Over-expression of SDF-1α on BMSCs Reduce the Injury Caused by PMN in Myocardial Ischemia/reperfusion

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Research

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Abstract

Background: To study the protective effect of BMSC overexpressing SDF-1α on myocardial ischemia/reperfusion (I/R), to improve the limitation that only part of BMSC is recruited to the site of myocardial injury in the treatment of ischemic heart disease. It provides a new scheme for stem cell therapy for clinical treatment of reperfusion injury.

Methods: Collect blood samples from PCI patients and healthy individuals to detect PMN and SDF-1α expression; Construction of BMSC overexpressing SDF-1α (oe-SDF-1α); In the case of no intervention or intervention by BMSC respectively, cell level: the migration ability of PMN to hypoxia/reoxygenation (H/R) cardiomyocytes and the expression of SDF-1α, CXCR4, apoptosis, oxidative stress and other indicators of cardiomyocytes were detected; In vivo level: PMN, SDF-1α, CXCR4, oxidative stress and inflammatory factor levels were detected in I/R mice. And carry out statistical analysis.

Results: In the clinic, compared with the control group, the expression levels of SDF-1α and PMN in the blood of PCI patients increased. Under H/R conditions, cardiomyocytes express and secrete SDF-1α, activate PMN migration and infiltration mediated by SDF-1/CXCR4 signal pathway, promote cardiomyocyte apoptosis and increase the level of oxidative stress; oe-SDF-1α has a stronger ability to migrate to H/R cardiomyocytes and has more repair ability than BMSC.It is more suitable as a tool cell for stem cell therapy. The expression levels of SDF-1α and PMN are increased in I/R mice. oe-SDF-1α can reduce the ability of PMN to reside to the damaged part of myocardial tissue significantly, thereby reducing myocardial tissue damage, oxidative stress, inflammatory factor levels in I/R mice.

Conclusions: The SDF-1/CXCR4 biological axis not only plays an important role in BMSC migration, but also helps to enhance the therapeutic effect of BMSC-based therapy. Oe-SDF-1α has a more repairing effect on reducing cell damage caused by PMN, and can be used as a new type of cell for the treatment of IRI.

Background

Ischemic heart disease is the first enemy threatening human health in the world [1]. Ischemia can initiate a series of pathophysiological processes, leading to the death of cardiomyocytes in the ischemic area. Therefore, blood reperfusion to the ischemic area as early as possible is the only effective way to prevent the death of cardiomyocytes [2, 3]. However, reperfusion itself initiates a series of new pathophysiological changes (including myocardial contraction, vascular endothelial dysfunction, microvascular collapse, blood supply disorder and myocardial cell death), which aggravates and enlarges myocardial cell damage after ischemia, that is, reperfusion injury [4–6]. Clinically, myocardial ischemia/reperfusion injury (IRI) often occurs after coronary artery disease and cardiac surgery. Among them, the implementation of reperfusion and the implementation of the reperfusion plan are started after the patient arrives at the hospital, so it is proactive and easy to control. If interventions can be made for the reperfusion process, reperfusion injury can be minimized, or it can be an effective way to treat ischemic cardiomyopathy.

It is well known that reperfusion initiates a typical inflammatory response during IRI, in which Polymotphonucleat Leukocytes (PMNs) are the main mediators mediating the inflammatory response [7–9]. Previous studies have shown that IRI can induce myocardial inflammation, resulting in the up-regulation of the expression of inflammatory cytokines and chemokines. PMN is mobilized, activated and recruited to inflammatory sites rapidly [10, 11]. Under the action of inflammatory factors, PMN adheres, aggregates and causes cardiomyocyte damage by releasing inflammatory mediators, toxic oxygen groups, proteolytic enzymes, etc. [12, 13]. However, the mechanism of how PMN is mobilized, activated and rapidly recruited to cardiomyocytes during IRI is still unclear. Therefore, it is very important to find a target that regulates PMN overactivation and chemotaxis to block its damage to cardiomyocytes and cause reperfusion injury.

Stromal cell derived factor-1α (SDF-1α) is a key member of the CXC family of chemokines and plays an important role in the chemotaxis of stem cells and immune cells PMN [14]. A large number of data show that bone marrow mesenchymal stem cells (BMSC) are a promising tool for the treatment of ischemic heart disease. The increased expression of SDF-1α after myocardial ischemia leads to the mobilization and migration of BMSC to damaged the damaged site, resulting in increased vascular density, tissue regeneration and improved cardiac function [15–17]. However, only part of BMSC recruited to the site of myocardial injury in the treatment of ischemic heart disease, which has limitations. In addition to promoting the gradient-guided homing of stem cells to the site of myocardial injury to stimulate myocardial repair, SDF-1α can also enhance the transduction of intracellular pro-survival signals to protect the myocardium directly. Studies have shown that SDF-1α treatment can promote myocardium 72 hours after myocardial infarction cell survival [18], but the specific mechanism is still unclear. So will BMSC overexpressing SDF-1α enhance the therapeutic effect of stem cell therapy on IRI?

Interestingly, in IRI, in addition to stem cell homing, SDF-1α is also related to the residence of immune cells such as PMN in the bone marrow and migration to the site of myocardial ischemia injury [19], but SDF-1α chemotactic stem cells have a protective effect on myocardium, chemotactic PMNs have a damaging effect on myocardium. So do stem cells interfere with PMN's damage to myocardium? In view of this, this study will establish tool cells BMSC overexpressing SDF-1α, comparing BMSCs and BMSCs overexpressing SDF-1α on PMN-induced IRI intervention from both in vivo and in vitro levels, so as to provide new ideas and treatment schemes for stem cell therapy of IRI.

Materials And Methods

Patient sample

The blood and general clinical data of 107 patients with percutaneous coronary intervention (PCI) and 68 healthy people who were treated in the Department of Cardiology of the Second Hospital of Shanxi Medical University from July 2020 to June 2021 were collected. The patient samples were approved by the ethics committee of the Second Hospital of Shanxi Medical University according to the Declaration of Helsinki. The confirmed patient's informed consent was obtained.

Animal feeding

SPF grade male C57BL/6 mice, aged 6-12 weeks, were raised in a light / dark cycle of 12 hours at 22-24 ℃, could eat and drink freely. All animal work was carried out in accordance with the recommendations in the National Institutes of Health Laboratory Animal Care and Use Guidelines. All studies involving mice were approved by the ethics committee of the animal center of Shanxi Medical University.

Isolation and culture of mouse cardiomyocytes and cardiac fibroblasts

Isolation and culture of cardiomyocytes: 12-week-old male C57BL/6 mice were taken. After anesthesia, the hearts were cut off and perfused retrogradely. Cut the digested ventricular tissue into small pieces, grind gently with a pipette, adjust the cell concentration to 5×10^4 cell/ml, add laminin (10 μg/mL) (sigma) to pretreat 60mm plates and place them in cultivate for 1 hour in an incubator containing 5% CO_2 at 37℃, remove non-adherent cells, add DMEM medium containing 2.5% fetal bovine serum to continue culturing for subsequent experiments.

Isolation and culture of cardiac fibroblasts: mouse hearts were isolated according to the above methods, and the ventricles were cut into $1mm^3$ tissue blocks. Add the digestion solution prepared by PBS (1g/L trypsin, 0.8g/L type IV collagenase) (Roche) to the tissue block, prepare the resuspended cells, transfer them to the culture dish and culture for 90min. After repeated cleaning with PBS at 37℃, the non adherent cells were removed. DMEM containing 10% FBS was added and cultured in an incubator containing 5% CO_2 at 37℃, the solution was changed once every 3 ~ 4 days.

Hypoxia/reoxygenation (H/R) of cardiomyocytes and fibroblasts

Resuspend primary mouse cardiomyocytes and cardiac fibroblasts, pretreat 60mm plates with laminin (10 μg/mL) for 1h, add 1×10^6 cells to each plate. H/R treatment group of cells were transferred to 37 ℃ incubator containing 1% O_2, 5% CO_2 and 94% N_2 for continuous culture for 6h, and then transferred to normal incubator for continuous culture for 18h. The cells were collected for related detection.

Isolation, culture and identification of mouse bone marrow mesenchymal stem cells

Take 12-week-old male C57BL/6 mice, sacrifice the mice by cervical dislocation, separate the mouse femurs and tibias aseptically, cut both ends of the bones with straight shears, expose the bone marrow cavity, flush out the bone marrow with a syringe, blow and suck several times with pipette, and filter the flushing solution into 50ml centrifuge tube. Slowly add the cell suspension to Percoll (density 1.082g/ml) separation solution, centrifuge 500g at room temperature for 30min, aspirate the upper layer of cells, wash them with PBS twice, culture them with DMEM containing 10% FBS, and culture them in an incubator containing 5% CO_2 at 37 ℃. In order to facilitate BMSC tracing, we transfected the cultured

second generation BMSC with lentivirus carrying EGFP gene to construct BMSC stably expressing EGFP gene and make them fluoresce green.

Isolation and identification of PMN from mouse bone marrow

The cell suspension method is the same as that of mouse BMSC. The cell suspension is slowly added to Histopaque-1077 PMN separation solution (Sigma-Aldrich, St. Louis, MO, USA), centrifuged, the supernatant is discarded. Resuspend the cell pellet in PBS, slowly add the cell suspension to Histopaque-1119 PMN separation solution, centrifuged, and the middle flocculent layer is PMN. Use DMEM medium containing 10% FBS to culture in an incubator containing 5% CO_2 at 37°C. Take PMN in good growth condition, count and adjust the cell concentration. If Ly6G is positive by flow cytometry, it is PMN.

