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Naringenin protects myocardial ischemia/reperfusion injury by regulating miR-24-3p to inhibit cell death-inducing p53 target 1 expression

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Abstract. Myocardial ischemia/reperfusion (I/R) causes serious threats to human life. Naringenin, a polyphenolic compound naturally occurring in citrus fruit, has cardioprotective effects against myocardial I/R injury. Besides, miR-24-3p is also reported to have cardioprotective effects. We intended to explore whether the cardioprotective effects of naringenin relate to miR-24-3p and its underlying mechanism. In this study, we used an *in vivo* rat myocardial I/R model and an *in vitro* cardiomyocyte H9c2 hypoxia/reoxygenation (H/R) model. Myocardial injury was detected by hematoxylin-eosin staining and ELISA for creatine kinase (CK), malondialdehyde (MDA), and lactate dehydrogenase (LDH). miR-24-3p and cell death inducing p53 target 1 (Cdip1) mRNA expressions were examined by RT-PCR. We find that naringenin pretreatment significantly relieves myocardial I/R injury, reduces LDH, CD, and MDA levels, and increases miR-24-3p expression. Furthermore, miR-24-3p alleviates myocardial I/R injury partially through regulating Cdip1. Moreover, naringenin protects myocardial I/R injury partially by regulating miR-24-3p to inhibit Cdip1 expression. In conclusion, our data suggest naringenin protects myocardial I/R injury partially through miR-24-3p/Cdip1 axis.

Key words: Myocardial ischemia/reperfusion injury — Naringenin — miR-24-3p — Cell death-inducing p53 target 1 — Apoptosis

Abbreviations: CCK-8, cell counting kit-8; Cdip1, p53 target 1; CK, creatine kinase; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; HE, hematoxylin-eosin; H/R, hypoxia/reoxygenation; I/R, ischemia/reperfusion; LDH, lactate dehydrogenase; Keap1, Kelch-like ECH-associated protein 1; MDA, malondialdehyde; miRNA, microRNA; NC, negative control; PBS, phosphate-buffered saline; PI, propidium iodide; PKG, protein kinase G; ROS, reactive oxygen species.

Introduction

Ischemic heart disease seriously threatens human health (Chen et al. 2021; Horváth et al. 2022). Nowadays, timely recovery of blood supply of ischemic myocardium is an effective therapeutic intervention (Saccaro et al. 2021). However, reperfusion inevitably triggers additional myocardial injury defined as ischemia/reperfusion (I/R) injury (Wu et al. 2021). Despite lots of efforts on interventional therapy for ischemic heart disease, effective drugs are still lacking (Zhou et al. 2021). Accordingly, further understanding of the pathological mechanism of myocardial I/R injury and developing effective drugs are urgently needed.

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MicroRNAs (miRNAs), consisting of 18–22 nucleotides, suppress target mRNA expression *via* complementary pairing (Shukla et al. 2011; Fang et al. 2022). As reported before, many miRNAs participate in regulating myocardial I/R injury (Gong et al. 2021; Yang et al. 2021; Li et al. 2022). For example, miR-218-5p promotes myocardial I/R injury by targeting myocyte enhancer factor 2C mRNA (Yang et al. 2021). miR-590-3p inhibits cardiomyocyte apoptosis by regulating hypoxia-inducible factor 1α under hypoxia/reoxygenation (H/R) condition (Gong et al. 2021). miR-29b protects the heart against I/R injury by targeting phosphatase and tensin homolog (Li et al. 2022). In addition, each miRNA could regulate many targets. For example, miR-24-3p inhibits cardiomyocyte apoptosis by targeting the Kelch-like ECH-associated protein 1 (Keap1) in myocardial I/R injury (Xiao et al. 2018). miR-24-3p alleviates cardiac fibrosis by suppressing cardiac fibroblast mitophagy *via* downregulating prohibitin 2 (Zhang et al. 2022). However, the molecular mechanism of miR-24-3p in myocardial I/R injury still needs further elucidation.

Naringenin is a polyphenolic compound naturally occurring in citrus fruit (Du et al. 2021). Up to now, naringenin exhibits extensive biological activities, including antioxidant, anti-inflammatory, and so on (Hämäläinen et al. 2007; Jayaraman et al. 2009). Some studies report that naringenin could decrease cardiac damage following I/R injury (Yu et al. 2019). For example, naringenin protects the heart against I/R injury *via* regulating protein kinase G (PKG) signaling (Yu et al. 2019). Naringenin suppresses myocardial I/R injury by regulating miR-126 (Li et al. 2021). Whether the cardioprotective effects of naringenin against I/R injury are associated with miR-24-3p is still unknown.

In this study, we explored whether the cardioprotective roles of naringenin against myocardial I/R injury relate to miR-24-3p in rats and cardiomyocyte H9c2 cells responding to I/R injury, and its underlying mechanism.

Materials and Methods

Cells

Rat cardiomyocyte H9c2 cells and Hek293 cells were obtained from Nanjing Cobioer Biosciences Co., LTD and were cultured in DMEM supplemented with 10% fetal bovine serum (FBS).

Rat myocardial I/R model

This study was approved by the Jinan University Laboratory Animal Ethics Committee. All experiments were performed *per* the National Institutes of Health Guide to minimize the experimental pain in conscious animals. 32 adult male Sprague-Dawley rats (age, 8–10 weeks; weight, 250 ± 10 g) were purchased from Guangdong Medical Laboratory Animal Center. Rats were randomly assigned into four groups (8 for each): Sham group, I/R group, I/R+NC (negative control, administered with dimethyl sulfoxide (DMSO), I/R+NAR (naringenin, purchased from Aladdin Biotechnology (N486154, Shanghai, China), the purity is above 98%). The scheme of the chemical structure of naringenin is shown in Figure 1A. Rats were gastric infused with 50 mg/kg/d naringenin (dispersed in DMSO) for 7 days preoperatively, and then subjected to a myocardial I/R model. The rat myocardial I/R model was established, according to the previously described (Li et al. 2022).

