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Original Article

SDF-1α promotes repair of myocardial ischemic necrosis zones in rats

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Abstract: Objective: To explore the repair effect of stromal cell-derived factor-1α (SDF-1α) on myocardial ischemic necrosis zones. Methods: Lentivirus (LV-SDF-1α-GFP) containing SDF-1α target gene was established, the separated and cultured neonatal rat cardiac fibroblasts were transfected, and caudal intravenous injection of isoproterenol was conducted to prepare a rat model of myocardial ischemia. Small animal ultrasound was used to evaluate the effect on cardiac functions. Morphology and immunofluorescence were used to observe the change of ischemic necrosis zones and expressions of stem cellular markers c-kit, CD34, nkx2.5, and nanog, and a quantitative analysis was performed. Results: The established LV-SDF-1α-GFP was used to transfect myocardial fibroblasts which presented GFP green fluorescent expression and could secrete SDF-1α. The small animal ultrasound system showed that rat cardiac functions of the lentivirus group and cell group were improved to different degrees, myocardial ischemic necrosis zones of lentivirus group and cell group were reduced, and differences had statistical significances ($P<0.05$). Immunofluorescence showed that expressions of stem cellular markers c-kit, CD34, nkx2.5 and nanog in myocardial tissue ischemic zones in both the lentivirus group and cell group increased, and differences through inter-group comparison had statistical significances ($P<0.05$). Conclusion: SDF-1α can promote migration and proliferation of stem cells into the myocardial ischemic necrosis zone, participate in repair of the myocardial necrosis zone, and improve cardiac function.

Keywords: Lentivirus vector, myocardial necrosis, myocardial repair, stromal cell-derived factor-1α

Introduction

Acute myocardial infarction (AMI), a clinically common myocardial ischemic disease, arises out of necrosis of myocardial cells in quantity based on coronary artery disease or due to abrupt reduction or interruption of blood supply to coronary artery occlusion. Even though the development of drug therapy, percutaneous coronary intervention, and coronary artery bypass surgery can narrow the scope of myocardial infarction to a certain degree and reduce the death rate of myocardial infarction, these therapeutic approaches are useless for necrotic myocardial cells. A large quantity of necrotic myocardial cells can not be regenerated after myocardial infarction, and scar tissue replacement causes left ventricular remodeling and cardiac dilation, and finally gives rise to cardiac failure [1]. It is believed that the heart is a terminally differentiated organ at all times. Myocardial cells withdraw from the cell cycle immediately after birth and canot be regenerated, so features of myocardial cells have become the "bottleneck" in the treatment of myocardial infarction.

With the rapid development of biologic therapeutic technologies of stem cells, researchers have studied ventricular muscle. Strem et al. [2] directly injected adipose-derived stem cells (ADSCs) differentiated without in-vitro induction into the peri-infarction zone of the AMI rat, and observed that transplanted ADSCs formed neonatal myocardial cells in the peri-infarction zone. Orlic et al. [3] transplanted Lin^-/c-Kit^+ bone marrow stem cells into the rat infarction border zone so that newborn myocardial cells could be generated and accounted for 86% of the area of the infarction zone, and as a result,

manifestations of the coronary artery disease were improved. Lim et al. [4] conducted intravenous injection of umbilical cord-derived mesenchymal stem cells (UC-MSCs), which could effectively protect left ventricular function of myocardial infarction and relieve cardiac functions of AMI pigs. Their myocardial protection mechanism was mainly manifested by paracrine factors which could enhance angiogenesis, restrict the inflammatory reaction, and protect Cx43 gap junctions. In addition, researchers have continuously studied myocardial damage repair using mesenchymal stem cells, amniotic stem cells, and placental stem cells [5-7]. Exogenous stem cells, when applied to damage repair, can improve cardiac function to different degrees but still with insurmountable difficulties such as low repair efficiency, uneasy determination of quantity of transplanted cells and transplantation time, and tumorigenicity.

In recent years, researchers have proposed the concept of cardiac stem cells (CSCs). In 2003, Beltrami et al. [8] reported separation of CSCs from myocardial tissues in adult rats. These CSCs express surface molecules of stem cells with features of self-renewal, colony growth, and pluripotent differentiation, and they can differentiate into three types of cells such as myocardial cells, smooth muscle cells, and endothelial cells. Stem cells are injected into the infarction zone, which can increase blood vessel density and repair infarction scars so as to improve cardiac function. Hierlihy et al. [9] separated side-population (SP) cells from neonatal rat myocardial tissues, which accounted for 1% of the total number of cardiac cells. Laugwitz et al. [10] verified that $Isl1^+$ cells existed in neonatal rat ventricular muscle. These cells expressed myocardial transcription factors nkx2.5 and Gata4 and did not express Sca-1, CD34 or c-Kit, and they could be differentiated into mature myocardial cells after being co-cultured with neonatal rat cardiac cells. Cardiosphere-derived cells (CDCs), separated from neonatal rat ventricular muscle for culturing, consisted of multiple cells, including $CD90^+$ cells, $c\text{-}Kit^+$ cells, and cells expressing pluripotent markers such as Sox2, Oct3/4, and Klf-4 [11-13]. When injected into ventricular muscle of the myocardial infarction rat, CDCs can express troponin, von Willebrand factor, and smooth muscle actin; with high myogenic performance and paracrine effect, CDCs can increase blood capillary density in the infarction border zone, reduce infarction area, and significantly increase cardiac ejection fraction. In the past, cells presenting colony growth could be separated from ventricular ventricular muscle of a neonatal rat. Cell colony and myocardial cells were jointly cultured and could be differentiated into beating cells [14], and these cells under colony growth expressed $c\text{-}Kit^+/CD34^-$ and $Nanog^+/CD34^-$. At present, great controversies exist over recognition of surface markers of cardiac stem cells. Different antigenic stem cells separated and cultured from all kinds of animal myocardia can be differentiated into myocardial cells to different degrees so as to improve cardiac functions, but they cannot allow complete regeneration of ventricular muscle in the infarction zone.

