Mesenchymal stem cells over-expressing SDF-1 promote angiogenesis and improve heart function in experimental myocardial infarction in rats

Junming Tang a,b,c,d,* , Jianing Wang a,b,d,* , Jianye Yang a,b , Xia Kong a,b , Fei Zheng a,b , Linyun Guo a,b , Lei Zhang a,b , Yong Zhang Huang a,b

a Institute of Clinical Medicine, Renmin Hospital, Yunyang Medical College, Shiyuan, Hubei 442000, People’s Republic of China
b Department of Cardiology, Renmin Hospital, Yunyang Medical College, Shiyuan, Hubei 442000, People’s Republic of China
c Key Lab of Human Embryonic Stem Cell of Hubei Province, Yunyang Medical College, Shiyuan, Hubei 442000, People’s Republic of China

Received 24 November 2008; received in revised form 13 April 2009; accepted 27 April 2009; Available online 12 June 2009

Abstract

Background: In addition to its multipotent capability, the mesenchymal stem cell (MSC) can secrete and supply a large amount of vascular endothelial growth factor (VEGF). The stromal-derived factor-1 alpha (SDF-1α) plays an important role in the homing of stem cells to the injured tissues of the heart. Therefore, the MSCs over-expressing SDF-1α could augment the angiogenesis pathway. Methods: In vitro, the differentiation of the MSCs into endothelial-like cells was induced by cultivation of cells in 10% foetal calf serum and 50 ng ml⁻¹ SDF-1α or in specific inhibitors for endothelial nitrous oxide synthase (enOS). In vivo, the differentiation of myocardial infarction was established by occlusion of the left anterior descending coronary artery. Seven days following surgery, 5.0 × 10⁶ pfu Ad-SDF-1α (adenoviral vector containing human SDF-1α gene under the control of the rous sarcoma virus (RSV) promoter), 5.0 × 10⁶ Ad-LacZ-MSC or 5.0 × 10⁹ pfu Ad-SDF-1α gene under the control of the left anterior descending coronary artery. Seven days following surgery, 5.0 × 10⁶ pfu Ad-SDF-1α (adenoviral vector containing human SDF-1α gene under the control of the rous sarcoma virus (RSV) promoter), 5.0 × 10⁶ Ad-LacZ-MSC or 5.0 × 10⁹ Ad-SDF-MSC suspension in a 0.2-ml serum-free medium was injected into four sites in infarcted areas (0.05 ml per site). The rats receiving Ad-SDF-MSC also received the nitrous oxide (NO) synthesis inhibitor N⁶-nitro-L-arginine methyl ester (L-NAME) in drinking water (1 mg kg⁻¹). The rats in the control group received the same volume of cell-free medium. Four weeks following transplantation, the heart function was assessed, and histological and molecular analyses were conducted. Results: The MSCs could differentiate into endothelial cells in the presence of SDF-1α, and the effect could be inhibited by L-NAME in vitro and in vivo. Western Blotting revealed an increased expression of VEGF, Akt and eNOS. Four weeks following transplantation, a reduced infarct size and fibrosis, greater vascular density and thicker left ventricular wall were observed in the Ad-SDF-MSC group. The measurement of haemodynamic parameters showed an improvement in the left ventricular performance in the Ad-SDF-MSC group as compared with other groups. Conclusion: The MSCs over-expressing the SDF-1α can produce effective angiogenesis, resulting in the prevention of progressive heart dysfunction after a myocardial infarction.

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Keywords: Angiogenesis; Myocardial infarction; SDF-1α; Stem cell; VEGF; eNOS

1. Introduction

Despite advances in re-vascularisation techniques, the treatment of ischaemic heart disease remains a challenge. Gene and stem cell-based therapies hold promise for the treatment of ischaemic diseases.

The development of blood vessels in adult tissues is a complex process in which growth factors, cytokines and cells act in concert. Two main mechanisms are known for the formation of new blood vessels: angiogenesis (sprouting existing primitive vasculature from differentiated cells) and vasculogenesis (developing new blood vessels in situ by differentiation from precursors) [1]. Vascular endothelial growth factor (VEGF) determines the activation, proliferation and migration of vascular endothelial cells, and could significantly enhance angiogenesis in ischaemic tissues [2, 3]. The VEGF alone may be insufficient to achieve the development of a functional and mature vasculature [2]. A possible mechanism is that angiocompetent stem/precursor cells, pericytes and smooth muscle cells may not entrap endothelial cells, that is, these cells could egress from VEGF-over-expressing tissues when stromal-derived factor-1 alpha (SDF-1α) expression was switched off [3].