Hematoxylin and eosin (HE) staining

Heart tissues were fixed with 4% paraformaldehyde, sliced into paraffin sections (5 μm), and then used for HE staining.

Cardiomyocyte H/R model

Rat cardiomyocyte H9c2 cells were cultured in the FBS-free and sugar-free DMEM medium under 5% CO_2 and 1% O_2 at 37°C for 6 h, and then cultured in DMEM with 10% FBS under 5% CO_2 for 24 h.

Measurement of creatine kinase (CK), lactate dehydrogenase (LDH), and malondialdehyde (MDA) content

CK, LDH, and MDA contents were examined by the Creatine Kinase Activity Assay kit (ab155901, Abcam), the LDH release assay kit (C0017, Beyotime, China), or the Lipid Peroxidation MDA Assay Kit (S0131M, Beyotime, China), respectively.

Cell viability assay

H9c2 cells were cultured in a 96-well plate and then treated with naringenin (80 μM) for 1 h. The cells were subjected to H/R injury and then cell viability was examined by the Cell counting kit-8 (CCK-8) (C0037, Beyotime, China).

Measurement of mitochondrial membrane potential

The mitochondrial membrane potential of H9c2 cells was measured by JC-1 mitochondrial membrane potential measurement kit (C2003S, Beyotime, China), according to the manufacturer's instructions. Briefly, after H/R injury, H9c2 cells were washed and then stained with 2 mm/l JC-1 for 15 min at room temperature in the dark. Next, the cells were washed with phosphate-buffered saline (PBS, Gibco, Grand Island, USA) 3 times and then were photoed with a fluorescence microscope. With the presence of non-apoptotic cells,

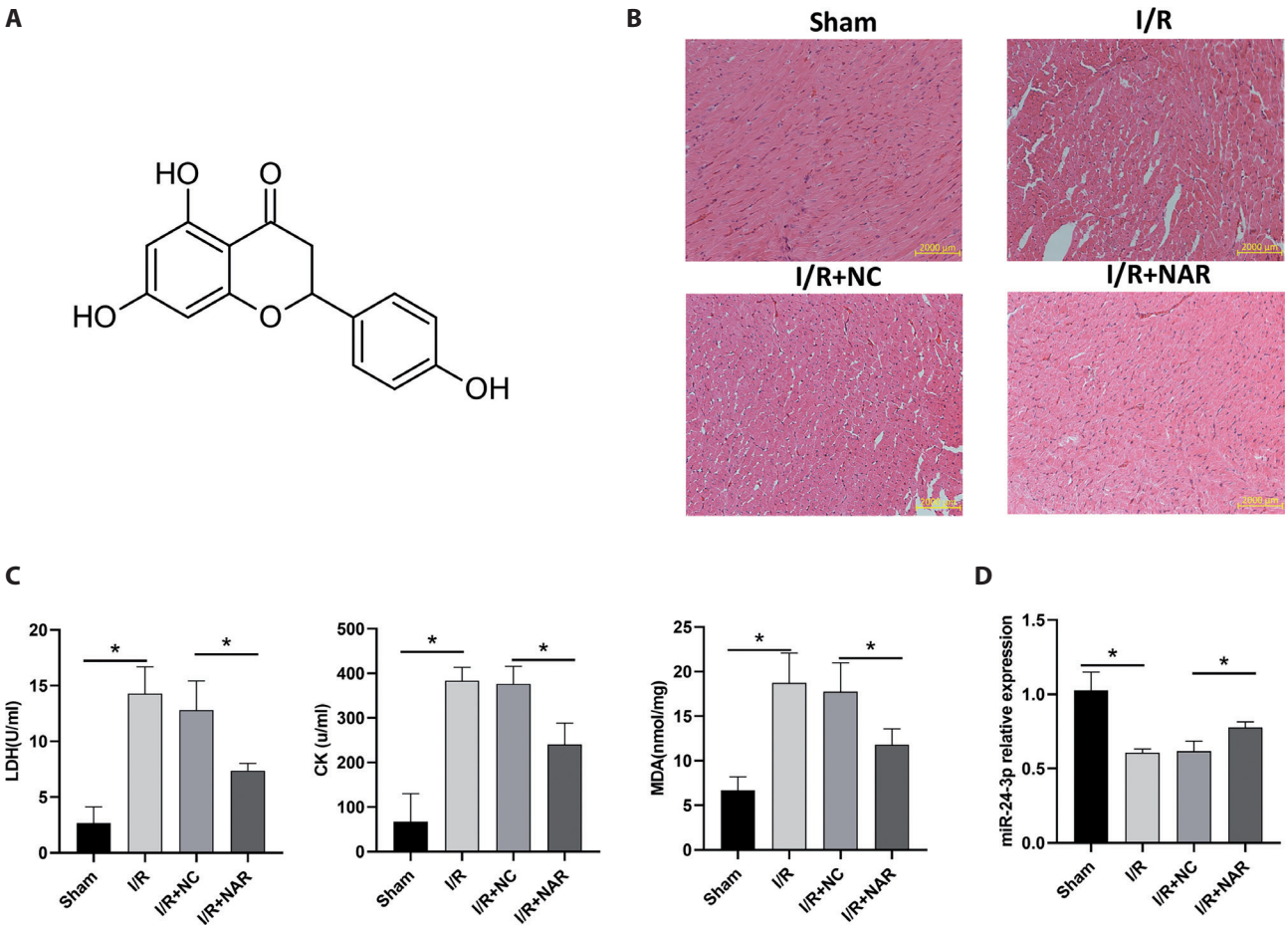


Figure 1. Naringenin alleviates I/R-induced myocardial injury and increases miR-24-3p expression. **A.** The scheme of the chemical structure of naringenin. **B.** HE staining was used to detect myocardial injury, scale bar = 2000 μm. **C.** The levels of LDH (serum), CK (serum), and MDA (myocardial tissues) were detected by ELISA kits. **D.** miR-24-3p expressions in the myocardial tissues were detected by RT-PCR. Data are means ± SD; $n = 3$; * $p < 0.05$. NAR, naringenin.

mitochondria appear red after JC-1 reagent aggregation. In apoptotic cells, the dye maintained its monomer form and showed green fluorescence.

