

Adenovirus-mediated stromal cell-derived factor-1 alpha gene transfer improves cardiac structure and function after experimental myocardial infarction through angiogenic and antifibrotic actions

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Abstract Stromal cell-derived factor 1 α (SDF-1) is not only a major chemotactic factor, but also an inducer of angiogenesis. The effects of SDF-1 α on the left ventricular remodeling in a rat myocardial infarction (MI) model were analyzed. Myocardial infarction was induced by ligation of the left coronary artery in rats. 0.5×10^{10} pfu/ml AdV-SDF-1 or 0.5×10^{10} pfu/ml Adv-LacZ were immediately injected into the infarcted myocardium, 120 μ l cell-free PBS were injected into the infarcted region or the myocardial wall in control, and sham group, respectively. We found that AdV-SDF-1 group had higher LVSP and \pm dp/dt_{max}, lower LVEDP compared to control or Adv-LacZ group. The number of c-Kit⁺ stem cells, and gene expression of SDF-1, VEGF and bFGF were obviously increased, which was associated with reduced infarct size,

thicker left ventricle wall, greater vascular density and cardiocytes density in infarcted hearts of AdV-SDF-1 group. Furthermore, the expression of collagen type I and type III mRNA, and collagen accumulation in the infarcted area was lower, which was associated with decreased TGF- β 1, TIMP-1 and TIMP-2 expression in AdV-SDF-1 group. Conclusion: SDF-1 α could improve cardiac structure and function after Myocardial infarction through angiogenic and anti-fibrotic actions.

Keywords Myocardial infarction · SDF-1 α · Stem cell · Angiogenesis · Remodeling · TIMP

Introduction

The transplantation of multiple stem cell types at the time of myocardial infarction has been shown to improve left ventricular perfusion and/or function in preclinical and clinical studies. Cardiac stem cell (CSC) is pluripotent, adult stem cells and mostly resides within the bone marrow or heart. In contrast to other stem cells, c-Kit⁺ or Sca⁺ Cardiac stem cells can differentiate not only into cardiomyocytes, but also into vascular endothelial cells [1]. In vivo, cardiac stem cells directly injected into an infarcted heart have been shown to induce myocardial regeneration and improve cardiac function. More importantly, when given intravenously after infarction, these cells targeted injured myocardium and differentiated into cardiomyocytes [2, 3]. These observations prompted a search for chemotactic factors that facilitate this “homing” process. If the effects of translocated stem cells prove important in improving cardiac function, the ability to harness or augment these effects may be possible through gene transfer strategies.

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It is interesting that the cytokine stromal cell-derived factor 1 α (SDF-1 α or CXCL12) is a major chemotactic factors. CXCR4 is the cell surface receptor for SDF-1, and is expressed on early hematopoietic stem cells (HSC), cardiac stem or precursor cells and endothelial progenitor cells (EPC) [1]. Ma and our study recently demonstrated that SDF-1 is expressed increased by the heart immediately after an acute myocardial infarction [4]. And the SDF-1 expression at a time of myocardial infarction (MI) can restore stem cell homing to damaged cardiac tissue [2, 5]. However, the effect of intrinsic SDF-1 upregulated expression is, transient and insufficient for cardiac repair [6]. Therefore, forced expression of SDF-1 α by adenoviral gene delivery and SDF-1 α protein increased the recruitment of stem cells to the infarcted heart. In addition, SDF-1 is also an inducer of angiogenesis. SDF-1 promotes not only EPCs (CD133 is one of its characteristic markers) recruitment into the ischemic muscles to promote angiogenesis, but also induces bone marrow derived-c-Kit⁺ stem cells to differentiate into EPCs and endothelial cells [7–10]. These findings raise the possibility that SDF-1 has the effects on myocardial structure and function via myogenesis and angiogenesis. However, little information is available about the role of SDF-1 on left ventricular remodeling after MI.

A model of MI in the rat has been created by Left anterior descending artery (LAD) ligation, which results in severe heart failure characterized by increased cardiac fibrosis and left ventricular (LV) dilation. The purpose of this study was to investigate the following topics: (1) whether SDF-1 results in homing of c-Kit⁺ cardiac stem cells to the injured myocardium and (2) whether the myoangiogenesis effects of SDF-1 mediated stem cells on left ventricular remodeling.

Methods

Myocardial infarction model and viral transduction

Myocardial infarction (MI) was performed by the ligation of LAD as described previously [7]. Briefly, male Wistar inbred rats (280–320 g) were anesthetized with ketamine (50 mg/kg IP) and xylazine (10 mg/kg IP) and tracheally ventilated with room air using a columbus ventilator (HX-300, Taimeng Instruments, and Chendu, China). After left lateral thoracotomy in the fourth intercostal space, LAD was ligated. Before chest closure, infarction was confirmed by observation of a demarcation of injury with blanching of the myocardium and electrocardiograph. Experimental animals were randomized for each group. After MI induction, 0.5×10^{10} pfu/ml of AdV.SDF-1 were

separately injected into six different sites (30 μ l per site) for each MI heart in the AdV.SDF-1 group with a 30-gauge tuberculin syringe. Two injection sites were in the myocardium peri-infarction area and two within the infarction area and Control or AdV.LacZ group received the same MI operation and was injected with an equivalent volume of the cell-free PBS or AdV.LacZ, respectively. The sham group underwent the identical surgery with neither ligation of the coronary artery nor adenovirus injection. Penicillin (150,000 U/ml) was given intravenously before each operation and buprenorphine hydrochloride (0.05 mg/kg) was administered subcutaneously twice a day for the first 48 h after operation.

Peripheral blood analysis

0.5 ml blood sample was serially (on days 3, 7, 14 and 28) drawn from the tail vein to measure peripheral mononuclear cell count. Total peripheral blood mononuclear cells (PBMNCs) count was determined using a Neubauer hemacytometer (Fisher Scientific) [11].

Western blotting and ELISA

Three, 7, 14, and 28 days after treatment (each time point, $n = 5$), rats were killed and peripheral blood (10 ml) was collected to measure plasma levels of stromal cell-derived factor 1 α (SDF-1 α) by enzyme-linked immunosorbent assay (ELISA). Myocardium tissue extracts of the infarction area and peri-infarction area were clarified and protein content quantified by Bradford method. Subsequently, the levels of SDF-1 α in the infarction area and peri-infarction area of Myocardium were measured by use of the SDF-1 α (R&D Systems). And left ventricle tissue extracts were clarified and protein content quantified by Bradford method. Thirty micrograms of the clarified extracts was run in 12% SDS-PAGE followed by transfer to a nitrocellulose membrane. Western analysis was carried out using the following primary antibodies: transforming growth factor- β 1 (TGF- β 1), tissue inhibitor of metalloproteinase-1 (TIMP-1), TIMP-2 (1:500, Santa Cruz). After extensive washing, immunocomplexes were detected with horseradish peroxidase-conjugated appropriate secondary antibodies followed by enhanced chemiluminescence reaction (KPL, American) [7, 8].

