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Role of SDF-1:CXCR4 in Impaired Post-Myocardial Infarction Cardiac Repair in Diabetes

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ABSTRACT

Diabetes is a risk factor for worse outcomes following acute myocardial infarction (AMI). In this study, we tested the hypothesis that SDF-1:CXCR4 expression is compromised in post-AMI in diabetes, and that reversal of this defect can reverse the adverse effects of diabetes. Mesenchymal stem cells (MSC) isolated from green fluorescent protein (GFP) transgenic mice (control MSC) were induced to overexpress stromal cell-derived factor-1 (SDF-1). SDF-1 expression in control MSC and SDF-1-overexpressing MSC (SDF-1:MSC) were quantified using enzyme-linked immunosorbent assay (ELISA). AMI was induced on *db/db* and control mice. Mice were randomly selected to receive infusion of control MSC, SDF-1:MSC, or saline into the border zone after AMI. Serial echocardiography was used to assess cardiac function. SDF-1 and CXCR4 mRNA expression in the infarct zone of *db/db* mice and control mice were quantified. Compared to control mice, SDF-1 levels were decreased 82%, 91%, and 45% at baseline, 1 day and 3 days post-AMI in *db/db* mice, respectively. CXCR4 levels are increased 233% at baseline and 54% 5 days post-AMI in *db/db* mice. Administration of control MSC led to a significant improvement in ejection fraction (EF) in control mice but not in *db/db* mice 21 days after AMI. In contrast, administration of SDF-1:MSC produced a significant improvement in EF in both control mice and *db/db* mice 21 days after AMI. The SDF-1:CXCR4 axis is compromised in diabetes, which appears to augment the deleterious consequences of AMI. Over-express of SDF-1 expression in diabetes rescues cardiac function post AMI. Our results suggest that modulation of SDF-1 may improve post-AMI cardiac repair in diabetes. STEM CELLS TRANSLATIONAL MEDICINE *2018;7:115–124*

SIGNIFICANCE STATEMENT

This study tested the hypothesis that the dysregulation of the SDF-1:CXCR4 axis is a critical mechanism for impaired post-acute myocardial infarction (AMI) cardiac repair in diabetes. Novel physiology and treatment strategies for post-AMI cardiac repair in diabetes will be developed based the detailed understanding of the mechanism. These findings can be directly translated to clinical populations and will affect the treatment of cardiac dysfunction in diabetic patients.

INTRODUCTION

Diabetes is one of the most prominent risk factors for ischemic heart disease and is associated with increased morbidity and mortality [1, 2] in the event of an event such as acute myocardial infarction (AMI). Compared to nondiabetics, the overall mortality following MI was four times and seven times higher in diabetic male and female patients, respectively [1]. The mechanisms involved in impaired post-AMI cardiac repair in diabetes remain unknown, but there are a myriad of hypotheses including inflammation, oxidative stress, and the occurrence of nephropathy and neuropathy. Stem cell-based therapies have the potential to improve myocardial healing after AMI in nondiabetic animal; however, how this approach translates to the diabetic state still requires further examination. Studies suggest that stem cells isolated from diabetic animals have a blunted reparative capacity [3, 4]. Diabetes leads to premature aging and impaired proliferation of stem cells along with deficient paracrine factor release [5, 6]. Diabetes impairs stem cell function in both wound healing [7] and limb ischemia [8]. Diabetes also reduces Granulocyte-colony stimulating factor (G-CSF)-mediated hematopoietic stem cell (HSC) mobilization despite an increased number of HSCs within the bone marrow (BM) due to the altered microenvironment [9, 10]. Impaired endothelial progenitor cells (EPC) homing in diabetes is linked to decreased expression of stromal cell-derived factor-1 (SDF-1) in the area of wound. SDF-1 overexpression can reverse the diabetic defect in stem cell homing in wound healing [11–13]. These findings suggest an impaired role for SDF-1 in diabetic

wound healing, which may extend to a deficit in post-AMI cardiac repair in diabetes. Our laboratory has focused on defining the molecular mechanisms responsible for stem cell based tissue repair following ischemic injury and demonstrated the importance of the SDF-1:CXCR4 axis in myocardial repair [14, 15] in a model of coronary occlusion but without diabetes. If this axis is attenuated in diabetes; perhaps this could be one mechanism by which the diabetic heart is more vulnerable to the consequences of ischemia.

SDF-1 is an important stem cell homing factor and SDF-1 signaling is initiated by ligation of the chemokine with its receptor CXCR4, a G-protein coupled receptor [16, 17]. SDF-1 expression is elevated in most tissues secondary to injury and is instrumental in mobilization and migration of stem cells from the BM to the site of tissue injury. In previous studies using rodent model systems, we demonstrated that exogenous SDF-1 delivered either through gene transfer or by infusion of MSC was sufficient to induce stem cell homing to injured myocardial tissue [14, 15]. We further demonstrated that re-establishment of SDF-1 expression at a time remote from AMI was sufficient to induce BM derived and cardiac stem cell recruitment to the infarct border-zone and led to neovascularization, ventricular remodeling, and improvement in cardiac function [14]. Our recent results demonstrate that the local trophic effects of MSC require cardiac myocyte CXCR4 (CM-CXCR4) expression and in the absence of CM-CXCR4 expression, there is a significant loss of functional benefit in MSC-mediated repair despite equal increases in vascular density [18]. In the current study, we aimed to test if SDF-1:CXCR4 expression is compromised in diabetes and if SDF:CXCR4 axis plays an important role in impaired post-AMI cardiac repair in diabetes.

Methods

Experimental Animals

The experimental procedures described in this study were approved by the Institutional Animal Care and Use Committee of Northeast Ohio Medical University. In brief, male homozygous B6.Cg-m +/+ Leprdb/J (*db/db*) mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 4 weeks of age and were housed within the Animal Facility with free access to food and water until 12 weeks of age in an American Association for Accreditation of Laboratory Animal Care-approved animal facility. Age- and gender-matched wild-type C57BL/6J mice were used as lean control. The animal experiments were performed conform the NIH guidelines (guide for the care and use of laboratory animals).

