformation and excess ECM deposition. Immunohistochemistry analysis showed that DM induced the expression of collagen-Ia1, collagen-IIIa1 and CTGF in myocardium, indicating the deposition of ECM. Insulin treatment significantly alleviated the increase in collagen-Ia1, collagen-IIIa1 and CTGF, while islet transplantation further reduced their expression (**Figure 5A-D**). Moreover, western blot analysis showed that islet transplantation was associated with a significantly lower levels of α-SMA compared with insulin treatment, suggesting lower cardiac fibroblasts differentiation (**Figure 5E**).

Additionally, real-time PCR revealed that the transcript levels of collagen-Ia1, collagen-IIIa1, CTGF and α-SMA were increased in the heart of diabetic rats. Consistently, islet transplantation further blunted the diabetic-induced expression of all aforementioned gene except collagen-IIIa1, when compared with insulin treatment (**Figure 5F**).

Effects of Islet transplantation depend on deactivation of the TGF-β_1/Smad3 signaling pathway

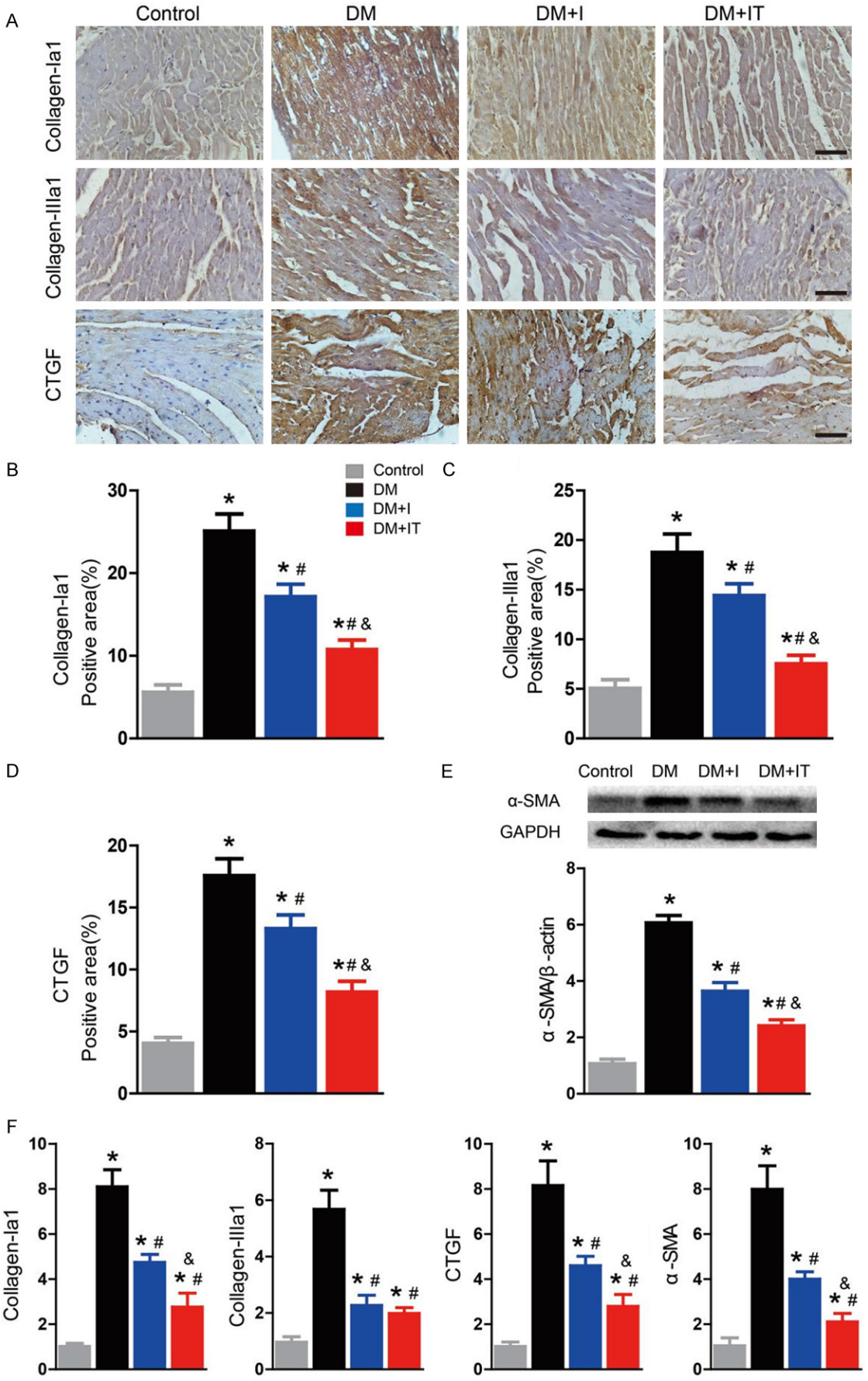
TGF-β_1/Smad signaling pathway was the well-established molecular mechanism underlie the cardiac fibrosis, thus we determined the effects of islet transplantation on the expression of TGF-β_1 and p-Smad3. DM increased the expression of TGF-β_1 and phosphorylated Smad3 with no impact on total Smad3 (**Figure 6A-D**), yet, insulin treatment reduced the protein levels of TGF-β_1 and p-Smad3, while islet transplantation further inhibited the activation of TGF-β_1/Smad3 signaling pathway.