Measurement of reactive oxygen species (ROS)

After treatment, H9c2 cells were stained with 10 mmol/l DCFH-DA (S0033S, Beyotime, China) and 5 mg/ml Hoechst 33342 for 20 min in the dark. Next, ROS fluorescence was detected with a fluorescence microscope (CX31-P; Olympus, Tokyo, Japan).

Flow cytometry

The apoptosis was detected using flow cytometry with the Annexin V-FITC Apoptosis Detection Kit (C1062M, Beyotime, China). Briefly, after treatment, H9c2 cells were collected, stained with Annexin V-FITC and propidium iodide (PI) using the Annexin V-FITC Apoptosis Detection Kit (C1062M, Beyotime, China), and then analyzed by flow cytometry (BD Biosciences).

Real-time PCR

Total RNA was extracted, and then reverse transcribed into cDNA using the PrimeScript RT reagent kit (Takara, Japan) or the miRNAFirst Strand cDNA Synthesis (Stem-loop Method) kit (Sangon Biotech, China). RT-PCR primers include: miR-24-3p, 5-ACACTCCAGCTGGG TGGCTCAGTTCAGCAG-3, and 5-TGGTGTCGTG-GAGTCG-3; U6, 5-AGAGAAGATTAGCATGGCCC-CTGC-3 and 5-ATCCAGTGCAGGGTCCGAGG-3; Cdip1, 5-GACTTCAGCCTTTTGTTCATGG-3, and 5-TCTTT-GCTGTTGATACTCCTGG-3; GAPDH, 5-TCAACAG-CAACTCCCACTCTTCCA-3 and 5- CCCTGTTGCTG-TAGCCGTATTCA-3.

Prediction of miRNA-targeted genes

The potential target genes of hsa-miR-24-3p were predicted by miRWalk (http://mirwalk.umm.uni-heidelberg.de/) and miRDB (http://www.mirdb.org/miRDB/download.html). Only genes predicted by both databases were used for the subsequent biological functional enrichment analysis by Metascape (https://metascape.org/gp/index.html#/main/step1). DNA damage-related genes were obtained from PathCards (https://pathcards.genecards.org/).

Transfection

H9c2 cells were transfected with miR-24-3p mimic, miRNA-negative control (NC), miR-24-3p inhibitor, inhibitor-NC, pCMV3-Cdip1 (RG81052-UT, SinoBiological, Beijing, China), or pCMV3-empty vector (CV011, SinoBiological) using Lipofectamine 2000.

Dual-luciferase reporter assay

The fragments of the Cdip1 3'UTR containing conserved miR-24-3p binding sites (wild type, WT) and corresponding mutant-binding sites (Mut) were synthesized and then inserted into the pmirGLO vector (Promega, Madison, WI). Hek293 cells were co-transfected with the constructed reporter plasmid (0.05 μg) and miR-24-3p mimics (50 nM) or the scrambled control (miR-NC) using Lipofectamine 2000 transfection reagent. After 48 h, a Dual-Luciferase Reporter Assay (Promega, Madison, WI) was performed to quantify the luciferase.

Western blot

Briefly, myocardial tissue or H9c2 cells were lysed, fractionated by 12% SDS-PAGE gels (Invitrogen), and then transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membrane was incubated with primary Cdip1 antibody (A14883, ABclonal, China) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 60004-1-Ig; Proteintech) overnight, and then incubated with secondary antibodies. After washing three times with PBS (containing 0.05% TWEEN 20; PBST), protein bands were then imaged with the enhanced chemiluminescence solution (ECL; Amersham Biosciences, Piscataway, NJ, USA).

Statistical analysis

Statistical analysis was detected by Prism GraphPad. All the data are presented as mean ± standard deviation (SD). Differences were performed using a one-way analysis of variance after Turkey's multiple comparisons. $p < 0.05$ was considered significant.

Results

Naringenin alleviates myocardial I/R injury and increases miR-24-3p expression

To investigate whether the cardioprotective mechanism of naringenin against myocardial I/R injury is related to miR-24-3p, a myocardial I/R rat model was used. As shown in Figure 1B, the myocardial I/R group showed serious myocardial pathological damage detected by HE staining, compared with the Sham group. Naringenin pretreatment significantly alleviated I/R-induced myocardial injury (Fig. 1B), which is published by Xu et al. (2021). Besides, naringenin pretreatment significantly relieved oxidative stress in rat myocardial I/R tissues, evidenced by reducing the LDH, CK, and MDA expressions in the serum of myocardial I/R groups (Fig. 1C). Furthermore, compared with the Sham group, miR-24-3p expression significantly decreased in the myocardial I/R-injured tissues (Fig. 1D), which is consistent with the previous study (Xiao et al. 2018). Whereas, naringenin pretreatment significantly inhibited the I/R-induced decrease of miR-24-3p expression in myocardial tissues (Fig. 1D). Collectively, our results show that naringenin alleviates I/R-induced myocardial injury and increases miR-24-3p expression.

