FDNC5/Irisin improves the therapeutic efficacy of bone marrow-derived mesenchymal stem cells for myocardial infarction

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Research

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Abstract

Background

The beneficial functions of bone marrow mesenchymal stem cells (BM-MSCs) decline with decreased cells survival, limiting their therapeutic efficacy for myocardial infarction (MI). Irisin, a novel myokine which is cleaved from its precursor fibronectin type III domain-containing protein 5 (FNDC5), is believed involved in a cardioprotective effect but little was known on injured BM-MSCs and MI repair yet. Here, we investigated whether FNDC5 or irisin could improve the low viability of transplanted BM-MSCs and increase their therapeutic efficacy after MI.

Methods

BM-MSCs, isolated from dual-reporter firefly luciferase and enhanced green fluorescent protein positive (Fluc + –eGFP +) transgenic mice, were exposed to normoxic condition and hypoxic stress for 12 h, 24 h, and 48 h, respectively. In addition, BM-MSCs were treated with irisin (20 nmol/L) and FNDC5 +/+ in serum deprivation (H/SD) injury. Furthermore, BM-MSCs were engrafted into infarcted hearts with or without FNDC5 +/+ .

Results

Hypoxic stress contributed to increased apoptosis, decreased cells viability and paracrine effects of BM-MSCs while irisin or FNDC5 +/+ alleviated these injuries. Longitudinal in vivo bioluminescence imaging illustrated that MSCs FNDC5+/+ treatment improved the survival of transplanted MSCs, which ameliorated the increased apoptosis and decreased angiogenesis of BM-MSCs in vivo . Furthermore, MSCs FNDC5+/+ therapy significantly reduced fibrosis and alleviated injured heart function.

Conclusions

The present study indicated that irisin or FNDC5 improved BM-MSCs engraftment and paracrine effects in infarcted hearts, which might provide a potential therapeutic target for MI.

Background

The treatments focus on retaining the function of the remaining cardiomyocytes, but myocardial infarction (MI) still remains a major cause of morbidity and mortality worldwide ^(1,2). Recently, mesenchymal stem cells (MSCs) transplantation has held great promise for multiple diseases, such as MI ⁽³⁻⁴⁾. The illustrated underlying therapeutic mechanisms include engraftment ⁽⁵⁾, paracrine signaling ⁽⁶⁾. Moreover, these mechanisms play a role on the premise that the engrafted MSCs survives for a certain period. However, many studies have shown that only a small number of transplanted MSCs eventually survive to play protective effects ⁽⁷⁻⁹⁾. Also, evidences indicated that under myocardial ischemia conditions, hypoxic stress caused increased apoptosis of MSCs and reduce its therapeutic effects ^(1,10).

Therefore, it is reasonable to assume that promoting the low engrafted MSCs survival in ischemia myocardial tissue may be the key to improving stem cells therapeutic efficacy after MI.

FNDC5 is a transmembrane protein that possesses two domains (fibronectin III and carboxy-terminal respectively) located in the cytoplasm⁽¹¹⁾. Previous study has indicated that FNDC5 can be cleaved by an unknown enzyme and then the extracellular domain of FNDC5 was named as irisin⁽¹²⁾. In addition, Irisin was recognized as a myokine secreted from skeletal muscle and heart^(13,14). Meanwhile, accumulating studies demonstrated that irisin was involved in cardioprotective effect, such as anti-apoptosis, increased cells viability and anti-oxidative stress by various pathways^(15–16).

However, the protective effect of FNDC5/Irisin in hypoxia-induced the low viability of transplanted BM-MSCs is still unclear. Therefore, we hypothesized that Irisin may protect against increased apoptosis and paracrine dysfunction of BM-MSCs induced by hypoxia. Moreover, genetically modified BM-MSCs overexpressing FNDC5 may promote the functional survival of transplanted BM-MSCs and increase the cardiac protective effect.