Construction and identification of BMSC overexpressing SDF-1α

SDF-1α-overexpressing lentivirus (Hanbio Technology (Shanghai) Co., Ltd.) was used to infect fluorescent BMSCs, puromycin (Sigma-Aldrich, St. Louis, MO, USA) was added to the culture medium to pass resistance screening BMSC stably expressing SDF-1α (oe-SDF-1α). Take BMSCs in good growth condition, count and adjust the cell concentration. The antibody is made by BD biosciences, and the flow cytometry test is negative for CD34 and CD45, and positive for CD29, CD90, CD73, and CD105 is oe-SDF-1α.

Transwell method to analyze the effect of mouse cardiomyocytes on the migration of BMSC and PMN

Add mouse cardiomyocytes to the lower chamber of the Transwell culture system (BD Falcon), adjust the density of BMSC/oe-SDF-1α or PMN to 1×10^5 cells/ml with complete medium, and add 200μl of cell suspension to the upper chamber of Transwell. Place it in a 37°C, 5% CO_2 incubator for culture. Cardiomyocytes under H/R conditions were cultured in hypoxia for 4 hours and then co-cultured with BMSC/oe-SDF-1α or PMN; cardiomyocytes under H/R+AMD3100 conditions were cultured in hypoxia for 4 hours and BMSC/oe-SDF-1α or PMN co-culture, while adding 40 μmol/L CXCR4 antagonist AMD3100 (abcam) to the medium. For BMSCs, the Transwell chamber was taken out after 24 hours, fixed with 4% paraformaldehyde and stained with crystal violet, photographed under a microscope, counted the number of cells penetrating the membrane. For PMN, the lower cells were collected 4 hours later, the number of Ly6G positive cells was detected by flow cytometry.

Flow Cytometry to Detect Intracellular Ros Levels

Resuspend the mouse cardiomyocytes in the lower chamber with the probe staining working solution (Solarbio, Beijing, China), use flow cytometry for computer detection, use 480nm wavelength excitation,

measure 525nm emission, ROS-positive cells It has strong green fluorescence, corresponding to the FL1 detection channel of BD flow cytometer.

Flow Cytometric Detection of Cell Apoptosis

Gently resuspend the mouse cardiomyocytes in the lower chamber with 200μl Annexin V-FITC binding solution, add 10μl propidium iodide staining solution, mix gently, incubate at room temperature (20-25℃) in the dark for 10-20 minutes, and then Performed on a flow cytometer (CytoFLEX; Beckman, USA) by AnnexinV-FITC Analysis Kit (Beyotime, Shanghai, China), and operated according to the instructions.

Animal Model

Take 12-week-old male C57BL/6 mice, fasted with water for 8h before operation. 1h before the operation, 2×10^6 EGFP-labeled BMSC cells were injected into the tail vein. Then intraperitoneal anesthesia. The left chest was depilated, the trachea was intubated, and the small animal ventilator was connected to maintain breathing. The airway pressure was 14.4cm H_2O, the respiratory rate was 88 times/min, and the ventilation volume was 1L/min. Connect the electrodes of the small animal ECG detector to observe the electrical changes in the operation center. The surgical area is prepared and disinfected. The skin is cut in the 4th and 5th intercostal space on the left side of the sternum, the subcutaneous tissue and muscle are bluntly separated, the thoracic cavity is opened, the pericardium is carefully separated, the left atrial appendage is exposed, and the LAD is ligated with 8.0 sutures. The electrocardiogram showed that the ST-segment was raised in the arched dorsal shape, maintained for 30 minutes, and the ligature was removed. The electrocardiogram showed that the ST-segment dropped slowly, indicating that the reperfusion was successful and the chest cavity was closed. For mice in AMD3100 treatment group, 2.5 mg/kg body weight of AMD3100 was immediately administered intraperitoneally. After 8 hours, the mice were sacrificed, and part of myocardial tissue was fixed with 4% neutral formaldehyde to prepare tissue paraffin blocks and paraffin sections for HE, tissue immunofluorescence and TUNEL Staining; take part of myocardial tissue to extract protein, perform fluorescence quantitative PCR, WB and biochemical detection. Blood samples are used to detect the levels of SDF-1α, PMN, IL-1β, IL-6, IL-10 and TNF-α.

RNA Extraction, Reverse Transcription and Fluorescence Quantitative PCR Detection

Collect cells and extract RNA with RNA easy kit. Use RT2 First Strand Kit (QIAGEN) to reverse transcribe RNA into cDNA. All operations are performed in accordance with the instructions. Amplification was carried out on the ABI StepOne Plus (Applied Biosystems, Inc.) system, and the reaction conditions were 95°C, 10min pre-denaturation; 95°C, 5s, 60°C, 40 s for a total of 40 cycles. GAPDH was used as an internal reference, and the relative expression of mRNA was calculated by the $2^{-\Delta\Delta CT}$ method. The primer sequences used for amplification are as follows: SDF-1α, F: 5'AGTCAGCCTGAGCTACCG 3'; R: 5'AAGGGCACAGTTTGGAGT 3'; CXCR4, F: 5'AGTCAGCCTGAGCTACCG 3'; R: 5'AAGGGCACAGTTTGGAGT 3'; GAPDH, F: 5'AATCCCATCACCATCTTC 3'; R: 5'AGGCTGTTGTCATACTTC 3'.

Western Blot

The cells were collected, and the cells were lysed with Cell Lysis Solution (Sigma-Aldrich, St. Louis, MO, USA), centrifuged at 4°C, 1000 rpm for 5 min, and the supernatant was collected. Add a protein electrophoresis loading buffer with a final concentration of 1×, boil it for 5 minutes, perform SDS-PAGE electrophoresis, transfer to a PVDF membrane (Amersham Biosciences, Piscataway, NJ, USA), use 5% skim milk at room temperature, and block on a shaker for 2 hours. Incubate overnight at 4°C with SDF-1α antibody (Abcam, Cambridge, UK; 1:1000), CXCR4 antibody (Abcam, Cambridge, UK; 1:100) or GAPDH antibody (Abcam, Cambridge, UK; 1:2000) After washing, add appropriately diluted HRP-labeled goat anti-rabbit IgG (Abcam, Cambridge, UK; 1:50,000) and incubate at room temperature for 1 hour. After the membrane was washed 3 times, ECL luminescent solution (Perkin-Elmer Inc.) was applied to the membrane and the results were observed on Imagequant LAS4000 (GE Healthcare, Japan).

Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labelling (TUNEL) Staining

The extent of apoptosis was measured by TUNEL staining (Roche, USA) according to the manufacturer's instructions. Briefly, the specimens were fixed with 4% paraformaldehyde for 15-24 min and prepared paraffin sections. Place the slices in trypsin solution, incubate for 40 minutes, and wash with PBS for 3 min×3 times. Add 50μl TUNEL solution dropwise to the sliced tissue, add it to a light-proof humidified box at 37°C for 1h; wash with PBS for 3min×3 times. Add 50μl POD dropwise to the sliced tissue, add it in a light-proof humidified box at 37°C for 30min, and wash with PBS for 3min×3 times. Add appropriate amount of DAB dropwise, keep it away from light at room temperature for 3-10 minutes, and counterstain with hematoxylin. Dehydrated, transparent, neutral resin sealing and sealing. The analysis of apoptosis (ratio of TUNEL-positive cells to all cells) was conducted using images of randomly selected fields obtained with a microscope (ECLIPSE Si, Nikon, Japan).

Determination of Oxidative Stress and Myeloperoxidase (MPO)

The levels of malondialdehyde (MDA), superoxide dismutase (SOD) and MPO in myocardial tissue homogenate were detected by a commercial kit (Jiancheng Biotech, Nanjing, China), and related operations were performed according to the kit instructions.

Detection of SDF-1α Content in Cell Culture Supernatant and Detection of Serum Il-1β, Il-6, Il-10 and TNF-α Levels

The content of SDF-1α in the cell culture supernatant was detected by the mouse SDF1 ELISA kit (abcam); the levels of L-1β, IL-6, IL-10 and TNF-α in the serum were detected by the commercial kit (abcam). Refer to the kit instructions for related operations.

Statistical Analysis

The data are expressed in the form of mean±SEM deviation, all analyses were performed on GraphPad Prism 8 software (GraphPad, San Diego CA). Use Student t test and one-way analysis of variance (ANOVA) to compare the differences between different groups, and P<0.05 indicates that the differences are significant.

