

miR-124 inhibits cardiomyocyte apoptosis in myocardial ischaemia-reperfusion injury by activating mitochondrial calcium uniporter regulator 1

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Received December 14, 2022; Accepted April 28, 2023

DOI: 10.3892/mmr.2023.13031

Abstract. Mitochondria-mediated apoptosis is the primary cause of cardiomyocyte death. Therefore, mitochondria are a key target for treating myocardial injury. Mitochondrial calcium uniporter regulator 1 (MCUR1)-mediated mitochondrial calcium homeostasis markedly promotes cell proliferation and resistance to apoptosis. However, whether MCUR1 is involved in regulation of cardiomyocyte apoptosis during myocardial ischaemia-reperfusion remains unknown. microRNA-124 (miR-124) is upregulated in cardiovascular disease, suggesting a key role for miR-124 in the cardiovascular system. Whether miR-124 affects cardiomyocyte apoptosis and myocardial infarction is not well understood. Western blot showed that miR-124 and MCUR1 were upregulated in cardiomyocyte apoptosis induced by hydrogen peroxide (H₂O₂). Flow cytometry assay of cell apoptosis showed that miR-124 inhibited cardiomyocyte apoptosis by activating MCUR1 following

H₂O₂ treatment. The dual-luciferase reporter assay confirmed binding of miR-124 to MCUR1 3'-UTR and subsequent activation of MCUR1. FISH assay revealed the entry of miR-124 into the cell nucleus. Therefore, MCUR1 was identified as a novel target of miR-124, and it was shown that the miR-124-MCUR1 axis modulated cardiomyocyte apoptosis induced by H₂O₂ *in vitro*. The results indicated induced expression of miR-124 during acute myocardial infarction and its transport to the nucleus. In the nucleus, miR-124 transcriptionally activated MCUR1 by binding to its enhancers. These findings reveal a role of miR-124 as a biomarker for myocardial injury and infarction.

Introduction

Ischaemic heart disease, particularly myocardial infarction (MI), is the leading cause of mortality worldwide (1,2). The risk of 1-year mortality is increased by 7.5% for each 30-min delay in primary angioplasty for ST-segment elevation myocardial infarction (1). Free calcium ion (Ca²⁺) concentration in mitochondria may serve a key role in the regulation of myocardial ischaemia-reperfusion (I/R) injury, especially in regulation of Ca²⁺ balance (3-6). Mitochondria, as Ca²⁺ storage organelles, regulate Ca²⁺ homeostasis (7,8). This is important in cellular homeostasis (9-11). Mitochondrial Ca²⁺ maintains dynamic balance of cytoplasmic Ca²⁺ concentration, which is a key regulatory factor of mitochondrial respiration (12,13). Mitochondrial calcium uniporter (MCU) is a key component of the mitochondrial Ca²⁺ channel (14-17). MCU regulator 1 (MCUR1) is a regulatory protein of MCU. MCUR1 is a key component of the mitochondrial unidirectional transporter complex required for mitochondrial Ca²⁺ uptake and maintenance of normal cell bioenergy. Normal Ca²⁺ expression and function are key for mitochondrial Ca²⁺ homeostasis (18-23). MCUR1-mediated remodelling of the mitochondrial Ca²⁺

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Key words: microRNA-124, cardiomyocyte, apoptosis, mitochondrial calcium uniporter regulator 1, myocardial ischaemia-reperfusion

environment promotes proliferation and resistance to apoptosis, facilitating malignant progression of hepatoma cells (24,25). Thus, MCUR1 is key in the development of hepatocellular carcinoma. Under pathological conditions, mitochondrial Ca^{2+} uptake mediated by MCU activates the mitochondrial permeability transition pore, resulting in cell death during myocardial I/R (26-33). However, the role of MCUR1 in myocardial I/R is unclear.

microRNAs (miRNAs or miRs) are small non-coding RNAs that mediate post-transcriptional gene modulation. miRNAs are key in aging and development of cancer and cardiovascular diseases. Increasing evidence indicates that miRNAs regulate cardiac balance and response to injury (34-37). miR-124 is the most abundant miRNA, with a range of biological functions in the central nervous system (38). Previous studies have showed that miR-124 was elevated in acute myocardial infarction and was correlated with myocardial pathophysiology and cardiac function (39-41). The expression of miR-124 is upregulated in smokers and is associated with increased risk of subclinical arteriosclerosis due to altered single-cell phenotypes (42). Randomised clinical trials have indicated that serum miR-124 levels may be a useful prognostic indicator of outcomes after cardiac arrest (43,44). Circulating miR-124 is upregulated in patients with acute coronary syndrome, with requirement for urgent coronary occlusion differentiating this syndrome from membranous inflammation (45). Although clinical and preclinical data indicate a key role of miR-124 in the cardiovascular system (46-48), data that substantiate this role are limited. Whether miR-124 affects cardiomyocyte apoptosis and MI requires further investigation.

To investigate the role of miR-124 in cardiomyocyte apoptosis and MI, cardiomyocyte apoptosis was induced using hydrogen peroxide (H_2O_2) to simulate oxidative stress induced by I/R injury. We investigated the mechanism of miR-124 regulated during H_2O_2 -induced cardiomyocyte apoptosis.