Methods

Animal

Adult male C57BL/6 mice were provided by the PLA Rocket Force Characteristic Medical Center (Beijing, China). Meanwhile, Fluc⁺–eGFP⁺ transgenic mice [Tg(fluc –egfp)] were purchased from Contag Laboratory (Stanford, CA, USA). Mice were created on the C57BL/6 background to stably express both firefly luciferase (Fluc) and enhanced green fluorescence protein (eGFP) in all tissues and organs. As alleged up front⁽¹⁾, mice were placed into a temperature-controlled animal facility with a 12-h light/dark cycle (light cycle, 8:00 a.m. to 8:00 p.m.), with tap water and rodent chow provided ad libitum. All animal experiments were performed by a protocol approved by the Animal Care and Use Committee of the PLA Rocket Force Characteristic Medical Center (ID: 5034) and were in compliance with the Guidelines for the Care and Use of Laboratory Animals, as published by the National Academy Press.

Isolation, culture and treatments of BM-MSCs

BM-MSCs were isolated and expanded as described⁽¹⁰⁾. Briefly, bone marrow was flushed from the femoral and tibia of adult Tg(fluc –egfp) mice with fetal bovine serum (FBS)-free Dulbecco's modified Eagle's medium (DMEM). After passing through a 70-μm strainer and centrifugation at 1200 rpm for 5 min at room temperature, the cell pellet was re-suspended in DMEM supplemented with 20% FBS and incubated at 37 °C in an atmosphere containing 5% CO₂. After 24 h, the medium was replaced to remove the nonadherent cells, and then was completely replaced every 3 days. Third-passage BM-MSCs with optimal growth at the third generation were applied for different treatments to avoid contamination with other types cell. Then, cells were treated with irisin (20 nmol/L) in the presence or absence of hypoxia for 48 h⁽¹⁵⁾.

FNDC5 over-expression

As described previously ⁽¹⁷⁾, FNDC5 cDNA was cloned from cells. The primer sequences were as follows: 5'-ATG CAC CCC GGG CCG CCC CG- 3' (forward) and 5'-GTC CCC TCT CTC CCT GAG C-3' (reverse). The PCR product and the pIRES2-EGFP vector (from Clontech) were digested by EcoRI and BamHI restriction sites. And the FNDC5 fragment was ligated into the pIRES2-EGFP vector that was used as control. Then, Lipofectamine and plasmids were diluted in Opti-MEM (Gibco), separately. The two solutions were then mixed in a ratio of 1:1 and incubated at 20–25 ° C for 5 minutes. Once BM-MSCs reached 60–70% fusion, the mixture was added into the cells, and then incubated and processed for the indicated time.

Hypoxia/serum deprivation injury

Hypoxia/ serum deprivation (H/SD) injury was performed for the hypoxic stress of BM-MSCs as described previously ⁽¹⁸⁾. Briefly, after being replaced in glucose-free DMEM without FBS, BM-MSCs were exposed to hypoxia (94% N₂/5% CO₂/1% O₂) with an anaerobic system (Thermo Forma) at 37 ° C for 12, 24, and 48 h, respectively. Also, BM-MSCs in the control group incubated under normoxic conditions (37 ° C in 95% air, 5% CO₂) with full medium for equivalent periods.

Measurement of BM-MSCs apoptosis

BM-MSCs apoptosis was determined by flow cytometry with an Annexin V-FITC/PI Kit (Merck) according to the manufacturer's instructions ⁽¹⁹⁾. In brief, cells were re-suspended in 200 µL of binding buffer. Cells were incubated with 10 µl of Annexin V solution and 5 µl propidine iodide (PI) at room temperature for 30 minutes respectively. The cells were immediately analyzed on a FACSC-LSR (Becton, Dickinson and Company, San Jose, CA). Caspase-3 activity was measured using a Caspase-3 Assay kit (Clontech, Mountain View, Calif) according to the manufacturer's instructions.

Cell viability assay

The cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously ⁽²⁰⁾. Briefly, BM-MSCs were plated in 96-well plates at 1 × 10⁵ cells/well. After an overnight incubation,

BM-MSCs with different treatments were incubated for 72 h. Then, MTT solution (Sigma) was added into a final concentration of 0.5 g/L to each well. These cells in 96-well plates were cultured in a 5% CO₂ incubator at 37 ° C for 4 h, and further the medium was aspirated. Next, 200 µL dimethyl sulphoxide (DMSO) was added into each well. The absorbance was determined at a wavelength of 490 nm. In addition, optical density (OD) values of each group were detected in six duplicate wells and their averages were calculated.