Naringenin alleviates H/R-induced cardiomyocytes injury and upregulates miR-24-3p expression

We further investigated the protective effect of naringenin on myocardial I/R injury *in vitro*, and found naringenin pretreatment increased the cell viability of cardiomyocyte H9c2 cells with H/R injury (Fig. 2A). Furthermore, naringenin pretreatment also significantly inhibited H/R-induced dysfunction of mitochondrial membrane potential (Fig. 2B), and decreased ROS production (Fig. 2C). Moreover, naringenin pretreatment also significantly inhibited H/R-induced upregulation of LDH, CK, and MDA in H9c2 cells (Fig. 2D). In addition, we found that naringenin pretreatment significantly upregulated miR-24-3p expression (Fig. 2E), and inhibited apoptosis of H/R-induced cardiomyocytes (Fig. 2F).

miR-24-3p targets cell death inducing p53 target 1 (Cdip1) gene in cardiomyocyte

Although miR-24-3p plays cardioprotective roles in myocardial I/R injury by targeting Keap1 (Xiao et al. 2018), whether there are other novel potential targets of miR-24-3p still needs to be explored. As shown in Figure 3A, 115 potential targets of miR-24-3p were identified through bioinformatic analysis using MiRWalk and MiRBD. Furthermore, the biological functional enrichment analysis of the 115 potential

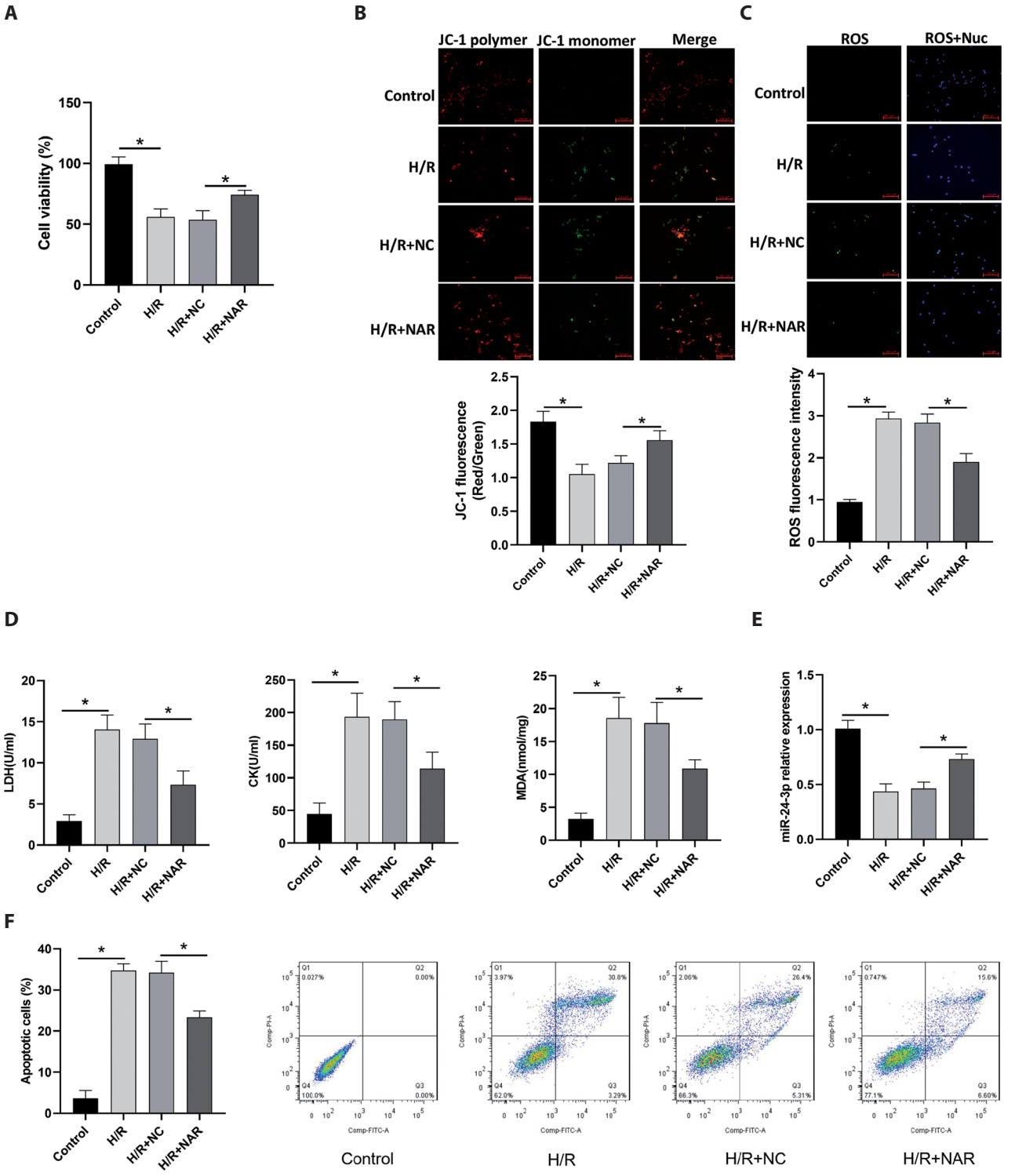
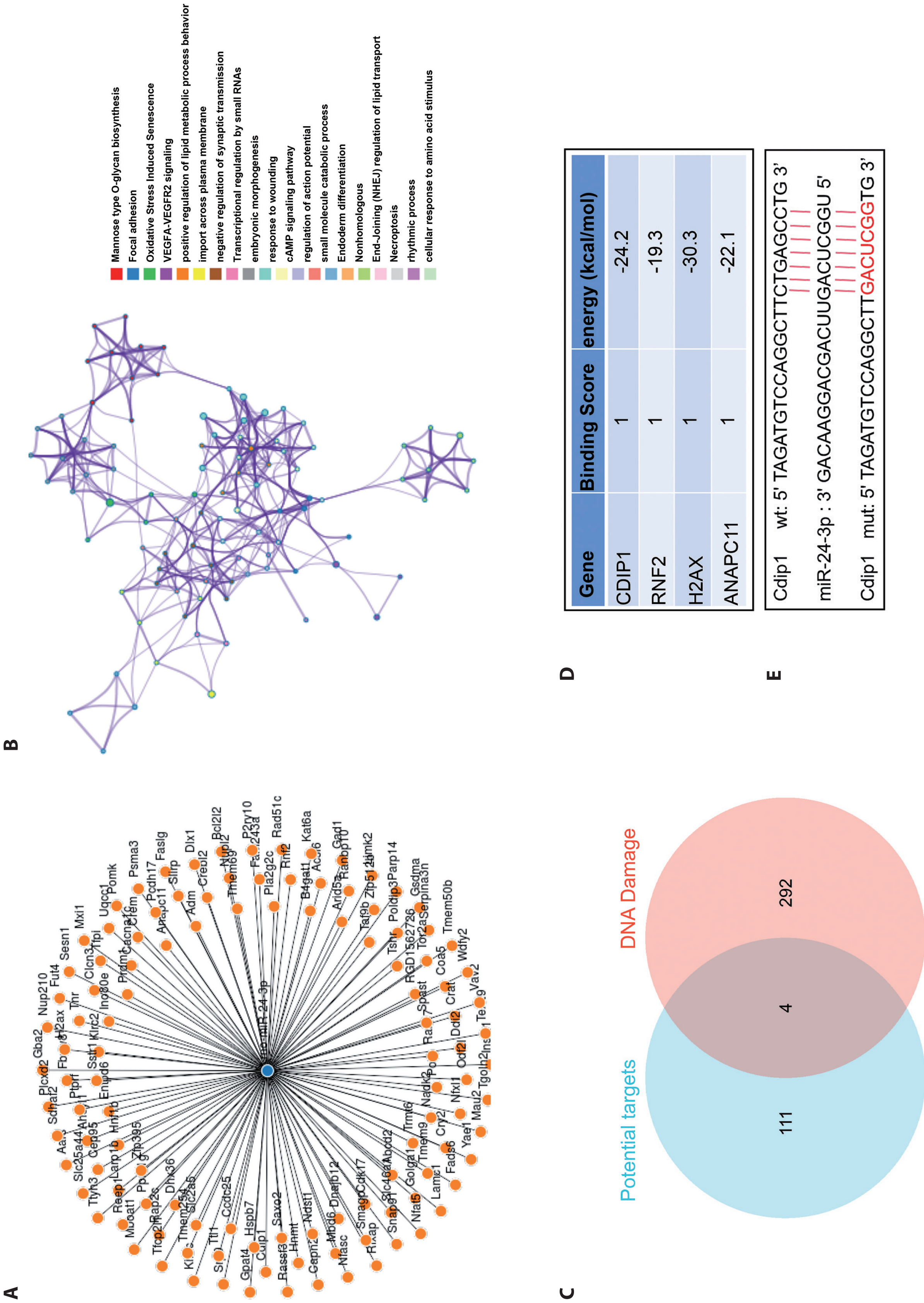