Results

Increased levels of SDF-1α and PMN in patients with coronary artery disease

According to the comparison of the basic information of the subjects, it was found that there was no significant difference in the age, male, Body Mass Index (BMI), ever smoker, and creatinine between PCI patients and the control group (P>0.05). It shows that there is no difference in basic information between PCI patients and the normal control group, which can be used as experimental group and control group to participate in the experiment. The serum SDF-1 level of 173 experimental participants was analyzed by ELISA, and it was found that the serum SDF-1 level of PCI patients was significantly increased. Through case data statistics, it was found that the amount of PMN in the blood of PCI patients was increased significantly. There was significant difference between them (*P*<0.05). See Tab. 1

Table 1
Basic characteristics of PCI patients and control group

	PCI patients (n=107)	Control (n=68)	P value
Age (years, mean±SD)	60.9±12.7	61.5±15.1	0.715
Male, n(%)	71 (66.4%)	66 (61.7%)	0.084
BMI	24.3 ± 3.9	23.5 ± 2.9	0.091
Ever smoker, n(%)	51 (47.7%)	49 (45.8%)	0.154
Creatinine (mg/dl)	1.2±0.3	1.1±0.3	0.592
SDF-1α (ng/L)	774.84±346.61	575.11±63.68	0.041
PMN(10^9/L)	7.68±2.84	4.82±1.41	<0.001

The expression levels of SDF-1α and PMN increase in I/R mice, the cardiomyocytes express and secrete SDF-1α in the H/R cell model

In order to verify the clinical data, I/R mice were constructed. Compared with the control group, the expression level of SDF-1α in serum and myocardial tissue of I/R mice was significantly increased, and the expression of its receptor CXCR4 in myocardial tissue was increased significantly(Fig. 1a-d), indicating that I/R mice activated the SDF-1/CXCR4 signaling pathway. Compared with control mice, the positive rate of Ly6G and MPO activity in the myocardial tissue of the I/R mice were increased significantly(Fig. 1e, f), indicating that the content of PMN in myocardial tissue of I/R mice increased and aggregated .

In order to understand which cells secrete and express SDF-1α under H/R conditions, we isolated and cultured primary mouse cardiomyocytes and cardiac fibroblasts. The supernatant of cultured cells was collected and tested by ELISA. It was found that the ability of H/R cardiomyocytes to secrete SDF-1α was increased significantly, while the ability of H/R cardiomyocyte fibroblasts to secrete SDF-1α did not change significantly (Fig. 1g). Through PCR and Western blot detection, it was found that SDF-1α, CXCR4 mRNA and protein expression in H/R cardiomyocytes increased, while H/R cardiomyocyte fibroblasts did not change significantly (Fig. 1e, f, h), indicating that H/R cardiomyocytes secrete and express SDF-1α.

Under H/R conditions, cardiomyocytes recruit PMN through SDF-1/CXCR4 signaling pathway to promote cardiomyocyte injury

In order to confirm that the increase of PMN in the myocardial tissue of I/R mice is related to the activation of SDF-1/CXCR4 signal pathway by cardiomyocytes, we co-cultured PMN with cardiomyocytes. Cardiomyocytes are located in the lower layer and PMN is located in the upper layer. According to the culture conditions of cardiomyocytes, they were divided into control group, H/R group and H/R+AMD3100 group. Compared with the control group, the number of PMN migration increased (Fig. 2a), the apoptotic rate of cardiomyocytes increased (Fig. 2b), the level of ROS in cardiomyocytes increased (Fig. 2c), and the activity of MPO increased (Fig. 2d), the content of MDA increases (Fig. 2e), and SOD activity decreases (Fig. 2f) in the H/R group, but these changes can be blocked by AMD3100. It is suggested that under H/R, PMN migration mediated by SDF-1/CXCR4 signal pathway promotes the accumulation of ROS and increases the level of apoptosis in cardiomyocytes.

Construction of BMSC strain overexpressing SDF-1α (oe-SDF-1α)

The oe-SDF-1α cell line was screened out by flow cytometry. The cells are negative for CD34 and CD45 and positive for CD29, CD73, CD90, CD105, which are in line with the characteristics of BMSC markers (Fig. 3a). The expression of EGFP in the cells was observed under a fluorescence microscope (Fig. 3b), PCR detection showed that the expression level of SDF-1α mRNA in the cells increased (Fig. 3c).

Overexpression of SDF-1α promotes the migration of BMSCs to cardiomyocytes under H/R conditions

In order to confirm that oe-SDF-1α has an enhanced ability to migrate to H/R cardiomyocytes than BMSC. We co-cultured BMSC and oe-SDF-1α with cardiomyocytes respectively. Cardiomyocytes are located in the lower layer and BMSC or oe-SDF-1α is located in the upper layer. According to the culture conditions of cardiomyocytes and the upper cell types, they are divided into Control+BMSC, Control+oe-SDF-1α, H/R+BMSC, H/R+oe-SDF-1α and (H/R+AMD3100)+BMSC group.

The results of the Transwell experiment showed that compared with normal conditions, the migration ability of BMSCs under H/R conditions was enhanced, but the migration ability of BMSCs could be blocked by AMD3100, suggesting that the SDF-1α/CXCR4 signaling pathway mediates the migration of BMSCs. Compared with Control+BMSC, the migration ability of oe-SDF-1α will be enhanced even when co-cultured with normal cardiomyocytes in Control+oe-SDF-1α. Compared with H/R+BMSC, the migration ability of oe-SDF-1α will be further enhanced significantly (Fig. 4a, c), this indicates that overexpression of SDF-1α can promote the migration of BMSCs to cardiomyocytes. The apoptotic ratio and ROS level of cardiomyocytes were significantly decreased in the H/R+oe-SDF-1α group (Fig. 4b, d, e), indicating that oe-SDF-1α has more repair ability to cardiomyocytes under H/R conditions and is more suitable as a tool cell for stem cell therapy.

BMSCs overexpressing SDF-1α can reduce PMN induced cardiomyocyte injury

Since the migration of BMSC and PMN to cardiomyocytes under H/R conditions are both related to SDF-1α/CXCR4 signal pathway, we further observed whether BMSC affects the injury of PMN to cardiomyocytes through Transwell experiment. We co-cultured BMSC and oe-SDF-1α with cardiomyocytes+PMN respectively. Cardiomyocytes and (BMSC or oe-SDF-1α) are located in the lower layer, PMN is located in the upper layer. According to the culture conditions of cardiomyocytes and the type of cells cultured with the cardiomyocytes in the lower layer, they are divided into Control+'BMSC, H/R+'BMSC, H/R+'oe-SDF-1α and (H/R+AMD3100)+'BMSC.

The results showed that compared with the H/R+'BMSC group, the H/R+'oe-SDF-1α group did not affect the migration of PMN to H/R cardiomyocytes (Fig. 5a, d), but it could reduce level of apoptosis in cardiomyocytes (Fig. 5b), reduce the level of ROS (Fig. 5c), reduce MPO activity (Fig. 5d), reduce the content of MDA (Fig. 5e), and enhance SOD activity (Fig. 5f) significantly. Interestingly, its effect on cardiomyocytes is equivalent to that of the (H/R+AMD3100)+'BMSC group, blocking PMN migration is equivalent, indicating that oe-SDF-1α can act as a stem cell without affecting PMN migration. The repairing effect of PMN also reduces the damage of cardiomyocytes caused by PMN.

Oe-SDF-1α reduce the resident ability of PMN in injured tissues and significantly reduce myocardial injury in I/R mice

In order to further observe the therapeutic effect of oe-SDF-1α on myocardial IRI, we established I/R mice model and administered BMSC treatment through the tail vein. According to the type of injected cells, it was divided into Control, I/R, I/R+BMSC, I/R+(oe-SDF-1α).

The results of HE staining showed that the myocardial fibers in the marginal area of MI in I/R group were disordered and broken, the myocardial structure was blurred, the inflammatory cell infiltration and congestion were obvious; In I/R+BMSC group, the myocardial inflammatory cell infiltration decreased significantly, but the myocardial fiber arrangement was still disordered; There was no obvious inflammatory cell infiltration in the myocardium of mice in I/R+(oe-SDF-1α) group and the myocardial fibers were arranged more orderly; AMD3100 can block the therapeutic effect of oe-SDF-1α, which can be seen in this group The obvious myocardial fiber arrangement is disordered and broken (Fig. 6a). TUNEL staining showed that BMSC can reduce cardiomyocyte apoptosis, but oe-SDF-1α can further reduce cardiomyocyte apoptosis. AMD3100 can block the inhibitory effect of oe-SDF-1α on cardiomyocyte apoptosis (Fig. 6b, e).

We used EGFP and Ly6G to observe the distribution of BMSC and PMN in the myocardial tissue of each group of mice. The results showed that compared with the I/R+BMSC group, the I/R+(oe-SDF-1α) group had more EGFP-positive cells in the injured myocardium, suggesting that the increased expression of SDF-1α promoted the migration of BMSC to the injured site, this effect can be blocked by AMD3100 (Fig. 6c, f). BMSC can inhibit the number of PMN infiltration in the myocardial tissue, especially in the I/R+(oe-SDF-1α) group, the number of PMN decreased significantly (Fig. 6d, g), but AMD3100 could not reduce the amount of PMN in the damaged myocardial tissue. Combined with in vitro experiments, it is shown that oe-SDF-1α can reduce the ability of PMN to stay at the injured site. Compared with the control group, SDF-1α and CXCR4 mRNA, protein expression levels in the I/R group were increased significantly. Compared with the I/R group, the mRNA and protein expression levels of SDF-1α and CXCR4 in the I/R+BMSC group were decreased, and the I/R+(oe-SDF-1α) group were lower than I/R+BMSC group. The mRNA and protein expression levels of SDF-1α and CXCR4 in the I/R+(oe-SDF-1α)+AMD3100 group were increased significantly.