Furthermore, the viability of cells was also assessed by bioluminescence imaging (BLI) with the IVIS Kinetic system (Caliper, Hopkinton, MA, USA) ⁽¹⁹⁾. In brief, BM-MSCs were plated in 24-well plates (5 × 10⁴ per well). Cells were given different treatments, and then the medium was removed. MSCs were incubated

with D-Luciferin reporter probe (4.5 µg/mL) and further measured by the IVIS Xenogen Kinetic system (Caliper Life Sciences, USA).

Determination of VEGF, bFGF, IGF-1, and HGF

Enzyme-linked immunosorbent assay (ELISA) was performed to determine the concentrations of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF)-1, and hepatocyte growth factor (HGF) secreted by MSCs following the manufacturer's instructions. All samples and standards were measured in duplicate.

Myocardial infarction model

MI was accomplished by ligation of the left anterior descending (LAD) artery as described previously^(3,21). In brief, mice were anesthetized with isoflurane and mechanically ventilated. The heart was exposed by left thoracotomy. Then, LAD artery was permanently ligated with a 6 – 0 silk suture. When the anterior wall of the left ventricle (LV) turned pale and characteristic electrocardiographic (ECG) changes were recorded, success of the ligation was confirmed. At last, the chest and skin were sealed, and mice were placed on the ventilator until they woke up. Mice in Sham-operated control group underwent the same operation except that the suture below the left coronary artery was not ligated.

BM-MSCs transplantation

As described previously^(3,19), BM-MSCs transplantation was performed immediately after MI. In brief, BM-MSCs were collected and randomly divided into the different groups separately. The suspended cells (1×10^6) were injected directly into the peri-infarcted areas [at 2 sites near the peri-infarct zone (medial and lateral zones)] by a Hamilton syringe with a 29-gauge needle (in 20 mice in every group).

In vivo evaluation of MSCs engraftment

BLI was performed to track transplanted BM-MSCs using an IVIS® Kinetic system (Caliper, Hopkinton, MA, USA)⁽¹⁾. After intraperitoneal injection with D-luciferin (375 mg/kg body weight), recipient mice were anesthetized with isoflurane and imaged for 10 min on days 1, 7, 14, 21, 28 until sacrificed. Peak signals (photons/s/cm²/sr) from a fixed region of interest (ROI) were analyzed with Living Image® 4.0 software (Caliper, MA, USA).

Histological analysis of fibrosis and angiogenesis

As described previously⁽²²⁾, Fast green/Sirius red stain was performed to detect fibrosis in cardiac muscle in 4-week post-procedure. Fibrosis was evaluated by measuring the collagen area as a proportion of the total left ventricular area with Imaging Pro Plus software. Meanwhile, the capillary density was determined by CD31 immunohistochemistry. Vessels in the peri-infarct zone were counted in randomly chosen high-power fields (HPFs, magnification x400). The results are expressed as vessels per HPF.

Echocardiographic measurements

Cardiac function was measured under anesthesia with 2% isoflurane by transthoracic echocardiography at baseline, 7 days, and weekly until sacrifice at 4-week post-operation with a 30-MHz transducer on a Vevo® 2100 ultrasound system (VisualSonics, CA, USA) ^(19, 21). Briefly, mice were anesthetized (2% isoflurane and oxygen) and put in a supine position. M-mode images and grayscale two-dimensional parasternal short-axis images at the mid-papillary level of each mouse were recorded. Measurements were carried out offline by a single observer in a group-blinded manner. The left ventricular end-systolic diameter (LVESD) and end-diastolic diameter (LVEDD) were measured from M-mode images. Meanwhile, left ventricular end-systolic volume (LVESV) and left ventricular end-diastolic volume (LVEDV) were also measured to calculate left ventricular ejection fraction (LVEF) and fractional shortening (FS) with the following equations: $LVEF = (LVEDV - LVESV)/LVEDV \times 100\%$, $LVFS = (LVEDD - LVESD)/LVEDD \times 100\%$.

