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Mediated protective effect of electroacupuncture pretreatment by miR-214 on myocardial ischemia/reperfusion injury

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Abstract

Background Electroacupuncture pretreatment plays a protective role in myocardial ischemia/reperfusion (I/R) injury and microRNAs (miRNAs) could act on various facets of cardiac function. However, the role of miRNAs in the cardioprotection by electroacupuncture pretreatment on myocardial I/R injury remains unknown. The purpose of the study was to examine whether miR-214 was involved in cardioprotection by electroacupuncture. **Methods** Using rat myocardial I/R model, we examined the role of electroacupuncture pretreatment in myocardial I/R injury and analyzed the changes in the expression of miR-214. In addition, I/R was simulated *in vitro* by performing oxygen-glucose deprivation (OGD) on H9c2 cell cultures, and the effect of electroacupuncture pretreatment on I/R injury as well as expressional level of miR-214 were examined *in vitro*. Furthermore, the miR-214 mimic was transfected into OGD-treated H9c2 cells, we analyzed the cell apoptosis, lactate dehydrogenase (LDH) and creatine kinase (CK) activities, intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) as well as the relative protein levels of sodium/calcium exchanger 1(NCX1), BCL2-like 11 (BIM), calmodulin-dependent protein kinase IIδ (CaMKIIδ) and Cyclophilin D (CypD). **Results** The *in vivo* results revealed that compared with the I/R group, the electroacupuncture pretreatment group showed significant decreased myocardial infarct size, as well as the increased indices of the cardiac function, including heart rate, mean arterial pressure, left ventricular systolic pressure and maximal rate for left ventricular pressure rising and declining (±dp/dt max). In addition, electroacupuncture pretreatment could inhibit the elevation of LDH and CK activities induced by I/R injury. The quantitative PCR (qPCR) results demonstrated electroacupuncture pretreatment could provide cardioprotection against myocardial I/R injury in rats with miR-214 up-regulation. In the meanwhile, *in vitro*, electroacupuncture pretreatment protected H9c2 cells from OGD-induced injury. Transfection of miR-214 mimic showed protective effects on OGD-induced injury to H9c2 cells by reducing apoptosis, decreasing LDH and CK activities, rescuing the OGD-induced Ca^{2+} and down-regulating elevated protein levels of NCX1, BIM, CaMKIIδ and CypD. **Conclusions** Our findings firstly demonstrated that electroacupuncture pretreatment promotes the expression of miR-214 in myocardial I/R injury and miR-214 contributes to the protective effect of electroacupuncture on myocardial I/R injury.

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Keywords: I/R injury; miR-214; Electroacupuncture; Protective effect

1 Introduction

Cardiovascular disease is the leading cause of death in the developed countries and even affects up to 80 million people in the United States.[1] Myocardial ischemia/reperfusion (I/R) injury contributes to adverse cardiovascular outcomes after myocardial ischemia, cardiac surgery or circulatory arrest.[2] The molecular mechanisms underlying myocardial I/R injury, however, are complex.[3]

Although clinical studies have convincingly demonstrated that myocardial ischemia can be effectively treated via acupuncture at the single Neiguan point,[4,5] the mechanisms in charge of the cardioprotective effect through acupuncture therapy remains undefined.[6]

The recent studies have revealed that microRNAs (miRNAs) play important roles in various aspects of cardiac function involved in the regulation of cardiovascular physiological and pathological processes, including cardiac hypertrophy, cardiac fibrosis, cardiac apoptosis and angiogenesis.[7–13] Moreover, it has been found that miR-214 is a sensitive marker of a variety of cardiac stresses, which is

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up-regulated in I/R-induced cardiac injury and plays a protective role against I/R injury by controlling Ca^{2+} overload.[14] Also, acupuncture-serum could decrease Ca^{2+} content in cultured rat myocardial cells,[15] and even electroacupuncture pretreatment was involved in maintaining cardiomyocyte Ca^{2+} homeostasis.[16,17] However, the role of miRNAs in the cardioprotection by electroacupuncture pretreatment on myocardial I/R injury remains unknown. Therefore, these encourage studies have stimulated us to investigate whether miR-214 is involved in cardioprotection by electroacupuncture pretreatment.