RNA preparation and RT-PCR

Three, 7, 14, and 28 days after treatment (each time point, $n = 5$), rats were killed, coronary blood was washed out with saline, the peri-infarction area (100 mg) and infarction area (100 mg) of the heart was removed

and minced, and total RNA was extracted by the guanidinium thiocyanate-phenol chloroform method. Reverse transcription-polymerase chain reaction (RT-PCR) was performed to determine expression of the myocardial cytokines VEGF, basic-FGF, TGF- β 1, SDF-1 α and its receptor CXCR4, stem cell factor receptor c-Kit, and collagen type I and III. The RT-PCR procedure was performed according to the manufacturer's recommendation (RT-PCR Kit, Fermentas Life Science, Ontario, Canada). The primers used in this study are listed in supplement 1. Quantitative RT-PCR was performed using SYBR Green Reaction Mix (Eurogentec) on an ABI PRISM 7900HT Detection System (Applied Biosystems). Each sample was run in duplicate. The expression of each gene within the different tissue samples was quantified relative to GAPDH expression [11].

Measurement of hemodynamics

Twenty-eight days after treatment, hemodynamic measurements in vivo were performed with the methods as following. Rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.). A carotid artery and femoral artery were isolated. The two catheters filled with heparinized (10 U/ml) saline solution were connected to Statham pressure transducer (Gould, Saddle Brook, NJ, USA). The carotid arterial catheter was advanced into the left ventricle to record ventricular pressure for a brief period of time. And the femoral artery catheter was inserted into an isolated femoral artery to monitor mean arterial pressure (BP), and heart rate (HR). These hemodynamic parameters were monitored simultaneously and recorded on a thermal pen-writing recorder (RJG- 4122, Nihon Kohden, Japan) and on an FM magnetic tape recorder (RM-7000, Sony, Tokyo, Japan). Tapes were later played back. After the measurements, the heart was rapidly removed from the killed rats (each group, $n = 10$ – 12) [12].

Histology and morphometric measurement

At day 28 after MI, rat wet weight measured, and then LV function was assessed. The hearts were quickly removed and was cut into six transverse slices from apex to base and processed for histology. Heart infarct size was measured using triphenyltetrazolium chloride (TTC). 28 days after MI (each group, $n = 6$). Briefly, left ventricles were sliced transversely into five to six sections approximately 2 mm in thickness and incubated for 30 min in a 1% solution of buffered TTC at 37°C. Slices were photographed with a digital camera with magnification in order to identify the infarcted myocardium

(unstained by TTC) and the non-ischemic zones (stained brick-red). All measurements were done by two experienced technicians who were blinded to the treatment group. The LV-wall thickness was measured at three widely spaced locations within the scar segment as well as the non-infarcted region for each slide and the average of these multiple measurements were calculated. To estimate an overall degree of ventricular remodeling, expansion index was computed by our previous method. The analyses of LV-wall thickness and expansion index were performed upon two middle slides and the averages were calculated for each heart (each group, $n = 6$) [12–14].

Immunohistochemistry analysis

Seven and 28 days after treatment (each time point, $n = 5$), rats were killed. After quick removal of the hearts, the hearts were immersion fixed in 4% paraformaldehyde and embedded in optimum cutting temperature (OCT) compound (Fisher Scientific, Fair Lawn). Serial transverse sections of 5 μ m were cut across the entire long axis of the heart and subsequently mounted on slides.

Double-staining immunofluorescence was performed with specific primary antibodies. Briefly, frozen tissue sections were incubated simultaneously with rabbit anti-human SDF-1 α (hSDF-1 α , 1:200, R&D Systems) and mouse anti-rat Troponin T (Cardiac isoform, cTnt, 2 μ g/ml, Neomarkers), mouse anti-rat c-Kit (1:250, Santa Cruz) and rabbit anti-rat CXCR4 (1:250, Santa Cruz) for 60 min at room temperature, respectively. After a washing with PBS solution, sections were incubated with a goat anti-mouse conjugated TRITC IgG (1:250, R&D Systems) for c-Kit and cTnt, and goat anti-rabbit conjugated FITC IgG for CXCR4 and hSDF-1 α (1:100, Santa Cruz). Cellular nucleus was stained with 4', 6-diamidino-2-phenylindole (DAPI, 50 μ g/ml). The numbers of c-Kit and CXCR4 positive cells were analyzed five randomly selected fields of the muscle tissue section per high power field (200 \times) by count method using image pro5.02 [12].

Immunohistology was performed with specific primary antibodies against factor vWF-VIII (1:250, Santa Cruz) and cTnt, respectively. The positive stains were shown as red fluorescence color with TRITC IgG. Capillary density was evaluated by counting the number of capillaries in five randomly selected fields of the muscle tissue section ($n = 5$, each). The value of cTnt positive red fluorescence photodensity was analyzed in five randomly selected fields of the muscle tissue section per high power field (200 \times) by count densitometric method using image pro5.02 ($n = 5$, each).

Collagen volume fraction (CVF)

To analyze collagen accumulation, Masson's trichrome staining was performed to delineate collagen content as percent of infarction and peri-infarction area. Sixteen separate areas of high power fields (200 \times) in each section were visualized under light microscope and photographed with the same exposure time. The collagen volume fraction (CVF) in the infarction and peri-infarction area was calculated as the percentage of stained tissue in the sum of muscle area and connective tissue by densitometric method using image pro5.02 (each group, $n = 6$) [15].

Data analysis

The data are expressed as mean \pm SD. Statistical significance between two groups was determined by paired or unpaired Student's *t*-test. Results for more than two experimental groups were evaluated by one-way ANOVA to specify differences between groups. *P* value 0.05 was considered significantly different.

Results

SDF-1 α expression assay and peripheral blood mononuclear cells (PBMNCs) count

Representative immunofluorescence-stained sections of the hSDF-1 α expression showed in the infarction area (Fig. 1a, b) of the AdV-SDF-1 group 7 days after MI. SDF-1 mRNA expression by RT-PCR after MI was increased in the infarcted cardiac tissues, and the level of SDF-1 mRNA expression was highest in 3 days after MI (Fig. 1c). Importantly, the level of SDF-1 protein expression by ELISA after direct myocardial injection of AdV.SDF-1 was significantly increased in the infarcted myocardium (Fig. 1d). At the same time, there was increase of the SDF-1 levels in the serum (Fig. 1e). Concomitantly, the number of PBMNCs was increased on day 3, and then decreased after MI. Furthermore, the number of PBMNCs in AdV-SDF-1 group showed a greater increase than the other group on day 3, and was elevated until day 7, then decreased thereafter (Fig. 1f). Thus, there was a prolonged