Figure 2. Naringenin alleviates H/R-induced cardiomyocyte injury and upregulates miR-24-3p expression. **A.** Cell viability was detected in the cardiomyocyte H9c2 cells with H/R injury. **B.** Mitochondrial membrane potential was detected by JC-1 staining, and the JC-1 fluorescence intensity was calculated, scale bar = 200 μm. **C.** ROS was detected by immunofluorescence staining, and the ROS fluorescence intensity was calculated, scale bar = 200 μm. **D.** The activity of LDH, CK, and MDA in the culture medium of H9c2 cells was detected by ELISA kits. **E.** miR-24-3p expressions in H9c2 cells were detected by RT-PCR. **F.** Apoptosis of H9c2 cells was detected using flow cytometry. Data are means ± SD; $n = 3$; * $p < 0.05$. NAR, naringenin.


A
B
C
D
E
hsa-miR-24-3p
Mannose type O-glycan biosynthesis
Focal adhesion
Oxidative Stress Induced Senescence
VEGFA-VEGFR2 signaling
positive regulation of lipid metabolic process behavior
import across plasma membrane
negative regulation of synaptic transmission
Transcriptional regulation by small RNAs
embryonic morphogenesis
response to wounding
cAMP signaling pathway
regulation of action potential
small molecule catabolic process
Endoderm differentiation
Nonhomologous
End-Joining (NHEJ) regulation of lipid transport
Necroptosis
rhythmic process
cellular response to amino acid stimulus
Potential targets
DNA Damage
111
4
292
Gene | Binding Score | energy (kcal/mol)
CDIP1 | 1 | -24.2
RNF2 | 1 | -19.3
H2AX | 1 | -30.3
ANAPC11 | 1 | -22.1
Cdip1 wt: 5' TAGATGTCCAGGCTTCTGAGCCTG 3'
miR-24-3p : 3' GACAAGGACGACUUGACUCGGU 5'
Cdip1 mut: 5' TAGATGTCCAGGCTTGACUCGGTG 3'