2 Methods

2.1 Myocardial I/R injury model establishment

All procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication no. 86-23, revised 1986). The present study was approved by the Ethics Committee of Southern Medical University. Male Sprague-Dawley rats weighing 280-320 g were obtained from Shanghai Slac Laboratory Animal Company (Shanghai, China). The rats were anesthetized by intraperitoneal injection of 3.5% chloral hydrate (50 mL/kg; ZhuJiang Hospital of Southern Medicine University). Then, the animals were placed in the supine position and intubated, ventilated artificially with a respirator (TKR-200C; Jiangxi Teli Anesthesia & Instrument, Nanchang, Jiangxi, China) with a tidal volume of 20 mL/kg body weight and 50 breaths per minute. Electrocardiographic electrodes were connected to the limbs through small needles inserted subcutaneously. Following the skin incision, the hearts were exposed through a left thoracotomy in the fourth intercostal space. The left anterior descending (LAD) coronary artery was ligated with 6–0 silk suture using a snare occluder. Ischemia was confirmed by visual observation (cyanosis) and continuous ECG monitoring. Evidence for a successful intervention was a cyanotic left anterior ventricular wall and local wall distension, as well as elevated ST segments and peak T waves on the electrocardiogram. After completion of 40 min of occlusion, the coronary artery was reperfused by releasing the knot. Following reperfusion for 180 min, the rat hearts were harvested. Sham surgery animals underwent the same procedures without occlusion of the LAD.

2.2 Application of electroacupuncture

The G6805-2 electric impulsers were obtained from Shanghai Medical Electronic Apparatus Company (Shanghai, China). Two stainless steel needles were inserted into "Neiguan" acupoints which were located in the interosseal muscles between the radius and the ulna of the distal medial thoracic limb at the level of 3 mm superior to the wrist joint in rats. These two needles were connected to positive and negative poles of the acupuncture apparatus. Application of electroacupuncture was continued for 30 min a day and for three consecutive days before the myocardial I/R experiment. The stimulatory frequency of electroacupuncture was 4/20 Hz, 0.5 ms duration and at an intensity of 1 mA, just strong enough to elicit a slight twitch of the foot. Electroacupuncture stimulation was applied to H9c2 cells according to the methods of Wu, *et al*.[18] The frequency was 50 Hz and the duration was 0.5 ms. Cells received electroacupuncture treatment daily for 30 min and continued for 10 days.

2.3 Measurement of myocardial infarct size

The hearts were injected with 1% Evans Blue solution (Sigma, St. Louis, MO, USA) and then harvested. The hearts were refrigerated at -4˚C overnight and sliced horizontally to yield five slices, the sections were about 2 mm thick. The slices were incubated in 1% triphenyltetrazolium chloride TTC (Sigma) at 37˚C for 30 min and then stirred to stain completely. Following staining, the slices were fixed by immersion in 4% paraformaldehyde solution. Area at risk (AAR) was determined as the percentage of Evans blue-unstained tissue area in the total left ventricular region. Infarct size was reported as a percentage of the AAR.

2.4 Quantitative PCR assay of miR-214

MicroRNAs were isolated from tissues or cultured cells with a miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The primers used in this study were as follows: miR-214: forward, 5′-ACAGCAGGCACAGACAGGCAG-3′; reverse, 5′-GTGCAGGGTCCGAGGT-3′. U6: forward, 5′-CTCGCTTCGGCAGCACA-3′; reverse, 5′-AACGCTTCACGAATTTGCGT-3′. Quantitative real-time PCR (qPCR) for miR-214 was performed on a 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using the TaqMan MicroRNA Assay kit (Applied Biosystems) in accordance with the manufacturer's instructions. U6 was used as an internal control. The relative quantities of each mRNA were calculated by the comparative CT methods.

2.5 Cell culture

The H9c2 cells were maintained as monolayers in high glucose (25 mmol/L) Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (FBS; Gibco), at 37℃ in a humidified atmosphere with 5% CO_2. Medium was changed

every three days. Osmotic control cells (to account for medium hyper-osmolarity) were exposed to mannitol (24.5 mmol/L) in normal medium containing glucose (5.5 mmol/L). For oxygen-glucose deprivation (OGD)-treated cells, the complete growth medium was replaced by DMEM without glucose (Gibco), and cells were incubated at 37°C with 5% CO_2 and 95% N_2 (v/v) for 10 h. After hypoxia, the culture medium was removed, replaced by fresh high glucose DMEM, supplemented with 10% FBS and cells were maintained in a regular 5% CO_2 incubator.