Determination of apoptosis of engrafted BM-MSCs

Apoptosis in the heart at 48 h after BM-MSCs transplantation was determined by Terminal-deoxynucleotidyl transferase mediated-dUTP nick-end labeling (TUNEL) assay as previously described ⁽²³⁾. In brief, cell nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Sections were imaged using confocal microscope (Fluo-View-FV1000, Olympus, Japan). At the same time, the percentage of apoptotic cells was calculated.

Western blot assay

Cells were collected and dissolved in protein lysis buffer (Sigma). Then, equivalent protein was separated by electrophoresis on 12% SDS-PAGE gels at 120 V for 1.5 h. Furthermore, they were transferred to PVDF membrane by 300 mV electrophoresis for 1.5 h. Cellular membranes were subjected to immune-blotting with primary antibodies overnight at the temperature 4 °C after blocked in 5% nonfat dry milk (BD Biosciences) with 1xTBST at room temperature for 1 h. After incubation with appropriate secondary antibodies binding to horseradish peroxidase, an enhanced chemiluminescence system (Amersham Bioscience) was used to visualize blots bands. Also, we determined densitometric analysis of Western blot with VisionWorks LS, version 6.7.1.

The following antibodies were used: rabbit anti-mouse FNDC5(1:1000, Cellular Signal Technology), and rabbit anti-mouse β -actin (1:1000, Abcal).

Statistics analysis

Data analysis was performed with Graph Pad Prism 5.0 (San Diego, CA, USA). All quantitative data are expressed as the Mean $\pm$ SEM. The different groups were compared by the homogeneity tests and one-way analysis of variance(ANOVA). P value less than 0.05 was considered as statistical significance.

Results

Hypoxia increased apoptosis of BM-MSCs

To analyze BM-MSCs apoptosis induced by hypoxic stress, flow cytometry and caspase-3 activity assays were performed. Annexin V is considered a marker for early stage apoptosis. Early stage apoptotic cells will only take up Annexin V stain but will remain PI negative. The late-stage apoptotic and necrotic cells will be positive for both Annexin V and PI. The representative flow cytometry results shown in Fig. 1a-c indicated that the percentage of early stage apoptotic cells under hypoxic condition significantly increased than that in normal condition ($p < 0.05$). Meanwhile, the expression of cleaved caspase-3 was increased under hypoxia time-dependently (Fig. 1d, $p < 0.05$). These data suggested that hypoxic stress increased the apoptosis of BM-MSCs.

Hypoxia exposure decreased MSCs viability

Representative in vitro BLI indicated a robust linear correlation between the cells number and average Fluc radiance ($r^2 = 0.98$; Fig. 2a), suggesting that BLI of Fluc could be reliably used to monitor the viability of engrafted MSCs^{Fluc+GFP+} quantitatively in vivo. Furthermore, in vitro BLI displayed a remarkable decline of BLI signal intensity in MSCs^{Fluc+GFP+} after H/SD injury compared with normoxia (Fig. 2b-c, $p < 0.05$). Concurrently, MTT results also showed that hypoxia exposure impaired viability of MSCs^{Fluc+GFP+} after H/SD injury (Fig. 2d, $p < 0.05$). Collectively, these data suggested that hypoxic stress decreased the viability of BM-MSCs.

Hypoxic stress inhibited growth factors secretions in MSCs

BM-MSCs involved in cardiac repair and regeneration at least in part by paracrine effects. Therefore, we evaluated the effect of hypoxia on cytokine secretion in BM-MSCs. Results demonstrated that H/SD injury suppressed VEGF secretion compared with that in normoxic group (Fig. 3a, $p < 0.05$). Also, the ELISA assay results illustrated that the bFGF, IGF-1 and HGF were restrained in MSCs with hypoxic treatment (Fig. 3b-c, $p < 0.05$). Taken together, these results showed that H/SD injury contributed to paracrine dysfunction of BM-MSCs.

H/SD injury reduced expression of FNDC5 in BM-MSCs

To explore the effect of hypoxic stress on the protein expression of FNDC5, Western blot was performed. The representative results shown in Fig. 4a indicated that compared with normoxic group, the protein expression level of FNDC5 was decreased in hypoxia exposure group. Meanwhile, semi-quantitative analysis demonstrated that expression level of FNDC5 was reduced in H/SD group ($p < 0.05$, Fig. 4b). In a word, these results showed that H/SD injury suppressed the expression of FNDC5 in BM-MSCs time dependently.