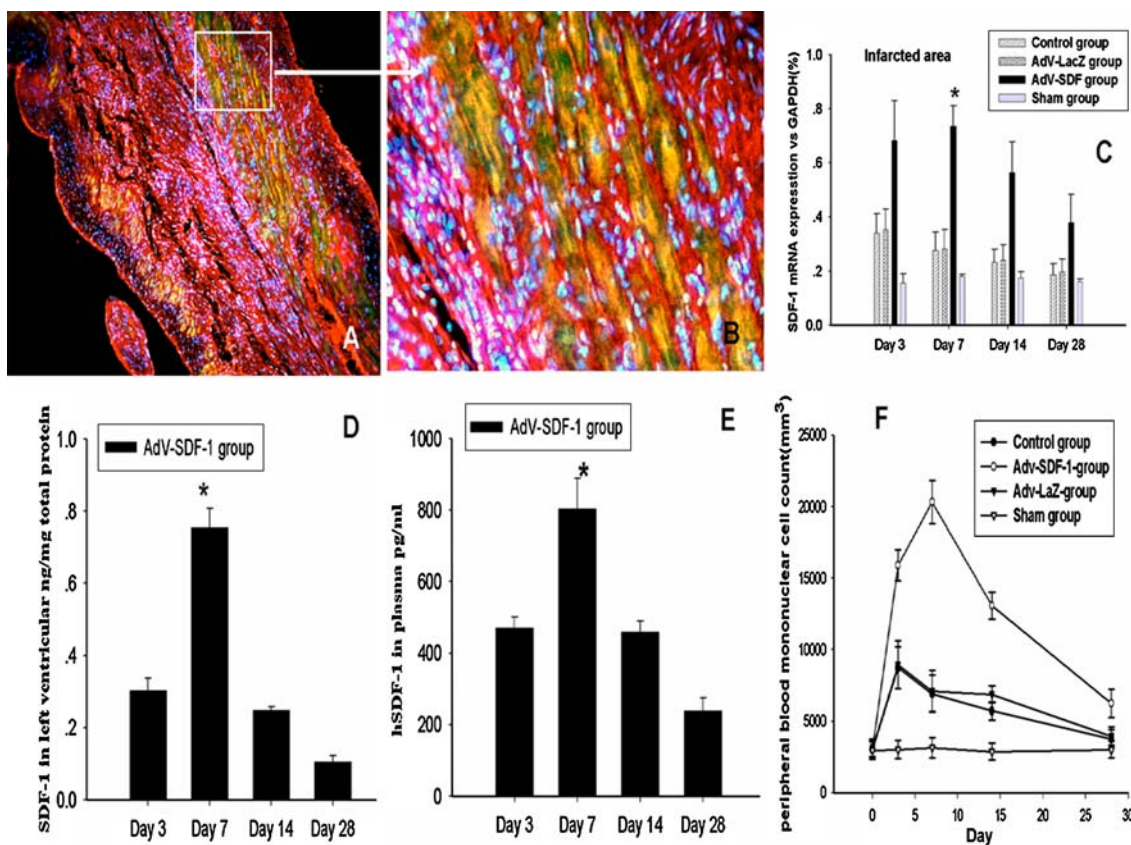


Fig. 1 SDF-1 expression and peripheral blood mononuclear cells (PBMNCs) count. **a, b** Immunofluorescence staining for hSDF-1 α expression in the infarction area and non-infarction area 7 day after MI. RT-PCR for SDF-1 mRNA expression in different time point after

MI (**c**). ELISA for SDF-1 levels in heart tissues (**d**) and serum (**e**) in the AdV.SDF-1-group. **f** PBMNCs counts after MI. Values are mean \pm SD. ($n = 5$, each). * Denotes $P = 0.005$ vs. control group. **a** 100 \times ; **b** 400 \times

augmented response of PBMNCs count to SDF-1 after MI induction.

Effect of SDF-1 on stem and progenitor cell homing into myocardium

CXCR4 and c-Kit (CD117) mRNA expression levels increased obviously in the infarcted area of AdV.SDF-1 group compared with control group or AdV.LacZ group. And CXCR4 mRNA expression was greater than c-Kit mRNA expression in the infarcted area of AdV.SDF-1 group (Fig. 2a, b). Simultaneously, the heart tissue sections were immunostained for the presence of c-Kit (CD117) and CXCR4 positive cells in different groups 7 and 28 days after treatment. The numbers of c-Kit⁺ and CXCR4⁺ cells were significantly higher in the infarct areas with overexpression of SDF-1 α in the heart of AdV-SDF-1 injection, as

compared with the other group (Fig. 2c–e). Furthermore, c-Kit⁺ cells were simultaneously CXCR4⁺ (Fig. 2f).

Effect of SDF-1 on surviving cardiocytes and angiogenesis in the heart

The data in Fig. 3 demonstrate that SDF-1, to a greater extent, increase the area and number of cardiac myocytes within the infarct zone (Fig. 3a–d). Semi-quantitation analysis of myocardium in the infarcted area showed cTnt density were higher in AdV.SDF-1-group ($P < 0.001$) as compared with the control group and AdV.Lac-Z group (Fig. 3j). The angiogenic effect of SDF-1 overexpression was measured in terms of blood vessel density in the infarct regions after immunostaining for vWF-VIII (Fig. 3e–h). The numbers of blood vessel per area were higher in infarct regions of the AdV.SDF-1-group ($P < 0.001$) as compared with the control group and AdV.Lac-Z group (Fig. 3i).