Oe-SDF-1α can inhibit oxidative stress and inflammatory factor levels in I/R mice

Detecting the levels of IL-1β, IL-6 and TNF-α in the serum of mice in different groups found that the levels of IL-1β, IL-6 and TNF-α were significantly reduced, while IL-10 The level has increased significantly in the serum of mice in the oe-SDF-1α group;

Detecting the levels of ROS, MPO, MDA and SOD in myocardial tissues also found that administration of oe-SDF-1α can significantly reduce the levels of ROS, MPO and MDA, enhance the levels of SOD in the myocardial tissues of I/R mice, This effect can be blocked by AMD3100. (Fig. 7). It indicates that the SDF-1α/CXCR4 signal pathway mediated the migration of BMSCs to the myocardium of I/R mice, can inhibit PMN retention, reduce the accumulation of ROS and inflammatory response, which is beneficial to the repair of the injured site.

Discussion

In recent years, the incidence rate and mortality rate of ischemic heart disease are increasing year by year. IRI often occurs in patients undergoing surgery for coronary artery disease, which can cause oxidative stress and trigger systemic inflammatory response, making patients face considerable surgical risk [20]. However, the molecular mechanism of IRI is not clear completely and needs to be further studied. Recent studies have found that PMN expression in peripheral blood increases after myocardial infarction, becoming the first cell to invade the heart [21]. In MI or heart failure, the expression level of SDF-1α is up-regulated, exerting stronger chemotaxis [22]. This is consistent with our study. Under the premise that there is no difference in basic information between PCI patients and the control group, compared with the control group, the expression levels of SDF-1α and PMN in the blood of PCI patients are increased, and both were statistical differences ($P<0.05$) (see Table 1). It shows that PMN and SDF-1α play an important role in the pathological mechanism of IRI.

In order to study how PMN induces myocardial damage after I/R, the specific mechanism of action of PMN and SDF-1α in IRI, the correlation between them, we established I/R mice model and H/R cells model.In I/R model mice, we found that the expression level of SDF-1α and CXCR4 in myocardial tissue increased significantly. It shows that SDF-1 and CXCR4 interact to form SDF-1/CXCR4 biological axis after IRI (Fig. 1A-D). As an important effector cell of inflammatory response, PMN can release MPO after activation, MPO is usually used as a marker of PMN aggregation [23]. Flow cytometry was used to detect the amount of water in cells positive for the PMN marker Ly6G to evaluate the migration of PMN. By testing the two indicators of MPO and Ly6G, we found that IRI promotes the massive migration and aggregation of PMN to myocardial tissue (Fig. 1E&F). Studies have shown that the SDF-1/CXCR4 signaling pathway is also an important pathway for chemotactic PMN migration [24]. Therefore, we speculate that the increased expression of SDF-1α can activate the SDF-1/CXCR4 signaling pathway, promote the migration and accumulation of PMN, lead to myocardial damage.In the H/R cell model, we further studied which cells secreted and enhanced SDF-1α expression under H/R conditions, we isolated and cultured primary mouse cardiomyocytes and cardiac fibroblasts. The supernatant of cultured cells was collected and tested by ELISA. It was found that the ability of H/R cardiomyocytes to secrete SDF-1α was increased significantly, while the ability of H/R cardiomyocyte fibroblasts to secrete SDF-1α did not change significantly (Fig. 1G). Through PCR and Western blot detection, it was found that SDF-1α, CXCR4 mRNA and protein expression in H/R cardiomyocytes increased, while H/R cardiomyocyte fibroblasts did not change significantly (Fig. 1E, F and H), indicating that H/R cardiomyocytes secrete and express SDF-1α, activate the SDF-1/CXCR4 signal pathway. Therefore, we speculate that the increased expression of SDF-1α in myocardial cells of I/R mice can promote PMN's damage. We have done the following research on the specific damage mechanism.

Studies have shown that PMN is the main source of reactive oxygen species (ROS) [25]. When a large amount of activated PMN is not cleared in time by the body, PMN extracellular trap net is attached to vascular endothelium, causing vascular endothelium apoptosis [26]. AMD3100, as a CXCR4 receptor blocker, can inhibit the interaction between SDF-1α and CXCR4 [27]. Therefore, by co-cultivating cardiomyocytes with PMN, we found that compared with the control group, the number of PMN migration increased in the H/R group (Fig. 2A), the recruitment of activated PMNs would cause cardiomyocyte

apoptosis and oxidative stress levels. Both are elevated, but these changes can be blocked by AMD3100 (Fig. 2B-F). It shows that PMN migrates to cardiomyocytes through the SDF-1α/CXCR4 signaling pathway under H/R conditions and causes its damage. Then intervention on the migration of PMN and the impact on PMN cardiomyocytes may reduce the reperfusion injury.

In recent years, the rise of stem cell therapy has become a promising tool for the treatment of ischemic heart disease [28]. Stem cells recruited to ischemic tissue or injected into the infarcted heart may secrete various cytokines and adjust the local microenvironment, thereby enhancing the survival of cardiomyocytes, angiogenesis and heart regeneration [29]. Although many chemokines are involved in tissue inflammation and post-injury processes, SDF-1α is considered to be the main stem cell chemokine. SDF-1α combined with CXCR of stem cell plays an important role in regulating BM homing, reproliferation and mobilizing stem cells into peripheral blood [30]. In addition to participating in the mobilization and migration of stem cells, SDF-1α can also participate in ischemia-related signaling pathways to directly protect cardiomyocytes. Therefore, we have successfully constructed BMSCs (oe-SDF-1α) overexpressing SDF-1α (Fig. 3A-C), and co-cultured BMSCs and oe-SDF-1α with cardiomyocytes. By testing the migration ability of stem cells, cardiomyocyte apoptosis and oxidative stress levels (Fig. 4A-E) have found that oe-SDF-1α is more suitable as a tool cell for the treatment of IRI.

In view of the fact that the migration of BMSC and PMN to cardiomyocytes under H/R conditions is related to the SDF-1α/CXCR4 signaling pathway. We speculate that when myocardial ischemia occurs, the SDF-1α/CXCR4 pathway may eliminate dead cardiomyocytes by recruiting PMN on the one hand, and reduce the killing effect of PMN on normal cardiomyocytes by recruiting stem cells on the other hand, so as to promote the repair of heart injury. However, BMSC is only partially recruited to the site of myocardial injury in the treatment of ischemic heart disease, which has limitations [31]. It may be because in the inflammatory response, cathepsin G and elastase released by PMN can remove the essential N-terminal amino acid residues that interact between SDF-1α and CXCR4, thereby inactivating SDF-1 [31, 32]. And oe-SDF-1α can effectively improve this phenomenon. The results showed that compared with the H/R+'BMSC group, the H/R+'oe-SDF-1α group did not affect the migration of PMN to H/R cardiomyocytes (Fig. 5A&D), but it could reduce level of apoptosis in cardiomyocytes (Fig. 5B), reduce the level of ROS (Fig. 5C), reduce MPO activity (Fig. 5D), reduce the content of MDA (Fig. 5E), and enhance SOD activity (Fig. 5F) significantly. Interestingly, its effect on cardiomyocytes is equivalent to that of the (H/R+AMD3100)+'BMSC group, blocking PMN migration is equivalent. It shows that oe-SDF-1α can reduce the damage of PMN to cardiomyocytes without affecting the phagocytic function of PMN and the ability to generate NETosis.

There have been a large number of reports on the protective effects of BMSC treatment on various ischemic tissue damage in I/R animal models [33–37]. Pan et al. used adenoviruses overexpressing the SOD gene to infect BMSCs and transplanted them into the myocardium of MI mice. They found that the survival rate of BMSCs was greatly increased, the oxidative stress at the injured site was reduced, which has a potential cardioprotective effect [38]. However, BMSCs overexpressing SDF-1α are rarely studied in I/R therapy. In this study, we gave I/R mice intravenously with BMSC, oe-SDF-1α and CXCR4 antagonist

AMD3100 to observe the effect of SDF-1α/CXCR4 signaling pathway in the treatment of myocardial IRI by oe-SDF-1α. We found that BMSC can improve cardiomyocytes (Fig. 6A), reduce the apoptotic rate of cardiomyocytes (Fig. 6B, E), inhibit the amount of PMN infiltration in myocardial tissue. Overexpression of SDF-1α in BMSC can promote BMSC to enter the site of myocardial injury (Fig. 6C and F), the number of PMN significantly decreases (Fig. 6D and G). Further promote its performance to improve cardiomyocytes and reduce the apoptotic rate of cardiomyocytes (Fig. 6A). Combined with the results of our in vitro experiments: oe-SDF-1α does not affect the migration of PMN to H/R cardiomyocytes. However, in I/R mice, oe-SDF-1α can reduce the amount of PMN, indicating that oe-SDF-1α can reduce PMN staying in the injury site of I/R mice. Although AMD3100 is used, it can prevent BMSC from accumulating to the site of myocardial injury. However, this study shows that AMD3100 cannot reduce the amount of PMN in the damaged myocardial tissue, suggesting that there may be other ways to promote the migration of PMN to the site of myocardial injury, which needs to be further studied.