MSC Preparation

MSCs were prepared as previously described [18]. Briefly, 6-week-old GFP mouse was sacrificed and the hind limbs were removed. The cells were isolated by flushing the femurs with flush medium (Alpha Medium with 2 g/l $NaHCO_3$, 10% horse serum, 10% fetal bovine serum (FBS), 1% L-Glutamine, 1% penicillin-streptomycin). The cells were filtered through a 70-μm nylon mesh filter followed by centrifugation for 5 minutes at 260 g and washed with phosphate-buffered saline (PBS). The washed cells were incubated at 37°C. Nonadherent cells were removed by replacing the medium after 24 hours. When cells reached 80% confluence, adherent cells were detached after incubation with 0.05% trypsin and 2 mM EDTA (Invitrogen, Carlsbad, CA) for 5 minutes. Cells were depleted of CD45+, CD34+ cells by negative selection using primary phycoerythrin (PE)-conjugated antibodies: mouse anti-CD45 (BD Biosciences, San Diego, CA) and mouse anti-CD34 antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). PE-positive cells were negatively selected using the EasySep PE selection kit according to the manufacturer's instructions (Stem Cell Technologies, Vancouver, BC, Canada). MSCs were replaced in medium and were subsequently passaged until passage 6.

Generation of MSCs Engineered to Overexpress SDF-1

MSC isolated from GFP transgenic mice were induced to overexpress SDF-1 by transduction with a lentivirus encoding SDF-1[15]. The Lentivirus was constructed using the pLenti6 V5/DEST plasmid Gateway System from the Virapower Lentiviral Expression System (Invitrogen). The SDF-1 gene was generated by reverse transcription from the mRNA of human foreskin fibroblasts and PCRed using the primers, Forward: 5′-GCTAGCGTCGACATGAACGCCA AGGTCGTGGTCGTGCTGGTC-3′; Reverse: 5′-GAAT TCTTACTTGT TTAAGGCTTTCTCCAGGTACTCCTGAAT-3′. The resulting product was digested with SalI and EcoRI and ligated it onto a SalI/EcoRI digested shuttle vector, pENTR 2B (Invitrogen, Carlsbad, CA). Following the Gateway System kit instructions, the pENTR2B/hSDF vector was clonased into the pLenti6.2 V5/DEST Vector, creating the pLenti6.2/hSDF-1 construct, lentiviral particles were produced and cells were transduced. Blasticidin selection was used to isolate a stably transfected cell population. To quantify SDF-1 overexpression of MSC, 50,000 control and SDF-1 overexpression MSC (SDF-1:MSC) were plated separately in T75 flask in serum-free dulbecco's modification of eagle's medium (DMEM). SDF-1 levels in the media were quantified using ELISA 24 hours later (R&D Systems, Minneapolis, MN). An equal cell number was verified by quantifying total protein per cell layer at the end of the experiment.

Annexin V-Cy5 Apoptosis Analysis

MSC and SDF-1:MSC were treated under different glucose concentrations (5 mM, 25 mM) with or without H_2O_2 (0.06 mM) under hypoxic conditions (1% oxygen) at 37°C for 24 hours. Cell apoptosis was measured by Annexin Cy5 apoptosis assay kit (abcam, ab14150, Cambridge, MA) according to the manufacturer's instructions. Apoptotic cells were quantified by flow cytometry.

Left Anterior Descending Ligation

Myocardial infarction was induced in mice as described [19]. Briefly, animals were anesthetized using Xylazine/Ketamine combination (ip injection, Ketamine 75 Mg/kg; Xylazine 7.5 Mg/kg; about 0.07 ml for 30 g mouse). Then the animals were endotracheal intubated and ventilated with room air at 100 breathes per minute using a rodent ventilator (Harvard Apparatus). Sternotomy was performed and the proximal left anterior descending (LAD) was identified using a surgical microscope (Leica M500) after retraction of the left atrium and ligated with 7-0 prolene. Blanching and dysfunction of the left ventricular wall verified LAD ligation. Control and *db/db* mice were randomly selected to receive injection of saline or 200k control MSC or SDF-1:MSC in the infarct border zone immediately after LAD ligation. The animals were monitored every hour for the first 3 hours and then daily after LAD ligation. Parameters that were monitored include body condition, hydration status, mentation, and activity level for evidence of pain or surgical complications, including infection. The animals received subcutaneously buprenorphine (0.05–0.1 mg/kg) immediately after surgery and then twice daily for 3 days. After LAD ligation, the animals were evaluated with echocardiography or sacrificed using an

overdose of Barbiturate (Intraperitoneal, 100 mg/kg) at different time points to collect cardiac tissue for analyses.

Echocardiographic Assessment of Heart Function

Two-dimensional (2D) echocardiography was performed using a 15-MHz linear array transducer interfaced with a Sequoia C256 (Acuson) as preciously described [19]. Briefly, baseline before LAD ligation as well as 3 days, and 21 days after LAD ligation, left ventricle (LV) dimensions were quantified by digitally recorded 2D clips and M-mode images from the mid-LV just below the papillary muscles to allow for consistent measurements from the same anatomical location in different mice. Ejection fraction (EF), fractional shortening, diastolic thicknesses of the LV posterior wall; systolic thicknesses of the LV posterior wall; diastolic LV internal dimensions (LVIDD); and systolic LV internal dimensions (LVIDS) were measured. Echocardiographic measurements were performed and analyzed by investigators who were blinded to the treatment and identity of the mouse.

SDF-1 and CXCR4 mRNA Expression in the Hearts of *db/db* Mice

To determine the effects of diabetes on SDF-1:CXCR4 axis following AMI, we quantified SDF-1 and CXCR4 mRNA levels in the hearts of *db/db* mice before AMI and in the infarct zone of *db/db* mice 1, 3, and 5 days after LAD ligation by quantitative polymerase chain reaction (qPCR). Briefly, the hearts were perfused with of saline and infarcted LVs were cut at a level just below the ligation. The RNA was harvested using TRIzol and cDNA was synthesized using SuperScript VILO cDNA synthesis kit from Invitrogen. The real-time PCR reaction for SDF-1, CXCR4, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was carried out using TaqMan primers on Applied Biosystems 7900HT Real-Time PCR System. qPCR primer probes for SDF-1, CXCR4, and GAPDH were purchased from Invitrogen.