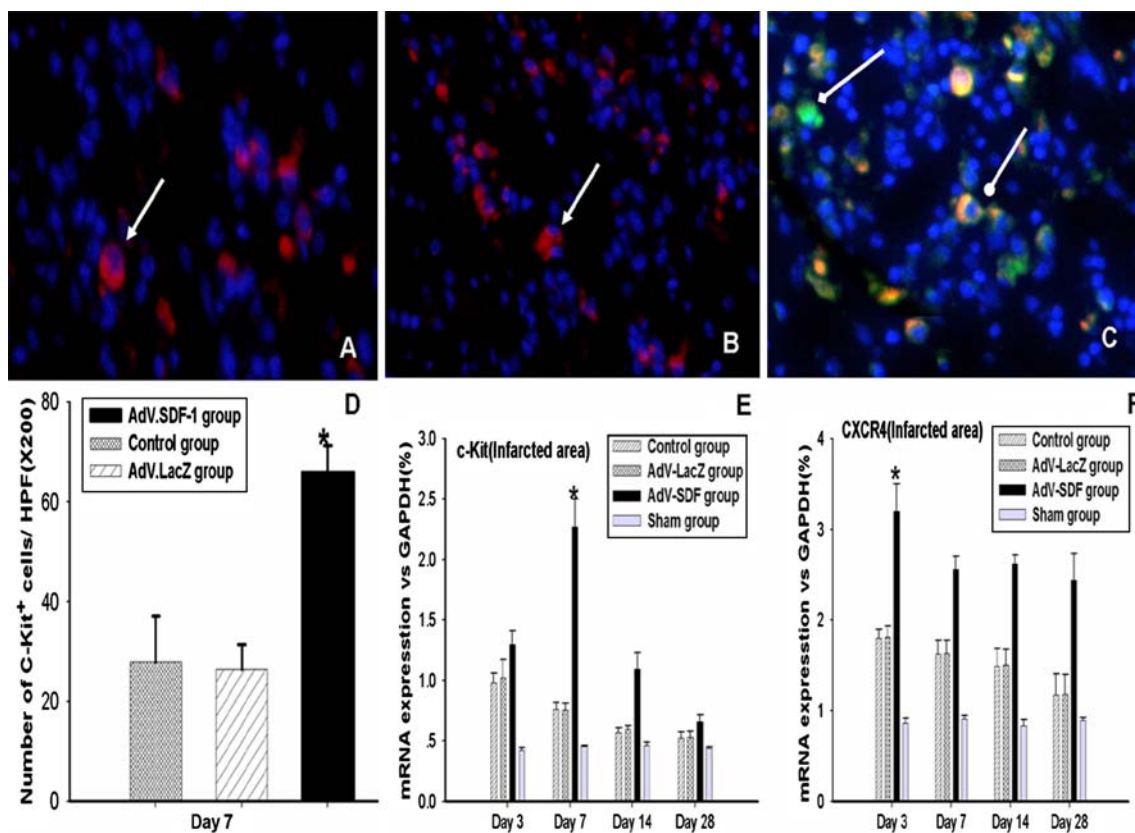


Fig. 2 Representative immunofluorescent staining of homing stem cells in the myocardium infarction area. **a, b** c-Kit staining 7 days after MI. c-Kit positive cells were observed in the infarction area of the AdV.SDF-1 (**a** 400 \times) and control groups (**b** 400 \times). **c** c-Kit and CXCR4 double-staining 7 days after MI (**c** 400 \times). c-Kit⁺ (arrow indicated), red fluorescence; cellular nucleus marked by DAPI, blue fluorescence; CXCR4⁺ (diamond arrow indicated), green fluorescence. c-Kit⁺ CXCR4⁺ cells, round arrow indicated. **d** The number

of c-Kit⁺ and CXCR4⁺ cells were analyzed per high power field (200 \times) by count method using image pro5.02, respectively. Data are mean \pm SD ($n = 5$ each). **e, f** RT-PCR analysis of c-Kit (**e**) and CXCR4 (**f**) mRNA expressions in the infarction area. Rat GAPDH served as an internal control. Data are the means of experiments carried out in duplicate. Values are mean \pm SD. ($n = 5$, each). * Denotes $P = 0.003$ vs. control or AdV.LacZ group

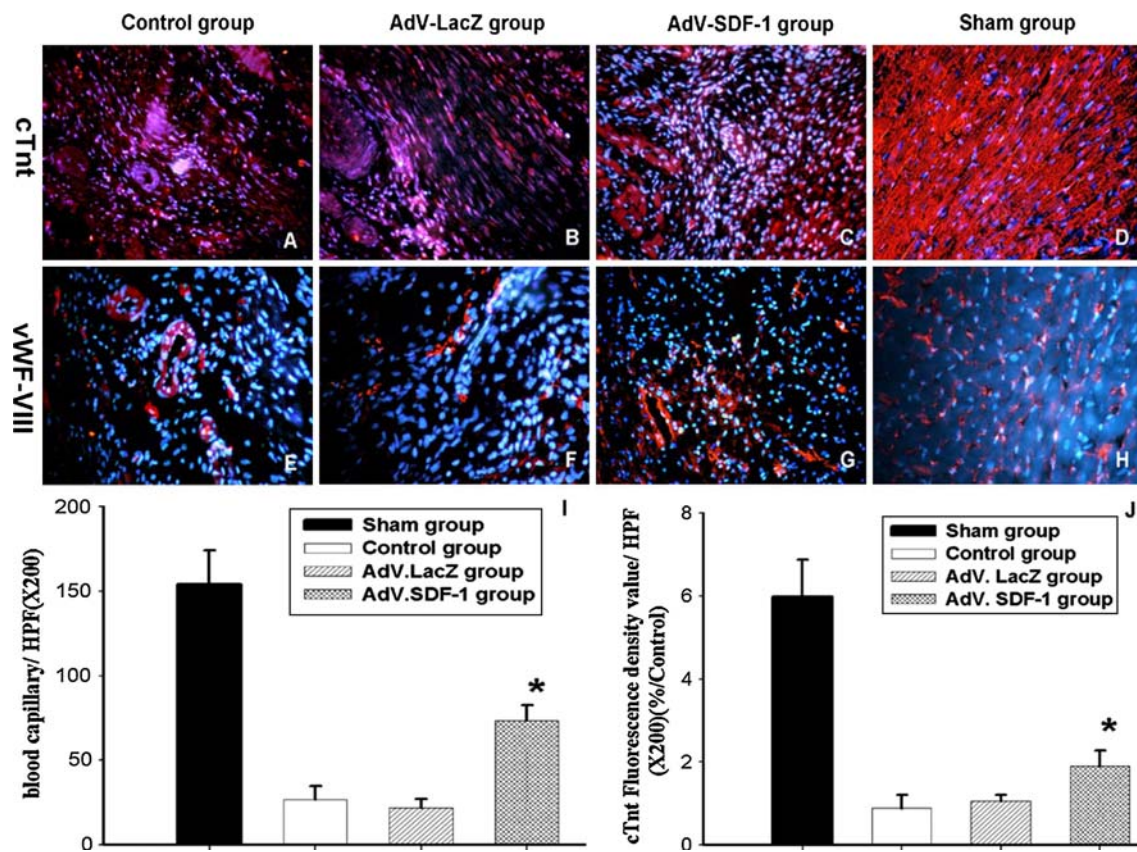


Fig. 3 Immunofluorescent staining of myocardium and blood vessel. **a–d** cTnt staining 28 days after MI. cTnt positive cells were observed in the infarction area of the control (**a**), AdV.LacZ (**b**), AdV.SDF-1 (**c**) and sham groups (**d**). **e–h** Von Willebrand factor (vWF) staining after MI. blood vessel was observed in the infarction area of the control (**e**), AdV.LacZ (**f**), AdV.SDF-1 (**g**) and sham groups (**h**), respectively. **i** The number of blood vessel was analyzed per high

power field (200 \times) by count method using image pro5.02, respectively. Data are mean \pm SEM ($n = 5$ each). **j** The value of cTnt positive red fluorescence photodensity was analyzed by per high power field (200 \times) by densitometric method using image pro5.02. Data are mean \pm SEM ($n = 5$ each). * Denotes $P = 0.001$ vs. control or AdV.LacZ group

However, these above changes were lower than sham group.

Measurement of hemodynamics

To confirm whether the over-expression SDF-1 in injured myocardium would even further improve cardiac function, we carried out measurement of hemodynamics 28 days after transplantation. Measurement of hemodynamics in vivo showed that LV function was significantly lower in AdV.SDF-1 group, AdV.LaZ group and control group than sham group. However, improvement of LV function was significantly greater in AdV.SDF-1 group than the control group or the AdV.LaZ group. The differences of LVSP ($P < 0.05$), $\pm dp/dt_{max}$ and LVEDP ($P < 0.05$) were statistically significant between the three groups (Table 1).