A paracrine mechanism was first proposed by Gnecchi et al. [15]. Research findings show that through mesenchymal stem cell transportation after myocardial infraction, neonatal cells and blood vessels at the injured focus are not sufficient to explain why cardiac function is significantly improved. Therefore, the paracrine mechanism hypothesis is proposed. Stromal cell-derived factor-1 (SDF-1/CXCL12) is a chemotactic factor secreted by tissue matrix cells and its receptor is CXC chemokine receptor 4 (CXCR4). Wang et al. [16] deemed that the SDF-1/CXCR4 axis played a significant role in many physiologic processes such as hematopoietic function, cardiogenesis, angiogenesis, and nerve cell development as well as migration of endothelial progenitor cells. Secretion of chemotactic factors (such as SDF-1) at the injured part will promote effective homing of cyclic $CXCR4^+$ stem cells and other stem cells ($c\text{-}Kit^+/GATA4^+$) as well as organogenesis and tissue repair. SDF-1α presents a high expression in tissues of the injured organ such as pulmonary epithelial injury zone, skin injury zone caused by diabetes, nerve injury zone, and fracture healing zone [17-19]. Moreover, multiple cells in the organism highly express CXCR4 and participate in injury repair, including neural stem cells, mesenchymal stem cells, peripheral blood mononuclear cells and endothelial progenitor cells [20-22]. Paracrine factors in the myocardial infraction zone and effective extracellular matrixes (ECM) constitute a specific environment for myocardial regeneration [23].

Since stem cell transplantation was used to treat myocardial infarction in 2001 [24], multi-

ple types of stem cell transplantation have been tried to promote myocardial regeneration. Especially in recent years, it has become apparent that adult ventricular muscle has pluripotent stem cells: adult myocardial stem cells, with specific myocardial differentiation potential, which have injected new force into stem cell transplantation to treat ischemic cardiomyopathies [1, 25]. Effectively driving migration of endogenous stem cells into the ischemic necrosis zone to improve repair of the infarcted zone has become the research topic in this study. This study plans to conduct tail intravenous injection of lentivirus (LV-SDF-1α-GFP) containing SDF-1α target gene and fibroblasts after successful transfection with LV-SDF-1α-GFP, and then explore the mechanism of SDF-1α-mediated endogenous stem cell migration and homing.

Materials and methods

Animals

Healthy SD newborn rats (1~3 d) were selected. Their weights ranged from 7.35±0.51 g, and male and female quantities were not restricted. Moreover, 40 SD male rats (150~200 g) were also included in this study. All animals were provided by the Experimental Animal Center (SYXK 2009-000). This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Bethesda, MD, USA). Eighth Edition, 2010. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Xinxiang Medical University.

Lentiviral vector construction

Rat SDF-1α gene sequence (NM-001033883) was obtained from GenBank. The cds zone length of the gene was 360 bp. GV287 was taken as the backbone vector. Its element sequence was: Ubi-MCS-3FLAG-SV40-EGFP. Primers were designed according to the target gene fragment, and primer sequences were as follows: SDF-1α forward: GAGGATCCCCGGGTACCGGTCGCCACCATGGACGCCAAGGTCGTC, SDF-1α reverse: TCCTTGTAGTCCATACCGTTTTTCTTTTTCTGGGC. The lentiviral vector LV-SDF-1α was constructed by Genechem Co., Ltd., Shanghai, China.

Primary culture of fibroblasts

1-3 d SD neonatal rats were taken, then the heart was extruded out after sterilization using ethanol, and cut into 1 mm^3 small blocks which were transferred to a 15 ml centrifuge tube. 2 ml II-type collagenase (Sigma, St. Louis, MO, USA) was added for blowing and blending. The mixture was digested under 37°C for 2 min, and the supernatant was discarded. 2 ml II-type collagenase was added. The mixture was put under 37°C for 8 min continuous digestion. An equivalent amount of low-sugar Dulbecco's modified eagle medium (DMEM) (Hyclone, Logan, Utah, USA) containing 10% fetal bovine serum (FBS) (Hyclone, Logan, Utah, USA) was added, containing penicillin and streptomycin (North China Pharmaceutical, Shijiazhuang, China). Digestion was terminated, the product was blown and blended, and the supernatant was absorbed out. The digestion was continued for several times according to the above steps until the tissue block was completely digested. The supernatant was centrifuged for 5 min at 1,000 rpm, the supernatant was discarded and added to a small quantity of culture medium for resuspension. The mixture was inoculated in a 25 cm^2 culture flask (Corning, Steuben County, New York, USA) after filtering through the 200-mesh filter screen and placed at 37°C and in a 5% CO_2 incubator for 90 min. The supernatant was discarded, and the culture medium was replaced for continuous culturing. Culture solution was replaced every 2 d.