Vessel Density Measurement

Mice were anesthetized and perfusion fixed with 10% phosphate-buffered formalin at physiological pressures 3 and 21 days after LAD ligation. Fixed hearts were embedded in paraffin and serially cut at 4 μm from the apex to the level just below the coronary artery ligation site. Antigen retrieval was performed using 10 mmol/l sodium citrate buffer (pH 6.0) and incubated with 1% normal blocking serum in PBS for 60 minutes to suppress nonspecific binding of IgG. Sections were stained with fluorescein-conjugated isolectin (Vector Laboratories, Burlingame, CA), which stains endothelium, and rhodamine-conjugated wheat germ agglutinin (Vector Laboratories), which labels myocyte membranes as previously described [18]. Three randomly selected fields for each sample were imaged at ×60 with confocal microscopy and vessel density within the infarct border zone was measured with ImageJ. Samples were blinded and randomized prior to analysis.

TUNEL Assay for Assessment of Cell Apoptosis

Three days after LAD ligation, mice were anesthetized and perfusion fixed with 10% phosphate-buffered formalin at physiological pressures. Fixed hearts were embedded in paraffin and serially cut at 4 μm from the apex to the level just below the coronary artery ligation site. TUNEL was performed to detect apoptotic nuclei using terminal deoxynucleotidyl transferase-mediated in situ fluorescein-conjugated, dUTP nick end-labeling technique according to the manufacturer's protocol (Roche, Indianapolis, IN). The sections were incubated again with a murine monoclonal anti-cardiac myosin heavy chain (Abcam, Inc., San Francisco, CA) to specifically recognize apoptotic cardiomyocytes. The fluorescence staining was viewed with a confocal laser scanning microscope. The number of apoptotic cells with TUNEL-positive nuclei from border zone was counted by two independent observers blinded to treatment group and expressed as a percentage of total myocyte population.

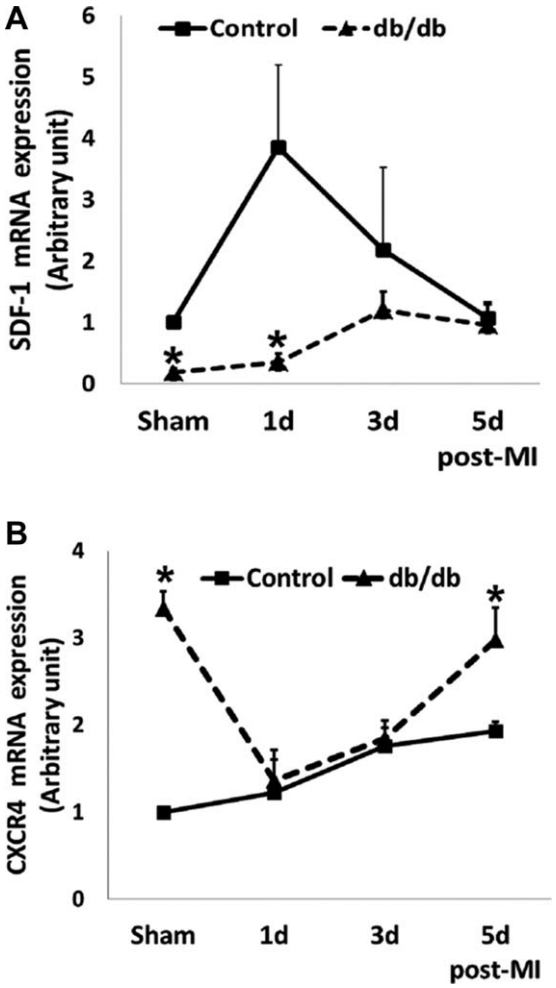

Figure 1. SDF-1 mRNA expression **(A)** and CXCR4 mRNA expression **(B)** in the infarct zone of *db/db* mice 1, 3, and 5 days after left anterior descending ligation. Data represent mean ± SEM. $n = 4$ per group. *, $p < .05$ versus corresponding control group. Abbreviations: MI, myocardial infarction; SDF-1, stromal cell-derived factor-1.

Statistical Analysis

Data are presented as mean ± SEM. Comparisons between two groups were made with a two-tailed Student *t* test. Comparisons among multiple groups were made with two-way analysis of variance (ANOVA) followed by the Tukey post hoc analysis. All differences were considered statistically significant at $p < .05$ levels.

Results

SDF-1 and CXCR4 mRNA Expression in the Hearts of *db/db* Mice

We quantified SDF-1 and CXCR4 mRNA levels in the hearts of control and *db/db* mice before AMI and in the infarct zone of *db/db*

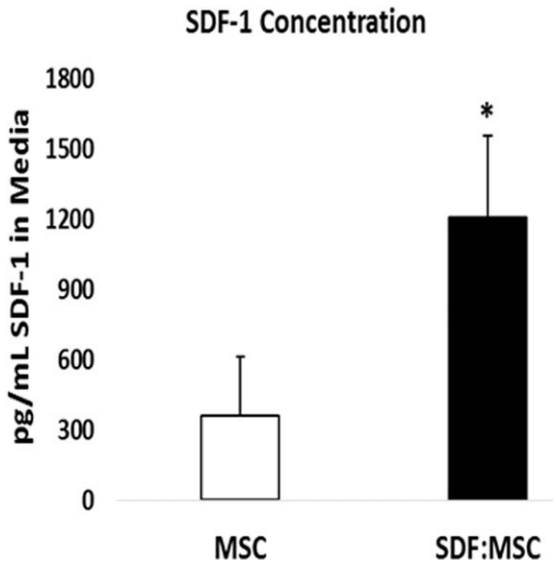

Figure 2. SDF-1 concentration in the media. Data represent mean ± SEM, $n = 4$. *, $p < .05$ versus MSC group. Abbreviations: MSC, mesenchymal stem cells; SDF-1, stromal cell-derived factor-1.