Quantitative RT-PCR analysis

mRNA expression of $TGF\beta 1$ in the infarcted area of AdV.SDF-1 group, control group and AdV.LacZ group increased obviously compared to that of sham-operated rats, and was peaked on day 3 or 7 up to approximately 2–3 fold compared to that of sham-operated rats, and then slowly declined. However, $TGF\beta 1$ mRNA expression in the infarcted area of AdV.SDF-1 group also showed no difference or lower levels compared with control group or AdV.LacZ group 7–28 days after treatment (Fig. 4a). Collagen type I and type III mRNA expression levels in the infarcted area of AdV.SDF-1 group, control group and AdV.LacZ group increased obviously compared to that of sham-operated rats. But, Collagen type I and type III mRNA expression levels in the infarcted area of AdV.SDF-1 group were lower than the control group or AdV.LacZ group (Fig. 4c, d). Over-expression SDF-1 in AdV.SDF-1

Table 1 Physiologic and hemodynamic parameters on days 28

	Sham group (<i>n</i> = 11)	Control group (<i>n</i> = 10)	Adv-SDF-1 group (<i>n</i> = 12)	Adv-LacZ group (<i>n</i> = 10)
HVW/BW (mg/g)	2.146 ± 0.30	1.88 ± 0.22	2.02 ± 0.43	1.85 ± 0.32
HR (BMP)	402 ± 11	375 ± 16	394 ± 15	379 ± 17
LVSP (mmHg)	120 ± 3.1	91 ± 3.71	106 ± 4.3*	92 ± 4.1
LVEDP (mmHg)	3.25 ± 0.53	14.86 ± 0.97	10.01 ± 0.71*#	13.97 ± 0.98
+dP/dt _{max} (mmHg/s)	7,781 ± 252	2,870 ± 129	4,330 ± 267*#	2,967 ± 131
−dP/dt _{max} (mmHg/s)	6,634 ± 231	1,927 ± 177	3,624 ± 295*#	2,023 ± 105

+dP/dt_{max} and −dP/dt_{max} rate in rise and fall of ventricular pressure, respectively. Data are mean ± SEM (*n* = 7–10)

HVW heart ventricular weight, BW body weight, LVSP left ventricular systolic pressure, LVEDP left ventricular end-diastolic pressure

* Denotes *P* < 0.005 vs. control group

Denotes *P* < 0.005 vs. sham group

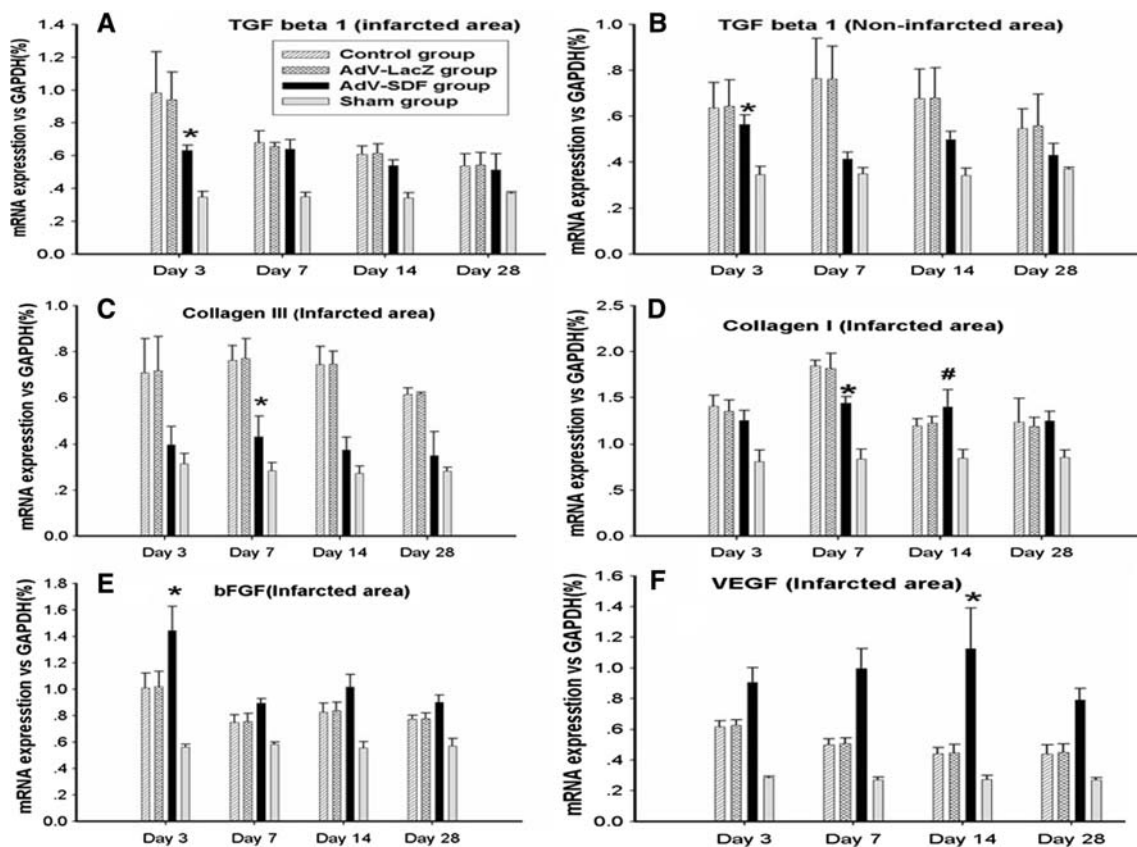


Fig. 4 SYBR Green real-time PCR analysis of TGF- β 1 in infarcted (a) and noninfarcted (b) myocardium, and of collagen type I (c), type III (d), bFGF (e) and VEGF (f) mRNA expression in infarcted myocardium. Data were processed with a specially designed software program based on Ct values of each sample and normalized to GAPDH. Bars represent mRNA levels relative to GAPDH mRNA