2.6 Transfection

The H9c2 cells were transfected with the miR-NC, or miR-214, using lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Subsequently, cells were washed and maintained in the medium until analysis.

2.7 Flow cytometry

Cell apoptosis rates was assessed with the Annexin V-FITC and propidium iodide (PI) apoptosis kit (KeyGEN Biotech.CO., LTD, Nanjing, Jiangsu, China) according to the manufacturer's protocol. After dual-staining with Annexin V-FITC and PI, the cells were immediately analyzed with flow cytometry (FC 500 MPL system; Beckman Coulter, Inc., Miami, FL, USA).

2.8 Measurement of lactate dehydrogenase (LDH) and creatine kinase (CK)

The blood samples were centrifuged at 1,000 r/min, 4°C for 15 min, and serum was extracted and stored at −80°C until analyzed. The LDH and CK activities in the serum or cellular supernatant were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China), respectively.

2.9 Measurement of intracellular calcium ($[Ca^{2+}]i$)

Fluo-3/AM (Beyotime, Shanghai, China) was used to detect intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). After washing with PBS, the cells were incubated with Fluo-3/AM solution for 45 min at 37℃. The fluorescence intensity of Fluo-3/AM-loaded cells were detected by confocal microscopy (Leica TCS SL, Heidelberg, Germany) at 488 nm excitation and 525 nm emission wavelengths.

2.10 Western blot analysis

Total protein was isolated from H9c2 cells using a total protein extraction kit (Applygen Technologies Inc, Beijing, China) according to the manufacturer's instructions. Protein concentrations were examined using the BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Shanghai, China). Protein samples were separated on 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (EMD Millipore Corporation, Billerica, MA, USA). Thereafter, the membranes were blocked via incubation with solution (Tris-buffered saline containing 5% nonfat milk and 0.05% Tween 20) at 4°C overnight. The blot was probed with the primary antibodies followed with IgG-horse radish peroxidase (HRP)-conjugated secondary antibody. The signal was detected by an ECL western blotting kit (Pierce Biotechnology, Inc., Rockford, IL, USA).

2.11 Statistical analysis

The results were analyzed using SPSS 19.0 statistical software. Data were expressed as the mean ± SD. Student's *t* test was used for the comparison of differences between the two groups. *P* values less than 0.05 were considered to be significant.

3 Results

3.1 Myocardial I/R injury was rescued by electroacupuncture pretreatment

We established the rat model of myocardial I/R and examined the effect of electroacupuncture (EA) pretreatment on myocardial I/R injury. Figure 1A shows the infarct size following myocardial I/R. Significant decreased myocardial infarct size was observed in the electroacupuncture pretreatment group relative to I/R group ($P < 0.05$).

The indices of cardiac function, including heart rate (HR), mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), maximal rate for left ventricular pressure rising and declining (±dp/dt) max in the groups of sham, sham+EA, I/R and I/R+EA are listed in Table 1. It has been shown that all the indices of the cardiac function were not significantly different between the sham and the sham+EA group. Compared with the sham group, all the indices of the cardiac function were decreased significantly in I/R group ($P < 0.05$). However, electroacupuncture pretreatment could reverse this cardiac function injury. I/R+EA group showed that all the indices of the cardiac function were increased compared with I/R group ($P < 0.05$).

As shown in Figure 1B, compared with the sham group, the activity of LDH was not altered in the sham+EA group, but significantly increased in the serum of I/R group ($P < 0.01$). Consistent with the result, the activity of CK was also increased following I/R ($P < 0.05$, Figure 1C), which indicated that electroacupuncture pretreatment could inhibit the elevation of LDH and CK activities induced by I/R injury.