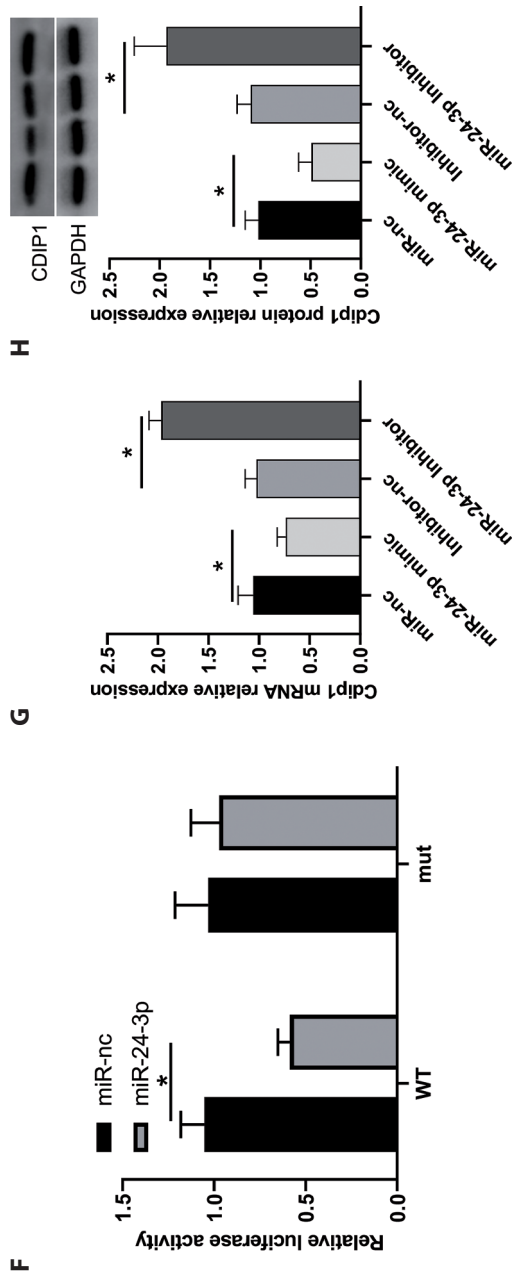


Figure 3. miR-24-3p targets cell death-inducing p53 target 1 (Cdip1) gene in cardiomyocytes. **A.** The potential targets of miR-24-3p were analyzed by MiRWalk and miRDB. **B.** The biological functional enrichment analysis of the potential target genes miR-24-3p was analyzed by Matascape. **C.** Venn analysis. **D.** The genes identified by Venn analysis. **E.** Target relations between miR-24-3p and Cdip1 gene. **F.** The double luciferase reporter assay analysis of the interaction of miR-24-3p with the Cdip1 3'UTR. **G.** Cdip1 mRNA expression in H9c2 cells was detected by RT-PCR. **H.** Western blotting was performed to determine the protein expression of Cdip1. Relative levels of Cdip1 protein normalized to GAPDH. Data are means ± SD; n = 3; * $p < 0.05$. WT, wild type; Mut, mutant.

target genes was performed by Metascape, and oxidative stress-induced senescence, response to wounding, and necroptosis were identified (Fig. 3B). Given that exploration of the cardioprotective mechanism of naringenin and that I/R stimulation generally induces myocardial DNA damage (Maulik et al. 1996), we then explored the potential targets through intersecting the 115 potential target genes with the 296 DNA damage-related genes, and identified 4 potential genes (Cdip1, Rnf2, H2ax, and Anapc11) (Fig. 3C,D). Among the 4 potential genes, H2ax has been reported as a target of miR-24 (Lal et al. 2009; Jeong et al. 2017). Moreover, The binding energy between miR-24-3p and Cdip1 was lower than those between miR-24-3p and Rnf2/Anapc11, indicating that the binding of miR-24-3p and Cdip1 was more closely (Fig. 3E). In addition, Cdip1 has been found as a pro-apoptotic protein which participates in myocardial I/R injury (Liao et al. 2021). Thus, we focused on the Cdip1 gene in the following study. Bioinformatics analysis indicated that miR-24-3p might bind to 3' UTRs of Cdip1 mRNA (Fig. 3E). Subsequently, luciferase reporter assay showed that miR-24-3p indeed targeted Cdip1 mRNA, as the relative luciferase activity of Cdip1-3'-UTR but did not significantly changed by co-transfection with miR-24-3p (Fig. 3F). Moreover, overexpression of miR-24-3p significantly inhibited Cdip1 mRNA and protein expression in cardiomyocytes, but miR-24-3p knockdown promoted (Fig. 3G,H). Collectively, miR-24-3p targets the Cdip1 gene in cardiomyocytes.

miR-24-3p alleviates I/R-induced myocardial injury partially by regulating Cdip1

To further explore whether the cardioprotective effect of miR-24-3p is related to regulating Cdip1 in myocardial I/R injury, miR-24-3p mimics were transfected into cardiomyocyte H9c2 cells, and then subjected to H/R injury. We found that Cdip1 mRNA and protein levels were significantly increased in H9c2 cells with H/R injury, and miR-24-3p overexpression notably inhibited Cdip1 expressions (Fig. 4A–C). Besides, miR-24-3p overexpression decreased H/R-induced LDH release and cell apoptosis, whereas co-transfection of pCMV3-cdip1 and miR-24-3p mimics partially terminated the cardioprotective effect on myocardial I/R injury (Fig. 4D,E). Collectively, miR-24-3p alleviates I/R-induced myocardial injury partially through regulating Cdip1.

Naringenin inhibits I/R-induced Cdip1 expression in vivo and in vitro

Finally, we investigated the effect of naringenin on Cdip1 expression in myocardial I/R injury. As shown in Figure 5A and B, naringenin pretreatment significantly inhibited

I/R-induced upregulation of Cdip1 mRNA and protein in myocardial tissues. Moreover, naringenin pretreatment also evidently decreased I/R-induced upregulation of Cdip1 mRNA and protein in cardiomyocyte H9c2 cells (Fig. 5C,D). Collectively, naringenin alleviates myocardial ischemia/reperfusion injury partially *via* regulating miR-24-3p to inhibit Cdip1 expression.