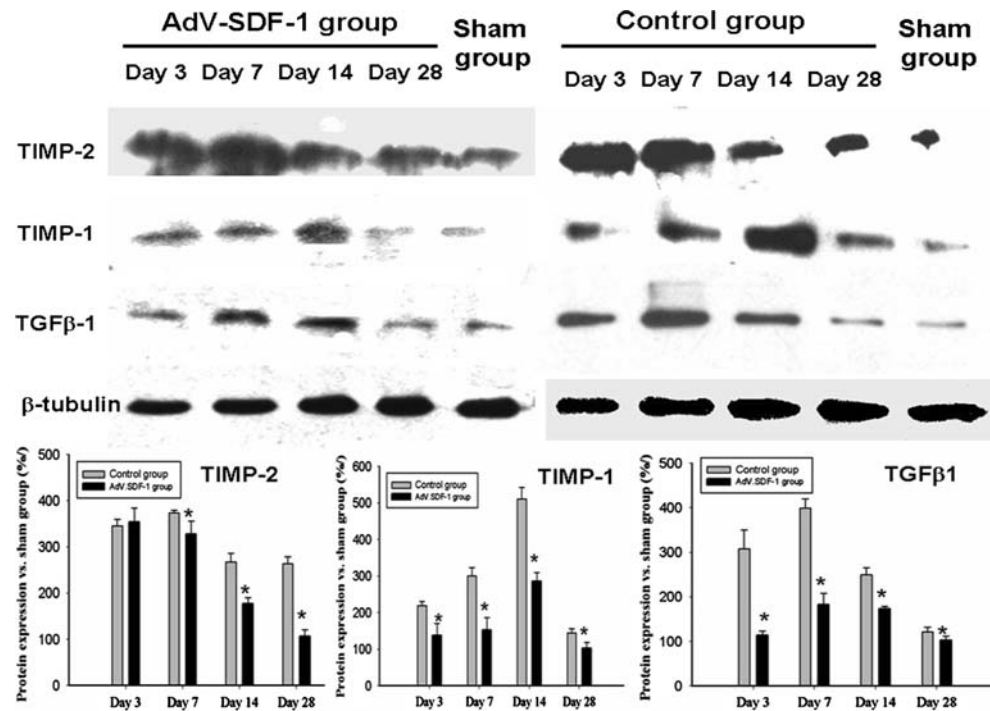
level for control, Adv.LacZ, Adv.SDF-1 and sham group. Data are the means of experiments carried out in duplicate. Values are mean ± SD. (*n* = 5 each). * *P* = 0.002 vs. control or Adv.LacZ group. VEGF vascular endothelial growth factor, basic-FGF basic fibroblast growth factor

group increased the expression of VEGF and bFGF mRNA analyzed in the myocardium infarction area (Fig. 4) after MI, and VEGF and bFGF expression increased obviously in 3 day after MI and continued until 7–28 days after MI.

Western blot analysis

Levels of TIMP-1, TIMP-2 and TGF- β 1 proteins in the infarcted area at different time point were analyzed by Western blotting. There was an increase in TIMP-1, TIMP-

Fig. 5 Western blotting analysis of TGF- β_1 , TIMP-1 and TIMP-2 in infarcted myocardium. Bars represent protein levels relative to β -tubulin for control group, sham group and AdV-SDF-1 group. Data are the means of experiments carried out in duplicate. Values are mean \pm SD. * Denotes $P = 0.004$ vs. sham or control group ($n = 5$ each)



2 and TGF- β_1 protein in the infarcted area in both the AdV.SDF-1 group and control group compared to sham group at each time point. Highest TIMP-1, TIMP-2 and TGF- β_1 expression was noted on day 14, 7 or 7 in different group, respectively. However, there was a decrease in TGF- β_1 , TIMP-1 and TIMP-2 protein in the infarcted area in the AdV.SDF-1 group compared to the control group at each time point (Fig. 5). And above these changes were no difference between the control group and AdV.LacZ group (data not shown).

Effect of SDF-1 on heart histology and morphology

Representative picture of myocardial infarction 28 days after surgery (Fig. 6a–d). As shown in Fig. 7g, k, new or surviving myocardium was located either in the middle of the scar or adjacent to the border zone. This type of pattern was not observed in any of the hearts that received medium injection only or AdV-LacZ. In contrast, the infarcted area in control group and AdV-LacZ group showed transmural and thin scar tissue composed of collagen (Fig. 5e, f). Representative Masson's trichrome-stained sections showed more collagen accumulation in the infarcted area and peri-infarction area 28 day after MI in control group or AdV-LacZ group (Fig. 5i, j) than AdV-SDF-1 group (Fig. 5k), semi-quantitative analysis indicated that collagen volume fraction in the infarcted area was significantly decreased in AdV-SDF-1 group compared to control group or AdV-LacZ group (Fig. 7a). Marked increases in the average LV wall thickness were also observed in the

AdV-SDF-1 hearts (Fig. 7b). Moreover, infarct size was significantly reduced in AdV-SDF-1 group as compared with the control group (Fig. 7c). Quantitative analysis of cavitory dilatation and wall thickness in all hearts of three groups revealed that AdV-SDF-1 injection animals displayed significantly less ventricular dilation than control group or AdV-LacZ group. The expansion index was significantly lower in AdV-SDF-1 group than in control group (Fig. 7d).

Discussion

Presently, isolation of cardiac stem cells was based exclusively on the expression of a stem cell-related surface antigen: c-kit and Sca-1. Freshly isolated c-kit⁺ Lin⁻ cells from heart were found to be self-renewing, clonogenic, and multi-potent, exhibiting biochemical differentiation into the myogenic cell, smooth muscle cell, or endothelial cell lineage [1–3, 16]. Interestingly, Sca-1⁺ c-Kit⁻ cells from mice hearts were induced in vitro to differentiate toward the cardiac myogenic lineage in response to 5'-azacytidine [17]. Obviously, cardiac stem cells appear to be a mixture of different subpopulations. In this study, we selected the marker: c-Kit as identification index of cardiac stem cell, and c-Kit⁺ cells in the damaged heart were observed. We found c-Kit⁺ cells increased significantly in the Adv-SDF-1 group than the other group, which were directly mediated by SDF-1/CXCR4.

It is interesting that Ratajczak et al. have reported that cells expressing early cardiac markers reside in the bone