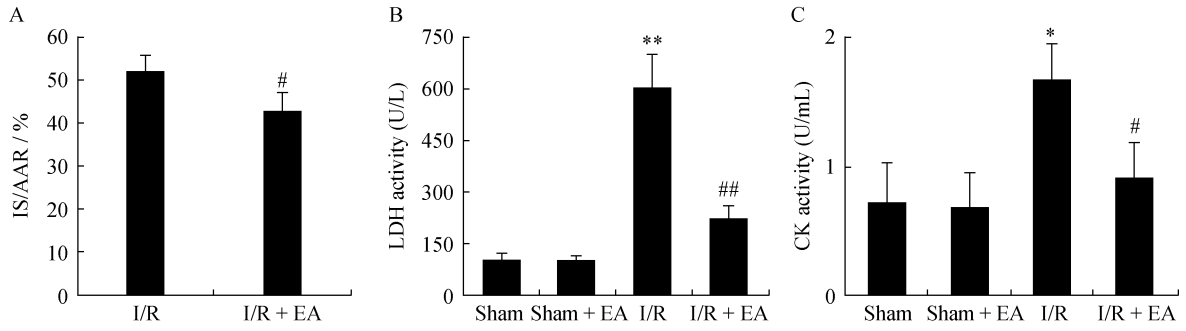

Figure 1. Electroacupuncture pretreatment protects against myocardial ischemia reperfusion injury. (A): Myocardial infarct size at risk; (B): serum LDH activity; and (C): CK activity. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs*. sham, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs*. I/R ($n = 6$). AAR: area at risk; CK: creatine kinase; EA: electroacupuncture; IS: infarct size; I/R: ischemia/reperfusion; LDH: lactate dehydrogenase.

Table 1. Indices of cardiac function in the groups of sham, sham+EA, I/R and I/R+EA.

	Sham	Sham+EA	I/R	I/R+EA
HR, beats/min	422.5 ± 39.6	442.1 ± 44.9	228.0 ± 85.7*	403.2 ± 42.1#
MAP, mmHg	105.2 ± 11.3	109.7 ± 12.9	78.0 ± 11.0*	100.5 ± 8.1#
LVSP, mmHg	159.1 ± 17.2	170.3 ± 19.6	111.4 ± 19.9*	149.2 ± 9.3#
+dp/dt max, mmHg/s	5389.2 ± 1087.5	5360.5 ± 1042.7	3146.9 ± 801.5*	5092.4 ± 861.3#

Data were expressed as the mean ± SD. $^{*}P < 0.05$ *vs*. sham, $^{\#}P < 0.05$ *vs*. I/R ($n = 6$). HR: heart rate; MAP: mean arterial pressure; LVSP: left ventricular systolic pressure; +dp/dt max: maximal rate for left ventricular pressure rising and declining; I/R: ischemia/reperfusion; EA: electroacupuncture.

3.2 miR-214 involvement in cardioprotection by EA against myocardial I/R injury

After qPCR analysis of the changes in expression of miR-214 following myocardial I/R, miR-214 could be detected in myocardium. As shown in Figure 2, the expression of miR-214 was increased in I/R group compared with the sham group ($P < 0.01$), also the expression of miR-214 in both the sham+EA and I/R+EA group was increased after electroacupuncture pretreatment.

3.3 Effect of electroacupuncture on I/R injury in H9c2 cells

To exclude the effects of hyper-osmolarity, the H9c2 cells were treated with 5.5 mmol/L D-glucose plus 24.5 mmol/L of mannitol, which did not affect cell apoptosis and injury (data not shown). To examine the effect of electroacupuncture on I/R injury *in vitro*, oxygen glucose deprivation was performed on H9c2 cells in order to simulate I/R. Compared with normal H9c2 cells, OGD-treated H9c2 cells showed increased LDH and CK activities in cellular supernatant ($P < 0.01$; Figure 3A). There were significant differences of the cell apoptosis rates between normal and OGD-treated H9c2 cells, with the higher of cell apoptosis rate in OGD cells than that in normal cells (Figure 3B). In addition, we examined the overload of intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) in H9c2 cells and it has been found that $[Ca^{2+}]i$ in OGD cells was significantly increased over control ($P < 0.01$; Figure 3C). As shown in Figure 3D and Table 2, the relative protein levels of NCX1, BIM, CaMKIIδ and CypD were also increased in the OGD group compared with the control group.

As shown in Figure 3A, there were no significant differences of LDH and CK activity in cellular supernatant between the control group and the control+EA group. LDH activity in cellular supernatant was decreased in OGD+EA group compared with the OGD group ($P < 0.01$). Similarly, the OGD+EA group showed decreased CK activity in cellular supernatant in comparison to the OGD group ($P < 0.05$). FCM results revealed that electroacupuncture did not affect the cell apoptosis rate of normal H9c2 cells. It was shown that the cell apoptosis rate in OGD group was 7.2%

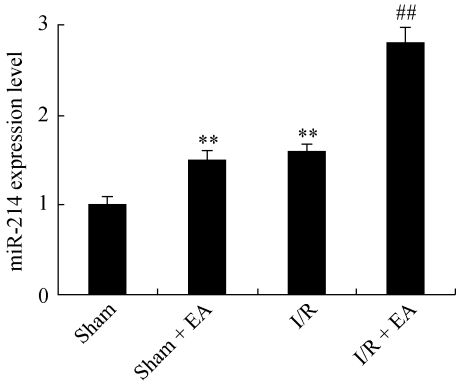

Figure 2. qPCR analysis of miR-214 expression levels in tissues. $^{**}P < 0.01$ *vs*. sham, $^{\#\#}P < 0.01$ *vs*. I/R ($n = 6$). EA: electroacupuncture; I/R: ischemia/reperfusion.