Although the migration pathway of chemotactic PMN to the site of myocardial injury cannot be fully described, the role of PMN activation in the pathogenesis of cardiovascular disease is clear. PMN was recruited to the infarct area very early, they play a role by adhering to vascular endothelial cells through molecules of selectins, integrins and immunoglobulin superfamily [39]. Once adhered to the infarct area, PMN will release ROS, cytokines and proteolytic enzymes, resulting in the increase of MDA level and the decrease of SOD level [40]. The increase of MPO produced by PMN activation has a great adverse effect on left ventricular remodeling and function in the infarcted myocardium [41]. This is also applicable to IRI. Our study shows that compared with normal mice, the levels of ROS, MPO, and MDA in the myocardium of I/R mice increase, while the levels of SOD decrease (Fig. 7F-I) and SDF-1α in the blood increases (Fig. 7E). Although the exact mechanism of IRI is not very clear, it is currently believed to be related to the essence of IRI, in addition to the increased production of free radicals leading to oxidative stress and excessive activation of the inflammatory response [42].

The inflammatory response induced by myocardial IRI involves PMN infiltration of macrophages, thus exhibiting high expression of pro-inflammatory cytokines (such as IL-1β, IL-6 and TNF-α) and suppressing immune regulatory factors such as IL-10 [43]. TNF-α can promote inflammation damage, induce the synthesis of chemokines and adhesion molecules in the myocardium [44]. IL-1β mediates the recruitment and activation of inflammatory leukocytes, at the same time delays the activation of myofibroblasts. In vitro studies have shown that inhibiting IL-1β can reduce cardiomyocyte apoptosis [45]. IL-6 is also up-regulated in infarcted myocardium through IL-6 receptor β Subunit and activate JAK/STAT cascade to regulate inflammation [46]. IL-10 has a strong anti-inflammatory effect and can prevent excessive inflammation [47, 48]. Yang and his colleagues believe that the inflammatory response is enhanced after I/R in IL-10 knockout mice, which is manifested by increased PMN recruitment, increased plasma TNF-α levels, and increased ICAM-1 expression in tissues [49]. Therefore, we have confirmed from both oxidative stress and immune inflammation: injecting BMSCs overexpressing SDF-1α into the tail vein of I/R mice, on the one hand, promotes the accumulation of BMCS to the injury site, on the other hand, it can reduce the PMN in the myocardial injury site. It can reduce the level of ROS at the injured site, reduce the level of

IL-1β, IL-6, and TNF-α in the serum, increase the level of IL-10, and play a role in inhibiting inflammation, thereby improving myocardial remodeling and function.

Conclusions

In conclusion, this study shows that H/R cardiomyocytes can up-regulate the expression of SDF-1 and CXCR4 from the in vitro level. Through SDF-1/CXCR4 signaling pathway, on the one hand, PMN is recruited to participate in the inflammatory response at the injury site, on the other hand, BMSC can reduce the excessive injury of cardiomyocytes caused by PMN. This study demonstrated for the first time that intravenous injection of SDF-1α gene-modified BMSC can protect the heart function of mice and reduce myocardial injury induced by cardiac IRI significantly, reduce the inflammatory infiltration at the injury site and the level of inflammatory factors throughout the body, have a potential protective effect on IRI. Among them, BMSC overexpressing SDF-1α not only improves the limitation of BMSC in treating myocardial injury, but also can reduce PMN's damage to myocardial cells without affecting the phagocytic function of PMN. It opens up a new program for the clinical treatment of myocardial IRI with stem cells.

Abbreviations

I/R: ischemia/reperfusion; PMNs: Polymotphonucleat Leukocytes; SDF-1α: Stromal cell derived factor-1α; BMSC: bone marrow mesenchymal stem cells; PCI: percutaneous coronary intervention; oe-SDF-1α: BMSC stably expressing SDF-1α; MDA: malondialdehyde, SOD: superoxide dismutase; (MPO: myeloperoxidase ; BMI: Body Mass Index; ROS: reactive oxygen species.

Declarations

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Author’s contributions

RW: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing-original draft preparation, Writing-review & editing, Visualization, Project administration. WW: Conceptualization, Methodology, Validation, Investigation. SR: Resources. TW:Investigation, Resources. BL: Conceptualization, Methodology, Validation, Investigation, Resources, Writing-review & editing, Visualization, Supervision, Project administration.

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Availability of Data and Material

Raw data will be provided upon reasonable request.

Ethics Approval and consent to participate

All animal experiments were approved by Animal Care committee of Shanxi Medical University and were performed in accordance with institutional guidelines (Approval number: IACUC2017-002). The human study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Second Hospital of Shanxi Medical University. Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Consent for Publication

Patient's legal representative signed informed consent regarding publishing their data.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

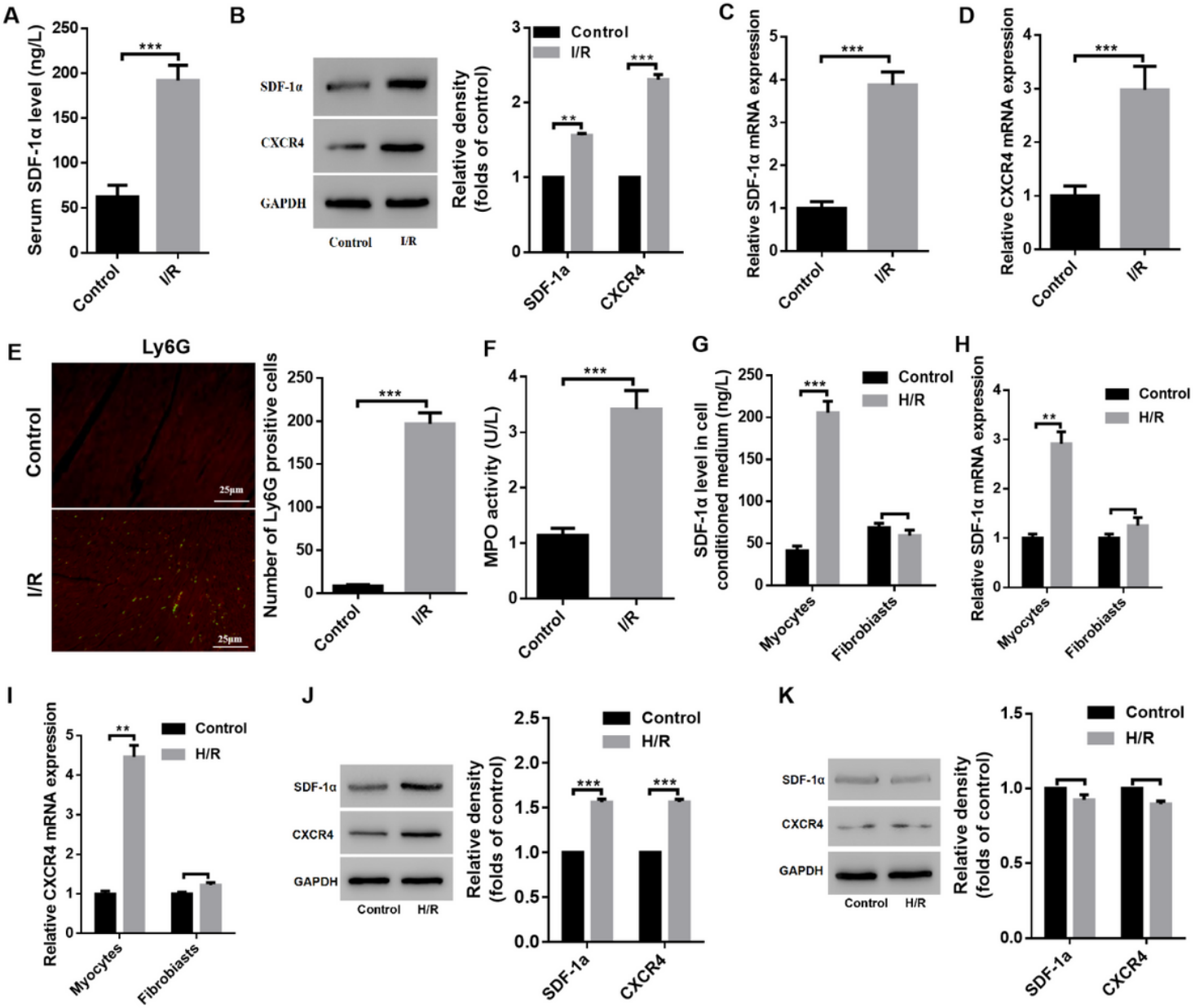

Figure 1

Under H/R conditions, the expression of SDF-1α and CXCR4 in cardiomyocytes increased. a Comparison of the level of SDF-1α in the serum of normal mice and I/R mice. b Western blot was used to detect the expression levels of SDF-1α and CXCR4 protein in myocardial tissues of normal mice and I/R mice. c,d PCR detection of SDF-1α and CXCR4 mRNA expression levels in normal mice and I/R mice. e Compare the positive rate of Ly6G in the myocardial tissues of normal mice and I/R mice (unit: 20μm). f Compare the activity of MPO in the myocardial tissues of normal mice and I/R mice. The results are expressed as mean±SEM (n=5 mice per group) g ELISA detects the level of SDF-1α in cardiomyocyte culture and the supernatant of cardiomyocyte fibroblasts. h,i PCR was used to detect the expression level of SDF-1α mRNA in cardiomyocytes and myocardial fibroblasts. j Western blot detection of SDF-1α and CXCR4 protein expression levels in cardiomyocytes. k Western blot detection of SDF-1α and CXCR4 protein expression levels in cardiac fibroblasts. The results are expressed as mean±SEM (n=4 in each group),

pairwise comparisons are analyzed by t-test, P<0.05 indicates statistical significance, T test is used, * is P<0.05, ** is P<0.01, *** is P<0.001.