To further increase the expression of FNDC5 in hypoxic BM-MSCs, FNDC5 was over-expressed by transfection. Results suggested that expression level of FNDC5 was promoted in MSCs^{FNDC5+/+} ($p < 0.05$, Fig. 4c and 4d).

FNDC5/Irisin ameliorated MSCs apoptosis induced by H/SD injury

Flow cytometry and caspase-3 activity assays were performed for confirming whether FNDC5/Irisin plays a protective role in hypoxia-induced apoptosis in BM-MSCs. The flow cytometry results illustrated that the percentage of early stage apoptotic BM-MSCs under hypoxia for 48 h significantly increased than that in normal condition (Fig. 5a-c, $p < 0.05$). Also, the expression of cleaved caspase-3 was promoted under hypoxia for 48 h (Fig. 5d, $p < 0.05$). These data suggested that hypoxic stress enhanced the apoptosis of BM-MSCs. Interestingly, FNDC5^{+/+} or irisin administration alleviated the increased apoptosis in hypoxia exposure for 48 h (Fig. 5a-d, $p < 0.05$).

FNDC5/Irisin alleviated the reduced viability of BM-MSCs induced by hypoxia

The in vitro BLI was performed to confirm the effect of FNDC5/Irisin on cells viability. Representative results in Fig. 6a displayed that the BLI signal intensity in MSCs Fluc + GFP + in FNDC5^{+/+} group and MSCs + irisin group was significantly increased compared with that in H/SD group (Fig. 6b, $p < 0.05$). Meanwhile, MTT results demonstrated that FNDC5^{+/+} or Irisin treatment promoted the impaired viability of MSCs^{Fluc+GFP+} under hypoxia condition for 48 h (Fig. 6c, $p < 0.05$). Collectively, these results suggested that FNDC5 or Irisin played a protective role in viability of BM-MSCs.

FNDC5/Irisin improved paracrine functions of hypoxic MSCs

To investigate the effects of FNDC5/Irisin on growth factors secretions in BM-MSCs, we performed ELISA assays. The representative results in Fig. 7a-d demonstrated that the secretions of VEGF bFGF, IGF-1 and HGF were increased after FNDC5^{+/+} or Irisin administration compared with that in hypoxia for 48 h ($p < 0.05$). Taken together, these results illustrated that FNDC5/Irisin ameliorated the paracrine dysfunction of BM-MSCs induced by hypoxia.

FNDC5 enhanced the retention and pro-angiogenic effect of engrafted MSCs

Longitudinal BLI was performed for determining the retention of BM-MSCs transplanted into infarcted hearts. Representative BLI results in Fig. 8a showed a progressive decay of BLI signal within 4 weeks after engraftment in MSCs group. By contrast, FNDC5^{+/+} promoted the retention of engrafted MSCs^{Fluc+GFP+} (Fig. 8b, $p < 0.05$). Consistently, the number of capillaries in myocardial tissue was evaluated by CD31 staining. The representative immunohistochemistry results demonstrated that the number of capillaries was reduced in MI group and in MI + MSCs group. And there was no statistical difference

between MI group and MI + MSCs group. Interestingly, MSCs^{FNDC5+/+} increased the number of capillaries compared with that of MI + MSCs group (Fig. 8c and 8d, $p < 0.05$). Collectively, FNDC5 over-expression improved the retention and pro-angiogenic effect after MSCs engraftment.