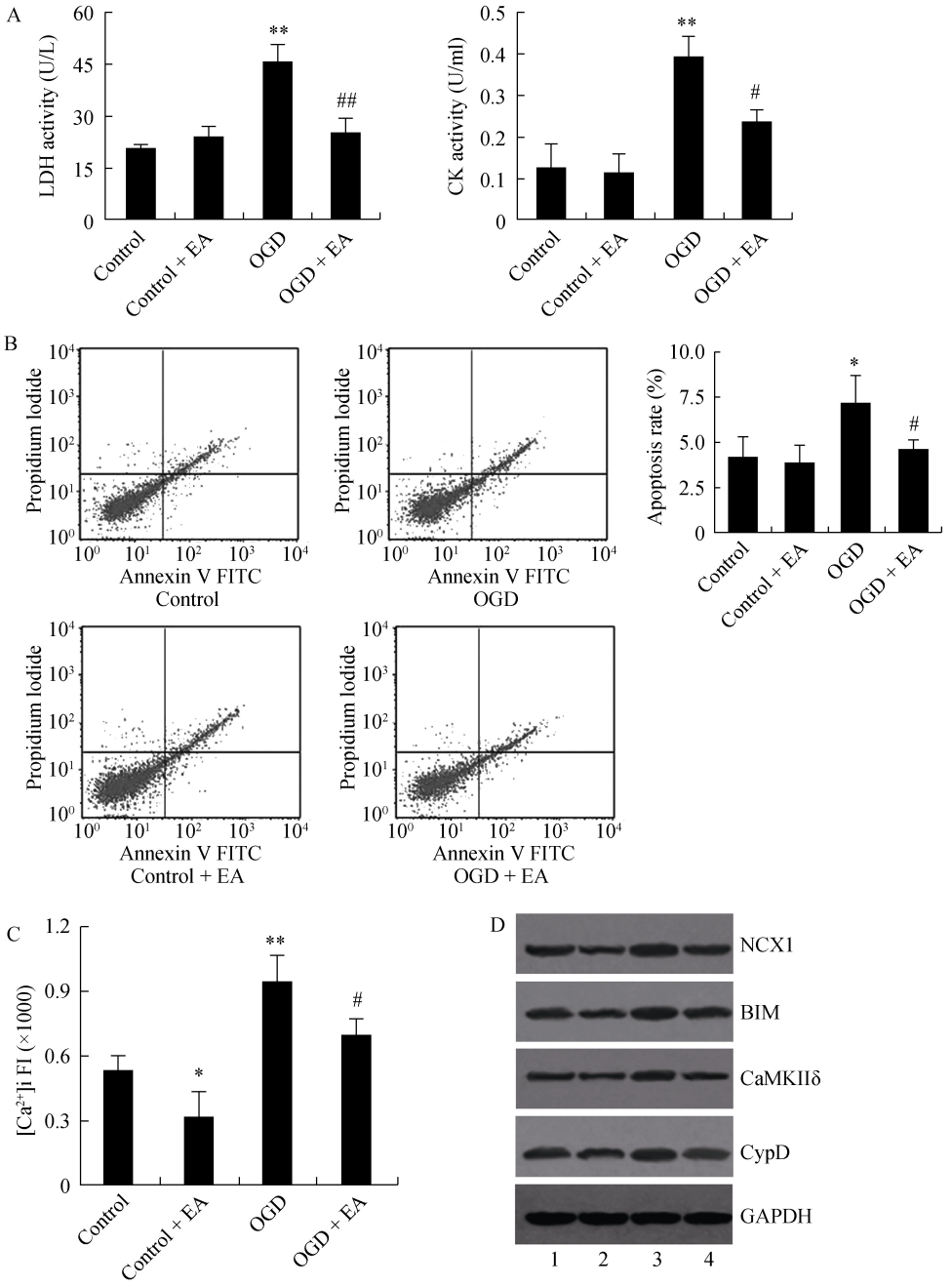

Figure 3. Electroacupuncture protects I/R injury in H9c2 cells. (A): LDH and CK activities in cellular supernatant; (B): cell apoptosis rates measured by FCM, the lower right quadrants contain apoptotic cells; (C): intracellular Ca^{2+} concentration ([Ca^{2+}]i) fluorescence intensity, and (D): representative western blot pictures for NCX1, BIM, CaMKIIδ and CypD. GAPDH was used as a control. Lane 1: control group; Lane 2: control+EA group; Lane 3: OGD group; Lane 4: OGD+EA group. *$P < 0.05$ and **$P < 0.01$ *vs*. control; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs*. OGD ($n = 3$). BIM: BCL2-like 11; CaMKII: calmodulin-dependent protein kinase IIδ; CK: creatine kinase; CypD: cyclophilin D; EA: electroacupuncture; FCM: flow cytometry; FI: fluorescence intensity; I/R: ischemia/reperfusion; LDH: lactate dehydrogenase; NCX1: sodium/calcium exchanger 1; OGD: oxygen-glucose deprivation.

± 1.5%, but it decreased to 4.6% ± 0.5% when OGD cells were treated with electroacupuncture ($P < 0.05$; Figure 3B). As shown in Figure 3C, [Ca^{2+}]i was down-regulated in cells treated with electroacupuncture for both the control and the OGD cells ($P < 0.05$). In addition, the relative protein levels of NCX1, BIM, CaMKIIδ and CypD were also decreased in cells treated with electroacupuncture compared with the untreated cells (Figure 3D and Table 2).

Table 2. Relative protein levels of NCX1, BIM, CaMKIIδ and CypD.

	Control	Control+EA	OGD	OGD+EA
NCX1	0.70 ± 0.10	0.42 ± 0.05*	0.94 ± 0.10*	0.72 ± 0.09$^{\#}$
BIM	0.66 ± 0.11	0.39 ± 0.04*	0.89 ± 0.08*	0.69 ± 0.07$^{\#}$
CaMKIIδ	0.31 ± 0.05	0.21 ± 0.04*	0.59 ± 0.08**	0.33 ± 0.04$^{\#\#}$
