

The proliferative and migratory effects of physical injury and stromal cell-derived factor-1 α on rat cardiomyocytes and fibroblasts

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Abstract. – **OBJECTIVE:** This study aims to explore the effects of physical injury and stromal cell-derived factor-1 α (SDF-1 α) on the proliferation of cardiomyocytes and chemotactic effects of cardiomyocytes on the migration of cardiac fibroblasts.

MATERIALS AND METHODS: Isolation and primary culture of rat cardiomyocytes and cardiac fibroblasts were performed; scratching was employed to induce physical injury on cells which were cultured with SDF-1 α at different concentrations; proliferation ability of cardiomyocytes was checked with CCK-8 assay and migratory ability of cardiac fibroblasts under the chemotaxis of cardiomyocytes was detected with Transwell assay.

RESULTS: SDF-1 α enhanced the proliferation ability of cardiomyocytes with physical injury, especially at the concentration of 80 μ g/L when the proliferation rate of cardiomyocytes increased most markedly. Moreover, physically injured cardiomyocyte that was cultured with SDF-1 α significantly elevated migratory ability of cardiac fibroblasts, which tended to be more obvious along with the chemotactic culture time.

CONCLUSIONS: SDF-1 α enhanced the proliferation ability of cardiomyocytes with physical injury, and physically injured cardiomyocyte that was cultured with SDF-1 α promoted the migration of cardiac fibroblasts.

Key Words:

Cardiomyocytes, Cardiac fibroblasts, Physical injury, Stromal cell-derived factor-1 α .

Introduction

The diagnosis of congenital heart defect (CHD), including atrial septal defect (ASD), ventricular septal defect (VSD) and patent ductus arteriosus (PDA), was followed by cardiac surgery in the past. Transcatheter closure, an intervention therapy rising up recently, although avoids the trauma and danger of thoracotomy, induces clinical

complications, such as occluder detachment, hemolysis and atrioventricular block^{1,2}. A new therapy for CHD with low complications is a novel topic. Our previous research demonstrated that small arterial duct in a diameter of 2 mm could heal itself without occluder through stimulating its arteriosus edge with conducting wire in the intervention therapy for CHD. We speculated that the injury promoted cell proliferation and tissue repair, which induced self-healing, but their specific mechanisms still require further investigation. Stromal cell-derived factor-1 α (SDF-1 α), is an important chemokine protein to promote stem cell homing and the recruitment of bone marrow-derived cells to injured cardiomyocytes after myocardial infarction (MI)³. However, it remains unclear whether SDF-1 α enhances self-healing of CHD induced by physical method. Our study aimed to explore the effects of physical injury and SDF-1 α on the proliferation of cardiomyocytes and chemotactic effects of cardiomyocytes on the migration of cardiac fibroblasts, and investigate its potential application value in the treatment of CHD.

Materials and Methods

Isolation and Primary Culture of Rat Cardiomyocytes and Cardiac Fibroblasts

Newborn experimental Sprague Dawley rats at 1-3 days old (The laboratory animal center of China Medical University, license key: SYXK [Liao] 2003-0013) were sacrificed by cervical dislocation. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Shenyang General Hos-

pital of PLA. Left ventricle was collected and washed with phosphate buffered saline (PBS) to remove residue blood and, then, cut into 1 mm³ pieces, which were repeatedly digested in 0.07% trypsin (Biosharp, China) and 0.05% Type II collagenase (Biosharp) at 37°C until the tissue completely disappeared. The digestion reaction was terminated by adding equal volume of medium with fetal bovine serum (FBS, Hyclone, Logan, UT, USA) into digestion supernatant. Cell suspension from digestion was centrifuged at 1000 rpm for 10 min and the supernatant was removed; the pellet was re-suspended in 1-2 ml of Dulbecco's Modified Eagle Medium (DMEM-Hi) glucose medium with 10% FBS (Hyclone) by gently pipetting, and seeded into 24-well plate after filtration with 200 mesh. Cardiac fibroblasts attached to the wells after 2h incubation, and the supernatant that contained relatively pure cardiomyocytes was re-seeded into another 24-well plate. The medium was replaced by DMEM-Hi glucose medium containing 0.1 mmol/L BrdU (bromodeoxyuridine) after cardiomyocyte attachment, while DMEM-Hi glucose medium with 10% FBS was added to cardiac fibroblasts. The culture plates were incubated in a humidified 37°C, 5% CO₂ incubator. Myocardial troponin I expression was detected in immunofluorescence assay to identify cardiomyocytes.