MSCs^{FNDC5+/+} reduced fibrosis and improved cardiac function after MI

To study the effects of FNDC5 on the therapeutic efficiency of transplanted BM-MSCs, sirius red/ fast green staining was performed to evaluate the fibrosis. Furthermore, echocardiography analysis was performed to evaluate the heart function. Representative sirius red/ fast green staining results in Fig. 9a showed that BM-MSCs decreased cardiac fibrosis after MI. However, there was no significant difference between MI group and MI + MSCs group statistically. Interestingly, MSC^{FNDC5+/+} significantly reduced myocardial fibrosis compared with MI and MI + MSCs group (Fig. 9a and b, $p < 0.05$). In addition, echocardiographic analysis revealed that the baseline parameters were similar in all groups. However, the left ventricle (LV) dimensions (LVEDD and LVESD) were increased after MI. Meanwhile, the LV dimensions were decreased in the MSC^{FNDC5+/+} group compared with the MI and MI + MSC groups (Fig. 9c-d, $p < 0.05$). Moreover, transplantation of BM-MSCs also manifested a trend towards improvement of cardiac performance over the 4 weeks after MI. Interestingly, the apparent benefit of BM-MSC transplantation was significantly promoted by MSC^{FNDC5+/+} (Fig. 9e and 9f, $p < 0.05$). Taken together, these data suggested that the therapeutic benefits of MSC transplantation after MI is enhanced by FNDC5.

MSCs^{FNDC5+/+} decreased apoptosis cardiomyocytes after MI

TUNEL assay was performed to confirm the protective effect of MSCs^{FNDC5+/+} on the apoptosis of cardiomyocytes after MI. As shown by representative immunofluorescence images in Fig. 10a, apoptotic cardiomyocytes, which were manifested by TUNEL positivity (in green), were more frequently observed in the MI group than that in the sham group ($41.70 \pm 0.69\%$ versus $10.97 \pm 0.58\%$; $p < 0.05$, Fig. 10b). Furthermore, the apoptosis positive cells were remarkably reduced in the MI + MSC^{FNDC5+/+} group, compared with that in MI and MI + MSCs group, ($41.70 \pm 0.69\%$ vs. $21.47 \pm 0.78\%$; $36.67 \pm 0.66\%$ vs. $21.47 \pm 0.78\%$, $p < 0.05$, Fig. 10b). Taken together, our data indicated that MSCs^{FNDC5+/+} decreased apoptosis cardiomyocytes after MI.

Discussion

In the present study, our data indicated that H/SD injury inhibited the protein expression levels of FNDC5 in BM-MSCs. Meanwhile, hypoxic stress involved in BM-MSCs paracrine dysfunction, increased cells apoptosis and viability. Furthermore, FNDC5 over-expression and irisin attenuated the BM-MSCs injury induced by hypoxia exposure. Interestingly, FNDC5 over-expression elevated the survival of BM-MSCs

after transplantation. Furthermore, MSCs^{FNDC5+/+} transplantation decreased the apoptosis and fibrosis of infarcted myocardium, increased capillary density and finally improved cardiac function. Collectively, our results suggested that FNDC5/irisin may be a proposed optimized strategy of MSCs therapy for MI (Fig. 11).

Previous studies in MSCs therapy for MI have achieved a promising stage, but studies also demonstrated that only marginal improvements in cardiac function were observed after the engraftment of MSCs into infarcted heart tissue^(22, 24). The reduced viability of implanted MSCs is considered to be a major limitation for stem cells therapy for clinical application. Our previous study has revealed a high level of MSCs apoptosis between day 3 and 7 after implantation⁽¹⁹⁾. In the present study, we tracked the transplanted BM-MSCs by BLI and also found similar acute cell death within 1 week after transplantation. Moreover, engraftment with BM-MSCs alone could not prevent cardiomyocytes death and improve cardiac dysfunction significantly, which was consistent with the results of Zhang et al.^(22, 25) Many factors contributed to the decreased the viability of MSCs after transplantation. Lacks of nutrients and oxygen may play important effects in ischemic myocardium⁽²⁶⁾. In present study, we found that H/SD injury reduced cells survival and caused paracrine dysfunction of BM-MSCs, which is similar to our previous study⁽¹⁰⁾. Therefore, promoting the low viability of MSCs under hypoxic conditions after transplantation is crucial for elevating the efficiency of cellular therapy.

FNDC5 was recognized as a transmembrane protein with a fibronectin III domain and a carboxy-terminal domain located in the cytoplasm⁽²⁷⁾. Once FNDC5 could be cleaved by an unknown enzyme, and the extracellular domain of FNDC5 was named as irisin^(12, 28). Fatouros et al.⁽¹³⁾ found that irisin was secreted by skeletal muscles. However, Yu Q. et al.⁽¹⁴⁾ indicated that the expression of irisin also increased in the heart of hypertrophic mice. They found that angiotensin II-induced myocardial cells hypertrophy was attenuated after administration of irisin in vitro, suggesting irisin also could be derived from heart and had a cardioprotective effect on cardiomyocytes dysfunction. In our study, we found for the first time that hypoxia exposure decreased the protein expression level of FNDC5 in MSCs. Meanwhile, MSCs^{FNDC5+/+} and irisin administration protected BM-MSCs from hypoxic injury. Concurrently, MSCs^{FNDC5+/+} significantly promoted beneficial functions of transplanted BM-MSCs and cardiac dysfunction after MI.