Materials and methods

Cell culture and transduction. The H9C2 cell line is a subclone of the original clonal cell line derived from embryonic BD1X rat heart tissue (49). H9C2 cells exhibit a number of skeletal muscle properties. Up to 95% of cells fused to form myotubes are characteristic of skeletal muscle (50). The length, diameter, and arrangement of sarcomeres and fine myofilaments in their myotubes are similar to those of developing skeletal and cardiac myofilaments *in vitro*. Cells were obtained from the Whole Gold Cell Bank (Beijing, China) and cultured in Dulbecco's Modified Eagle's Medium (Merck KGaA) containing 10% FBS (Thermo Fisher Scientific, Inc.) in an atmosphere of 95% oxygen and 5% carbon dioxide at 37°C. After starvation in serum-free DMEM (37°C) for 12 h, oxidative stress was induced by treatment at 37°C with 200 μM H_2O_2 for 0, 2, 4, 6, 8 and 10 h. Cells were collected at each time point for subsequent analysis.

RNA constructs miR-124 mimics (5'uaagcagcg-gugaugcc3'), anti-miR-124 (5'ATCAAGGTCCGCTGTG3') and corresponding negative controls (NCs) were purchased from GenePharma and transfected into H9C2 cells using FuGENE® HD (Promega Corporation) at room temperature for 15 min, according to the manufacturer's instructions.

miRNA (10 μM) and 10 μl FuGENE co-transfected in H9C2 cells. Following incubation at 37°C for 48 h, subsequent experiments were performed. In addition, miR-124 mimic, MCUR1 3'-untranslated region (UTR) plasmid, an MCUR1 overexpression plasmid vector [pEX-3(pGCMV/MCS/Neo)] (GenScript), NC (mimic Ctrl and pcDNA3.1) and an MCUR1 small interfering RNA (siRNA, 5'GCCAGAGACAGACAAUACUTT3'; GenePharma) were transfected using FuGENE® HD (Promega Corporation).

Cell Counting Kit-8 (CCK-8) proliferation assay. Cells were harvested in the exponential growth phase and suspended in complete medium. Medium containing cells (100 μl) were added to the wells and incubated at 37°C for 24 h. Subsequently, 200 μM (12.5 μl) H_2O_2 was added. Each well was incubated with 10 μl CCK-8 (Beijing Solarbio; cat. no. CA1210) for 1-4 h. The signal was measured by microplate reader at an absorption wavelength of 450 nm and a reference wavelength of 600-650 nm. Six samples was tested in triplicate.

Dual-luciferase reporter assay. MCUR1 3'-UTR was cloned into the gp-mirGLO vector (GenScript) and mutant 3'-UTR was obtained by target mutation (GenScript). The luciferase reporter plasmid (gp-mirGLO, GenePharma) and miR-124 mimic (GenePharma; 5'-uaagcagcg-gugaugcc3')/miR-NC (5'UUCUCCGAACGUGUCACGUTT3', GenePharma) were co-transfected into H9C2 cells (2×10^4) using FuGENE® HD (Promega Corporation) into H9C2 cells. Following incubation at 37°C for 48 h, a Dual-Glo® Luciferase Assay System (Promega Corporation, E2920) was used according to the manufacturer's instructions and luciferase activity was detected. *Renilla* luciferase activity was compared with that of the control group.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from H9C2 cells using TRIzol® (Thermo Fisher Scientific, Inc.) at room temperature according to the manufacturer's instructions. RNA was reverse-transcribed to cDNA using Titanium One-Step RT-PCR Kit (Clontech). qPCR was performed using SYBR® Premix Ex Taq™ II (Takara Bio, Inc.) and detected using an ABI 7,500 Fast Real-Time PCR System (Thermo Fisher Scientific, Inc.) with 50 ng cDNA and 500 nM each forward or reverse primers (Table I). The thermocycling conditions were as follows: 50°C for 1 h, 94°C for 5 min, 25-40 cycles (94°C for 30 sec, 65°C for 30 sec, 68°C for 1 min) and 68°C for 2 min. Data were normalized according to the ΔC_q method (51). The reference genes is U6 or GAPDH.

Western blotting. Protein was extracted from H9C2 cells using the RIPA extraction buffer (Beijing Solarbio Science & Technology Co., Ltd.) containing phenylmethylsulfonyl fluoride for 30 min and prepared to measure protein concentration by BCA. The same amount (15-40 μg) of protein was separated by 15% SDS-PAGE, transferred to a pre-treated PVDF membrane, blocked with 5% milk at room temperature for 1 h and incubated with primary antibody at 4°C (MCUR1 1:500, BOSTER, cat. no. A08547-1; β -actin 1:5,000, Beyotime, AF5003) over night. After washing with

Table I. Sequences of primers used for reverse transcription-quantitative PCR.

Gene	Sequence, 5'→3'
microRNA-124-5P	F: CGTGTTACAGCGGAC R: ATCAAGGTCCGCTGTG
U6	F: CTCGCTTCGGCAGCACATATACT R: ACGCTTCACGAATTTGCGTGTC
MCUR1	F: GCTCTACTTTGACACCCACGCTCTGG R: GTCTCCGTAATCTTGACCAATGC
GAPDH	F: GGACATTGTTGCCATCAACGA R: GGGTAGAGTCATACTGGAACATGT

MCUR1, mitochondrial calcium uniporter regulator 1; F, forward; R, reverse.

TBST (1% Tween-20) three times, the washed membrane was incubated with HRP-conjugated, goat anti-Rabbit IgG (Abbkina, cat. no. A21020, 1:10,000) at room temperature for 2.5 h and visualised using a chemiluminescence kit (Vazyme, E412-02). The results were visualized with a chemiluminescence analyser. Protein quantification was analysed using ImageJ software (ImageJ 1.51j8, National Institutes of Health).