Immunofluorescent assay

0.01 mol/L PBS was used to rinse the cultured fibroblasts for three times, 5 min each time. They were fixed using 4% paraformaldehyde for 30 min and washed using PBS (ZSGB-BIO, Beijing, China) for three times, 5 min each time. Specimen was hyalinized using 0.3% Triton-X-100 for 15 min and then washed using PBS for three times, 5 min each time. Goat serum blocking solution (Beyotime, Shanghai, China) was used for 3 min at 37°C, and rabbit anti-rat vimentin monoclonal antibody (Abcam, Cambridge, UK) (dilution: 1:400) was added for overnight incubation at 4°C. Next day, it was rewarmed for 30 min and washed using PBS, 5 min each time, and FITC-labelled goat anti-rabbit secondary antibody (dilution: 1:500) (Invitrogen, Carlsbad, CA, USA) was added for 1

h incubation under 37°C in the dark. DAPI nucleus staining was conducted for 10 min, then washed, and mounting was carried out using anti-quenching mounting medium. Negative control was set in the experiment. PBS was used to replace primary antibody, and other steps were copied. Laser scanning confocal microscope (FV-1000, Olympus, Tokyo, Japan) was used to observe and collect images.

Lentiviral transfection of fibroblasts

1 d before the experiment, primarily cultured fibroblasts with good growth conditions were prepared into a single-cell suspension, myocardial fibroblasts were inoculated into the 24-well plate at 3~5×10^6/ml, and the volume was 500 μl. 24 h later, transfection could be prepared when cell density reached 30% and cell growth was good. The supernatant was absorbed out and washed using PBS for three times. Serum-free incomplete medium was added for synchronization treatment, and it was continuously cultured for 12 h. According to conditions screened through the preliminary experiments, lentivirus LV-SDF-1α-GFP was added with a multiplicity of infection of 10:1, and an equivalent quantity of PBS was added in the blank group. Complete media was added 2 h later. It was cultured in 37°C in a 5% CO_2 incubator. Cell status was observed 12 h later, and fresh culture medium was used to replace the old medium. After 3-4 d infection, fluorescent expression was observed.

Preparation of the myocardial ischemic necrosis model

Isoproterenol (Meilun Biotechnology Co., LTD., Dalian, China) was continuously injected for 7 d at a dose of 4 mg/kg to prepare the rat myocardial ischemia model. After confirming that the myocardial ischemia model was successfully prepared through limb lead electrocardiogram (ECG), 24 surviving rats were randomly divided into 3 groups with 8 rats in each group. The first group received intravenous injection of 100 μl SDF-1α target gene lentivirus (LV-SDF-1α-GFP, LV group), the second group received intravenous injection of 100 μl cardiac fibroblasts after successful transfection with LV-SDF-1α-GFP (Cell group), and the third group received tail intravenous injection of 100 μl empty vector virus (CON145) as the control (Control group). Vevo®2100 imaging system was used to evaluate cardiac functions of animals in all groups before injection and at 1 w and 2 w after injection respectively, and measurements taken including left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), and left ventricular end-systolic volume (LVESV).

Sampling of ventricular muscle, specimen preparation, and GFP fluorescence observation

10% chloral hydrate was used to conduct intraperitoneal injection to anesthetize animals. The chest was rapidly opened, and the heart was rinsed using PBS. Atrium and blood vessels were cut off along the coronary sulcus, and the heart was cut transversely along the vertical major axis. Ventricular tissues were kept and embedded using optimal cutting temperature (OCT) compound (Sakura, Torrance, CA, USA), followed by liquid nitrogen quick freezing. 8 μm frozen sections were prepared using a frozen microtome, and the section was fixed using acetone for 15 min, and then preserved in a -20°C refrigerator for later use. Two slides were selected for ventricular muscle of each animal. GFP fluorescent expression was observed using a laser scanning confocal microscope (Olympus, Tokyo, Japan).

Masson staining and calculation of ischemic area

The frozen section was taken out from the -20°C refrigerator, and it was rewarmed for 30 min and hydrated for 3 min; Masson stained for 1 min, rinsed using 0.2% acetic acid I and II once; rinsed using 1% phosphomolybdic acid for 5 min; rinsed using 0.2% acetic acid I and II, and 95% ethanol for 1 s; hematoxylin stained for 2 min and soaked in tap water for 30 min; conventionally dehydrated, hyalinized and mounted. It was observed and photographed under an optical microscope. The necrosis zone was determined, and two Masson staining samples were selected for ventricular muscle of each animal. An image was acquired under the optical microscope, and Image J image analysis system was used to calculate area percentages of myocardial ischemic necrosis zones in all groups (ratio of area of ischemic necrosis zone to total observed area).

Sampling and treatment under transmission electron microscope

Ventricular muscle sampling was done as above. Precooled 2.5% glutaraldehyde fixing solution was dropwise added on the specimen. About 1 mm^3 ventricular tissue (ventricular muscle close to endocardium) was rapidly taken using a double-edge blade and placed in 2.5% glutaraldehyde, and it was placed in a 4°C refrigerator overnight. It was washed using PBS the next day, fixed using 1% osmic acid, conventionally dehydrated, and hyalinized It was soaked using Epon812 epoxy resin, and then embedded and polymerized. Ultrathin sectionswas prepared through a ultramicrotome (Leica, Bensheim, Germany). The specimen was stained using uranyl acetate and lead nitrate, and observed under transmission electron microscope (TEM) (H-7500, Hitachi, Tokyo, Japan), and images were collected.