Conclusions

Collectively, our in vitro and in vivo data embodied the FNDC5/irisin ameliorated hypoxia injury of BM-MSCs. Furthermore, gene modified with FNDC5 over-expression increased the survival of BM-MSCs after engraftment and improved the cardiac functions after MI, suggesting that FNDC5/irisin may be a potential optimizing target for BM-MSCs based cellular therapy for MI.

Abbreviations

FNDC5: Fibronectin type III domain-containing protein 5; BM-MSCs: Bone marrow mesenchymal stem cells; MI: Myocardial infarction; H/SD: Hypoxia and serum deprivation; Fluc: Firefly luciferase; eGFP: enhanced green fluorescence protein; DMEM: Dulbecco's modified Eagle's medium; FBS: Fetal bovine serum; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO: Dimethyl sulfoxide; OD: Optical density; BLI: Bioluminescence imaging; ELISA: Enzyme-linked immunosorbent assay; VEGF: Vascular endothelial growth factor; bFGF: basic fibroblast growth factor; IGF: Insulin-like growth factor; HGF: Hepatocyte growth factor; LAD: Left anterior descending; LV: Left ventricle; ECG: Electrocardiography; ROI: Region of interest; HPFs: High-power fields; LVESD: Left ventricular end-systolic diameter; LVEDD: Left ventricular end-diastolic diameter; LVESV: Left ventricular end-systolic volume; LVEDV: Left ventricular end-diastolic volume; LVEF: Left ventricular ejection fraction; FS: Fractional shortening; TUNEL: Terminal-deoxynucleotidyl transferase mediated-dUTP nick-end labeling; DAPI: 4,6-diamidino-2-phenylindole

Declarations

Funding

Not applicable

Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files.

Authors' contributions

JYD, NZ, ZZ and FC designed the study, drafted the manuscript, and approved its final version. ZZ, JYD and FC acquired data and approved the final version. YC, CX, JMZ and ZTJ revised the article's intellectual content. ZZ and FC are responsible for the integrity of this work. All authors read and approved the final manuscript.

Ethics approval

All procedures were performed in accordance with the institutional guidelines for animal research and were approved by the Animal Care and Use Committee of PLA Rocket Force Characteristic Medical Center.

Consent for publication

Not applicable.

Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files.

Competing interests

All the authors declare that they have no competing interests.

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Figures

Figure 1

Hypoxia exposure increased apoptosis of MSCs time-dependently. a Representative results of the flow cytometry analyses in MSCs under hypoxia for 12h, 24h and 48h. Viable cells: Annexin V-/PI-; Early apoptosis: Annexin V+/PI-; late apoptosis: V+/PI+; Necrotic: V-/PI+. (Scale bars: 20μm). b-c Quantification of the apoptotic MSCs. d Histogram illustrated the caspase-3 enzymatic activity in MSCs Fluc+GFP+ after H/SD injury. Data are expressed as the means ± SEM; n = 5; *p < 0.05

Figure 2

Effect of hypoxic treatment on the viability of MSCs Fluc+GFP+. **a** In vivo BLI showed a linear relationship between cells number and Fluc reporter gene activity. **b** In vitro BLI results of BM-MSCs Fluc+GFP+ under normal conditions and after HS/D injury for 12 h, 24 h and 48 h. **c** Representative the quantification of BLI assays. **d** MTT assay indicated the effects of hypoxia (0 h, 12 h, 24 h and 48 h) on viability of MSCs Fluc+GFP+. Data are expressed as the means $\pm$ SEM; $n = 5$; * $p < 0.05$