Fluorescence in situ hybridisation (FISH). Cell samples were fixed in paraformaldehyde (4%) at room temperature for 24 h and embedded in paraffin at 42°C, 4~8 h. Paraffin sections (3-4 µm) were removed using xylene, dehydrated and washed. FISH was performed according to a previously described protocol (52). Briefly, the slides were permeabilized by proteinase K (Solarbio, P1120) at 37°C for 20 min. The slides were incubated with pre-hybridization buffer 5xSSC (saline sodium citrate) for 1 h at 37°C. The hybridization buffer 5xSSC (Solarbio, S1030) was transferred to the sample, and a single gene probe (working solution, 50 ng/µl, synthesis by Shenggong Biotechnology Company) was added. Slides were incubated at 37°C overnight to hybridise the probe with target DNA. The slides were washed with 37°C preheated washing buffer (2xSSC) for 10 min, then washed with 37°C preheated washing buffer (1xSSC) for 10 min and 37°C preheated washing buffer (0.5xSSC) for 10 min. DAPI was incubated in the dark for 8 min and sealed with anti-fluorescence quenching sealing agent (Solarbio). The signal from each probe was observed under a Leica TCS SP8 MP laser scanning confocal microscope.

Flow cytometry measurement of apoptosis using Annexin-V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining. H9C2 cells were treated with 200 µM hydrogen peroxide at 37°C for 6 h and transfected with miRNA. Treated fresh cells were collected resuspended in 100 µl PBS. PI or Annexin-V-FITC or PI plus Annexin-V were added for staining. After 15 min incubation at 4°C, C6 flow cytometry was performed using a flow cytometer (BD accuri, C6 flow cytometry) equipped with FITC signal filtered by a FL1 detector at 530/30 nm and PI signal filtered

by a FL2 detector at 585/42 nm. The flow cytometry data are expressed as percentages of initial cell count. The data was analyzed by FLOWJO v10 (Becton, Dickinson & Company) software.

Flow cytometry-based measurement of mitochondrial membrane potential. Cells were digested with trypsin, resuspended in PBS and treated with rhodamine 123 (5 mg/l) at 37°C for 30 min, and washed once with PBS followed by centrifugation at 300 g, 10 min) at room temperature. Flow cytometric analysis (BD Accuri C6 Flow Cytometer, BD Company) was used to detect cells at an excitation wavelength of 488 nm. The data was analyzed by FLOWJO v10 (Becton, Dickinson & Company) software.

Online databases analysis. TargetScan was used to predict miRNA-binding sites in mammals (targetscan.org/vert_80/). MiRanda (bioinformatics.com.cn/local_miranda_miRNA_target_prediction_120) and miRDB (mirdb.org/) online databases analysis was performed according to previously described protocol (53,54).

Statistical analysis. The experimental data were analysed using the GraphPad Prism 6.02 software (Dotmatics). All experiments were repeated three times (n=3). The data are presented as the mean ± SD. Independent-sample t test was used for comparisons between two groups. One-way ANOVA followed by Dunnett's post hoc test was used for comparisons between >2 groups. Pearson's correlation coefficient was used for correlation analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-124 and MCUR1 are upregulated in H₂O₂-induced cardiomyocyte apoptosis. Cardiomyocytes undergo apoptosis and necrosis induced by hypoxia and oxidative stress during coronary artery occlusion and subsequent MI (55,56). CCK-8 viability assay was used to test cell activity following H₂O₂ treatment (Fig. 1A). To determine the involvement of caspase-3 in H₂O₂-induced apoptosis in cardiomyocytes, cleaved caspase-3 was measured by western blotting (Fig. 1B). The release of cytochrome c into the cytoplasm is a key step in the apoptotic process and plays an important role in the apoptotic mechanism. We therefore measured the release of cytochrome c by western blot (Fig. 1B). Flow cytometry (Fig. 2G) showed H₂O₂ induced both apoptosis and necrosis in cardiomyocytes, and H₂O₂ was used to generate oxidative stress to mimic I/R injury. The results indicated that H₂O₂ decreased cardiomyocyte viability, induced apoptosis and decreased mitochondrial membrane potential (Figs. 1A-D and 2G-I). H₂O₂ treatment significantly increased mRNA expression of miR-124 in H9C2 cells (Fig. 1E). These data suggested that upregulation of miR-124 may be related to H₂O₂-induced cardiomyocyte apoptosis. The online databases TargetScan, miRanda and miRDB were used to predict the potential targets of miR-124. Computational prediction (Fig. 2A) showed miR-124 binding sites exist in the 3' UTR of MCUR1. MCUR1 was identified as a potential target gene of miR-124 in cardiomyocyte apoptosis. A prior study demonstrated that MCUR1-mediated