Immunofluorescent assay

The section was rewarmed and rinsed in PBS, for 5 min each time; hyalinized using 0.3% Triton-X-100 for 15 min; goat serum blocking liquid was added, and the mixture was placed in 37°C for 30 min. Rabbit CD34 primary antibody (dilution: 1:200; Cat no. 14486-1-AP; Proteintech, Wuhan, China), mouse c-kit primary antibody (dilution: 1:200; Cat no. 18696-1-AP; Proteintech, Wuhan, China), rabbit nanog primary antibody (dilution: 1:200; Cat no. 14295-1-AP; Proteintech, Wuhan, China) and goat anti-rat nkx2.5 primary antibody (dilution: 1:400; Cat no. 13921-1-AP; Proteintech, Wuhan, China) were added, and they were placed at 4°C overnight. The next day, the section was rewarmed for 30 min and rinsed in PBS, incubated in fluorescent secondary antibody in the dark as follows. Cy3-labeling goat anti-rabbit secondary antibody (dilution: 1:1000; Cat no. A0516; Beyotime, Shanghai, China), Cy3-labeling goat anti-rat secondary antibody (dilution: 1:1000; Cat no. A0507; Beyotime, Shanghai, China) and FIFC-labeling donkey anti-goat secondary antibody (dilution: 1:500; Cat no. A16000; Invitrogen, Carlsbad, CA, USA) were added. The mixture was incubated under 37°C for 1 h, rinsed in PBS, nucleus was stained by DAPI for 10 min. Section was rinsed in PBS and mounted through anti-quenching mounting medium. Negative control was set in the experiment, whereby PBS was used to replace the primary antibody, and other steps were copied. The section was observed under laser scanning confocal microscope (Olympus, Tokyo, Japan) and images were collected. 15 fields of view were taken in each group to measure positive fluorescence intensity, and the fluorescence intensity was analyzed by Image J software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

SPSS 17.0 statistical software (SPSS Inc, Chicago, IL, USA) was used to analyze experimental data which were expressed as mean ± standard deviation. Independent-sample t test was used to conduct a comparative analysis of data between the two groups, and $P<0.05$ was considered significant.

Results

LV-SDF-1α construction

The lentiviral vector LV-SDF-1α-GFP 200 μL (5×10^8 TU/ml) was constructed and obtained from Genechem Co., Ltd., Shanghai, China.

Culture and assay of primary fibroblasts in neonatal rats

The quantity of fibroblasts obtained through enzyme-digestion combined method was large. After 60 min adherence to the well, their extensions were gradually stretched out, and fibroblasts could reach logarithmic phase 48 h later. Cells presented triangular or shuttle-shapes with large somas, transparent cytoplasm and large, and with 2-3 large oval nuclei. Cells had no spontaneous beating with vigorous cell division. Passaged to the 4th generation, their purity and diopter were high and follow-up experiments could be conducted. Immunofluorescence labeling for vimentin showed strong positive expression (**Figure 1**).

Lentivirus-transfected fibroblast GFP expression under different titers

A small quantity of GFP green fluorescence could be seen on the 2nd day after transfection, and through observation under a laser scanning confocal microscope until the 4th day of continuous culturing. GFP expression in group A and B showed strong expressions, GFP expression was not found in group C, and cell growth in all groups was good under the white-light channel (**Figure 2**).

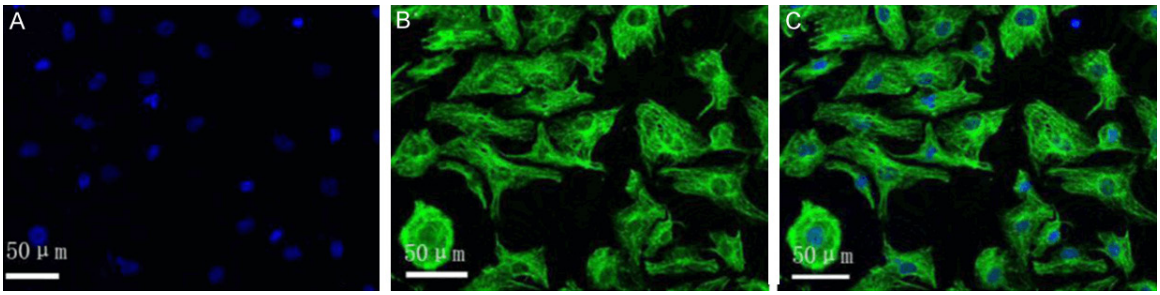

Figure 1. Vimentin-labeled cardiac fibroblasts (immunofluorescence). A. DAPI staining; B. Vimentin; C. Merged staining.