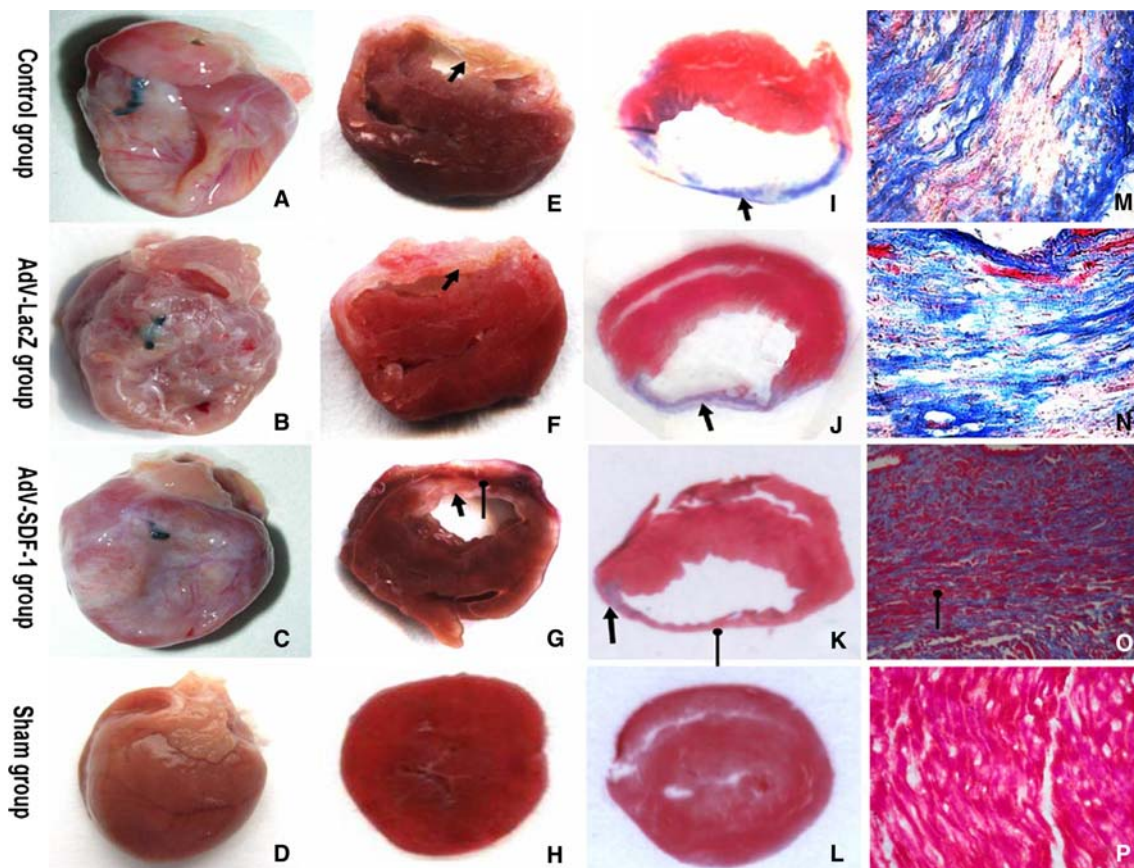


Fig. 6 Effects of SDF-1 on histology and form. **a–d** Representative picture of a myocardial infarction 28 days after surgery, the heart was exercised, and infarction size was measured by TTC (3 \times). **e–h** The infarcted myocardium was unstained by TTC (white, arrow indicated), and the non-ischemic zones stained brick red. **i–p** Representative Masson's trichrome-stained sections in the area of infarcted left

ventricular tissue 28 days after MI (blue, collagen, black arrow indicated; **i–l** 3 \times ; **m–p** 400 \times). There was a decreased infarct size, thicker LV wall in the Adv.SDF-1 group. The new or surviving myocardium (black round arrow indicated) typically appeared as red-positive cells wedged between an inner and outer layer of collagen (each group, $n = 6$) (Color figure online)

marrow and are mobilized into the peripheral blood after myocardial infarction. Furthermore, Cimini' study indicated that interventions that increase the infiltration of bone marrow derived c-kit⁺ cells to the infarcted heart could potentiate this endogenous repair response, prevent infarct expansion, and improve the recovery of cardiac function after MI [18]. However, some studies showed the characteristic of c-Kit⁺ cells derived from heart and bone marrow is different [1–3, 16, 17]. And in this study, we found cardiogenic c-Kit⁺ cells could spontaneously differentiate into cardiomyocytes in vitro, but bone marrow derived c-Kit⁺ cells could not spontaneously differentiate into cardiomyocytes, bone marrow derived c-Kit⁺ cells could differentiate into cardiomyocytes when co-cultured with cardiomyocytes in vitro [supplement 3]. Although we did not confirm whether these c-Kit⁺ cells derived from bone marrow or heart or not, cardiocytes in infarction and peri-infarction area increased significantly in the Adv-SDF-1 group than the control or Adv-LacZ group. These data

suggested the part of these cardiocytes could derive from c-Kit⁺ cardiac stem cells. In addition, SDF-1 has been recently shown to have additional non-stem cell-recruiting effects, including increasing stem cell survival. Our and Zhang' studies showed SDF-1 to the cell surface of injured cardiac myocytes could lead to inhibition of cardiocytes apoptosis via SDF-1 inducing up-regulation of prosurvival signaling [19, 20]. Therefore, these changes could lead to decrease scar sizes, reduce remodeling and improve cardiac function, which is a consequence of the increased cardiocytes in damaged heart sites.

It is interesting that our study have showed c-Kit⁺ stem cells differentiation into endothelial cells were related with the presence of SDF-1 in vitro [supplement 3]. Recently, Falco et al. [21] have shown that SDF-1 plays an important role in differentiation of bone marrow-derived c-kit⁺ stem cells into EPCs and endothelial cells. More importantly, Fazel et al. [22] have shown that bone marrow c-kit⁺ cells act as key regulators of the angiogenic switch in infarcted

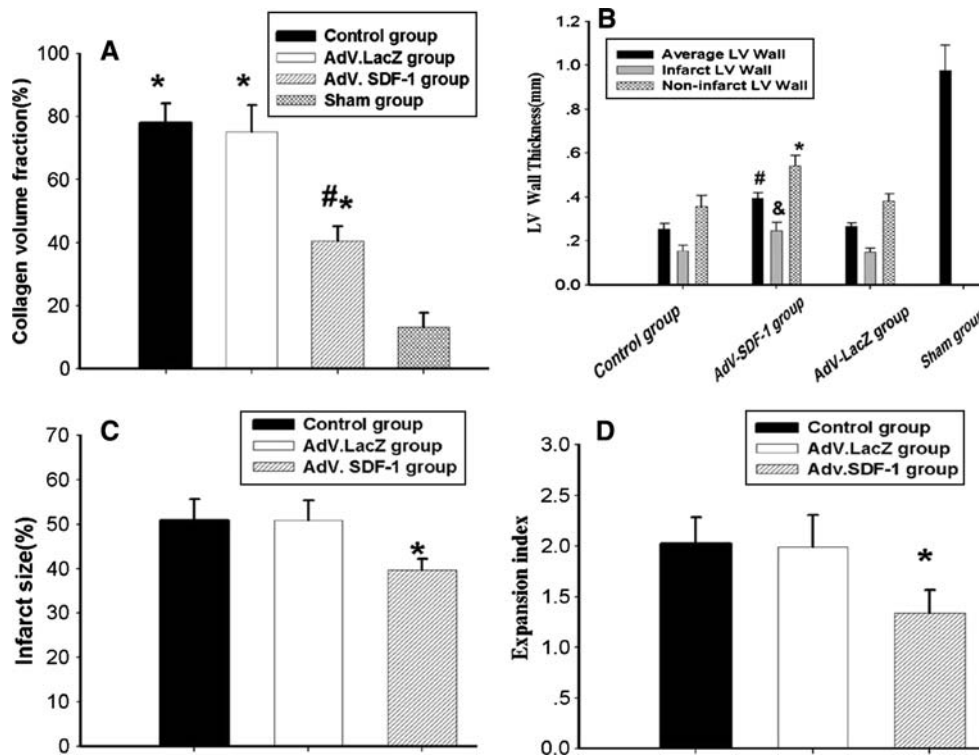


Fig. 7 Effects of SDF-1 on infarct size and left ventricle wall thickness. **a** Quantitation of collagen content in percent of infarct and noninfarcted area in multiple fields. **b** Thickness of infarct LV wall in AdV.SDF-1 group is obviously thicker than the infarct LV wall in the control and AdV.Lac-Z group. Thickness of infarct LV wall in AdV.SDF-1 group is thinner than the non-infarct LV wall ($^{\&} P = 0.001$) and is obviously thicker than the infarct LV wall in the control or AdV.Lac-Z group ($^{\&} P = 0.0005$). The average LV wall in the AdV.SDF-1 group is thicker than in the control or