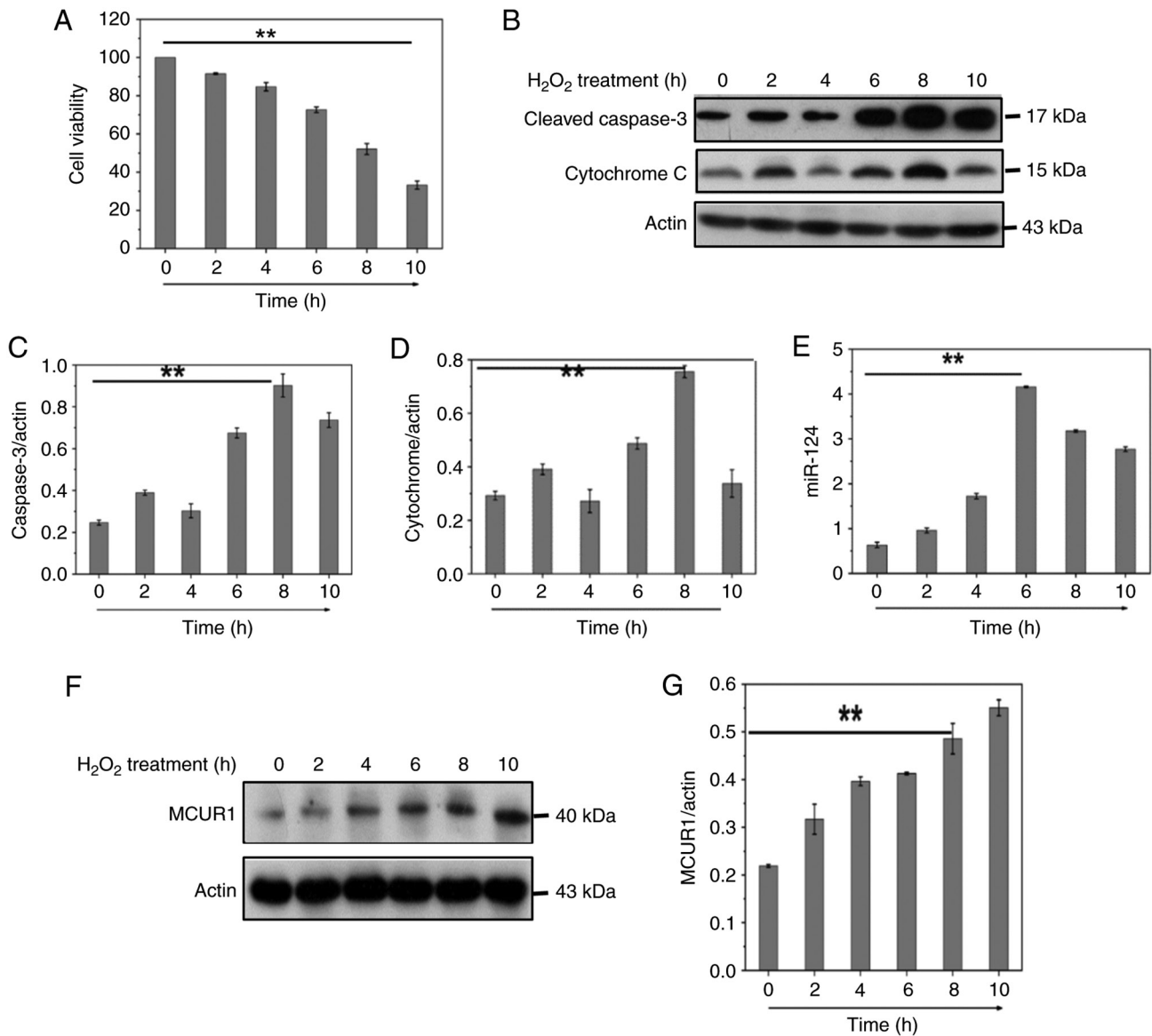


Figure 1. miR-124 and MCUR1 are upregulated in H_2O_2 -induced cardiomyocyte apoptosis. (A) Cell Counting Kit-8 analysis of H9C2 viability. (B) Western blotting of (C) cleaved caspase-3 and (D) cytochrome C in cytoplasm. (E) Reverse transcription-quantitative PCR was used to analyse the expression of miR-124. (F) Western blotting revealed (G) MCUR1 expression following treatment with H_2O_2 . ** $P < 0.01$. MCUR1, mitochondrial calcium uniporter regulator 1; miR, microRNA.

mitochondrial Ca^{2+} environment remodelling markedly promotes proliferation and apoptosis resistance of hepatoma cell lines (25). Therefore, expression of MCUR1 was investigated during cardiomyocyte apoptosis induced by H_2O_2 . MCUR1 protein expression increased in a time-dependent manner following H_2O_2 treatment (Fig. 1F and G). These results indicated that the MCUR1 protein expression was increased during cardiomyocyte apoptosis.

miR-124 binds to MCUR1 3'-UTR and inhibits cardiomyocyte apoptosis by activating MCUR1. Compared with miR-NC, miR-124 overexpression increased the protein expression of MCUR1 compared with miR-NC (Fig. 2B-D). Treatment with H_2O_2 increased the protein expression of MCUR1 and miR-124 further increased this following treatment with H_2O_2 (Fig. 3C and D). Simultaneously, the addition of the anti-124 in presence of H_2O_2 significantly decreased

expression of MCUR1 (Fig. 3A and B). MCUR1 siRNA treatment (100 nM) decreased the expression of MCUR1 (Fig. 2E and F). In the presence of H_2O_2 , miR-124 inhibitor (anti-124), or MCUR1 siRNA inhibited MCUR1 protein expression. Simultaneous treatment with miR-124 inhibitor and MCUR1 siRNA further inhibited MCUR1 expression (Fig. 3E and F). Flow cytometry showed that H_2O_2 enhanced apoptosis, while miR-124 significantly decreased apoptosis induced by H_2O_2 . However, when cells were co-treated with MCUR1-siRNA and miR-124, H_2O_2 -induced apoptosis was restored (Fig. 2G and H). These results indicated that miR-124 inhibited cardiomyocyte apoptosis by activating MCUR1 following H_2O_2 treatment. Overexpression of miR-124 restored mitochondrial membrane potential in H_2O_2 -treated cells (Fig. 2I). Moreover, when MCUR1-siRNA and miR-124 were applied simultaneously in H_2O_2 -treated cells, mitochondrial membrane potential was significantly decreased