Establishment of Physical Injury Model in Rat Cardiomyocytes

Primary cultured rat cardiomyocytes were isolated and incubated with DMEM-Hi glucose medium containing 10% FBS in a humidified 37°C, 5% CO₂ incubator. Serum starvation 24h well synchronized cells in G0 phase. The 200 mesh was cut into 2.5 cm long, which was sterilized to induced injury on cells by scratching cell culture plate along the same direction.

The effects of Physical Injury and Sdf-1 α on the Proliferation of Rat Cardiomyocytes Cultured In Vitro

Trypsinization was performed to collected normal and scratch-injured cells, which were seeded in 96-well plate and divided into normal group that was cultured in normal condition, scratching group without SDF-1 α and scratching combined with SDF-1 α group, in which injured cells were incubated with 10, 20, 40, 80, 160 g/L SDF-1 α (Peprotech, Rocky Hill, NJ, USA). Proliferation ability of all the cells were tested after 24h culture by measuring cell OD450 value after adding

10 l of Cell Count Kit-8 (CCK-8) reagent (Biyuntian Bioengineering Institute) into each well (five wells for each group). The effective concentration of SDF-1 α was identified according to cell proliferation.

The Effects of Cardiomyocytes on the Migration of Cardiac Fibroblasts

There were four groups, including control group that was cultured in normal condition, scratching group, SDF-1 α culture group in which the concentration of SDF-1 α was determined according to CCK-8 assay and scratching combined with SDF-1 α group. The basal Transwell chamber (Costar, Loughborough, Leicestershire, UK) was seeded with the above experimental cells in advance; 24h after the cell attachment, apical chamber was loaded with 0.1 ml of 1 $\times$ 10⁶/ml cardiac fibroblasts, which were cultured in a humidified 37°C, 5% CO₂ incubator for 24h, 48h and 72h. Transwell chamber was taken out and washed with PBS, followed by wiping off the upper cells on the microporous membrane with swab. The remaining cells were fixed with paraformaldehyde at room temperature for 20 min and stained with hematoxylin for 3min; after washing with distilled water, the cells migrated to the lower layer of microporous membrane were counted under inverted microscope (200 $\times$). Three fields of each sample were selected for cell counting and the average was calculated.

Statistical Analysis

Statistical analysis was performed with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA); data was presented as $\pm$ s; comparison between groups used t test and $p < 0.05$ was considered statistically significant.

Results

Cell Culture and Identification

The cultured cells were spindle-shaped or polygonal (Figure 1); positive expression of myocardial troponin I was shown by immunofluorescence (Figure 2), which indicated that the isolated cells were cardiomyocytes.

Comparison of Proliferation Ability of Cardiomyocytes Between Groups

After adding SDF-1 α , the proliferation rate of myocardial cells significantly enhanced (Figure 3), suggesting that cell proliferation ability in-

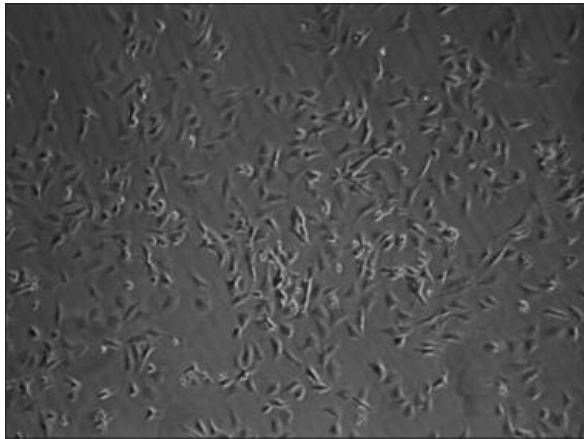


Figure 1. Appearance of cardiomyocytes (100×).

creased especially at the concentration of 80 $\mu\text{g/L}$, when the proliferation rate of cardiomyocytes elevated most markedly. As the concentration of SDF-1 α continued to rise, the increase of proliferation rate reached a plateau. Therefore, 80 $\mu\text{g/L}$ SDF-1 α was used in the following study.