CypD	0.45 ± 0.06	0.25 ± 0.04**	0.68 ± 0.11*	0.42 ± 0.07$^{\#}$

Data were expressed as the mean ± SD. GAPDH was used as a control. *P < 0.05 and **P < 0.01 *vs*. control, $^{\#}P$ < 0.05 and $^{\#\#}P$ < 0.01 *vs*. OGD (n = 3). BIM: BCL2-like 11; CaMKII: calmodulin-dependent protein kinase IIδ; EA: electroacupuncture; NCX1: sodium/calcium exchanger 1; OGD: oxygen-glucose deprivation.

3.4 I/R induced expression of miR-214 in H9c2 cells by electroacupuncture

As shown in Figure 4, qPCR results revealed that miR-214 expression was significantly up-regulated in normal H9c2 cells-treated with electroacupuncture and OGD cells compared with the normal H9c2 cells (P < 0.01). Furthermore, OGD cells-treated with electroacupuncture showed an increased miR-214 expression in comparison to the untreated OGD cells (P < 0.01).

3.5 miR-214 protection of I/R injury in H9c2 cells

In order to examine the role of miR-214 in protection against I/R injury *in vitro*, miR-214 mimic was transfected into OGD-treated H9c2 cells. The qPCR results showed that the expression level of miR-214 mRNA in miR-214 mimic transfected cells was up-regulated about 3-fold over miR-NC transfected cells (P < 0.01; Figure 5). As shown in Figure 6A, LDH and CK activities in cellular supernatant were decreased in the miR-214 mimic group compared with the miR-NC group (P < 0.01), and the miR-214 mimic also down-regulated the cell apoptosis rate (Figure 6B). In addition, the miR-214 mimic group showed a significant decrease in [Ca^{2+}]i compared with the miR-NC group (P < 0.05; Figure 6C). As shown in Figure 6D, the relative protein levels of NCX1, BIM, CaMKIIδ and CypD were also significantly decreased in miR-214 mimic group compared with the miR-NC group.