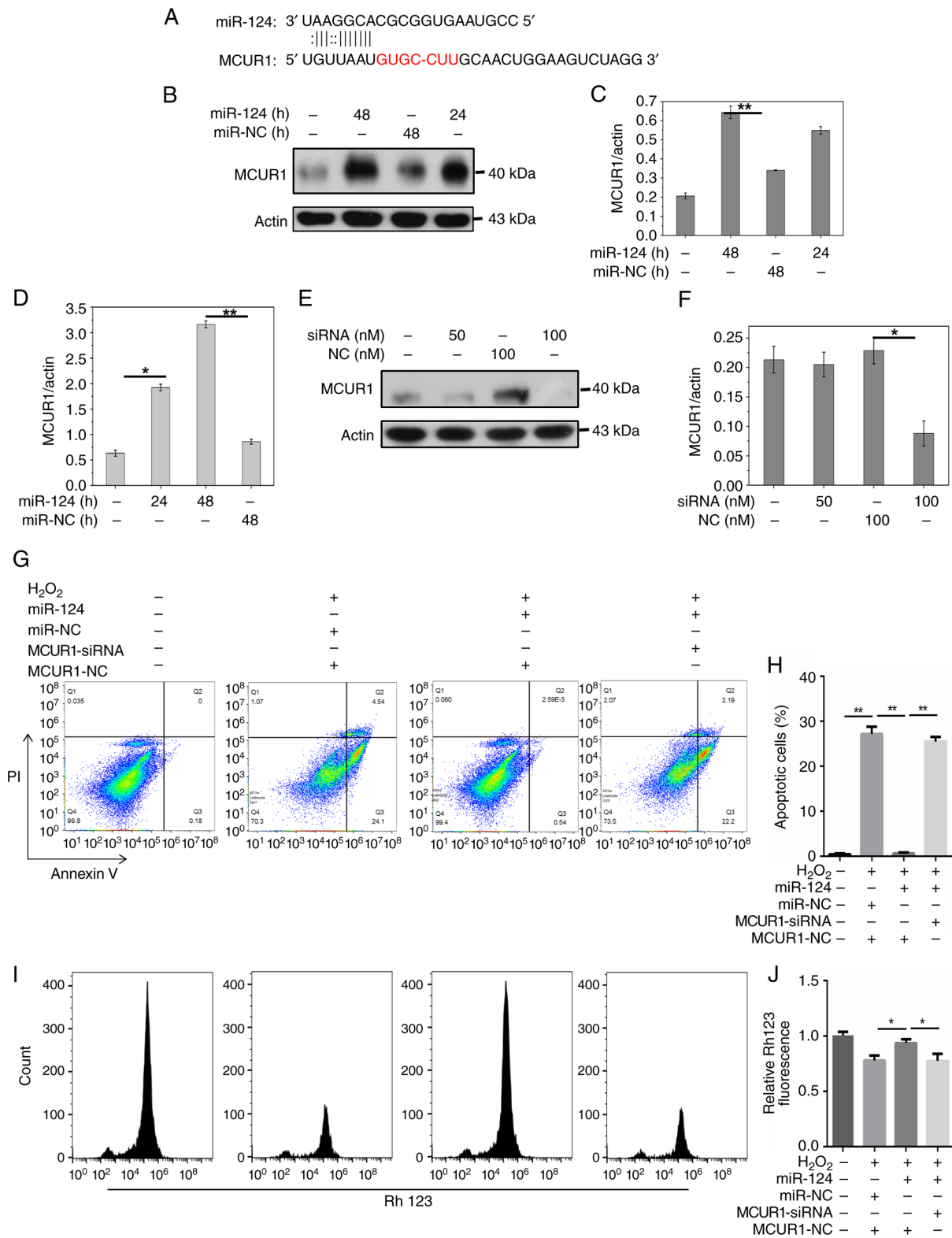


Figure 2. miR-124 may activate MCUR1 expression and inhibit H₂O₂-induced apoptosis of cardiomyocytes. (A) Predicted miR-124 binding site in the 3'-UTR of MCUR1. (B) Western blotting of (C) MCUR1. (D) Reverse transcription-quantitative PCR of MCUR1 expression after transfection of H9C2 with miR-124 mimic and NC. (E) Western blotting of (F) MCUR1 after transfection of H9C2 cells with MCUR1 siRNA and its NC. (G) Apoptosis of H9C2 cells after H₂O₂ treatment assessed by annexin V-FITC/PI flow cytometry. (H) Percentage of apoptotic cells. (I) Flow cytometry analysis of H9C2 mitochondrial membrane potential induced by H₂O₂, miR-124 mimic and MCUR1-siRNA. (J) Relative rh123 fluorescence analysis. *P<0.05, **P<0.01. NC, negative control; miR, microRNA; MCUR1, mitochondrial calcium uniporter regulator 1; rh123, rhodamine 123; si, small interfering; UTR, untranslated region.