Comparison of the Chemotactic Effects of Cardiomyocytes on the Migration of Cardiac Fibroblasts Between Groups

Compared with control group, scratching group, SDF-1 α culture group and scratching combined with SDF-1 α group showed increased number of migratory cardiac fibroblasts (Figure 4), which were 1.67, 2.32 and 3.13 times as many as control group after 24h incubation (Table I). The migratory cardiac fibroblasts tended to be more along with the chemotactic culture time, which demonstrated that scratching com-

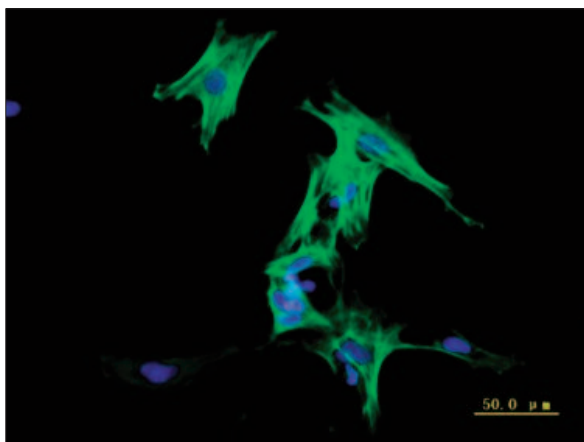


Figure 2. Immunofluorescence with cardiac muscle caldesmon protein I (400×).

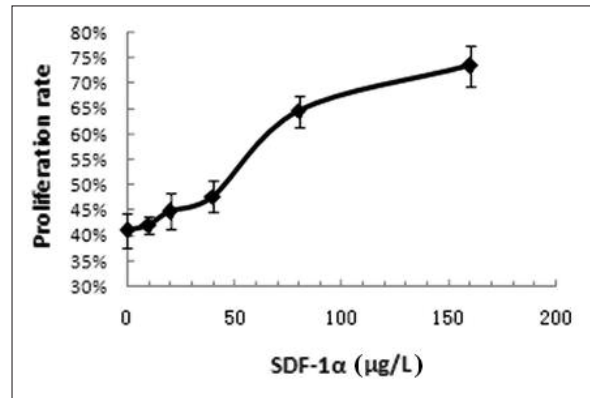


Figure 3. Detection of proliferation of cardiomyocytes ($\bar{x} \pm s$, $n = 5$).

bined with SDF-1 α culture distinctly enhanced the chemotactic ability and migration rate of cardiac fibroblasts.

Discussion

Early thoracotomy for patients with CHD induces big trauma and a certain risk. Transcatheter closure, an intervention therapy rising up recently, has been extensively accepted by patients and widely used in clinic due to its smaller trauma and danger compared with thoracotomy. However, placement of metal occluder complicates with occluder device embolization, postoperative residual leaks, arrhythmia and myocardium perforation, as well as rupture of chordae tendineae of the tricuspid valve^{4,5}. Along with the development of medical science and technology, research on “non-invasive technique” instead of “invasive technique” to treat CHD has become a new tendency of development in this field. Eroglu et al⁶ reported one case of VSD closure, in which the patient developed severe arrhythmia and the surgery was terminated by removing the occluder; self-healing of VSD was observed in the review three month later. The reason might be that new wound on the defective edge induced in the intervention therapy promoted cell proliferation, tissue repair and self-healing. According to the biological characteristics of cardiomyocytes and cardiac fibroblasts, processing on the defect sites in CHD patients to enhance cell proliferation and migration and promote defect repair, is likely a potential treatment for CHD.