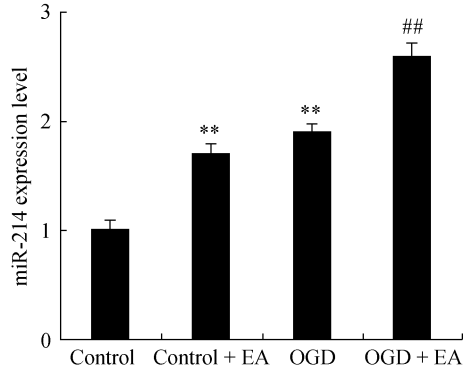

Figure 4. qPCR analysis of miR-214 expression levels in H9c2 cells. **P < 0.01 *vs*. control, $^{\#\#}P$ < 0.01 *vs*. OGD (n = 3). EA: electroacupuncture; OGD: oxygen-glucose deprivation.

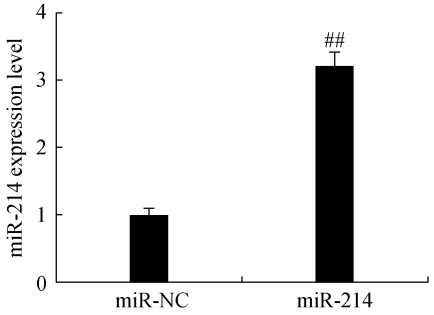

Figure 5. qPCR analysis of miR-214 expression levels in OGD-treated H9c2 cells following transfection of miR-NC or miR-214. $^{\#\#}P$ < 0.01 *vs*. miR-NC (n = 3). OGD: oxygen-glucose deprivation.

4 Discussion

In this study, using *in vivo* and *in vitro* myocardial I/R models, we uncovered the cardioprotective effect of electroacupuncture pretreatment by up-regulating miR-214 expression.

Using a rat model of myocardial I/R, we observed that electroacupuncture pretreatment could reduce the infarct size, reverse cardiac function injury as well as decrease serum LDH and CK activities, which were consistent with the previous *in vivo* studies,emphasized that electroacupuncture has a cardioprotective effect against myocardial ischemia.[19–21] In addition, we used a model of cardiac I/R, OGD in H9c2 cells to examine, *in vitro,* the effect of electroacupuncture pretreatment on I/R injury, it has been found that electroacupuncture pretreatment could protect H9c2 cells from OGD-induced injury by reducing apoptosis, decreasing LDH and CK activities, and rescuing the OGD-induced Ca^{2+} and elevated protein levels of NCX1, BIM, CaMKIIδ and CypD, which suggested that electroacupuncture pretreatment protects against myocardial I/R injury *in vitro*. Taken together, both the *in vivo* and *in vitro* results confirm that electroacupuncture pretreatment provides cardioprotection against myocardial I/R injury. In addition, electroacupuncture pretreatment could affect Ca^{2+} and protein levels of NCX1, BIM, CaMKIIδ and CypD of rats and H9c2 cells in normal conditions.

To further explore the mechanisms underlying the cardioprotective effect of electroacupuncture pretreatment, we focused on miRNAs which exert effects on cardioprotection. Since miR-214 is a sensitive marker of a variety of cardiac stresses, its expression is up-regulated in cardiac hypertro-