Discussion

The present study provided novel insights into the molecular mechanisms underlying the cardioprotective effect of islet transplantation in diabetic rats. Data from our study showed that STZ injection successfully induced hyperglycemia, and that insulin treatment partially reduced the blood glucose, whereas islet transplantation almost restored the function of β cell, which was evidenced by normalization of the glucose levels in the blood. Additionally, we demonstrated that islet transplantation protected the rats from diabetic-induced myocardial injury, inhibited myocardial hypertrophy and interstitial fibrosis, and decreased accumulation of ECM thus attenuating adverse ventricular remodeling. Furthermore, these beneficial effects were associated with decreased activation of TGF-β_1/Smad2/3 signaling pathway.

Islet transplantation has been developed as a promising endocrine replacement treatment for diabetic patients, and especially for those patients with type I diabetes mellitus. It provides an alternative to pancreatic transplantation due to minimal invasiveness, which makes it appropriate for patients with high risk of surgical complications [9, 16]. Compared with exogenous insulin treatment, the main advantage of islet or pancreatic transplantation is that it restores the function of β cells and mimics the physiological adjustment of insulin secretion, therefore maintaining normal fluctuations of blood glucose and ameliorating the cytotoxicity of hyperglycemia. Unexpectedly, in the present study, diabetic rats with islet transplantation almost got their normoglycaemia levels fully restored, whereas insulin treatment only slightly reduced the blood glucose. Consequently, it may be responsible for the long-term efficacy of islet or pancreatic transplantation in preventing diabetic-induced complications, thus providing the rational for clinical application.

Cardiovascular disease is the most important complication in patients with diabetes mellitus,


A
Control
DM
DM+I
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Collagen-Ia1
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B
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DM
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Collagen-Ia1 Positive area(%)
C
Collagen-IIIa1 Positive area(%)
D
CTGF Positive area(%)
E
Control DM DM+I DM+IT
α-SMA
GAPDH
α -SMA/β -actin
F
Collagen-Ia1
Collagen-IIIa1
CTGF
α-SMA

Figure 5. Effect of islets transplantation on the expression of collagen-Ia1, collagen-IIIa1, CTGF and α-SMA. A. Representative immunohistochemistry images of collagen-Ia1, collagen-IIIa1 and CTGF staining in the myocardium. Bar = 25 μm. B-D. Quantitative analysis of the expression of collagen-Ia1, collagen-IIIa1 and CTGF (n = 6-8 for each). Immunohistochemistry staining showed a larger area of collagen-Ia1, collagen-IIIa1 and CTGF in the myocardium of diabetic rats which was significantly reduced by insulin treatment. Surprisingly, islet transplantation was associated with a smaller area of collagen-Ia1, collagen-IIIa1 and CTGF than insulin treatment. E. Representative WB and quantitative analysis of α-SMA in the myocardium (n = 6-8 for each). The protein expression of α-SMA was remarkedly increased in the myocardium of diabetic rats, insulin treatment signifiantly alleviated the increase of α-SMA, and further reduced by islet transplantation. F. Transcription levels of collagen-Ia1, collagen-IIIa1, CTGF and α-SMA in the myocardium (n = 4-6 for each). DM induced the mRNA expression of collagen-Ia1, collagen-IIIa1, CTGF and α-SMA in the myocardium, islet transplantation significantly decreased the transcription levels of collagen-Ia1, CTGF and α-SMA but collagen-IIIa1 when compared with insulin treatment. DM, diabetes mellitus. I = insulin, IT = islets transplantation. *P < 0.05 vs. Control. #P < 0.05 vs. DM. #P < 0.05 vs. DM + I.