mice 1, 3, and 5 days after AMI (Fig. 1) by qPCR. Compared to control mice, there is an 82% decrease in SDF-1 expression before AMI, SDF-1 levels are decreased 91% and 45% in diabetic mice 1-day and 3-day post-AMI, respectively (Fig. 1A). We observed increased baseline gene expression of CXCR4 (more than twofold vs. control). CXCR4 levels are increased 54% 5 days post-AMI in *db/db* mice compared to control mice (Fig. 1B). To find out if MSC treatments can restore the SDF-1 expression, we quantified SDF-1 mRNA levels in the infarct zone of *db/db* mice 1 and 5 days after cell therapy (Supporting Information Fig. S1) by qPCR. At 1-day post-MI, when compared to control + saline group, there is a significant decrease in SDF-1 expression in *db/db* + saline group. It was also observed that in the *db/db* mice the SDF-1:MSC treatment restored the SDF-1 level whereas the MSC alone treatment did not. At 5 days after cell delivery, SDF-1 levels are significantly increased in both control and *db/db* mice compared to control + saline group (Supporting Information Fig. S1).

Generation of MSCs Engineered to Overexpress SDF-1

MSC were induced to overexpress SDF-1 by transduction with a lentivirus encoding SDF-1. Our data showed that SDF-1 level in SDF-1/MSC group increased 235% compared to control MSC group (Fig. 2).

Effects of SDF-1 on MSC Survival

To determine whether SDF-1 overexpression could improve MSC survival, we cultured control MSC and SDF-1:MSC for 24 hours with low glucose (5 mM) or high glucose (25 mM) and/or H_2O_2 (60 μM). The data in Figure 3 demonstrate that SDF-1:MSC had significant decreased apoptosis compared to control MSC under different glucose concentrations (5 mM, 25 mM) with or without H_2O_2.

LV Function Following Cell Therapy in *db/db* Mice

We found significantly increased LVIDD and LVIDS 3 weeks post MI in *db/db* mice compared to control group (Table 1). The infusion of MSC derived from control euglycemic GFP mice led to a significant improvement in EF in control mice (87%, $p < .01$) but not in *db/db* mice (33%, $p > .05$) 21 days after AMI. The infusion of SDF-1:MSC led to a significant improvement in EF in both control mice (114%, $p < .01$) and *db/db* mice (75%, $p < .05$) 21 days after AMI (Fig. 4). These data are consistent with the fact that the responsiveness of the SDF-1:CXCR4 axis is blunted but functional in diabetes.

Diabetes Is Associated with Decreased Cardiac Myocyte Survival After AMI

To investigate role of SDF-1:CXCR4 axis on cardiac myocyte preservation following MI in diabetes, we performed TUNEL to measure CM apoptosis. The number of TUNEL+ cardiac myocytes in the infarct border zone 3 days after MI was quantified. The number of TUNEL positive cardiac myocytes was significantly higher in the diabetic group compared with the control group (34%, $p < .05$). The infusion of control MSC led to a significant decrease in the number of TUNEL+ cardiac myocytes in control mice (49%, $p < .01$) but not in *db/db* mice (21%, $p > .05$) 3 days after AMI. The infusion of SDF-1:MSC led to a significant decrease in the number of TUNEL+ cardiac myocytes in both control mice (58%, $p < .01$) and *db/db* mice (45%, $p < .01$) 3 days after AMI (Fig. 5).

Role of SDF-1:CXCR4 Axis on Vascular Density in Diabetes After AMI Following Cell Therapy

To investigate if SDF-1:CXCR4 axis may affect vascular density following MSC infusion in diabetes after AMI, we evaluated vascular density 21 days after AMI. The myocardial vascular density was significantly lower in the diabetic group compared with the control group (27%, $p < .05$). The infusion of control MSC led to a significant increase in the number of capillaries and small arterioles in control mice (28%, $p < .01$) but not in *db/db* mice (11%, $p > .05$) 21 days after AMI. A significant increase in the number of capillaries and small arterioles was observed in both control mice 54%, $p < .01$) and *db/db* mice (41%, $p < .01$) 21 days after AMI after the infusion of SDF-1:MSC (Fig. 6).

Discussion

Diabetes Is Associated with Worse Outcomes

Diabetic patients are associated with worse outcomes such as increased morbidity and mortality following AMI [1, 2]. Diabetes is also associated with impaired stem cell function [3, 4]. We found significantly increased LVIDD and LVIDS 3 weeks post MI in *db/db* mice compared to control group (Table 1). Our data also demonstrated that the apoptotic cardiac myocytes was significantly higher and myocardial vascular density was significantly lower in the diabetic mice compared with the control mice (Figs. 5, 6).

Decreased Benefit of MSC Isolated from Nondiabetic Animal on Cardiac Repair in Diabetes

Several studies tested the effects of stem cells isolated from nondiabetic animal on cardiac dysfunction in diabetics. It was reported that intramyocardial transplantation of nondiabetic MSC has a protective effect on diabetic hearts [20, 21]. However, not all studies have consistently shown that stem cells from nondiabetic animals can improve the function of diabetic hearts [22]. In this study, we tested the effects of diabetes on post-AMI myocardial repair in the presence of stem cell therapy. First, we used stem cells from euglycemic animals to see if they could improve cardiac dysfunction in diabetics. Our results show that, in