Discussion

Myocardial I/R injury is the major problem for the clinical treatment of ischemic cardiomyopathy (Saccaro et al. 2021; Horváth et al. 2022). Although great effects have been made for alleviating myocardial I/R injury, effective drugs are still lacking. Here, we explored the cardioprotective mechanism of naringenin against myocardial I/R injury *in vivo,* and *in vitro*, and found that naringenin protects against myocardial I/R injury *via* promoting miR-24-3p expression to inhibiting Cdip1 expression, and provides the scientific basis for the possibility of naringenin for preventing and treating myocardial I/R injury.

Naringenin, predominantly derived from citrus fruits, exhibits extensive biological activities, such as including antioxidant, anti-inflammatory, anti-cancer, and so on (Hämäläinen et al. 2007; Jayaraman et al. 2009). Recently, some studies have reported that naringenin could decrease cardiac damage following I/R injury *via* regulating cGMP-PKG signaling, and activation of mitochondrial BK potassium channel (Testai et al. 2017). Consistent with these studies, our data also confirmed the cardioprotective effects of naringenin. Furthermore, our data found that naringenin alleviated I/R-induced myocardial injury partially *via* increasing miR-24-3p expression. Interestingly, naringenin is reported to play protective roles in various diseases by regulating different miRs (Shi et al. 2016; Yan et al. 2016; Li et al. 2020). For example, naringenin alleviates spinal cord injury-induced activation of neutrophils through decreasing miR-223 expression (Shi et al. 2016). Naringenin ameliorates kidney injury through upregulat-

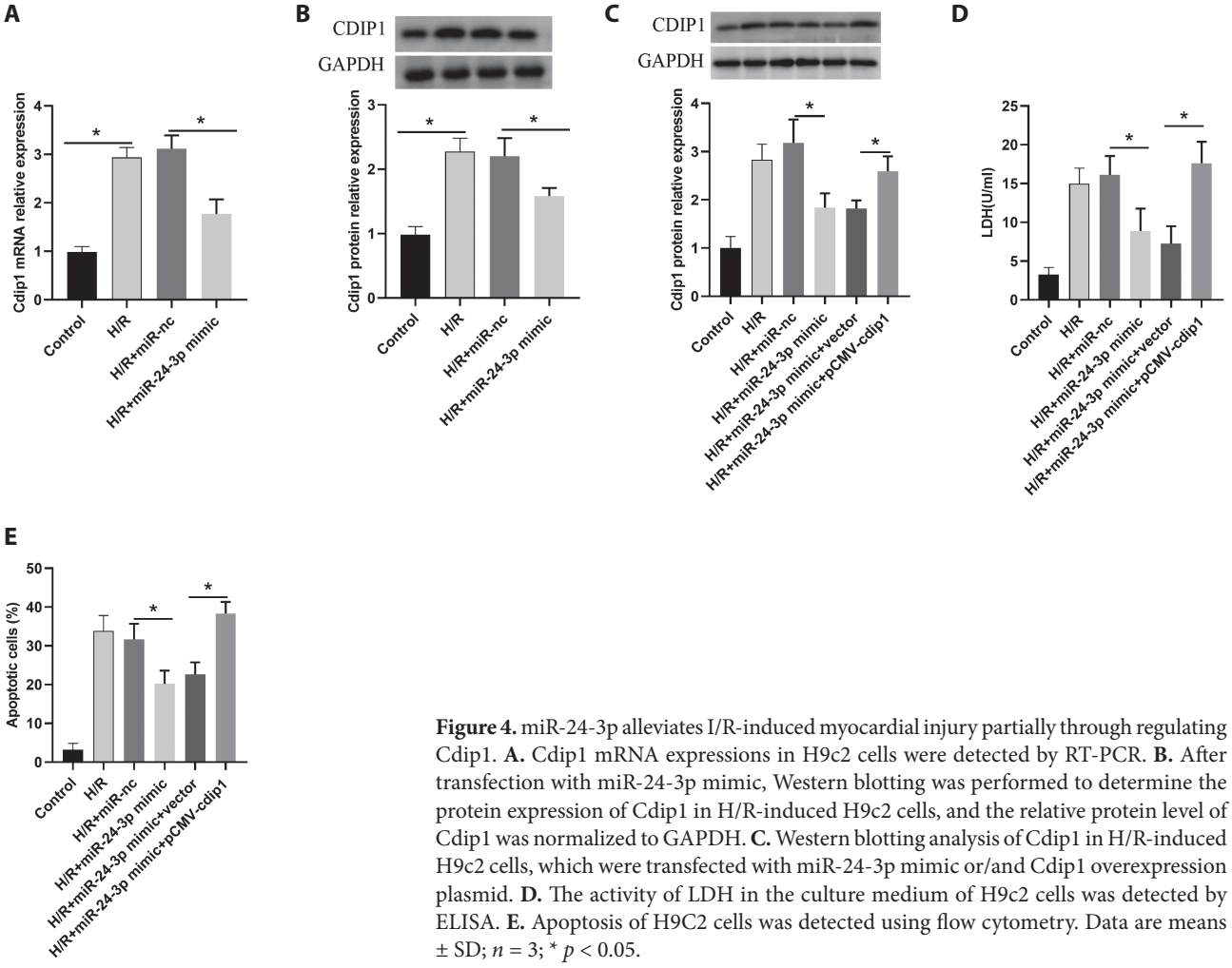


Figure 4. miR-24-3p alleviates I/R-induced myocardial injury partially through regulating Cdip1. **A.** Cdip1 mRNA expressions in H9c2 cells were detected by RT-PCR. **B.** After transfection with miR-24-3p mimic, Western blotting was performed to determine the protein expression of Cdip1 in H/R-induced H9c2 cells, and the relative protein level of Cdip1 was normalized to GAPDH. **C.** Western blotting analysis of Cdip1 in H/R-induced H9c2 cells, which were transfected with miR-24-3p mimic or/and Cdip1 overexpression plasmid. **D.** The activity of LDH in the culture medium of H9c2 cells was detected by ELISA. **E.** Apoptosis of H9C2 cells was detected using flow cytometry. Data are means ± SD; $n = 3$; * $p < 0.05$.