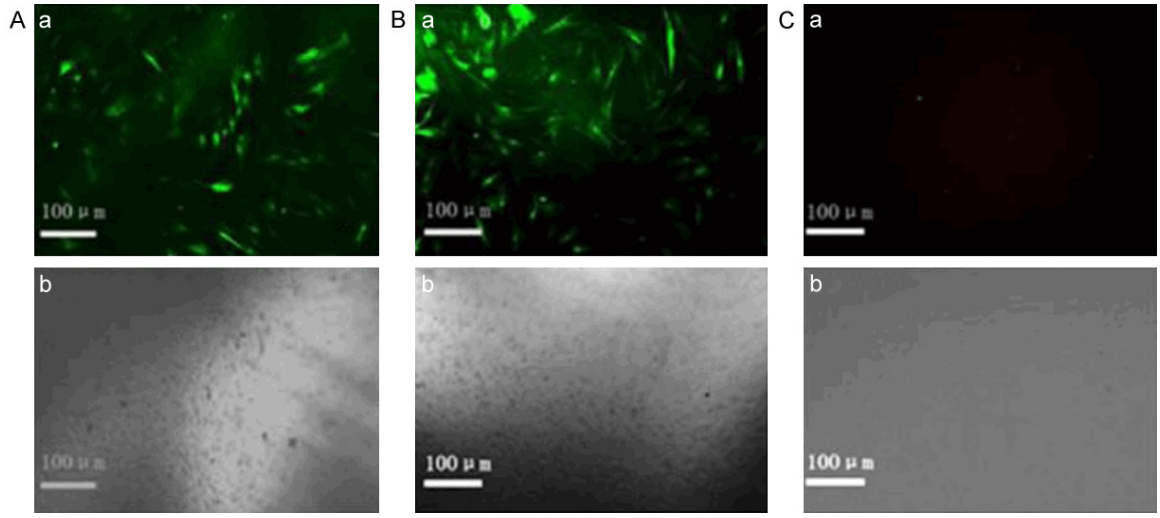

Figure 2. Lentivirus-transfected fibroblast GFP expression under different titers. A. MOI=100; B. MOI=10; C. MOI=1. The upper indicates GFP expression, and the lower indicates white-light channel.

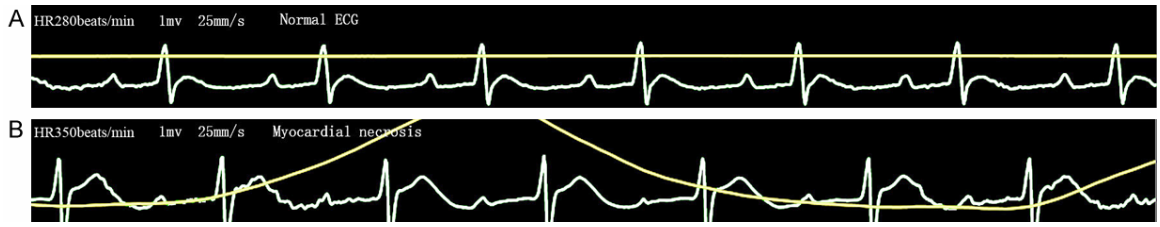

Figure 3. The results of rat limb lead ECG. A. Normal rat; B. Rat after injection of isoproterenol.

Rat limb lead ECG

Before intraperitoneal injection of isoproterenol, rat ECG showed the following: ventricular rate was 280 beats/min and there was no elevation of the ST segment. After intraperitoneal injection of isoproterenol, rat ECG showed: ventricular rate reached 350 beats/min, ST segment was arched and elevated upward, T wave amplitude was enlarged, and typical myocardial ischemic change was observed. This indicated that the myocardial necrosis model was successfully prepared (**Figure 3**).

Evaluation of cardiac functions

Animal ultrasound was used to detect cardiac function (**Figure 4A**). Statistical analysis showed that under the circumstance in the same group, LVEF, LVFS and LVESV before and after injection in the control group had no obvious differences ($P>0.05$). LVEF and LVFS in the

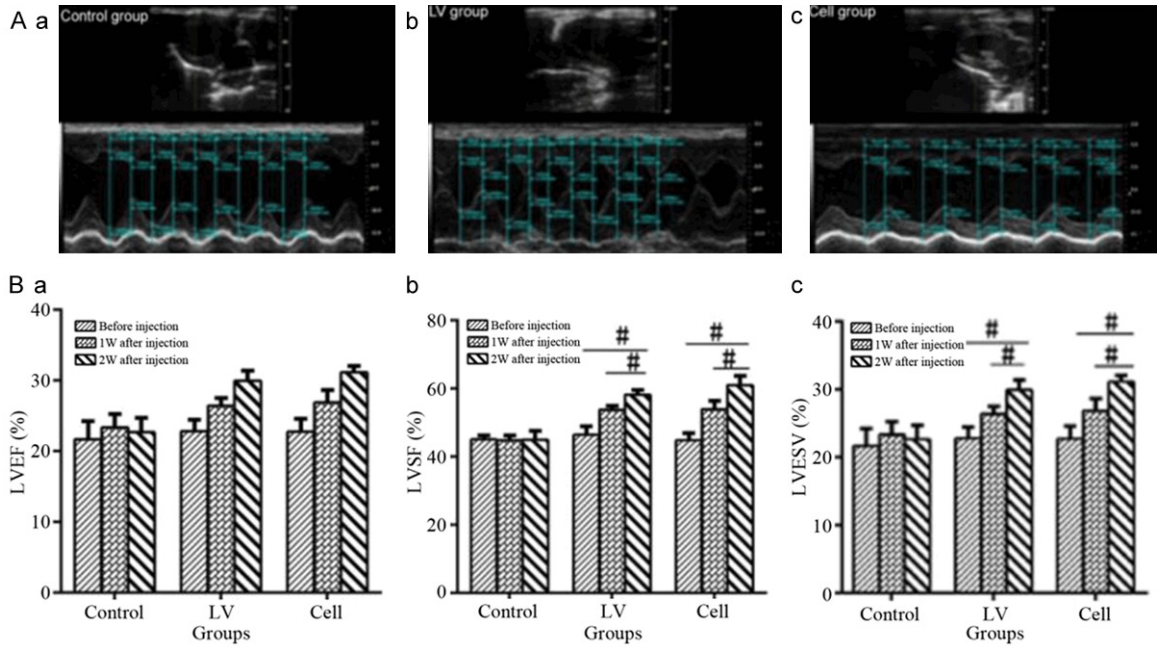

Figure 4. Evaluation of cardiac functions through the ultrasound system and statistical analysis of functional parameters. A. Ultrasound cardiogram results in three groups; B. The statistical analysis results of functional parameters in different groups, #P<0.05.