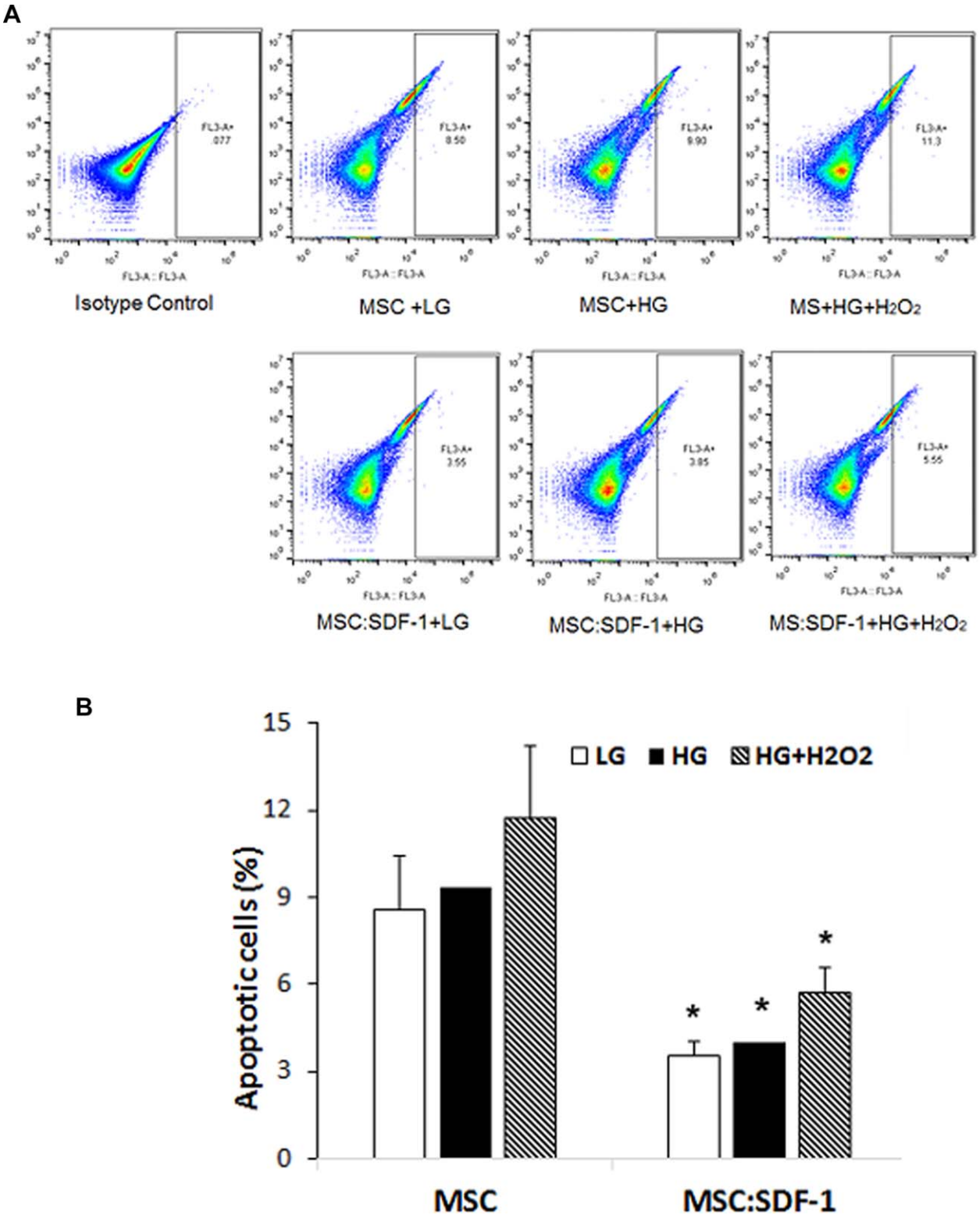

Figure 3. Annexin V-Cy5 apoptosis analysis of MSC. **(A):** Representative FACS analyses for Annexin V-positive cells. Fluorescence intensities were measured by flow cytometry using FL3 (Annexin V). The values shown in the left and right of each panel represent the percentage of viable and apoptotic, respectively. **(B):** Summary of percentage of apoptotic cells. Data represent mean ± SEM, $n = 4$. *, $p < .05$ versus corresponding MSC control group. Abbreviations: FACS, fluorescence-activated cell sorting; HG, high glucose (25 mM); LG, low glucose (5 mM); LH, 0.06 mM H_2O_2; MSC, mesenchymal stem cells; SDF-1, stromal cell-derived factor-1.

Table 1. LV function at baseline and 21 days post-MI

	LVIDD (mm)	LVPWD (mm)	LVIDS (mm)	LVPWS (mm)	EF (%)	FS (%)
Baseline						
Control						
Saline	3.02 ± 0.24	0.83 ± 0.07	1.36 ± 0.10	1.47 ± 0.09	88.3 ± 1.0	54.6 ± 1.0
MSC	3.02 ± 0.34	1.0 ± 0.14	1.45 ± 0.28	1.43 ± 0.14	83.4 ± 4.3	53.4 ± 6.5
SDF-1/MSC	3.74 ± 0.12	1.05 ± 0.20	1.78 ± 0.17	1.33 ± 0.13	83.4 ± 3.3	52.4 ± 3.8
db/db						
Saline	3.53 ± 0.21	0.96 ± 0.11	1.82 ± 0.18	1.35 ± 0.10	80.5 ± 2.3	48.9 ± 2.2
MSC	3.59 ± 0.25	1.27 ± 0.18	1.89 ± 0.18	1.53 ± 0.09	79.8 ± 1.9	47.7 ± 1.8
SDF-1/MSC	4.63 ± 0.82	1.04 ± 0.21	2.61 ± 0.69	1.71 ± 0.23	75.9 ± 6.4	46.1 ± 5.6
21d post-MI						
Control						
Saline	4.71 ± 0.15	0.57 ± 0.13	4.23 ± 0.18	0.79 ± 0.17	28.7 ± 2.1	12.4 ± 1.2
MSC	4.32 ± 0.47	1.12 ± 0.15*	3.14 ± 0.36*	1.32 ± 0.13*	53.8 ± 1.9*	27.5 ± 1.1*
SDF-1/MSC	4.33 ± 0.28	1.00 ± 0.07*	2.92 ± 0.40*	1.21 ± 0.15	61.7 ± 5.5*	33.3 ± 3.6*
db/db						
Saline	5.27 ± 0.14*	0.85 ± 0.09	4.71 ± 0.17*	0.95 ± 0.16	22.6 ± 3.3	10.5 ± 1.6
MSC	5.69 ± 0.44	1.48 ± 0.21	4.83 ± 0.30	1.71 ± 0.21	30.1 ± 5.0	14.6 ± 2.8
SDF-1/MSC	5.87 ± 0.59	0.81 ± 0.12	4.77 ± 0.71	1.11 ± 0.19	39.5 ± 7.4#	19.8 ± 4.1#

Data are expressed as means ± SEM ($n = 4$–8 in each group). *, $p < .05$ versus Control + Saline treatment group. #, $p < .05$ versus *db/db* +Saline group.