Figure 3

Hypoxia decreased paracrine mechanism of MSCs. Representative ELISA assay illustrated the levels of vascular endothelial growth factor (VEGF) (a), basic fibroblast growth factor (bFGF) (b), insulin-like growth factor-1 (IGF-1) (c), and hepatocyte growth factor (HGF) (d) within MSCs under normal conditions and hypoxia for 12 h, 24 h and 48 h. Data are expressed as the mean $\pm$ SEM; n = 5; *p < 0.05

Figure 4

Hypoxic injury decreased the expression of FND5 in MSCs. a and c Representative Western blot results in MSCs. b and d Semi-quantification of FND5 expression. Data are expressed as the mean $\pm$ SEM; n = 5; *p < 0.05

Figure 5

FDNC5/Irisin reduced increased apoptosis of MSCs with hypoxia for 48 h. a Results of the flow cytometry analyses in MSCs under normoxia, hypoxia for 48h, H/SD+MSCs^{FNDC5+/+} and HS/D+Irisin (Scale bars: 20µm). b-c Representative quantification of the apoptotic MSCs under different treatments. d Histogram illustrated the caspase-3 enzymatic activity in MSCs after various treatments. Data are expressed as the means $\pm$ SEM; n = 5; *p < 0.05

Figure 6

FDNC5+/+/Irisin improved the viability of MSCs under hypoxia for 48 h. a Representative in vitro BLI results of MSCs Fluc+GFP+ with normoxia, hypoxia for 48h, H/SD+MSCsFND5+/+ and HS/D+Irisin. b Representative the quantification of BLI assays in different groups. c MTT assay demonstrated the effects of FDNC5+/+/Irisin on viability of MSCs Fluc+GFP+. Data are expressed as the means $\pm$ SEM; n = 5; *p < 0.05

Figure 7

FDNC5+/+/Irisin ameliorated paracrine dysfunction of MSCs. Representative ELISA assay illustrated the levels of VEGF (a), bFGF (b), IGF-1 (c), and HGF (d) in MSCs under various groups. Data are expressed as the mean $\pm$ SEM; n = 5; *p < 0.05

Figure 8

FND5 promoted the retention and pro-angiogenic effect of engrafted MSCs. a Longitudinal BLI spatiotemporally tracked MSCs Fluc+GFP+ survival in MI with preconditioned mesenchymal stem cells (MPCMSCs) (top row, n = 10) and MSCsFND5+/+ preconditioned MSCs (MFPCMSCs) (second row, n = 10). Color scale bar values are in photons/s/cm²/sr. b Representative quantitative analysis of Firefly luciferase (Fluc) optical signals on fixed regions of interest (ROI). c Capillaries in the infarct border zone were determined by immunohistochemical staining for CD31-positive cells in different groups (Scale bars= 50 μ m). d Representative capillaries in the infarct border zone. Data are expressed as means $\pm$ SEM; n = 5; *p< 0.05

Figure 9

Evaluation of fibrosis and heart function after different groups. a Masson's trichrome staining indicated left ventricular fibrosis 4 weeks after various treatments (magnification 4x). b Representative quantitative analysis of the fibrotic area. Histograms illustrating heart function parameters: left ventricular end-diastolic diameter (LVEDd, c), left ventricular end-systolic diameter (LVESd, d), left ventricular fractional

shortening (e) and left ventricular ejection fraction (f). Data are expressed as means $\pm$ SEM; n = 5; *p < 0.05

Figure 10

Effects of MSCs FNDC5+/+ on cardiomyocytes apoptosis. a Confocal microscopy of TUNEL staining for myocardial cells apoptosis in border zone around infarcted area 48 h after LAD ligation in mice hearts. Apoptotic nuclei were identified as TUNEL positive (green fluorescent) and total nuclei by DAPI

counterstaining (blue fluorescent). Scale bar represents 20 μm . b Representative TUNEL-positive apoptotic cells. Data are expressed as means $\pm$ SEM; n = 5; *p < 0.05

Figure 11

Proposed hypothesis of FNDC5/Irisin improving the therapeutic efficacy of MSCs for MI. FNDC5/Irisin may protect against promoted apoptosis and paracrine dysfunction of MSCs induced by hypoxia. Furthermore, FNDC5/Irisin may contribute to enhance the low survival of engrafted MSCs and ameliorate cardiac dysfunction after MI.