(Fig. 2I and J). The dual-luciferase reporter assay confirmed binding of miR-124 to MCUR1 3'-UTR. The relative luciferase activity was increased in H9C2 cells co-transfected

with MCUR1 3'UTR and miR-124 mimic compared to 3'UTR and miR-NC co-transfected (Fig. 3G). These findings indicated that miR-124 may activate expression of MCUR1

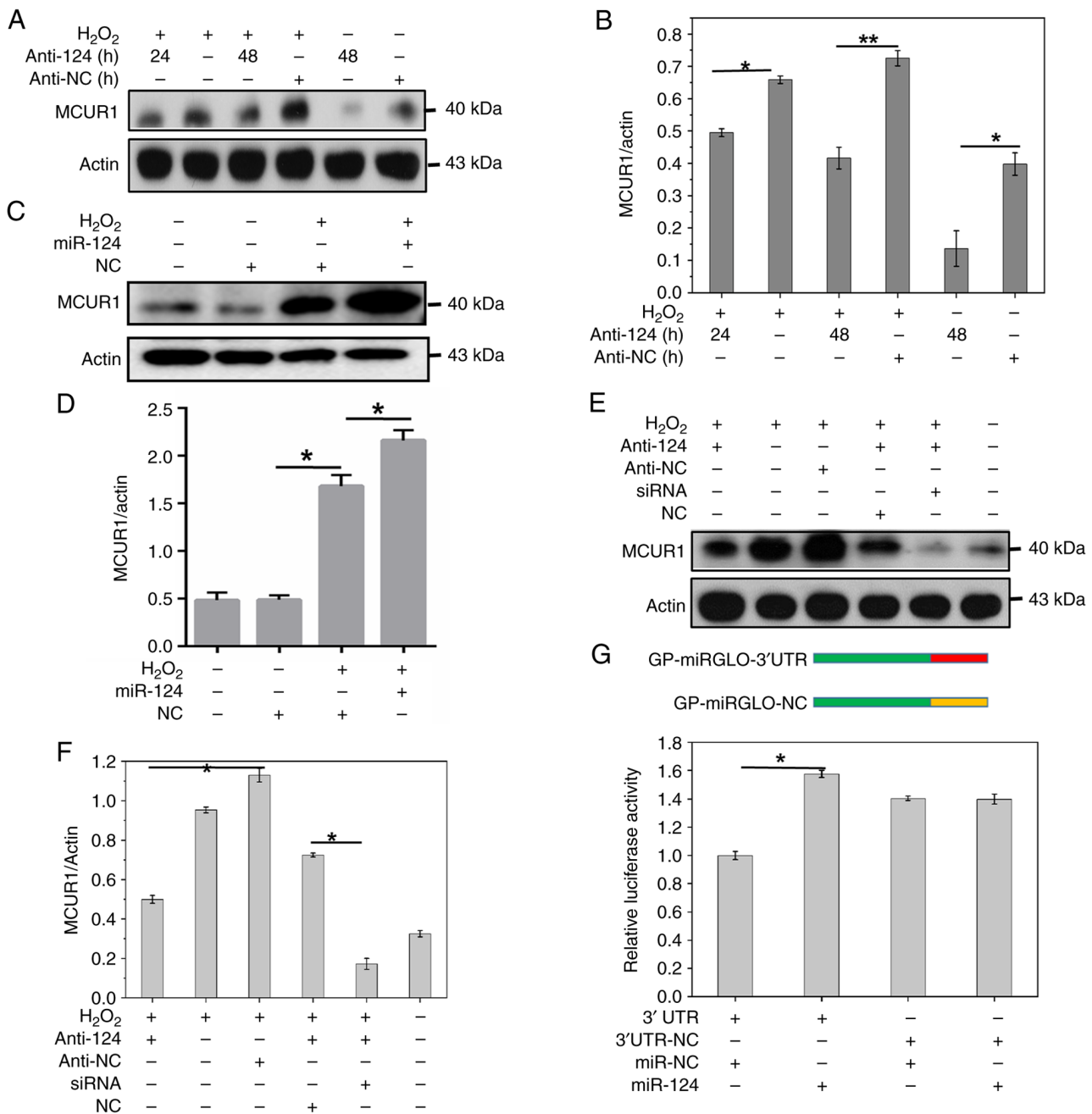


Figure 3. miR-124 binds to MCUR1 3'-UTR and activates MCUR1 expression in H₂O₂-induced cardiomyocyte apoptosis. (A) Western blotting of (B) MCUR1 under H₂O₂ stimulation after adding miR-124 inhibitor anti-124. (C) Western blotting of MCUR1 in H9C2 following miR-124 mimic and H₂O₂ treatment. (D) Quantitative analysis of MCUR1 expression in (C). (E) Western blotting of MCUR1 protein after addition of MCUR1-siRNA and miR-124 inhibitor under H₂O₂ stimulation. (F) Quantitative analysis of MCUR1 expression in (E). (G) Dual-luciferase assay following co-transfection of H9C2 cells with miR-124 mimic and wild-type MCUR1 3'-UTR reporter plasmid GP-miRGLO-3'-UTR or GP-miRGLO-NC. *P<0.05, **P<0.01. NC, negative control; miR, microRNA; MCUR1, mitochondrial calcium uniporter regulator 1; UTR, untranslated region; si, small interfering.

by binding to MCUR1 3'-UTR, decreasing cardiomyocyte apoptosis induced by H₂O₂.