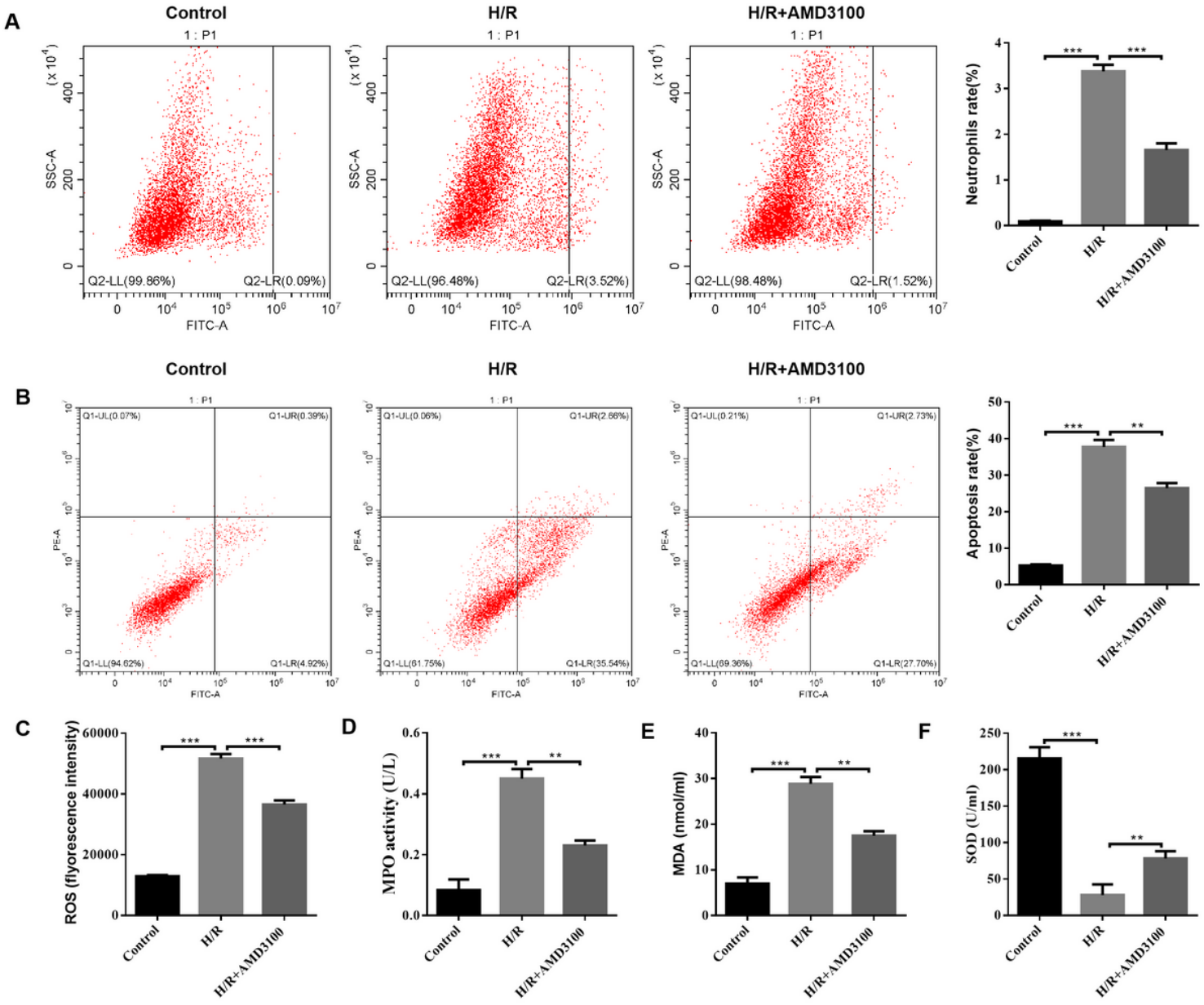

Figure 2

Under H/R condition, mouse cardiomyocytes recruit PMN through SDF-1/CXCR4 signaling pathway to promote cardiomyocyte injury. a Flow cytometry to detect the number of migrating PMN and comparison of the number of PMN migration in each group. b Flow cytometry to detect the ratio of cardiomyocyte apoptosis and comparison of the ratio of cardiomyocyte apoptosis in each group. c Comparison of ROS levels of cardiomyocytes in each group. d Comparison of MPO activity of each group. e Comparison of MDA level of each group of cardiomyocytes. f Comparison of SOD activity of each group of cardiomyocytes. The results are expressed as the mean±SEM (n=4 per group); P<0.05 indicates statistical significance, T test is used, * is P<0.05, ** is P<0.01, *** is P<0.001.

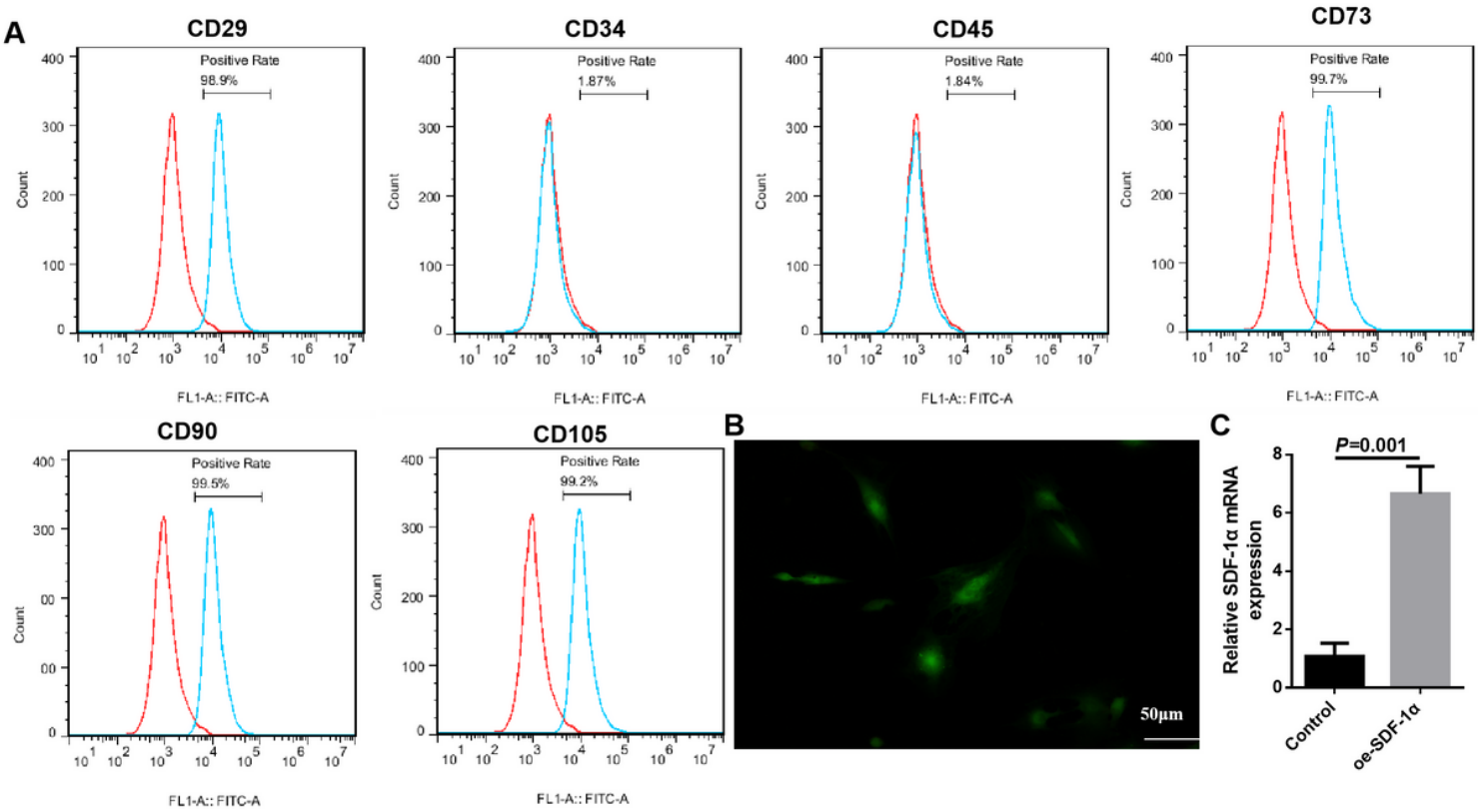

Figure 3

Identification of oe-SDF-1α. a Flow cytometry to detect the characteristic markers of BMSC. b Indirect immunofluorescence to observe the expression of EGFP in cells. c PCR to detect the expression level of SDF-1α mRNA in BMSC.The results are expressed as the mean±SEM (n=4 per group); P<0.05 indicates statistical significance, T test is used, * is P<0.05, ** is P<0.01, *** is P<0.001.