This study was supported by grants from the National Natural Science Foundation of China (30700306), Hubei Natural Science Foundation (305ABA079), Hubei Health Department Science Foundation (JX3B29), Hubei Education Department Science Foundation (Q200524003, B200624006) and the YunYang Medical College Science Foundation (2005QDJ01).

* Corresponding author. Address: Institute of Clinical Medicine, Renmin Hospital, Yunyang Medical College, Shiyuan, Hubei 442000, People’s Republic of China. Tel.: +86 719 8637170; fax: +86 719 8637011.

E-mail addresses: tangjm1416@163.com (J. Tang), rwyjn@vip.163.com (J. Wang).
As SDF-1α was initially described as a product of bone marrow stromal cells, its involvement in haematopoiesis was also suggested. In the meantime, SDF-1α has been found to be expressed in a wide variety of organs, such as the heart, liver, spleen and kidney. Several studies have revealed an instrumental role of SDF-1α in the homing of stem cells to the bone marrow and injured tissues or organs [4]. Besides its multipotent capability, mesenchymal stem cells (MSCs) can secrete and supply a large amount of VEGF, and transplanted MSCs enhanced angiogenesis in infarcted hearts [5–7]. However, the low survival of transplanted MSCs in infarcted hearts reduces the therapeutic effects [8]. In recent times, several studies have reported that the SDF-1α promoted the survival of cardiomyocytes, MSCs and other cells via Akt activation [9–11]. Therefore, we hypothesise that the MSCs that secrete VEGF in situ could be transfected with the SDF-1α, and their combination could greatly augment the angiogenesis pathway. We investigated the effect of the MSCs transfected with the SDF-1α gene on the neovascularisation of the myocardium in a murine model of myocardial infarction.

2. Methods

2.1. Isolation and culture of MSCs and multidifferentiation of the MSCs

The isolation and primary culture of the MSCs were carried out by our previously described method [7]. In brief, the rat MSCs were isolated from the bone marrow with density-gradient centrifugation and were cultured in low-glucose Dulbecco’s modified Eagle medium (DMEM; Gibco Laboratories, USA) supplemented with 10% foetal calf serum (FCS; Hyclone), 100 U ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin at 37 °C in a humidified atmosphere containing 5% carbon dioxide (CO₂). The culture medium was removed and replaced with fresh medium twice a week. At confluence, the cells were harvested for passage with 0.25% trypsin (Solen, OH, USA) containing 0.02% ethylenediaminetetra-acetic acid (EDTA; Sigma, St Louis, MO, USA).

Adipogenic and osteogenic differentiations were carried out by our method that has been described earlier [7]. For the differentiation of endothelial cells, the MSCs were kept after 48 h, and the culture medium (DMEM +10% foetal bovine serum (FBS)) was supplemented with recombinant human SDF-1α (hSDF-1α) only (50 ng ml⁻¹); SDF-1 (50 ng ml⁻¹), SDF-1 (50 ng ml⁻¹) and AMD3100 (10 μg ml⁻¹); SDF-1 (50 ng ml⁻¹) and Nω-nitro-o-arginine methyl ester (L-NAME; 100 μm) or without additives. The medium was changed every 3 days with the same formulation across 14 days.

2.2. MSCs transfected with adenoviral vectors and evaluation

Transduction of the MSC with adenovirus was carried out as described earlier [10]. In brief, the MSCs were plated at a density of 10 000 cells cm⁻² in six-well plates or T75 flasks, and were cultured overnight. The cells were exposed to a fresh culture medium for the MSCs containing Ad-SDF-1α (an adenoviral vector containing human SDF-1α gene under the control of the rous sarcoma virus (RSV) promoter) or Ad-LacZ (an adenoviral vector containing the nuclear-targeted β-galactosidase gene LacZ under the control of RSV promoter) at 100 multiplicities of infection (defined as pfu per cell) for 48 h. The cells were analysed for transgene expression or used for ex vivo gene therapy and cell viability was determined by the Trypan blue exclusion method. The expression of the hSDF-1α transgene in the MSCs was evaluated by immunohistology under fluorescence microscopy. The green fluorescent cells found in 2–3 microscopic fields were counted. Blue fluorescence showed 4,6-diamino-2-phenylindole (DAPI; 50 μg ml⁻¹) counterstaining of the cell nucleus as the total number of cells in the same visual field (transfection efficiency of MSCs with Ad-SDF-1 = merging of green and blue double-positive cells per blue fluorescence-positive cell nucleus).