Abbreviations: EF, ejection fraction; FS, fractional shortening; LV, left ventricle; LVIDD, diastolic LV internal dimension; LVIDS, systolic LV internal dimension; LVPWD, diastolic thicknesses of the LV posterior wall; LVPWS, systolic thicknesses of the LV posterior wall; MI, myocardial infarction; MSC, mesenchymal stem cells; SDF-1, stromal derived factor-1.

nondiabetic animals, the infusion of MSC after AMI led to a significant improvement in EF as well as decreased myocyte apoptosis and increase in vascular density. However, there was no significant benefit following MSC infusion in *db/db* mice (Figs. 4–6). These data indicate that the therapeutic effects of MSC from nondiabetic animal are decreased when delivered to diabetic mice. The differences between our results and previous reports from other labs may be due to several reasons such as different disease models (diabetic cardiomyopathy vs. AMI), different mouse models (high fat diet induced diabetic mice vs. *db/db* mice), and different stem cells sources that are studied.

Over-Expression of SDF-1 Could Rescue the Lack of Benefits of MSC in Diabetes

Emerging evidence suggests that dysfunction of SDF-1:CXCR4 axis may be responsible for delayed wound healing in type 2 diabetes mellitus. Diabetic animals show reduced SDF-1 expression and circulating CD34 + CXCR4+ cells compared with nondiabetic animals and impaired wound healing due to blunted SDF-1 [12, 23, 24]. These findings suggest that disordered chemokine expression in diabetes may reduce the ability to recruit BM derived cells to sites of injury. Interestingly, the administration of SDF-1 to wounds enhances EPC homing and improved wound healing in the setting of diabetes [13]. In our previous study, we observed that SDF-1 responsiveness was required for the benefits from MSC and activation of SDF-1:CXCR4 signaling and prolonged expression of SDF-1 following AMI led to preservation of cardiac myocytes in nondiabetic mice [15, 18]. In our current study, we found significantly decreased SDF-1 and increased CXCR4 expression in diabetic mice before and after LAD ligation compared to control mice (Fig. 1). Therefore, we hypothesized that the over-expression of SDF-1

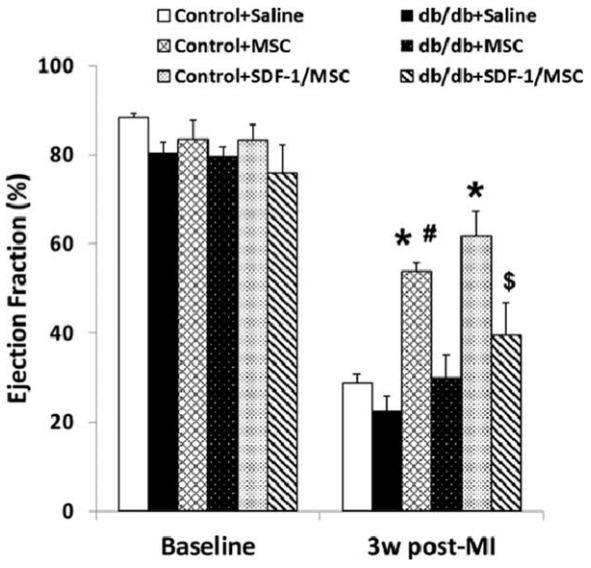

Figure 4. Ejection fraction at baseline and 21 days after left anterior descending ligation in control and *db/db* mice. Data are means ± SEM ($n = 4$–8 per group). *, $p < .05$ versus control +saline group; #, $p < .05$ versus *db/db* +MSC group; $, $p < .05$ versus *db/db* +saline group. Abbreviations: MI, myocardial infarction; MSC, mesenchymal stem cells; SDF-1, stromal cell-derived factor-1.

could rescue the lack of benefit of MSC. To confirm the role of over-expression of SDF-1 in impaired cardiac repair in diabetes, we developed MSC engineered to over-express SDF-1. These transduced cells produced significantly greater amounts of SDF-1 (more than double) than control (non-transduced) cells (Fig. 2).