miR-124 enters the nucleus and targets MCUR1 enhancer. Next, the association between miR-124 overexpression and increased MCUR1 expression was investigated. By screening miRNA database, the positions of numerous miRNAs including miR-24-1, lin-4 in the genome were coincident with enhancer regions (57). Most miRNAs are localized in the nucleus. These miRNAs bind to enhancers and activate gene expression at the genome level (57-60). miR-24-1 activated gene transcription

by targeting enhancers (45). It was reported that overexpression of miR-26a-1 increased the transcription of neighboring *ITGA9* and *VILL* genes (57). TargetScan (61), miRanda (53) and miRDB online databases are computational approaches have been used to predict mRNA-miRNA interaction and microRNA targets (54). In this study, these online databases were used to predict MCUR1 enhancers. Comparison of the miR-124 sequences revealed binding of MCUR1 enhancer to miR-124 (Fig. 4A). FISH at the cellular level revealed the entry of miR-124 into the nucleus (Fig. 4B), which may have increased MCUR1 expression.

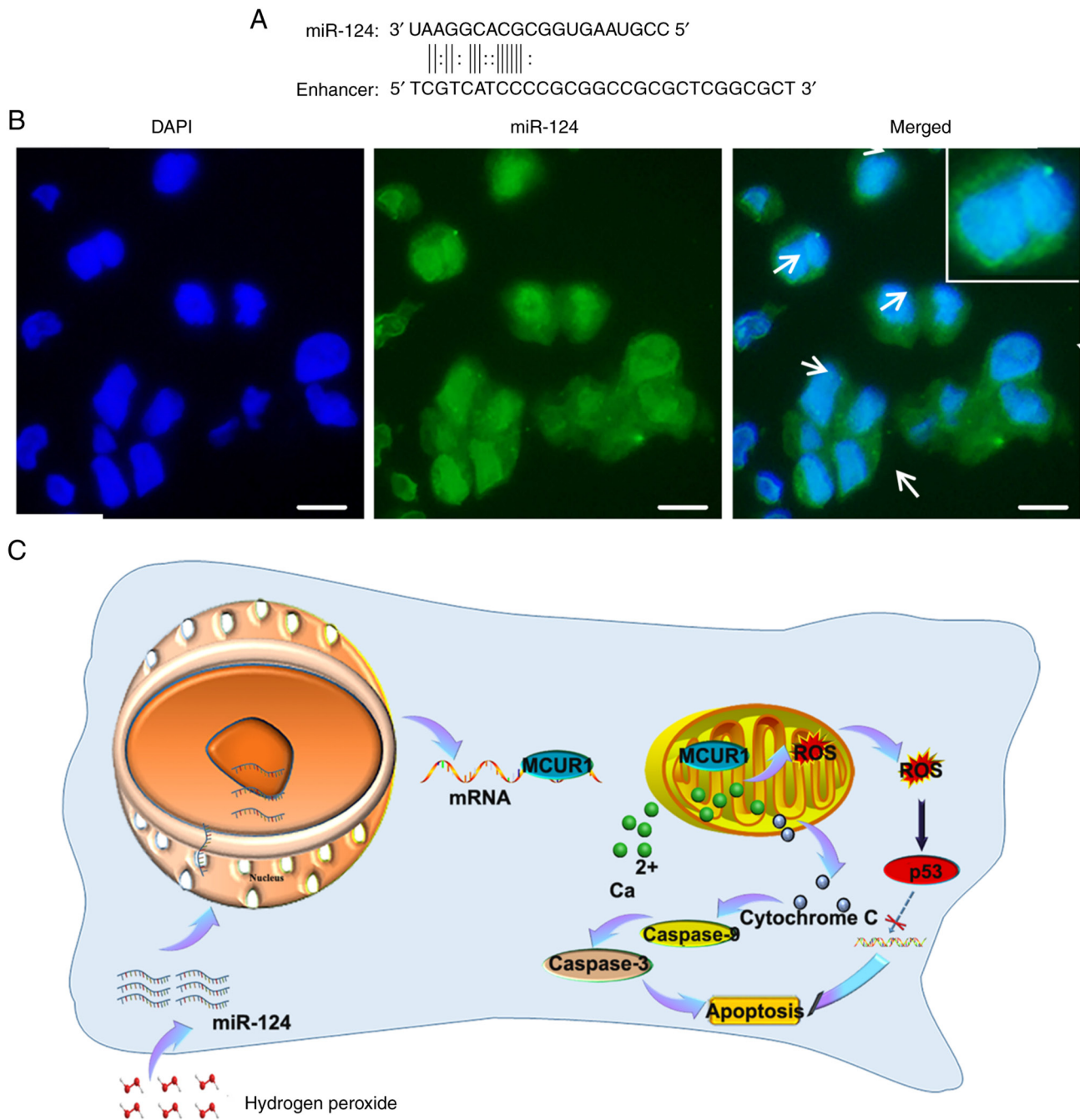


Figure 4. miR-124 enters the nucleus and targets the MCUR1 enhancer. (A) Schematic diagram of miR-124 targeting the MCUR1 enhancer. (B) Fluorescence *in situ* hybridization analysis of nuclear localisation of miR-124. Scale bar, 20 μ m. Arrow represents the miR-124 colocalization with the nucleus. (C) Schematic diagram of activation of MCUR1 expression by H₂O₂ and inhibition of apoptosis following interaction with MCUR1 enhancer. miR, microRNA; MCUR1, mitochondrial calcium uniporter regulator 1; ROS, reactive oxygen species.