AdV.Lac-Z group ($^{\#} P = 0.001$) and thinner than in the sham group ($^{\#} P = 0.0003$). The non-infarct LV wall in the AdV.SDF-1 group is thinner than in the sham group ($* P = 0.002$) and thicker than in the control or AdV.Lac-Z group ($* P = 0.0003$). **c** There were statistically significant differences in infarct size among the control, AdV.Lac-Z and AdV.SDF-1 groups (mean \pm SD). ($* P = 0.0006$). **d** Expansion index analyses in AdV.SDF-1 group showed lower than control or AdV.Lac-Z group (each group, $n = 6$)

myocardium, and SDF-1 promote angiogenesis by enhancement of EPCs incorporation into new vessels [23]. Furthermore, these stem cells produce a wide array of arteriogenic cytokines and improve perfusion and remodeling in mouse models of hindlimb ischemia, and these effects appear to be mediated through paracrine mechanisms associated with local release of the arteriogenic cytokines [22, 24–26]. In our study, VEGF expression level in the AdV-SDF-1 group obviously increased. Increased VEGF could involve in or trigger angiogenic progress of stem cells, even might contribute to postnatal neovascularization by mobilizing bone marrow-derived stem cells [7–9, 21, 24, 27, 28]. Of course, besides VEGF, released angiogenic cytokines after MI include fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and so on [28]. For example, Rosenblatt-Velin et al. [29] recently reported that bFGF controls the differentiation of resident cardiac precursors into functional cardiomyocytes. And other angiogenic factors contribute to angiogenesis in the damaged sites [30, 31]. Therefore; these cytokines could

contribute to stem cell-mediated regeneration repair of infarcted myocardium.

In our study, we evaluated the functional improvement seen in AdV.SDF-1 group by cardiac catheterization to detect ventricular pressure. The AdV.LacZ group and control group showed the expected decline in $\pm dp/dt_{max}$ and LVSP, and markedly increase in LVEDP. In contrast, and as expected, The AdV.SDF-1 group showed the lower decline in $\pm dp/dt_{max}$ and LVSP, and the lower increase in LVEDP. Overexpressing SDF-1 contributed to preservation of $\pm dp/dt_{max}$, LVSP and LVEDP in infarct hearts. Also, histology and morphometric measurement showed the expected decrease in infarcted size (IS), and obviously increase in LV wall thickness in AdV.SDF-1 group. And SDF-1 overexpressing hearts were less dilated and a combination of less dilatation and a trend toward less paradoxical systolic bulging probably led to the improved ventricular pressure. Furthermore, overexpressing SDF-1 could inhibit LV remodeling, augment or preserve the myocardial elasticity, improve heart function and reduce

the expression of extracellular matrix genes (eg: Collagen, et al.). These change of morphous and structure is related with the increasing of the area and number of cardiac myocytes within the infarct zone.

More Recently, Shao et al. [23] reported that SDF-1 promoted angiogenesis by enhancement of EPCs incorporation into new vessels, which was related with upregulation and activation of matrix metalloproteinase-2 (MMP) and MMP-9. However, TIMP-2 bound to the membrane in a hydroxamate insensitive manner specially inhibits MMP-2 activated by the membrane, but not other MMPs. Similarly, TIMP-1 inhibits MMP-9 activation [32]. Interestingly, TIMP-1 overexpression in pancreatic cancer cells reduced angiogenesis and cell implantation, this anti-angiogenic activity of TIMP-1 involved in part MMP-inhibition [33]. TIMP-2 mediated inhibition of angiogenesis was an MMP-independent mechanism [34]. Furthermore, TIMPs can block the angiogenic process at different stages and the acquisition of an angiogenic phenotype may involve the suppression of TIMPs expression [32]. For example, TIMPs can inhibit bFGF-induced human endothelial cell growth [35]. In our study, we observed that expression of Tissue inhibitors of metalloproteinases (TIMP1, 2) decreased in infarction area of myocardium in the AdV-SDF-1 group, which was concordance with much more stem cells and blood vessels. These data suggested that angiogenesis induced by SDF-1 could involve in TIMPs down-regulation and MMPs up-regulation, which would not only contribute to create a favorable tissue environment for stem cells migration, but also promote angiogenesis.

Moreover, TIMPs as multifunctional proteins involved in LV remodeling. Koch et al. reported that SDF-1 through transendocardial delivery promoted the degradation of collagen in peri-infarct area [18]. Collagen synthesis, which is promoted by TGF- β 1, has different significance in infarcted and noninfarcted areas [36]. Our current study suggested that TGF- β 1 down-regulation caused by SDF-1 was associated with decreased collagen accumulation and down-regulated TIMP-1 expression in the infarction area. Although production of fibrosis after MI prevents structural fragility. Previous studies reported that a MMP family was increased in the postinfarction heart failure models with permanent occlusion and large infarction and that the inhibitors beneficially affected cardiac remodeling and function [36, 37]. Furthermore, myocardial TIMP-1 plays a regulatory role in post-MI remodeling and the accelerated myocardial remodeling induced by TIMP-1 gene deletion can be pharmacologically “rescued” by MMP inhibition [38]. Thus, it is suggested that a decrease in TIMPs has an aggravating effect on heart failure via collagen degradation. However, it is well known that the volume of reactive granulation and/or scar tissues after skin injury after burn, surgery, etc, frequently becomes excessive, and this is

called hypertrophic scarring. The excessive extent of fibrosis without contractility or relaxation would accelerate cardiac remodeling and decrease cardiac function, as seen in ischemic or idiopathic dilated cardiomyopathy. Furthermore, an increase in MMPs by hepatocyte growth factor or G-CSF was beneficial on post-MI heart failure via its anti-fibrotic action; reduction of scar tissue and improvement of remodeling were observed simultaneously in myocardial regeneration therapy [23, 39–41]. In addition, in the setting of liver injury, MMP-9 is associated with increased CXCR4 expression on stem cells, and MMP inhibitors reduce homing of these cells to the liver [42, 43]. Although SDF-1 is cleaved by exopeptidases and matrix metalloproteinase-2, a new, protease-resistant SDF-1 and nanofiber-mediated delivery promoted recruitment of stem cells and improved cardiac function after myocardial infarction [44]. Thus, in the present study, the beneficial effect of SDF-1 over expression relies on a higher number of cardiocytes and blood vessels and down-regulation of TIMPs and increased participation of mobilized stem cells, which supports the concept of myocardial repair mediated by stem cells after infarction.

Conclusion

our data are consistent with the concept that there is a naturally occurring regenerative repair process that occurs in infarcted myocardium that can be enhanced through the over-expression of SDF-1 α within the myocardium after MI. Over-expressing SDF-1 α improved cardiac structure and function through the symphyisial effect of myogenesis and angiogenesis.

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