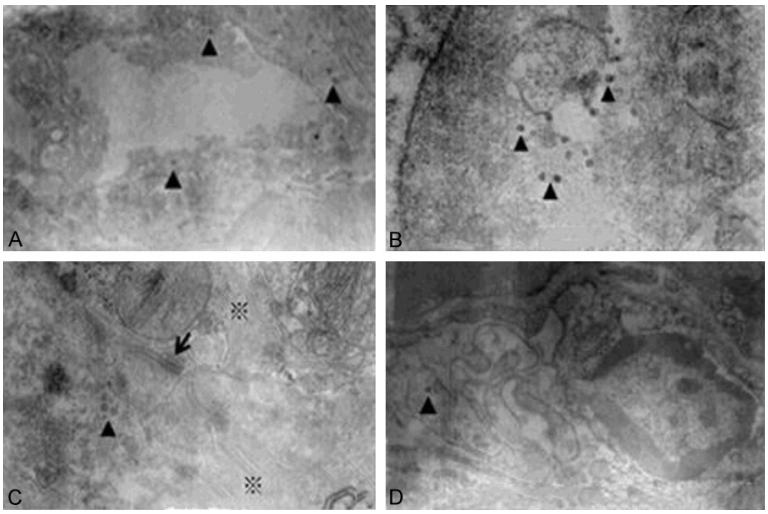

Figure 5. Ultrastructure of myocardial tissues (Magnification ×20,000). (A-C) lentivirus group: (A) shows virions in blood vessels and surrounding mesenchyme. (B) Shows virions in non-myocardial cells. (C) Shows virions in myocardial cells. (D) Cell group shows cells in blood lumens and virions in cells. Arrowhead represents virions, arrow represents specialized connection between myocardial cells, and asterisk shows myofilaments.

lentivirus group and cell group were slightly increased while LVESV was slightly reduced 1 w after injection, but the difference was not significant (*P*>0.05). However, 2 w after injection, LVEF and LVFS significantly increased while LVESV obviously declined, and the difference was significant (*P*<0.05). Compared with the control group, LVEF and LVFS in the lentivirus group and cell group slightly increased while LVESVs slightly declined 1 w after injection (*P*>0.05). However, LVEF and LVFS significantly increased while LVESV was obviously reduced 2 w after injection, and the difference was significant (*P*< 0.05). Comparative difference between the lentivirus group and cell group both 1 w and 2 w after injection was not significant (*P*>0.05) (**Figure 4B**).

Ultrastructural observation of ventricular muscle tissues

TEM observation showed that virions existed in blood capillaries and surrounding mesenchyme as well as myocardial cells and non-myocardial cells in the lentivirus group (**Figure 5A-C**). Virions were seen in blood vessels in the cell group (**Figure 5D**).

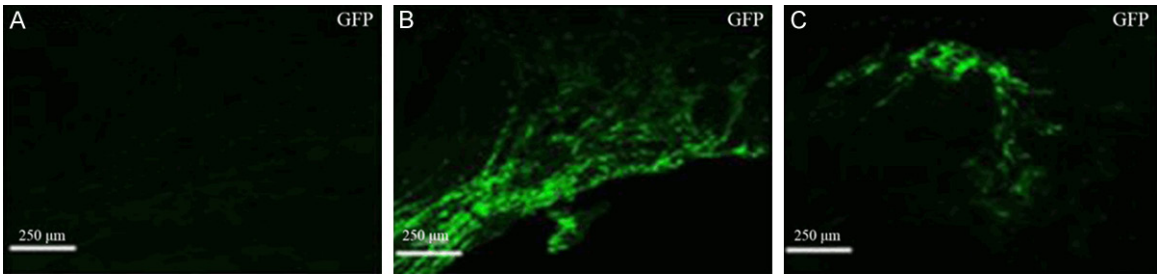

Figure 6. GFP fluorescent expression in myocardial tissues. A. Control group; B. LV group; C. Cell group.

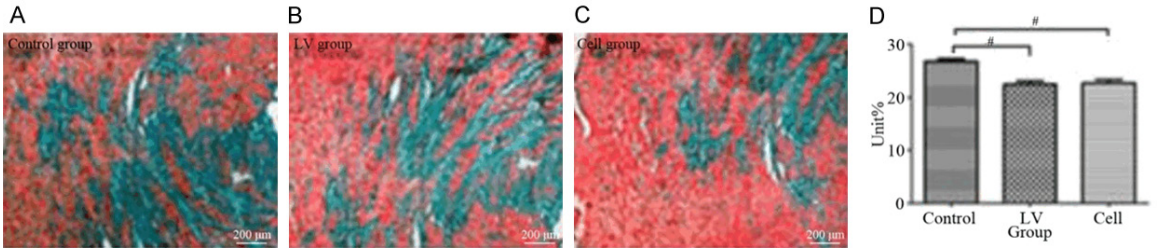

Figure 7. Myocardial Masson staining and quantitative analysis of necrosis areas. A-C. Masson staining: green color shows collagen in the ischemic necrosis zone. A. Control group; B. LV group; C. Cell group. D. Statistical analysis of percentages in ischemic necrosis zones.