Discussion

The present study revealed the key role of miR-124 in both cardiomyocyte apoptosis and MI and its underlying mechanism. The findings further demonstrated that MCUR1 was a novel target of miR-124, and that the miR-124-MCUR1 axis modulated cardiomyocyte apoptosis induced by H₂O₂. The findings were consistent with the hypothesis that the expression level of miR-124 increases during oxidative stress and that miR-124 enters the nucleus to combine with the MCUR1 enhancer. The subsequent high expression level of MCUR1 confers resistance to apoptosis in cardiomyocytes (Fig. 4C).

Thus, miR-124 may be a biomarker of myocardial injury and MI. Increasing the expression of MCUR1 may provide a new pathway to decrease effects of MI and subsequent dysfunction.

miR-124 has been reported to be involved in various cellular physiological and pathological processes (62-64). Accumulating evidence has indicated that the expression of miR-124 facilitates cell death and differentiation under pathological stress (41,46). Increasing evidence has shown that miR-124 is associated with cardiovascular disease (39,65,66). miR-124 modulates cardiomyocyte differentiation into bone marrow-derived stem cells (65). miR-124 overexpression decreased oxidative stress in doxorubicin-induced

cardiac injury and was a hopeful therapeutic target in doxorubicin-related cardiomyopathy (47). Previous studies have described the marked increase of miR-124 expression in smokers and patients with acute coronary syndrome, suggesting that miR-124 may be a biomarker of coronary heart disease (42,43). miR-124 has been proposed to be a cell cycle regulator that regulates cell survival and apoptosis (67-69), consistent with the finding of the current study that miR-124 regulated cardiomyocyte apoptosis.

MCUR1 is a regulatory protein of the MCU that plays an important role in mitochondrial Ca^{2+} [(Ca^{2+})m] uptake and maintenance of normal cellular bioenergy (18). MCUR1 knockout in HeLa cells causes a decrease in ATP synthesis, which is dependent on protein kinase activity and leads to autophagy (70). In addition, knockout of MCU and MCUR1 in vascular endothelial cells impaired [Ca^{2+}]m uptake, thus weakened mitochondrial biosynthesis and cell migration, decreases cell proliferation and induces autophagy (71). Disorders in mitochondrial Ca^{2+} homeostasis are associated with occurrence and development of various types of tumour such as melanoma and MCUR1 plays an important role in mitochondrial Ca^{2+} homeostasis (72). The role of MCU in I/R has attracted increasing interest (73). In the present study, MCUR1 expression increased, leading to resistance to apoptosis induced by oxidative stress.

miRNAs negatively regulate gene expression primarily by targeting the 3'-UTR of mRNA transcripts in cytoplasm to achieve instability or translation inhibition (74-76). Nuclear miRNAs exert gene activation functions. Human miR-373 was the first activator of gene transcription, which induced both E-cadherin (CDH1) and cold-shock domain-containing protein 2 (CSDC2) transcription (77). The analysis of 1302 breast cancer samples indicated that miRNAs and neighbouring genes may be positively associated (78). Thus, miRNAs have the dual functional ability to activate transcription in the nucleus and inhibit transcription in the cytoplasm. A number of studies have shown that microRNAs at enhancer sites exert transcriptional activation functions (48,79,80). miR-24-1 activates enhancer RNA (eRNA) expression and promotes the enrichment of p300 and RNA Pol II at enhancer sites (48). Nuclear miRNAs serve as enhancer triggers by modifying chromatin states that facilitate activation of transcriptional genes (81). miR-24-1 activates gene transcription by targeting enhancers (81). The present study identified a novel mechanism for the miR-124-MCUR1 axis during cardiac injury in cardiomyocytes. The present results revealed the mechanism of MCUR1 activation by miR-124 binding to MCUR1 enhancers and supported the hypothesis that miR-124 alters chromatin status and increases MCUR1 expression, leading to apoptosis resistance and inhibition of MI. However, further investigation is required to confirm these preliminary results. Further studies should investigate the interaction between miR-124 and MCUR1 and how miR-124 affects the chromosomal status of MUCR1 enhancers.

In summary, the present study demonstrated that high expression of miR-124 was induced under oxidative stress conditions. miR-124 enters the nucleus and combines with the enhancer of MCUR1 to activate expression of MCUR1. Mitochondrial Ca^{2+} environment remodelling significantly promotes resistance of cardiomyocytes to proliferation and apoptosis.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Project of Shandong Province Higher Educational Science and Technology Program (grant nos. J18KA250 and J18KA127), research project of Qingdao University Medical Group (grant no. YLJT20202039), Major Research Program of the National Natural Science Foundation of China (grant no. 91849209), National Natural Science Foundation of China (grant no. 81602353), Natural Science Foundation of Jiangsu Province (grant no. BK20171145), China Postdoctoral Science Foundation (grant nos. 2019M652314 and 2020T130333), Qingdao Applied Basic Research Project (grant no. 19-6-2-39-cg) and Major Science and Technology Project of Wenzhou Institute and University of Chinese Academy of Sciences (grant no. WIUCASQD2021028).

Availability of data and materials

The datasets used and/or analysed in the current study are available from the corresponding author upon reasonable request.

Authors' contributions

LG and HD conceptualized the study. LG and HD confirm the authenticity of all the raw data. CL and CJ designed the methodology. LHHA used software to process the data. LG, HD and YG performed experiments, wrote the manuscript and supervised the study. HD and YG collected data and reviewed and edited the manuscript. YD and CL analyzed and interpreted the data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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