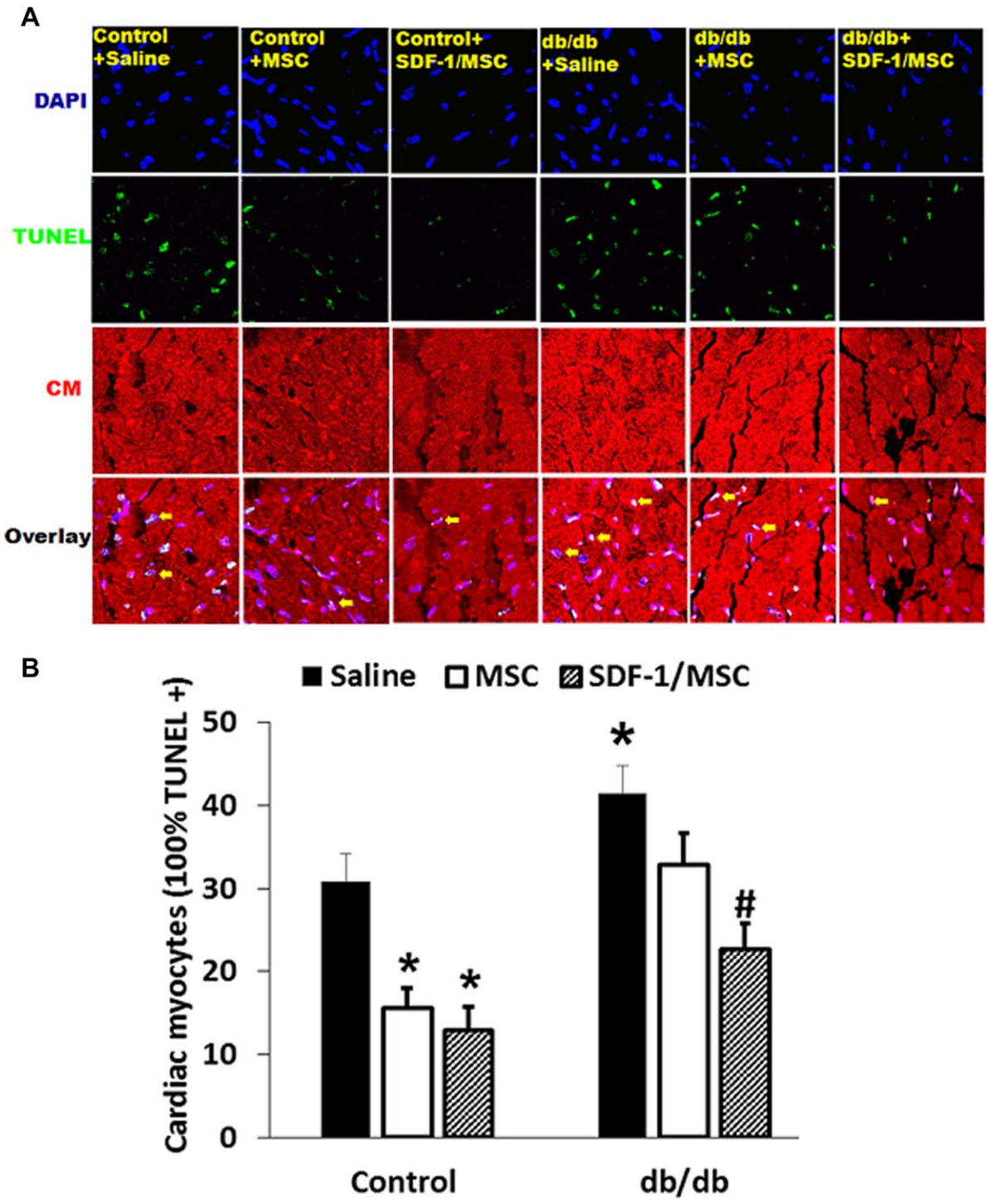

Figure 5. TUNEL assay for assessment of cell apoptosis. **(A):** Confocal image of representative immunofluorescent staining for cardiac myosin heavy chain (Alexa Fluor 594, red), TUNEL (Alexa Fluor 488, green), nuclei (DAPI, blue), and merged image from animals 3 days after left anterior descending (LAD) ligation (×60). **(B):** Number of TUNEL-positive cardiomyocytes in the infarct border zone 3 days after LAD ligation in animals that received Saline, MSC, or SDF-1 over-expressing MSC. Data represent the mean percent TUNEL-positive cells ± SEM ($n = 6–8$ per group). *, $p < .05$ versus Control + Saline group; #, $p < .05$ versus *db/db* + Saline group. Abbreviations: CM, cardiac myocyte; DAPI, 4′,6-diamidino-2-phenylindole; MSC, mesenchymal stem cells; SDF-1, stromal cell-derived factor-1.

Administration of the transduced MSC had far more beneficial effects than the control cells (Figs. 4–6). In diabetic animals, we found significant improvement in cardiac function, significant reduction in myocyte apoptosis, and significant increase in vascular density. We also observed greater benefits in euglycemic animals compare to the control cells. These results are somewhat surprising in that a conventional line of thought maintains diabetes senesces cells, and therefore, SDF-1, as a stem cell homing factor, will not work in diabetes. However, our results demonstrate that restoration of the SDF-1:CXCR4 signaling by MSC that over-