The MSCs were incubated with Ad-SDF-1 for 48 h. The cell lysate was assayed for hSDF-1α, endothelial nitric oxide synthase (eNOS; 1:250; Santa Cruz) and phosphorylated Akt (1:250; Cell signalling) by Western Blot analysis. A total of 100 μl of media was obtained 1, 6, 12, 24 and 48 h later. The levels of SDF-1α and hSDF-1α in the media were quantified using enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA).

The media (600 μl) were obtained 1, 6, 12, 24 and 48 h after the MSCs were incubated with Ad-SDF-1 or Ad-LacZ. The indicated concentration was put into the lower chamber of a Costar 24-well transwell (Millipore, USA). The human umbilical vein endothelial cells (HUVECs; 1–2 × 10⁵) in 200 μl of medium were placed in the upper chamber (pore size: 8 μm). The cells were collected from both chambers after 12 h of migration at 37 °C. The membrane was removed and scraped to remove non-migrating cells from the upper surface. The membrane was fixed and stained. The number of HUVECs cells that had migrated to the lower surface of the membrane was counted in 10 random high-power fields (HPFs) by light microscopy. The migration index was calculated to express stimulated migration using the following equation:

\[ \text{Migration index} = \frac{\text{Stimulated migration}}{\text{Random migration}} \]

Each assay was performed in triplicate wells.

2.3. Model of myocardial infarction and cell implantation

Male Sprague-Dawley rats were anaesthetised with ketamine (50 mg kg⁻¹, intraperitoneally (i.p.)) and xylazine (10 mg kg⁻¹, i.p.). Tracheal ventilation with room air using a Colombus ventilator (HX-300, Taimeng Instruments, Chendu, China) was done. Myocardial infarction was achieved by the ligation of the left anterior descending coronary artery 2–3 mm from the tip of the left auricle with a 6/0 silk suture. The successful performance of the coronary occlusion was verified by blanching of the myocardium distal to the coronary ligation, and electrocardiography [10]. The experimental animals were randomised into five groups. One week following their myocardial infarction, 5.0 × 10⁹ pfu Ad-SDF-
1, 5 × 10^6 Ad-LacZ-MSC or 5 × 10^6 Ad-SDF-1-MSC suspension in serum-free medium (0.2 ml) was injected separately, a 30-gauge tuberculin syringe, into four sites (0.05 ml per site) for each infarcted heart. The two injection sites were in the myocardium bordering the ischemic area, and two within the ischemic area. The control group underwent an identical procedure, but only an equivalent volume of the cell-free medium was injected.

2.4. Western Blotting and ELISA

The expression of VEGF, eNOS, SDF-1α, and Akt was detected by the Western Blot test. The total protein was prepared from the infarcted myocardial tissues 7 days after the treatment. Fifty micrograms of protein were electrophoresed on a 7.5% sodium dodecyl sulfate—polyacrylamide gel and transferred to polyvinylidene fluoride membrane (Millipore). After blocking with 0.2% Tween in Tris-buffered saline (TBS-T) containing 5% milk at room temperature for 2 h, the membranes were incubated for 2 h at room temperature with rabbit polyclonal anti-TnT (1:250, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membrane was washed thrice with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. The signal was detected by the addition of enhanced chemiluminescence reaction solution (KPL; American). The quantitative analysis of cardiac tissue contents of the rat VEGF and hSDF-1α (R&D Systems, Minneapolis, MN, USA) was measured by ELISA [10].

2.5. Heart function and morphometric measurement

The haemodynamic measurements in vivo were done 28 days following the treatment. The catheters filled with heparinised (10 U ml⁻¹) saline solution were connected to a stethoscope pressure transducer (Gould, Saddle Brook, NJ, USA). The carotid arterial catheter was advanced into the left ventricle to record ventricular pressure. These haemodynamic parameters were monitored 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). The heart was removed rapidly from the sacrificed rats (each group, n = 10–12) after the measurements were taken. Histology and morphometric measurement methods are shown in Supplement 1 [7].