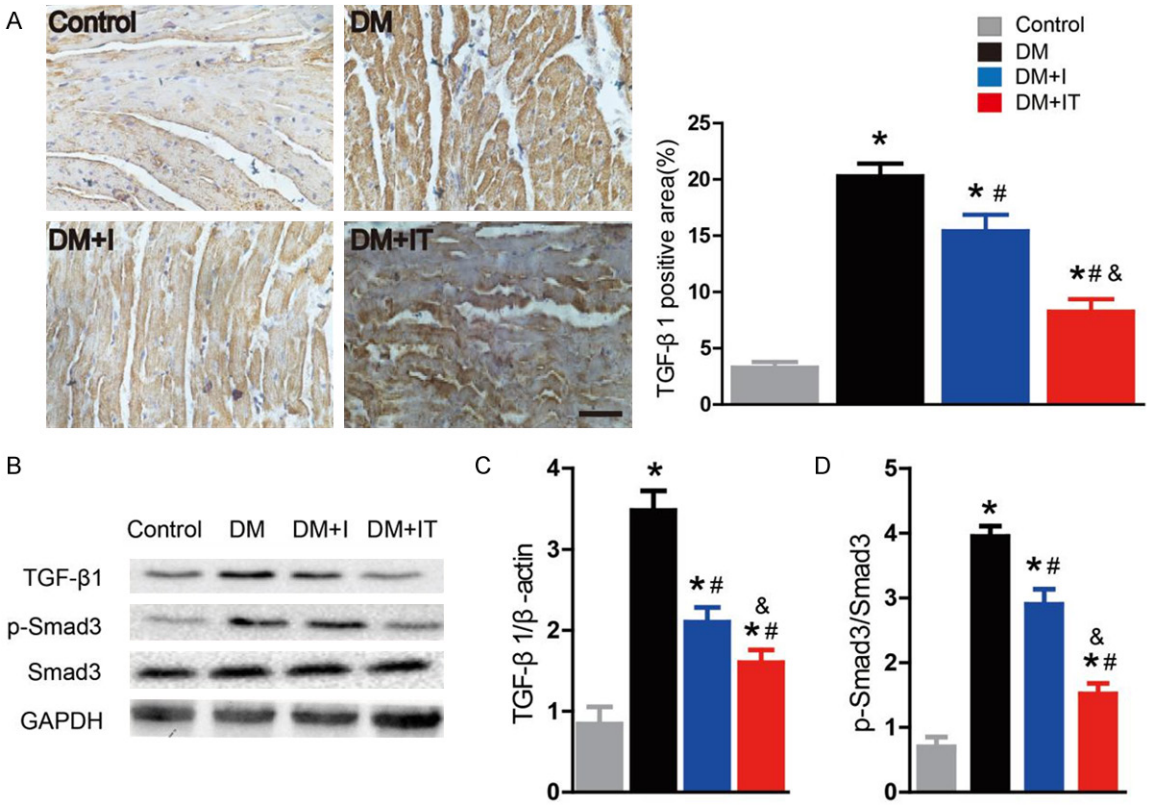

Figure 6. Islets transplantation inhibits the activation of TGF-β_1/Smad3 signaling pathway. A. Representative immunohistochemistry images and quantitative analysis of TGF-β_1 in myocardium (n = 6-8 for each). The TGF-β_1 positive area was significantly larger in the myocardium of diabetic rats than that of control, insulin treatment showed a significantly smaller TGF-β_1 positive. Intriguingly, islet transplantation further reduced the TGF-β_1 positive area than insulin treatment. Bar = 25 μm. B. Representative WB images of TGF-β_1, p-Smad3, Smad3. C and D. Quantitative analysis of the expression of TGF-β_1, p-Smad3, Smad3 (n = 6 for each). DM induced the protein expression of TGF-β_1 and phosphorylated Smad3 with no impact on the expression of total Smad3, however, islet transplantation significantly reduced the expression levels of TGF-β_1 and phosphorylated Smad3 in comparision with insulin treatment. DM, diabetes mellitus. I = insulin, IT = islets transplantation. *P < 0.05 vs. Control. #P < 0.05 vs. DM. #P < 0.05 vs. DM + I.

since uncontrolled hyperglycemia irreversibly impairs diastolic and systolic function, which inevitably deteriorate into heart failure [17]. Epidemiological evidence has shown that diabetic population is associated with a twofold to fivefold increased risk of congestive heart failure compared with non-diabetic counterparts, and even with higher all-cause mortality [18, 19]. Numerous studies have reported that inflammatory response, oxidative stress, apoptosis, and adverse ventricular remodeling are involved in the pathogenesis of diabetic-