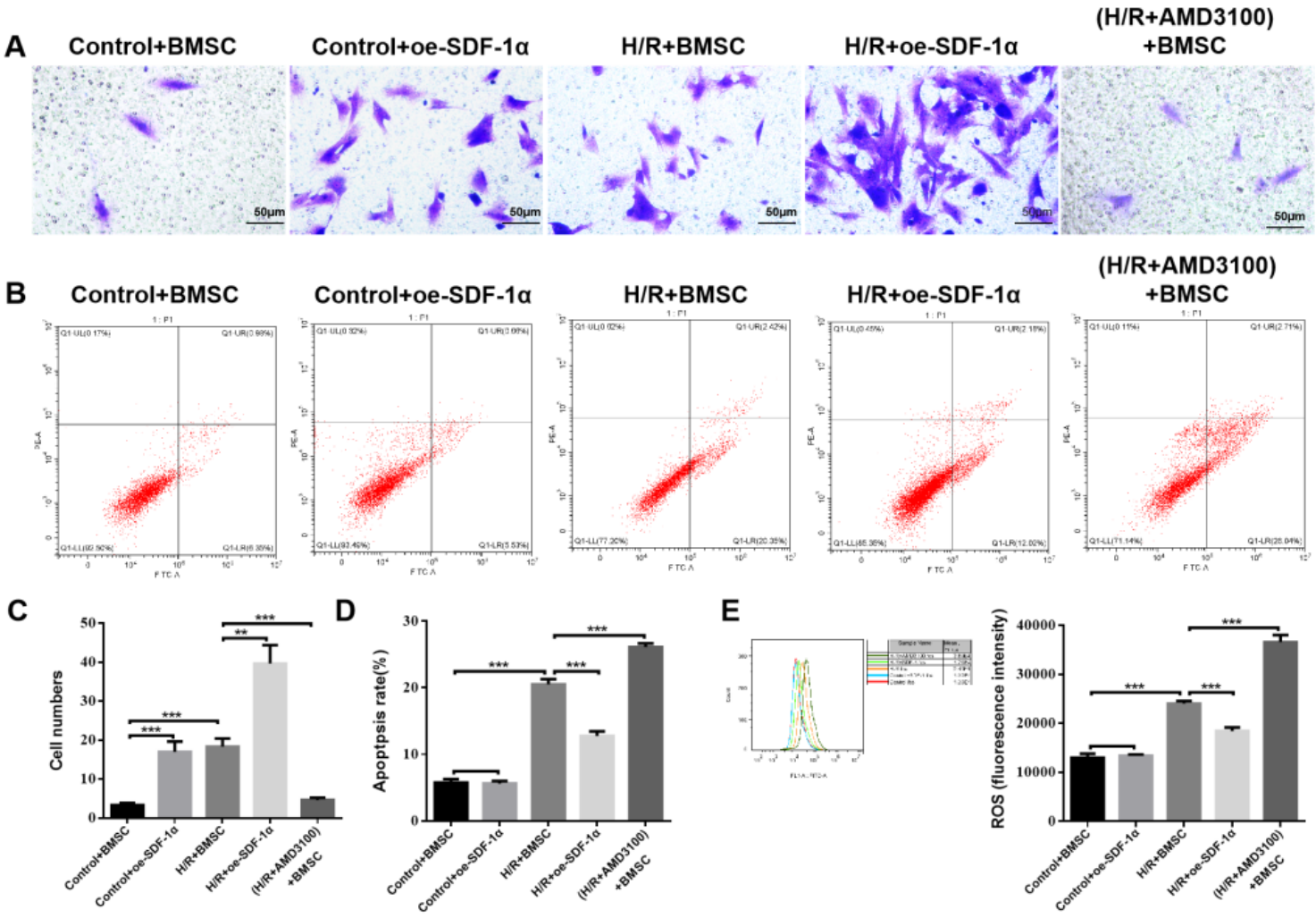

Figure 4

Overexpression of SDF-1α in BMSC promotes its migration to H/R cardiomyocytes and reduces cardiomyocyte damage. a Transwell test to detect the migration ability of BMSC. b Flow cytometry to detect the proportion of cardiomyocyte apoptosis. c Comparison of the number of cell migration in each group. Flow cytometry to detect the level of ROS in cardiomyocytes. d Apoptosis of each group Comparison of proportions. e Flow cytometry to detect ROS levels in cardiomyocytes and comparison of ROS levels in cells of each group. The results are expressed as the mean±SEM (n=4 per group); P<0.05 indicates statistical significance, T test is used, * is P<0.05, ** is P<0.01, *** is P<0.001.

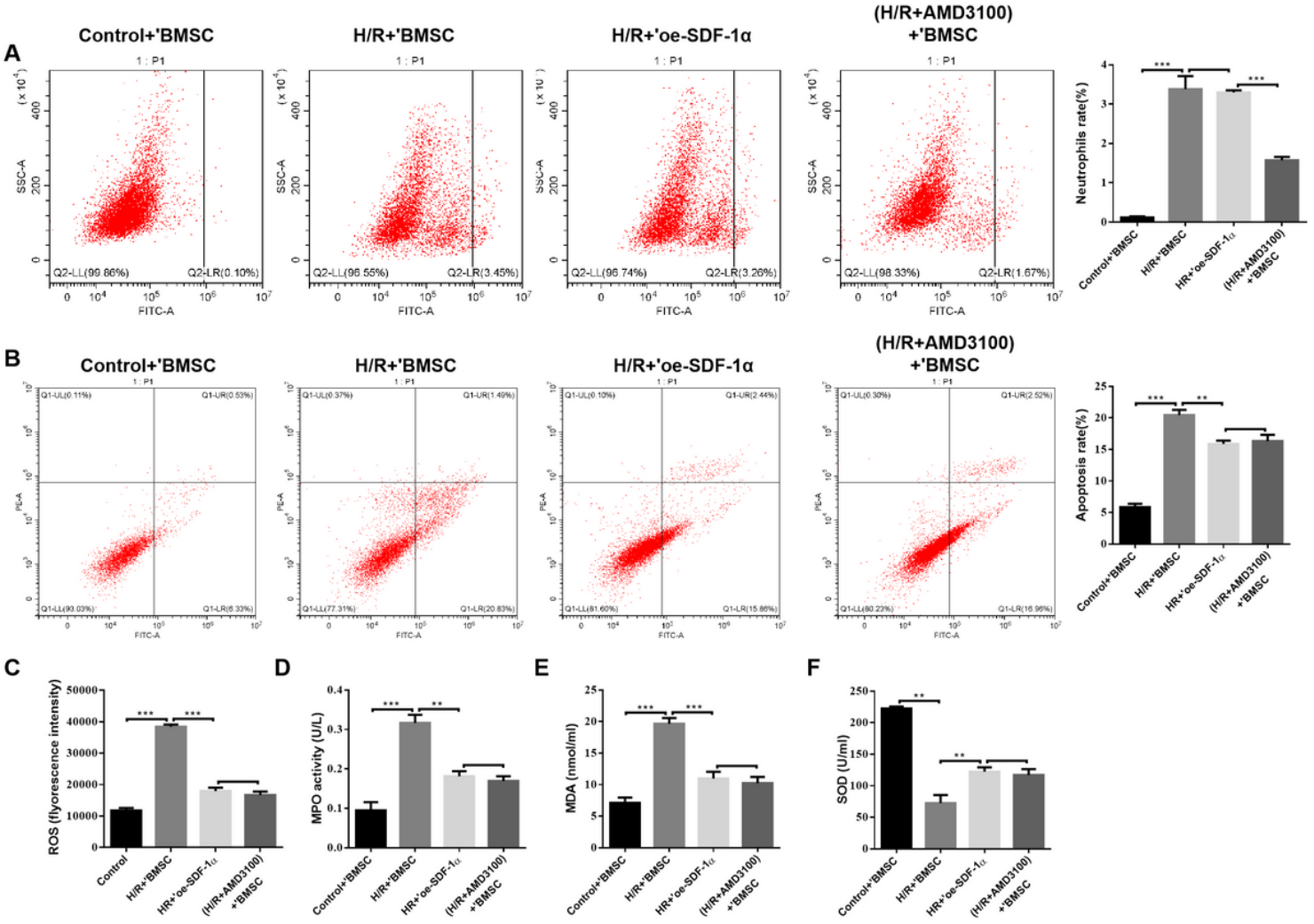

Figure 5

Oe-SDF-1α can reduce the damage of cardiomyocytes caused by PMN a Flow cytometry to detect the number of migrating PMN and comparison of the number of PMN migration in each group. b Flow cytometry to detect the ratio of cardiomyocyte apoptosis and comparison of the ratio of cardiomyocyte apoptosis in each group. c Comparison of ROS levels of cardiomyocytes in each group. d Comparison of MPO activity of each group. e Comparison of MDA level of each group of cardiomyocytes. f Comparison of SOD activity of each group of cardiomyocytes. The results are expressed as the mean±SEM (n=4 per group); P<0.05 indicates statistical significance, T test is used, * is P<0.05, ** is P<0.01, *** is P<0.001.