2.6. Immunohistochemical analysis

At Day 7 and Day 28 after treatment (each time point, n = 5), rats were sacrificed. After quick removal, the hearts were immersion-fixed in 4% paraformaldehyde and embedded in an optimum cutting temperature compound (Fisher Scientific, Fair Lawn). Serial transverse sections measuring 5 μm were cut along the entire long axis of the heart, and mounted on slides. The specimens were incubated overnight with a monoclonal anti-mouse CD31 (1:200, ABCAM) antibody, α-smooth muscle actin (α-SMA) (1:250, Santa Cruz) antibody or anti-cTnt (1:250, NeoMarkers) antibody at 4 °C. Positive staining was demonstrated by red fluorescence colour tetrarhodamine isothiocyanate immunoglobulin G (IgG).

2.7. Data analysis

The data are mean ± standard deviation (SD). The statistical significance between two groups was determined by the unpaired Student’s t-test. The results for more than two experimental groups were evaluated by one-way analysis of variance (ANOVA) to specify differences between groups. p < 0.05 was considered statistically significant difference.

3. Results

3.1. hSDF-1α expressed in transduced MSCs is biologically active

Ninety percent of the MSCs stained positive for hSDF-1α 48 h after transduction (Fig. 1A and B). The cell viability was 90%, and the percentage of the hSDF-1α-positive cells declined over a 28-day period in the Ad-SDF-1 transduced MSC, but was 50% on Day 28 (Fig. 1C). The Ad-SDF-1-transduced MSCs (Ad-SDF-1-MSC) expressed 4.15 ± 1.3-fold greater SDF-1α mRNA and protein than the wild-type MSC or the Ad-LacZ-transduced MSCs (Ad-LacZ-MSC) 2 days after transfection (Fig. 1D and E). In addition, the MSCs over-expressing SDF-1α had greater phosphorylated Akt than the MSCs or Ad-LacZ-MSC (Fig. 1E). However, transfection with the SDF-1α expression vector did not change CXCR4 expression in the MSCs. To determine whether the hSDF-1α expressed in the Ad-SDF-1-MSC is biologically active, the constitutive hSDF-1α activity in the Ad-SDF-1-MSC culture media was measured using the migration efficiency of the...
hSDF-1α protein in HUVECs. As shown in Fig. 1F, the hSDF-1α protein from the Ad-SDF-1-MSC promoted significantly greater migration of HUVECs compared with the protein from the wild-type MSCs or the Ad-LacZ-MSC (Fig. 1F).

3.2. SDF-1α promotes MSC differentiation towards the endothelial cell in vitro

The MSCs did not express CD133, CD31, factor VIII-related antigen and alpha-myosin heavy chain (αMHC), but expressed c-Kit and CXCR4 (see Supplement 2). To test the effect of the SDF-1α on the MSCs differentiation into the endothelial cells, the cells were cultured for 14 days in the presence of the SDF-1α. After Day 14 of treatment, the MSCs started to express endothelial cell markers such as the CD31 and the γ-factor-related antigen (Fig. 2). The effect of the SDF-1α on the differentiation of the MSCs into endothelial cells was partly blocked when the MSCs were pre-treated with the CXCR4 inhibitor AMD3100, VEGF antibody or the non-selective NOS inhibitor L-NAME (Fig. 2). We did not find that the MSCs could differentiate into cardiomyocytes by real-time polymerase chain reaction (RT-PCR), Western Blot and immunohistochemistry (Supplement 3).

3.3. Measurement of haemodynamics

The measurement of haemodynamic parameters in vivo showed that the differences in left ventricular systolic pressure, the rate of rise and fall of ventricular pressure and left ventricular end-diastolic pressure were statistically significant among the five groups. In addition, the left ventricular function was significantly greater in the Ad-SDF-1-MSC and Ad-LacZ-MSC groups than in the Ad-SDF-1, control and Ad-SDF-1-MSC-L-NAME groups. Furthermore, improvement of the left ventricular function was significantly greater in the Ad-SDF-1-MSC group than in the Ad-LacZ-MSC group (Fig. 3).