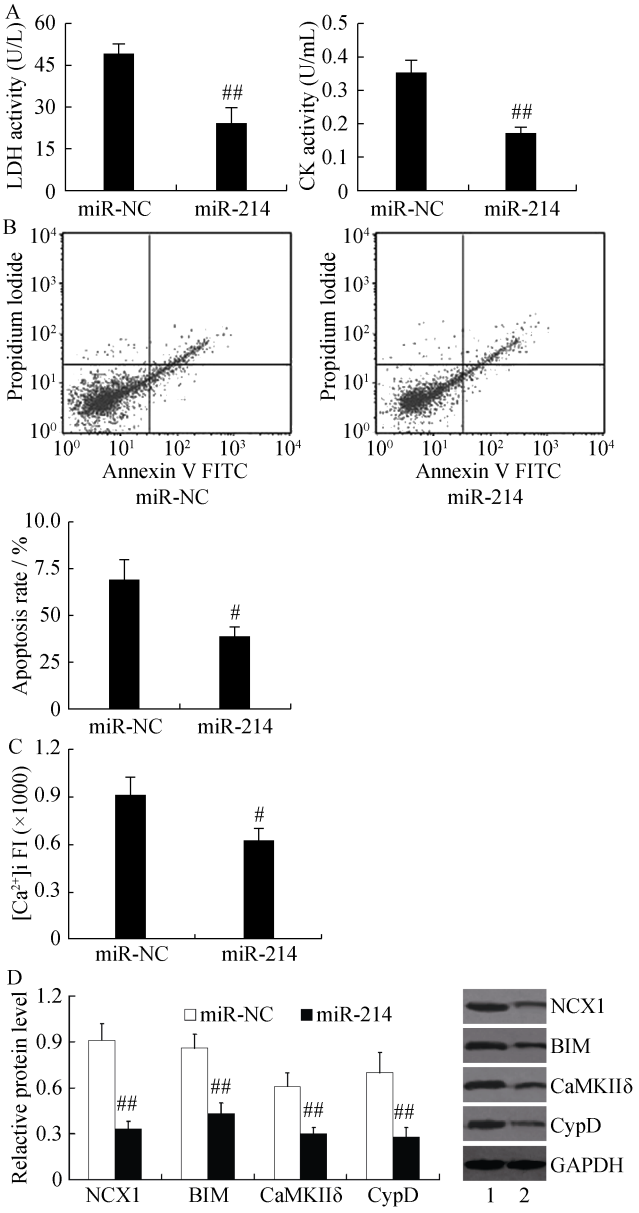

Figure 6. miR-214 protects I/R injury in H9c2 cells. (A): LDH and CK activities in cellular supernatant; (B): cell apoptosis rates measured by FCM, the lower right quadrants contain apoptotic cells. vs. miR-NC (n = 3); (C) intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) fluorescence intensity; and (D): relative protein levels of NCX1, BIM, CaMKIIδ and CypD. Lane 1: miR-NC group; Lane 2: miR-214 group. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs*. miR-NC (n = 3). GAPDH was used as a control. BIM: BCL2-like 11; CaMKII: calmodulin-dependent protein kinase IIδ; CypD: cyclophilin D; CK: creatine kinase; FCM: flow cytometry; FI: fluorescence intensity; I/R: ischemia/reperfusion; LDH: lactate dehydrogenase; NCX1: sodium/calcium exchanger 1.

phy, myocardial infarction and heart failure.[22,23] Recently, the study of Aurora, *et al*.[14] suggests that miR-214 is up-regulated in I/R-induced cardiac injury in mice and it plays a protective role against I/R injury. In the present study, we established I/R model in rats, it has been observed that miR-214 was increased in the hearts of rats subjected to 40 min of ischemia and 180 min of reperfusion. Furthermore, we examined the changes of miR-214 expression in the *in vitro* I/R model. The I/R model was simulated *in vitro* by performing OGD on H9c2 cell cultures, and consistent with the *in vivo* results, miR-214 was increased in OGD-treated H9c2 cells.

The overload of intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) is an important cause of cell injury.[24,25] It is well known that following myocardial I/R injury, intracellular Ca^{2+} overload occurs in cardiomyocytes, causing activation of NCX1 protein and downstream effectors of Ca^{2+} handling, including BIM, CaMKIIδ and CypD, and leads to cell death ultimately.[26–31] Several studies have identified that electroacupuncture pretreatment was involved in maintaining cardiomyocyte Ca^{2+} homeostasis, and this may be an important mechanism underlying the protective effect of electroacupuncture pretreatment on myocardial I/R injury.[16,17] Recently, miR-214 has been shown to protect against I/R-induced cardiac injury in mice by attenuating Ca^{2+} overload induced cell death.[14] Therefore, we hypothesized that miR-214 may mediate the effect of electroacupuncture pretreatment on attenuating myocardial I/R injury. Both *in vivo* and *in vitro* results demonstrated that miR-214 expression was up-regulated by electroacupuncture pretreatment. Furthermore, we assessed the hypothesis by transfecting the miR-214 mimic into OGD-treated H9c2 cells, and it has been confirmed that miR-214 could protect against I/R injury *in vitro* by reducing apoptosis, decreasing LDH and CK activities, and rescuing the OGD-induced Ca^{2+} and elevated protein levels of NCX1, BIM, CaMKIIδ and CypD.

To our knowledge, this is the first systematic study to examine whether miR-214 is involved in the mechanism of electroacupuncture on protecting against I/R injury *in vivo* and *in vitro*. Our findings demonstrated that electroacupuncture pretreatment promotes miR-214 expression in myocardial I/R injury and miR-214 contributes to the protective effect of electroacupuncture on IR injury. This study may help to provide a rational target for treatment of myocardial I/R injury.

Acknowledgements

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