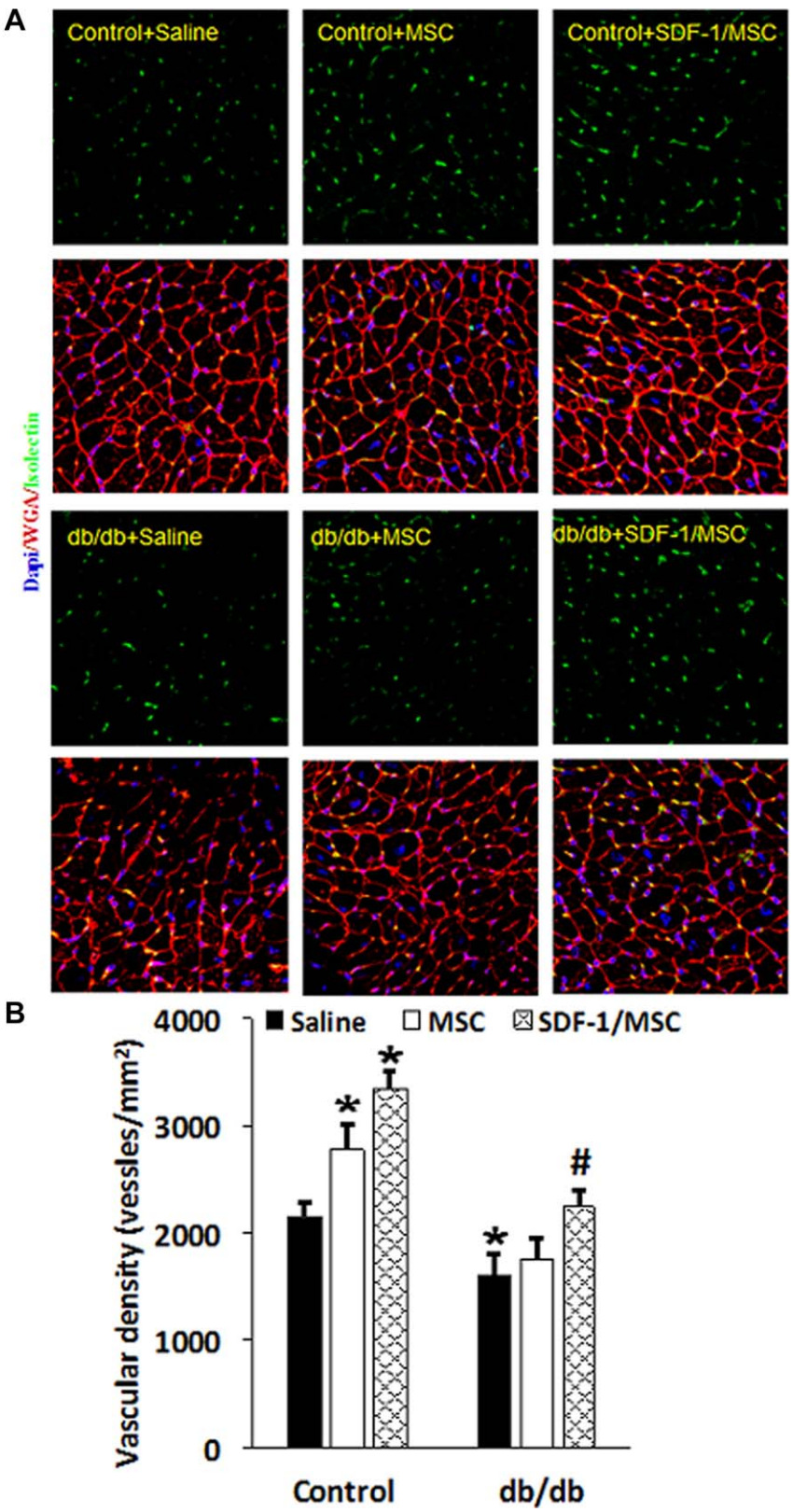

Figure 6. Vascular density in the border zone 21 days post-myocardial infarction. **(A):** Confocal image of representative immunofluorescent staining (×60). Endothelial cell staining with isolectin (green). Corresponding merged image: wheat germ agglutinin (red) and DAPI (blue). **(B):** Number of vascular density. Data represent mean ± SEM (vessels/mm^2, $n = 6–7$ per group). *, $p < .05$ versus Control + Saline group; #, $p < .05$ versus *db/db* + Saline group. Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; MSC, mesenchymal stem cells; SDF-1, stromal cell-derived factor-1; WGA, wheat germ agglutinin.

expression of the SDF-1 in diabetes recovers cardiac function. Based on these results, we think that impaired cardiac repair in diabetic mice maybe at least partially due to impaired SDF-1:CXCR4 axis. Our results showed that the infusion of SDF-1:MSC led to a significant improvement in EF in both control mice and *db/db* mice 21 days after AMI. However, the benefit appears less in *db/db* mice than in control mice. One of the reasons might be due to the hostile micro-environment in *db/db* mice (hyperglycemia, hyperinsulinemia, increased oxidative stress, etc.). Although the SDF-1:MSC had significant decrease in apoptosis compared to the control MSC, when comparing the micro-environments between the control mice and the *db/db* mice, the *db/db*'s may suppress the survival and function of transplanted SDF-1:MSC. Therefore, one of the aims of our future study is to compare the engineered SDF-1 overexpression MSC transplantation therapy to the infusion of SDF-1 plasmid therapy to determine which is the better method to upregulate SDF-1 in diabetes.

Relationships Among Apoptosis, Vascular Density, and SDF-1

An important dilemma in the field of stem cell therapy is the low survival of transplanted cells in the ischemic region, especially in hostile inflammatory environment with a high glucose and/or high reactive oxygen species level that is present in diabetes with or without acute tissue injury. We found SDF-1:MSC decreased apoptosis compared to control MSC under different glucose concentrations (5 mM, 25mM) with or without H_2O_2 (Fig. 3). Our data indicate that stem cell survival can be improved by increased SDF-1 expression, this may be one of the reason why SDF-1:MSC produced a better outcome (in terms of cardiac function) than control MSC in *db/db* mice. Our current study further supports the concept that the degree of cardiac apoptosis in AMI is modulated by the levels of SDF-1 in both control and diabetic mice (Fig. 5). A question of how this occurs still remains unanswered but some of the anti-apoptotic effects of SDF-1 may be mediated by the phosphoinositide 3-kinase/Akt signaling pathway [25]. Our previous study showed an increase in phosphorylated Akt in SDF-1:MSC than in control MSC [15]. SDF-1 can protect cardiac function through myocardial STAT3 signaling [26]. SDF-1/CXCR4-mediated STAT3 signaling pathway is also involved in the dipeptidyl peptidase-4 inhibitor induced post-AMI cardiac protection [27].

Similar to the relationships between apoptosis and SDF-1, there appears to be a direct relationship between the number of capillaries and small arterioles in both control and *db/db* mice with SDF-1 (Fig. 6). One suggestion from these data is that impaired cardiac repair in diabetes may be a consequence of increased vessel rarefaction and cardiac myocyte apoptosis. Although at this point, we cannot resolve if the vessel density controls levels of myocyte apoptosis, or preservation of myocytes also is associated with vascular preservation. Regardless of this conundrum, the SDF-1:CXCR4 axis is impaired and blunted in diabetes, and modulation of SDF-1 expression is a potential strategy to improve post-AMI cardiac repair and outcomes in diabetes.

Conclusion

In summary, our study revealed that the impaired SDF-1:CXCR4 axis in diabetic mice following AMI may lead to increased cell death and decreased vascular density at the sites of injury, as well as decreased cardiac function. Moreover, we observed that rescue of the functionality of the SDF-1:CXCR4 axis via over-expression of SDF-1 can improve cardiac function in the diabetic heart. That is, diabetes shifts the dose response relationship of SDF-1 on myocardial repair and that greater SDF-1 levels rescue the phenotype. These data suggest that a compromised SDF-1:CXCR4 axis is a critical mechanism in impaired post-AMI cardiac repair in diabetes and an important therapeutic target.

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Author Contributions

M.E.M.: collection and/or assembly of data, data analysis and interpretation, manuscript writing; M.K.: collection and/or assembly of data, manuscript writing; P.M.C.: collection and/or assembly of data, data analysis and interpretation; F.F., J.O., and K.W.: collection and/or assembly of data; W.C.: conception and design, administrative support, manuscript writing; M.S.P.: conception and design, financial support, provision of study material or patients, data analysis and interpretation, manuscript writing, final approval of manuscript; F.D.: conception and design, financial support, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

Dr. Penn has intellectual property rights and is a founder of Juventas Therapeutics, Inc. The other authors indicated no potential conflicts of interest.

Note Added in Proof

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