3.4. Histology and morphometric measurement

Twenty-eight days following the treatment, representative Masson’s trichrome-stained sections showed much more myocardium and decreased collagen accumulation in the left ventricular wall of the infarcted and peri-infarction area in the Ad-SDF-1-MSC and Ad-LacZ-MSC groups than the Ad-SDF-
1, control and Ad-SDF-1-MSC-NAME groups. This change was more typical in the Ad-SDF-1-MSC group than in the Ad-LacZ-MSC group (Fig. 4A—D). The semi-quantitative analysis indicated that the collagen content in the infarcted area was significantly decreased in the Ad-SDF-1-MSC group compared with the Ad-SDF-1, control, Ad-SDF-1-MSC-NAME and MSC groups (35.8 ± 5.2% vs 55.7 ± 7.8%, 86.5 ± 7.6%, 65.2 ± 8.9% and 45.7 ± 6.5%) (Fig. 4E—H). The marked increases in the average left ventricular wall thickness were also observed in the Ad-SDF-1-MSC hearts (Fig. 4I). The infarct size was significantly reduced in the Ad-SDF-1-MSC group compared with other groups (Fig. 4J). Quantitative analysis of the cavitory dilatation in all the hearts of the four groups revealed that rats injected with the Ad-SDF-1-MSC displayed significantly less ventricular dilation than other groups. The expansion index was significantly lower in the Ad-SDF-1-MSC group than in Ad-LacZ-MSC group (Fig. 4K).

3.5. Angiogenesis and myogenesis induced by Ad-SDF-1-MSC

Immunofluorescence demonstrated that the partly transplanted MSCs were positive for the cardiac marker cardiac troponin-T, suggesting that a small number of the transplanted MSCs can differentiate into cardiomyocytes (Fig. 5). The efficiency of differentiation of the injected Ad-SDF-1-MSCs was higher than in other groups. The efficiency of differentiation of the total MSCs was <5% per 200× field. The area and number of cardiomyocytes within the infarct zone increased two- to fourfold in the Ad-SDF-1-MSC group than in the Ad-LacZ-MSC or Ad-SDF-1 group.

Simultaneously, the transplanted MSCs formed vascular structures in the myocardium and were positive for CD31. The Ad-SDF-1-MSC in the injured heart was positive for α-SMA and participated in vessel formation as mural cells (Fig. 5). The efficiency of differentiation of the total MSCs was <4% per 200× field. The capillary density in the Ad-SDF-1-MSC group was more than in the MSC, Ad-SDF-1, Ad-LacZ-MSC or control groups (Fig. 6).

3.6. Effect of SDF-1α on expression of eNOS, VEGF, and phospho-Akt

Immunofluorescence cytochemical and Western Blot analysis showed that the eNOS expression was observed in the MSCs before the initiation of cell differentiation (Fig. 7A and D), eNOS expression clearly increased in the MSCs 3 days after the Ad-SDF-1 transfection (Fig. 7B and C) and the level of expression increased over time, with the highest level detected at Week 1 (Fig. 7D and E). Gene expression of VEGF, phospho-Akt and eNOS were increased...
in infarcted hearts of the Ad-LacZ–MSC, Ad-SDF-1, Ad-SDF-1–MSC and Ad-SDF-1–MSC-L-NAME groups, and were greater in the Ad-SDF-1–MSC group than in other groups (Fig. 7E and F). Furthermore, the Ad-SDF-1–MSC-induced increases in the VEGF, phospho-Akt and eNOS protein levels were blunted by L-NAME (Fig. 7E).

4. Discussion

In this study, we investigated the therapeutic angiogenic effects of transplantation of the Ad-SDF-1–MSC, Ad-LacZ–MSC and Ad-SDF-1 delivery only, and the possible mechanisms of the therapeutic effects of the Ad-SDF-1–MSC. These results showed that the Ad-SDF-1–MSC transplantation induced and enhanced myocardial neovascularisation, and prevented progressive scar formation and heart dysfunction in a rat model of myocardial infarction.