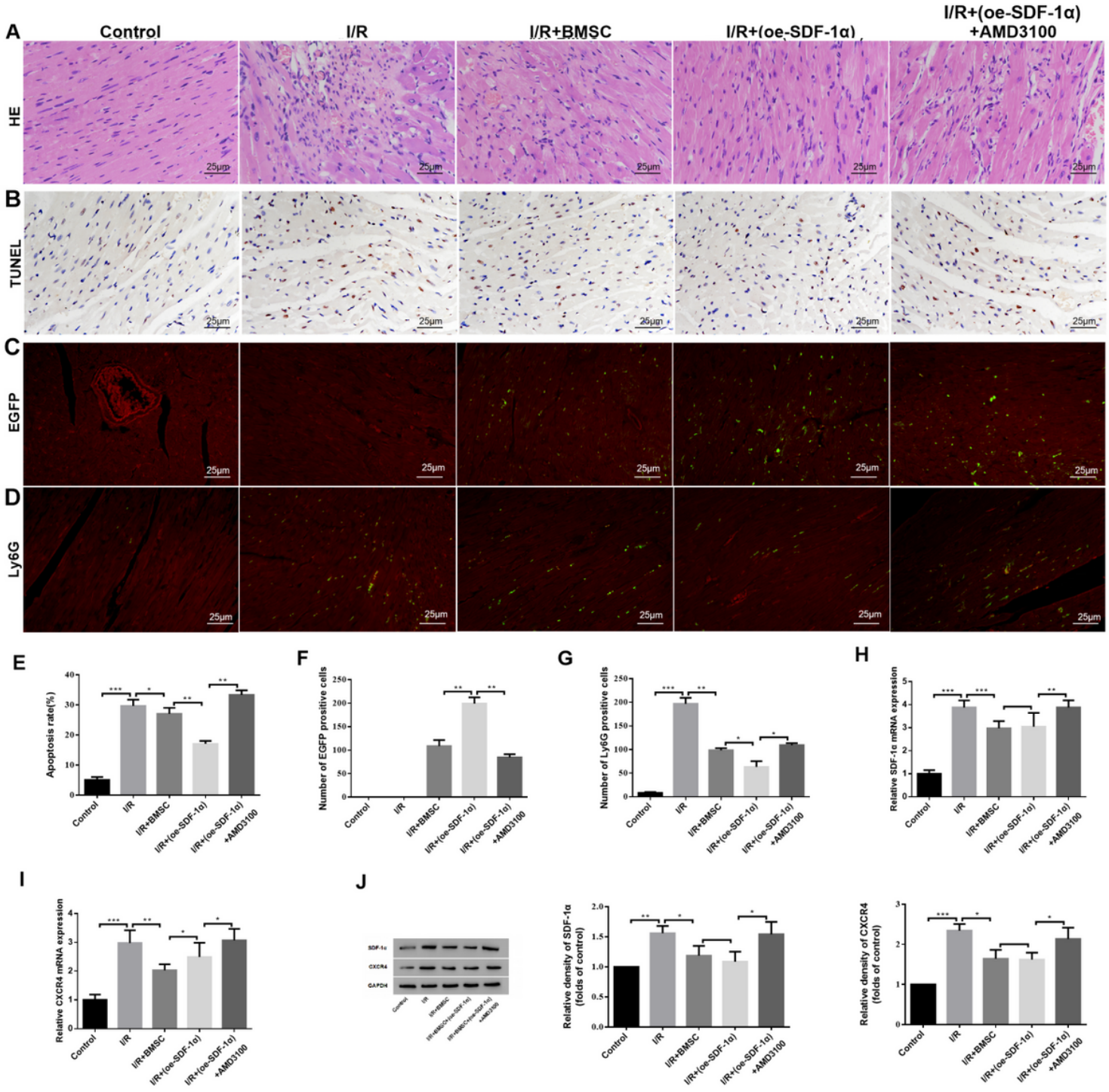

Figure 6

The effect of oe-SDF-1α on myocardial injury in I/R mice. a HE staining to analyze the myocardial pathological changes of mice in each group. b TUNEL staining to analyze the apoptosis level of myocardial cells in each group of mice. c Observe the distribution of EGFP-labeled BMSC in the myocardial tissue of each group of mice under an immunofluorescence microscope. d Observe the distribution of Ly6G-positive cells (PMN) in the myocardial tissue of each group of mice by immunofluorescence. e the proportion of TUNEL-positive cells in the myocardial tissue of each group of mice. f the proportion of EGFP-positive cells in the myocardial tissue of each group of mice . g the

proportion of Ly6G positive cells in the myocardial tissue of each group of mice. h,i PCR detection of SDF-1α and CXCR4 mRNA expression levels in normal mice and I/R mice. j Western blot was used to detect the expression levels of SDF-1α and CXCR4 in myocardial tissues of normal mice and I/R mice.The results are expressed as the mean±SEM (n=4 per group); P<0.05 indicates statistical significance, T test is used, * is P<0.05, ** is P<0.01, *** is P<0.001.

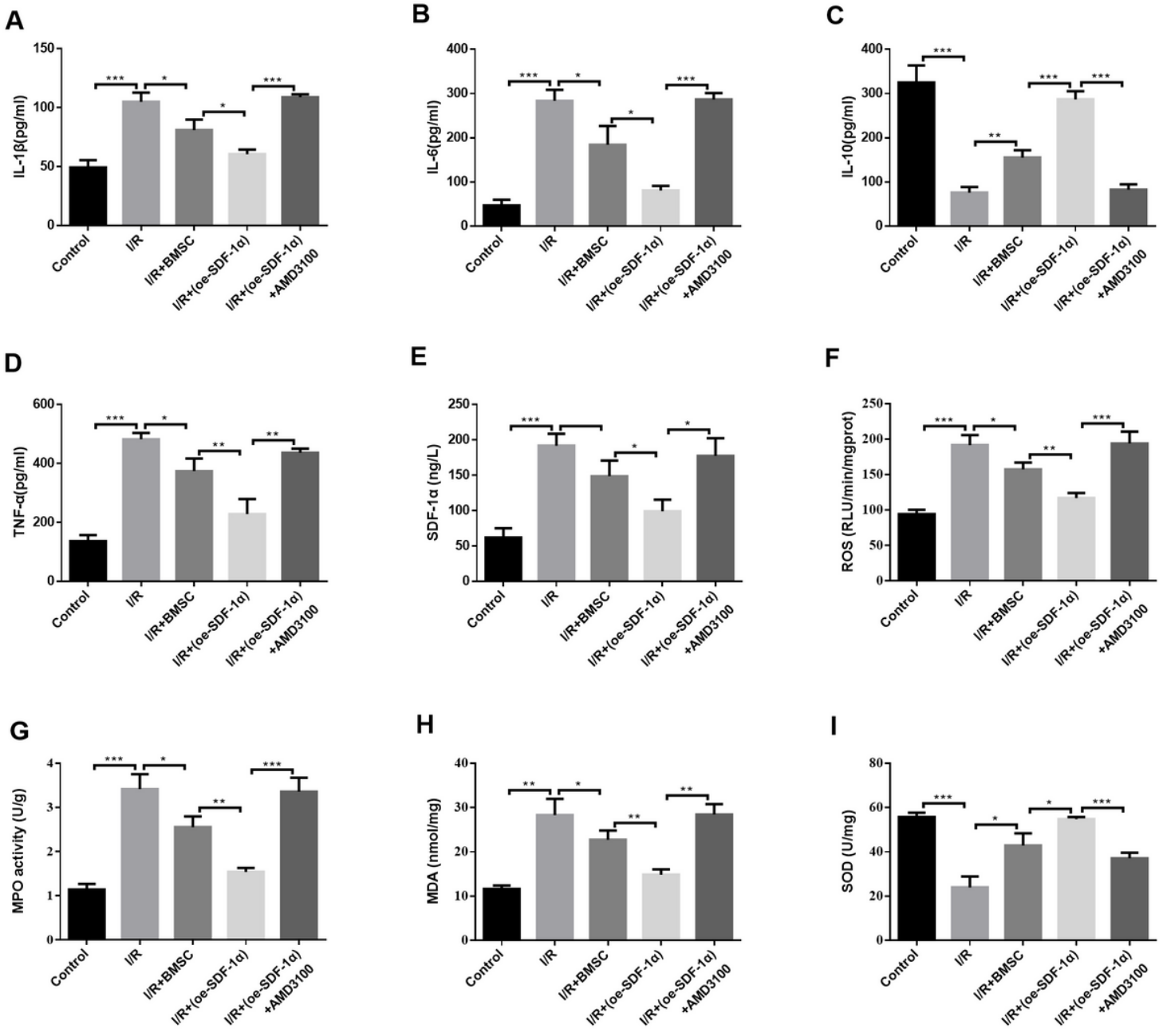

Figure 7

Detection of serum inflammatory factor levels and oxidative stress levels in myocardial tissues of mice in each group. a ELISA to detect IL-1β level in mouse serum; b ELISA to detect IL-6 level in mouse serum; c ELISA to detect IL-10 level in mouse serum; d ELISA to detect TNF-α in mouse serum Level; e ELISA to detect the level of SDF-1α in mouse serum; f detection of ROS level in myocardial tissue; g detection of MPO activity in myocardial tissue; h detection of MDA level in myocardial tissue; i detection of SOD level

in myocardial tissue.The results are expressed as the mean±SEM (n=4 per group); P<0.05 indicates statistical significance, T test is used, * is P<0.05, ** is P<0.01, *** is P<0.001.