Myocardium is relatively complex, which consists of three integrated components: cardiomy-

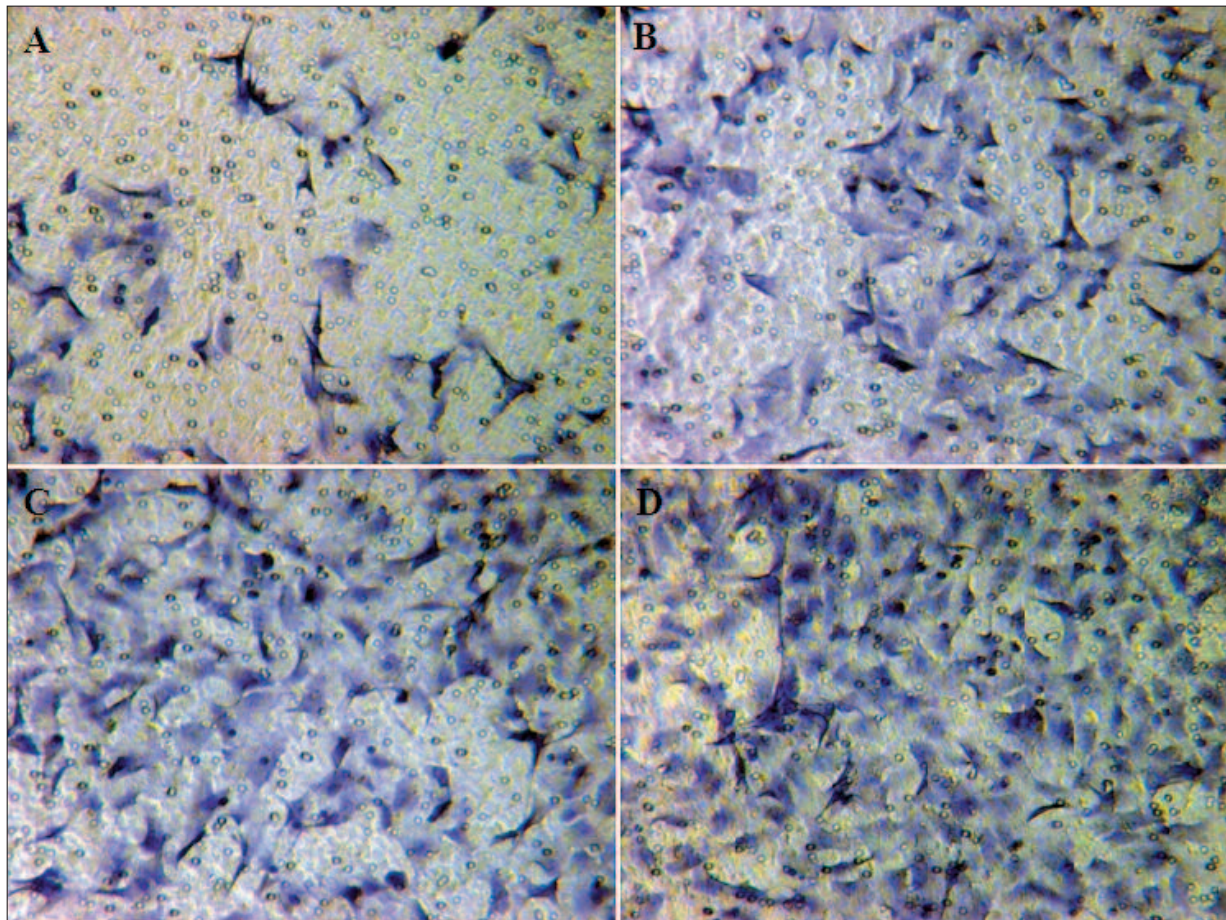


Figure 4. Migration of cardiac fibroblasts with cardiomyocyte effect (24h). **A**, Control group. **B**, Scraping group. **C**, SDF-1 α group. **D**, Scraping with SDF-1 α group.