induced myocardial damage and progression toward heart failure; nevertheless, the pathophysiological mechanism is far from being fully elucidated [3-5]. It is noteworthy that cardiac fibrosis has a critical role in structural remodeling of diabetic hearts and the consequential development of diabetic cardiomyopathy [20, 21]. Cardiac fibrosis has been detected and quantified in interstitial myocardium of diabetic patients by using MRI with diffusion weighted imaging and T1 mapping techniques, and further confirmed by autopsy or biopsy [22]. Interstitial fibrosis was initially a reparative process in response to cardiomyocytes loss or direct hyperglycemic insult, whereas subsequent excessive fibrosis resulted in diastolic dysfunction. Meanwhile, perivascular fibrosis reduced the blood flow and hindered the oxygen diffusion which contributed to systolic dysfunction. Furthermore, fibrotic remodeling in diabetic heart was accompanied with cardiac hypertrophy, a structural change of myocytes exposed to long-standing biological or pathological stimulation. Yet, hypertrophic hearts and cardiomyocytes were associated with an increased oxygen consumption, which aggravated myocardial ischemia and cardiac dysfunction in the setting of diabetes mellitus. Prior animal experiments have suggested that genetic regulation or pharmacological intervention targeting the molecular mechanism underlying adverse ventricular remodeling could retard or even reverse the progression of diabetic-induced heart failure [23, 24]. Consistent with these studies, diabetes induced myocardial injury and apoptosis, followed by adverse ventricular remodeling with cardiac hypertrophy and interstitial fibrosis in present study, whereas islet transplantation or insulin treatment significantly attenuated the diabetic-induced structural changes. Myofibroblasts are a hallmark of fibrotic process, which mainly derive from the differentiation of resident cardiac fibroblast, bone marrow-derived cells or epithelial-mesenchymal transformation [25]. It possessed contractile properties by expression of α-SMA and was characterized by accelerated synthesis of ECM. Accordingly, both therapeutic approaches decreased the protein levels of α-SMA and deposition of collagen-Ia1and-IIIa1. Furthermore, islet transplantation also alleviated the diabetic-induced expression of CTGF, an important mediator in fibroblasts activation such as proliferation, differentiation, adhesion and ECM synthesis. Intriguingly, our study demonstrated that diabetic rats that underwent islet transplantation had significantly less interstitial and perivascular fibrosis and ECM accumulation, along with lower levels of α-SMA and CTGF expression compared to insulin treatment. The preferable effect of islet transplantation in retarding diabetic-induced ventricular remodeling could attribute to restored β cell function and more optimal blood glucose regulation. However, whether transplanted islet exerted cardioprotective effect other than improving hyperglycemia remained unknown and thus needs to be further investigated.

TGF-β is the well-established cytokine/growth factor that regulates a wide variety of cell functions [26]. Evolving evidence has suggested that TGF-β is deeply implicated in the pathogenesis of cardiac remodeling and fibrosis through activation of cardiac fibroblasts and accumulation of ECM [27, 28]. By using TGF-β_1 heterozygous mice, Brooks et al have demonstrated that TGF-β1 promotes the deposition of collagen and extracellular matrix synthesis thus contributing to age-associated cardiac fibrosis [29]. Administration of a neutralized anti-TGF-β antibody markedly reduced collagen deposition following pressure overload or ischemia [30, 31]. By contrast, transgenic overexpression of TGF-β_1 led to a significantly increased expression of hypertrophy-related proteins and interstitial fibrosis [32]. TGF-β signaling activation is mainly dependent on the phosphorylation of downstream intracellular Smad proteins. Mechanistically, TGF-β_1 binds to type II and type I receptor, and then activates type I receptor phosphorylated Smad2 and Smad3, which interact with Smad4 and translocate into nucleus to induce transcription of fibrotic related gene, such as Fn, Ctgf, Col1a1 and Col3a1 [33, 34]. Genetic ablation of Smad3 protects against fibrotic remodeling in the kidney, eye and vasculature. Importantly, global deletion of Smad3 has been associated with a reduced ventricular fibrosis in myocardium of mice subjected to pressure overload or myocardial infarction [35]. While Smad3-/- fibroblasts present decreased proliferation, migration and attenuated TGF-β1-induce α-SMA expression when compared with wild-type cells, indicating a critical role of Smad3 underlying the phenotypic differentia-

tion and functional enhancement [36]. Consistent with the results of recent experiments, hyperglycemia increased the expression of TGF-β_1 and phosphorylated Smad2 and Smad3, nonetheless, islet transplantation markedly inhibited the activity of TGF-β1 and Smad2/3 in diabetic heart. Similarly, islet transplantation showed a more suppressed activation of this signaling pathway than insulin treatment, thus suggesting that the favorable effect of islet transplantation in attenuating diabetic-induced cardiac remodeling and fibrosis was mainly depended on TGF-β_1/Smad2/3 cascade.

Our study demonstrated that islet transplantation reduced diabetic-induced myocardial injury, subsequently inhibited cardiac hypertrophy, interstitial fibrosis and ECM deposition through deactivation of TGF-β_1/Smad2/3 signaling pathway, therefore protecting against DCM. These mechanistic findings supported the therapeutic potential of islet transplantation for the treatment of cardiac fibrosis in diabetic rats and highlighted its preferable role in clinical setting.

Acknowledgements

This work was supported the Natural Science Foundation of China (NSFC) Grant No.81170-302.

Disclosure of conflict of interest

None.

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