Detection of GFP fluorescent expression in myocardial tissues

Laser scanning confocal microscope was used to observe GFP fluorescent expression, and results showed that strongly positive GFP expression was present in ischemic necrosis zones in both the lentivirus group and cell group. GFP expression was weak in normal myocardial tissues and negative in the control group (**Figure 6**).

Masson staining and percentage of ischemic necrosis zone

Collagenous fibers presenting green cord-like distributions could be seen in the lentivirus group, cell group, and empty vector control group, and connective tissues were proliferated in quantity in ischemic necrosis zones (**Figure 7A**). Compared with the control group, percentages of myocardial ischemic necrosis zones in lentivirus group and cell group declined, and the difference was significant ($P<0.05$). The difference between the lentivirus group and cell group was not significant ($P>0.05$) (**Figure 7B**).

Immunofluorescent assay of stem cell markers in myocardial tissues

In the myocardial ischemic necrosis zone, c-kit was expressed in the cytoplasm around the cell nucleus, and CD34 presented a linear distribution in the necrosis zone with strong positive expression. Compared with the empty vector control group, fluorescence intensities in the cell group and lentivirus group were enhanced, and the difference was significant ($P<0.05$). The difference between the cell group and lentivirus group had no statistical significance ($P>0.05$). Myocardial early transcription factor nkx2.5 was expressed in the cytoplasm in the ischemic necrosis zone. Compared with the control group, fluorescence intensities in the cell group and lentivirus group were enhanced ($P<0.05$). Nanog presented strong positive expression in the cytoplasm in the myocardial ischemic necrosis zone. Compared with the control group, fluorescence intensities in cell group and lentivirus group were enhanced, and the difference was significant ($P<0.05$) (**Figure 8**).

Discussion

The present studies regarding myocardial regeneration mainly concentrated on cell types such as myocardial cells, smooth muscle cells, myocardial fibroblasts and stem cells. As for research strategies of myocardial regeneration, first of all, injured ventricular muscle is repaired through transplantation of exogenous stem cells [1], namely in-vitro transplantation meth-

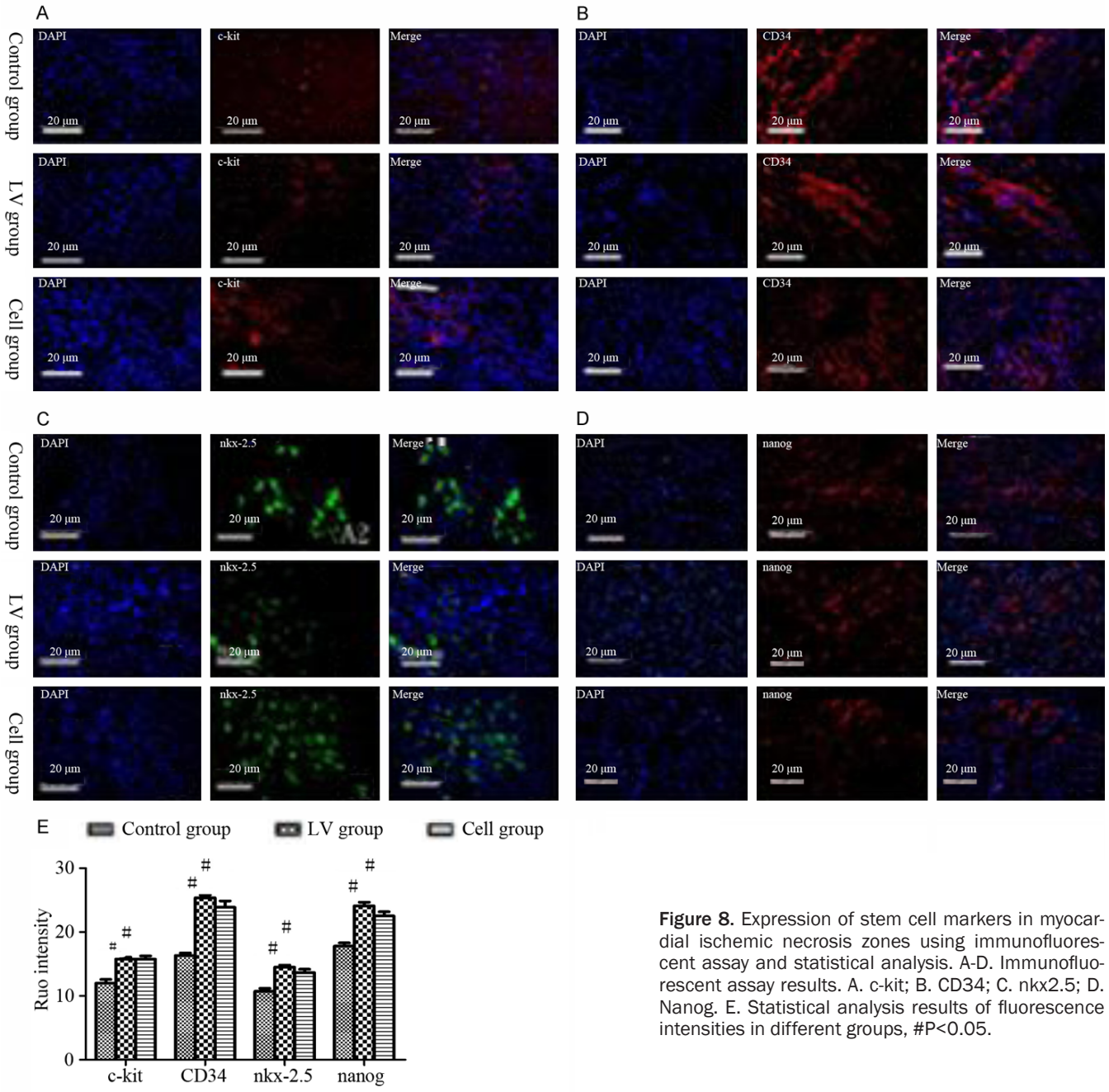

Figure 8. Expression of stem cell markers in myocardial ischemic necrosis zones using immunofluorescent assay and statistical analysis. A-D. Immunofluorescent assay results. A. c-kit; B. CD34; C. nkx2.5; D. Nanog. E. Statistical analysis results of fluorescence intensities in different groups, #P<0.05.