We initially found that the MSCs transduced with the SDF-1α increased the synthesis of the SDF-1α protein than the Ad-LacZ–MSC alone in vitro, and enhanced activation of the prosurvival factor, Akt. The increased SDF-1α suppressed the apoptosis of the MSCs and enhanced the survival ability of the MSCs via the activation of the Akt. The MSCs alone could produce many angiogenic cytokines such as the SDF-1α, angiopoietin 1 (Ang1), basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF) besides VEGF [5]. In the present study, overexpressing SDF-1α MSCs showed better survival ability than Ad-LacZ–MSC-alone transplantation in the infarcted heart (Supplement 4). We also demonstrated that the Ad-SDF-1–MSC transplantation enhanced the VEGF expression in infarcted hearts better than injections of Ad-LacZ–MSC or Ad-SDF-1 alone in vivo. We presumed that one of the possible mechanisms for this enhancement is related to the MSCs. The transplanted MSCs increased heart-tissue VEGF expression in the infarcted heart through the paracrine mechanism of the MSCs [7,12]. A greater number of surviving MSCs could produce more cytokines in the infarcted heart if the survival ability of the MSCs was enhanced [13]. The increased SDF-1α could promote expression of endogenous VEGF in infarcted hearts [14,15]. These increased cytokines could be involved in, or trigger, the angiogenic progress of myocardial neovascularisation. They could even recruit and activate cardiac stem/progenitor cells, endothelial progenitor cells (EPCs) and MSCs to enhance angiogenesis in the infarcted hearts [16]. These changes could lead to a decrease in scar sizes, reduce remodelling and improve cardiac function, which is a consequence of improved blood supply in the damaged heart sites.

In our study, we evaluated the functional improvement seen in the Ad-SDF-1–MSC group by cardiac catheterisation to detect ventricular pressure. Other groups showed the expected decline in the rate of rise and fall of ventricular pressure and left ventricular systolic pressure, and markedly increased left ventricle end-diastolic pressure. In contrast, and as expected, the Ad-SDF-1–MSC group showed lower decline in the rate of rise and fall of ventricular pressure and left ventricular systolic pressure, and the lower increase in the left ventricle end-diastolic pressure. The SDF-1-over-expressing MSC contributed to preserve the rate of rise and fall of the ventricular pressure, left ventricular systolic pressure and left ventricle end-diastolic pressure in the infarcted hearts. In addition, the histology and morphometric measurement showed the expected decrease in the infarct size, and an obvious increase in the left ventricular wall thickness, in the Ad-SDF-1–MSC group. Moreover, hearts in the Ad-SDF-1–MSC group were less dilated and a combination of lower dilatation and a trend towards a less paradoxical systolic bulging probably led to the improved ventricular pressure. Furthermore, the Ad-SDF-1–MSC transplantation could inhibit the left ventricle remodelling, augment or preserve the myocardial elasticity, improve heart function and reduce the expression of extracellular matrix genes (e.g. collagen). These changes of shape and structure are related to the increase in the area and number of cardiac myocytes within the infarct zone.

It is interesting that the present study showed the MSCs differentiating into endothelial cells was related to the presence of the SDF-1α. Recently, several studies showed that the SDF-1α not only recruited cardiac stem/progenitor cells into the infarcted hearts, but also activated the cardiac stem/progenitor cells to differentiate into endothelial and smooth muscle cells, and that the SDF-1α was critical to the conversion of cardiac stem/progenitor cells to their vascular fate [17,18]. More importantly, the involvement of Akt and eNOS in angiogenesis is mediated by the VEGF and SDF-1α [2,3,7,10,12,14,19,20]. These results demonstrate that the MSCs transplanted with Ad-SDF-1 increased gene expression of the SDF-1α and VEGF, and enhanced Akt phosphorylation and eNOS activity in vitro and in vivo. The up-regulation of the Akt, eNOS and angiogenic factors may result partly from increased mobilisation of the EPCs as well as neovascularisation [2,3,14,16,18]. Simultaneously, the SDF-1α could also stimulate the proliferation, suppress apoptosis and promote the survival of various cells (e.g. endothelial and cardiac cells) [9–11,16]. These changes could benefit the survival and recovery of cardiomyocytes, and aid neovascularisation in ischaemic and infarcted sites.

Interestingly, several studies reported that the SDF-1α promoted the in vitro differentiation of MSCs to cardiomyocyte phenotypes [21,22]. In the present study, the engrafted Ad-SDF-1–MSC could also differentiate into cardiomyocytes and endothelial cells in the ischaemic myocardium. However, Zhang and his colleague reported that injected over-expressing SDF-1α MSCs did not differentiate into mature cardiac myocytes immediately or 24 h after the myocardial infarction [9]. We presumed that this difference is related to the time of cell transplantation after the myocardial infarction. Several studies showed that cell transplantation at 1–2 weeks post-infarction exerted better effects on increases in the survival of engrafted cells, angiogenesis and functional cardiomyocytes in the injured hearts than immediately, 24 h or at 4 weeks following the infarction. At these time-windows, scar formation has not occurred and the inflammation is reduced, which should facilitate the integration of transplanted cells and functional recovery [23]. The controversy may be related to differences in cell origin, cell preparation and detection methodology.
Acknowledgements

We thank Professor Yongsheng Ren for his helpful instruction in heart-function evaluation. We are grateful to Long Chen for help with immunofluorescence staining. We thank the international scientific editorial company for the helpful instruction in editing this article.