ocytes, cardiac fibroblasts and capillary microcirculation. Cardiomyocyte rapidly develops in the embryonic and neonatal period, and then well differentiates into thesocyte that is mainly responsible for systolic function⁷. Cardiac fibroblast has proliferation ability and controls the production and deposition of extracellular matrix of the heart⁸, while activated cardiac fibroblast has stronger migratory ability, which migrates to cardiac injury area and rapidly proliferates under the

action of multiple inflammatory factors and cytokines^{9,10}. *In vitro* cultured cardiomyocytes and cardiac fibroblasts can preserve many aspects of their structure and function. The cardiomyocytes cultured in our study could spontaneously contract; therefore, *in vitro* intervention on myocardial cells and cardiac fibroblasts provides an excellent model for the research on physiopathologic mechanisms of CHD. Some studies successfully imitated *in vivo* myocardial ischemia-

Table I. Migration number of cardiac fibroblasts with cardiomyocyte effect ($\bar{x} \pm s$, n = 3).

Number of migrating cells	Control group	Scraping group	SDF-1 α group	Scraping with SDF-1 α group
24 h	61 $\pm$ 5	101 $\pm$ 4**	141 $\pm$ 6**	190 $\pm$ 8**
48 h	78 $\pm$ 8	125 $\pm$ 9**	153 $\pm$ 4**	211 $\pm$ 9**
72 h	71 $\pm$ 4	135 $\pm$ 5**	170 $\pm$ 6**	238 $\pm$ 8**

Compared with the control group, * p < 0.05, ** p < 0.01.

reperfusion injury with anoxia/reoxygenation model of neonatal rat myocardial cells¹¹ and myocardial hypertrophy with angiotensin II-induced hypertrophy model in cardiomyocyte¹². Moreover, ischemic cardiac fibroblasts can build engineered cardiac tissue¹³. All above reports have demonstrated that research on the role of myocardial cells and cardiac fibroblasts in the pathogenesis and treatment of heart disease has made some progress and it can be employed as useful model for heart disease.

Cell proliferation and migration are regulated by a variety of cytokines. SDF-1 belongs to the family of chemokine proteins and is produced in two forms, SDF-1 α and SDF-1 mainly by stroma cells and expressed in multiple tissues. Liehn et al¹⁴ and Tang et al¹⁵ have confirmed that SDF-1 α closely correlates with the repair after MI, improves the angiogenesis and cardiac function, as well as inhibits hypoxia and low serum-induced apoptosis of mesenchymal stem cells through regulating PI3K/Akt and ERK signaling pathway¹⁶, and simultaneously adjusts endogenous stem cells homing and recruitment^{17,18}. In addition, SDF-1 has been proved to promote the proliferation and migration of endothelial progenitor cells and breast cancer cells in other research^{19,20}. Our study utilized cardiomyocytes and cardiac fibroblasts as *in vitro* culture model, imitated physical injury by scratching to induce cell defect and added SDF-1 α to the medium to explore the effects of physical injury and SDF-1 α on the proliferation of cardiomyocytes and chemotactic effects of cardiomyocytes on the migration of cardiac fibroblasts. The experiment results showed that SDF-1 α enhanced the proliferation ability of cardiomyocytes with physical injury; physically injured cardiomyocyte that was cultured with SDF-1 α promoted the chemotactic effects on cardiac fibroblasts and markedly increased their migratory ability and rate, which indicating that physical injury and SDF-1 α could activate cardiomyocytes and cardiac fibroblasts and enhance the migration of cardiac fibroblasts via various ways. We hypothesized upon the above results that the cardiac fibroblasts migrated to cardiac injury area to perform tissue repair and promote self-healing during the treatment for CHD, while the effects of physical injury and SDF-1 α on cardiomyocytes and cardiac fibroblasts, such as the mechanism of activating quiescent fibroblasts and their effects on cell proliferation, migration/ chemotaxis, synthesis of extracellular matrix (imitating tissue repair in defect

area) and the expression of nuclear transcription factors, proinflammatory factors and anti-inflammatory factor still required further investigation.

Conclusions

SDF-1 α could enhance the proliferation ability of physically injured cardiomyocytes, and the combination of SDF-1 α culture with physical injury on cardiomyocytes promoted the migration of cardiac fibroblasts.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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