od of stem cells, but it presents many difficulties such as thrombus formation, low repair efficiency, intolerance of immunosuppression, and tumorigenicity. Second, injured ventricular muscle is repaired by an activating and amplifying endogenous mechanism of myocardial cell regeneration [26]. Past isoproterenol-induced myocardial ischemic necrosis rat model research disclosed that c-Kit$^+$/Nanog$^+$ stem cells exist in the ischemic necrosis zone, which can express myocardial cell transcription factors. Myocardial cell markers are positive, and immature myocardial cells are observed under the ultrastructure, but necrotic tissues still present scar repair [27]. So far, there are still many problems related to repair of the infarction zone. To sum up, cardiogenic stem cells can obviously improve cardiac function and mobilize endogenous stem cell proliferation and homing, and this is a key factor for treatment of myocardial infarction. Therefore, how to rapidly activate endogenous stem cells in situ, promote stem cell proliferation, migration and homing, and explore into key time-space "window" and molecular targets for treatment of myocardial infarction is the focus of our research group.

As a member of the chemotactic factor subfamily CXC, SDF-1α causes chemotaxis. Abbott et al. [28] found that SDF-1α mRNA expression increased 48 h and 72 h after myocardial infarction, and it recovered 7 d later. SDF-1α protein level reached a peak at 72 h and kept rising at a doubled rate at 96 h. Malik et al. [29] found that SDF-1α had a myocardial protective effect in ischemia-reperfusion injury and could be blocked by AMD3100. However, Boyle et al. [30] found that the SDF-1α expression level declined in peripheral blood of myocardial infarction patients and a myocardial infarction rat model. SDF-1α is a substrate of DPP4. Chiazza et al. [31] found that DPP4 blocking agents (Trajenta) improved functional stroke regarding dependence on SDF-1α/CXCR4.

The early-stage study showed that lentivirus containing SDF-1α target gene transfected into fibroblasts in vitro could generate a secreting-type SDF-1α protein in vitro [32]. This study indicated that for fibroblasts with tail intravenous infection of lentivirus or transduced with lentivirus, green fluorescent expression mainly concentrated in rat myocardial ischemic necrosis zones. Under the microscope, virions in the lentivirus group were not restricted to blood vessels. The virus could infect myocardial and non-myocardial cells, and cells containing virus could be seen in blood vessel lumens in the cell group. Therefore, we speculate that because of large and permeable blood vessels in the infarction zone, virus infection mainly concentrates in the infarction zone. Hence, high-fluorescence expression is present in the infarction zone. Keeping a high SDF-1α level through intervention can compensate for an SDF-1α decline after myocardial infarction so as to promote myocardial repair.

In this study, tail intravenous injection of SDF-1α gene-modified lentivirus and virus-transfected cardiac fibroblasts could effectively improve cardiac functional parameters, and morphologic structural observation indicated that myocardial infarction areas were all obviously reduced. Virions in the lentivirus group mainly existed in myocardial cells, endothelial cells, blood vessel lumen and mesenchyme, indicating that virions could infect multiple cells in the body. Virions could be seen in cells in the blood vessel lumen in the cell group, but no virions were seen in myocardial cells, or endothelial cells. Binding of GFP fluorescent protein expression occurred in the myocardial ischemic necrosis zone, indicating that virus infection concentrated in that zone, possibly owing to the enlarged permeability of blood vessels in the ischemic necrosis zone. These results showed that GFP fluorescent expression in the ischemic necrosis zone observed under the laser scanning confocal microscope might derive from different cells.

Immunofluorescent assay of stem cell markers showed that fluorescence intensities of c-kit, nanog, and CD34 increased, and myocardial early transcription factor nkx2.5 was observed in the myocardial necrosis zone. C-kit, which is one of the earliest identified stem cell markers, has the stem cell features: self-renewing, multidirectional differentiation, and colony growth. As a surface marker of hematopoietic stem cells, CD34 can be selectively expressed on surfaces of hematopoietic stem cells or progenitor cells in mammals and can also be transformed into endothelial cells to form blood vessels [33]. A large quantity of CD34 appears in the myocardial necrosis zone. We propose that stem cells from hematopoietic sources migrate into the necrosis zone to participate in myocardial repair. Positive nanog expression is detected in the infarction zone, and we propose that nanog cells may participate in myocardial repair. As one of markers for differentiation of

cardiac precursor cells, nkx2.5 is the earliest expressed transcription factor in the heart with specific myocardial expression features [34, 35]. This indicates that parenchymal cells are generated in the ischemic necrosis zone, and SDF-1α can promote effective migration of myocardial stem cells and other endogenous stem cells.

In conclusion, SDF-1α overexpression can induce recruitment of endogenous stem cells in the necrosis zone, thus providing a new strategy for clinical treatment of myocardial infarction. However, its repair mechanism remains unclear and needs more study.

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Disclosure of conflict of interest

None.

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