References

[1] Carmeliet P. VEGF gene therapy: stimulating angiogenesis or angioma-
M. Bone marrow mononuclear cells are recruited to the sites of VEGF-
induced neovascularization but are not incorporated into the newly
S, Landsman L, Abramovitch R, Keshet E. VEGF-induced adult neovascular-
ization: recruitment, retention, and role of accessory cells. Cell
2006;124:175–89.
[5] Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE.
Marrow-derived stromal cells express genes encoding a broad spectrum of
arteriogenic cytokines and promote in vitro and in vivo arteriogenesis
Effects of myocardial transplantation of marrow mesenchymal stem cells
transfected with vascular endothelial growth factor for the improvement
of heart function and angiogenesis after myocardial infarction. Cardiol-
participate in angiogenesis and improve heart function in rat model of
myocardial ischemia with reperfusion. Eur J Cardiothorac Surg 2006;30:
353–61.
[8] Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, Dzau VJ.
[9] Zhang M, Mal N, Kiedrowski M, Chadok M, Askari AT, Popovic ZB, Koc ON,
Penn MS. SDF-1 expression by mesenchymal stem cells results in trophic
support of cardiac myocytes after myocardial infarction. FASEB J 2007;21:
3197–207.
derived-factor-1alpha gene transfer induces cardiac preservation after
infarction via angiogenesis of CD133+ stem cells and anti-apoptosis.
Srivastava D. Stromal cell-derived factor-1alpha is cardioprotective after
enhances the effects of autologous mesenchymal stem cell transplanta-
tion on vascular regeneration in rat model of myocardial infarction. Ann
LG, Pratt RE, Ingwall JS, Dzau VJ. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J 2006;20:
661–9.
T, Takeshita A, Egashira K. Gene transfer of stromal cell-derived factor-
1alpha enhances ischemic vasculogenesis and angiogenesis via vascular
endothelial growth factor/endothelial nitric oxide synthase-related
pathway: next-generation chemokine therapy for therapeutic neovascu-
H, Ko YD. Stromal cell-derived factor 1alpha (SDF-1alpha) induces
gene-expression of early growth response 1 (Egr-1) and VEGF in human
arterial endothelial cells and enhances VEGF induced cell proliferation.
Cell Prolif 2003;36:75–86.
[16] Nakashishi C, Yamagishi M, Yahara I, Mori H, Sava Y, Yagihara
T, Kitamura S, Nagaya H. Activation of cardiac progenitor cells through
paracrine effects of mesenchymal stem cells. Biochem Biophys Res
cardiac stem cell-like cells that depolarize in vivo. Cell Transplant
Parolin C, Yasuzawa-Amano S, Muraski J, De Angelis A, Lecapitaine N,
Siggins RW, Loredo C, Bauri B, Urbanek K, Leri A, Kajstura J,
Anversa P. Formation of large coronary arteries by cardiac progenitor
[19] Duda DG, Fukushima D, Jain RK. Role of eNOS in neovascularization: NO for
Chae IH, Oh BH, Park YB, Kim HS. Akt is a key modulator of endothelial
Z. Stem cell plasticity revisited: CXCR4-positive cells expressing
CD34+ and CD133+ CD117- cell surface antigens. Blood 2006;108:
3536–47.
[22] Chen M, Xie QY, Deng L, Li XQ, Wang Y, Zhi W, Yang ZM. Stromal
[23] Vanderheyden M, Vercauteren S, Mansour S, Delrue L, Vandekerckhove B,
Heyndrickx GR, Van Haute I, De Bruyne B, Timmermans F, Wijns W,
Bartunek J. Time-dependent effects on coronary remodeling and epi-
cardial conductance after intracoronary injection of enriched hemato-
epoietic bone marrow stem cells in patients with previous myocardial