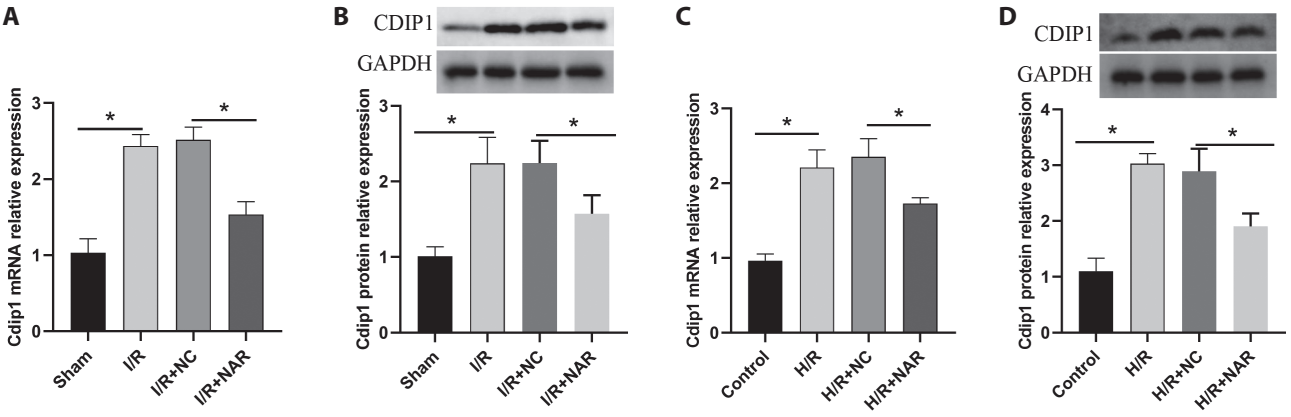


Figure 5. Naringenin inhibits I/R-induced Cdip1 expression *in vivo* and *in vitro*. **A.** Cdip1 mRNA expressions in the myocardial tissues were detected by RT-PCR. **B.** Cdip1 protein expressions in the myocardial tissues were detected by Western blotting. Relative levels of Cdip1 protein normalized to GAPDH. **C.** Cdip1 mRNA expressions in H9c2 cells were detected by RT-PCR. **D.** Western blotting was performed to determine the protein expression of Cdip1. Relative levels of Cdip1 protein normalized to GAPDH. Data are means ± SD; $n = 3$; * $p < 0.05$.

ing Let-7a expression in diabetic nephropathy. Naringenin protects against homocysteine-induced PC12 cells *via* regulating superoxide dismutase 1-miR-224-3p axis (Yan et al. 2016). Nowadays, more and more studies have shown that miRs could be a potential biomarker and treatment strategy for I/R Injury (Cao et al. 2021; Makkos et al. 2021; Li et al. 2022). Besides miR-24-3p, whether other miRs are also involved in the therapeutic action of naringenin against myocardial I/R injury needs to be further explored in the following studies.

Cdip1, a pro-apoptotic protein that is up-regulated upon DNA damage (Brown et al. 2007), promotes cell death by interacting with ESCRT-I (the endosomal sorting complex required for transport-I) and VAPA/B and transduces apoptotic signals from endoplasmic reticulum to mitochondria (Namba et al. 2013). miR-210-3p has been identified to specifically target Cdip1, thereby attenuating ROS and apoptosis in Angiotensin II-injured rat adipose-derived stem cells *via* targeting Cdip1 (Lai et al. 2020). Here, we found that miR-24-3p specifically targeted Cdip1 mRNA, and overexpression of miR-24-3p significantly inhibited Cdip1 expression and decreased I/R-induced myocardial injury. Given that Cdip1 mRNA could be regulated by various miRs, whether these miRs, including miR-133a-3p, miR-210-3p, and miRNA-21-5p are also involved in the protective effects of naringenin against myocardial I/R injury needs further investigation. In this study, through bioinformatic analysis, many other potential targets of miR-210-3p, such as Bcl2l2 (Bcl-2 like 2) and PARP14 (the poly ADP-ribose polymerase 14), were identified. Bcl2l2 plays not only an anti-apoptotic role but also promotes cell migration and invasion (Hartman and Czyz 2020), and decreases the myocardial I/R injury (Long et al. 2018). PARP14 has been reported to promote murine primary B cell survival *via* inhibiting a caspases-involved apoptotic process (Cho et al. 2009). Whether Bcl2l2 and PARP14 mRNAs are also regulated by miR-24-3p in the myocardial I/R injury needs further investigation. In conclusion, we first report that naringenin alleviates myocardial I/R injury through regulating miR-24-3p to inhibit Cdip1 expression and propose that naringenin may be potentially used for preventing or treating myocardial I/R.

Availability of data and materials. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions. XJ, LJ, and BW conducted the experiments and drafted the manuscript. XJ, LJ, BW, and DX were responsible for data integrity and analysis. XJ, LJ, and DX designed the study and reviewed the manuscript.

Ethics approval. This study was approved by the Jinan University Laboratory Animal Ethics Committee. All experiments were performed *per* the National Institutes of Health Guide to minimize the experimental pain in conscious animals.

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Conflict of interest. The authors have no conflicts of interest to disclose.

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