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Original Article

MiR-139-5p protect against myocardial ischemia and reperfusion (I/R) injury by targeting autophagy-related 4D and inhibiting AMPK/mTOR/ULK1 pathway

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Abstract: This study aimed at investigating the effect and underlying mechanism of miR-139-5p in myocardial ischemia and reperfusion (I/R) injury. A hypoxia/ reoxygenation (H/R) model was established in H9c2 cardiomyocytes. The level of miR-139-5p was detected in H/R-treated cardiomyocytes, and subsequently, the level of miR-139-5p or its target gene autophagy-related 4D (ATG4D) was up- or downregulated. Furthermore, the cell viability, apoptosis, and autophagy, as well as the expression levels of the proteins related to adenosine 5'-monophosphate-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR)/uncoordinated 51-like kinase 1 (ULK1) signaling pathway were determined. The MiR-139-5p was downregulated in H/R-treated cardiomyocytes in comparison to the untreated cells ($P < 0.05$). H/R treatment significantly decreased the cell viability but increased the cell apoptosis ratio, and autophagy-related proteins levels ($P < 0.05$). The overexpression of MiR-139-5p significantly promoted cell apoptosis and inhibited cell autophagy induced by H/R ($P < 0.05$); however, the effects of miR-139-5p on cell apoptosis and cell autophagy were inhibited by its target gene ATG4D ($P < 0.05$). Furthermore, the upregulated miR-139-5p remarkably inhibited the expression of p-AMPK, p-Raptor, and ULK1, but increased that of p-mTOR ($P < 0.05$) in H/R-treated cardiomyocytes. The MiR-139-5p has the potential of regulating cell apoptosis and cell autophagy by inhibiting AMPK/mTOR/ULK1 signaling pathway and thereby protecting against myocardial I/R injury.

Keywords: miR-139-5p, ischemia and reperfusion injury, hypoxia/reoxygenation, cell autophagy

Introduction

Myocardial ischemia is one of the most common cardiovascular diseases. It is caused by reduced hemoperfusion and may lead to reduced oxygen supply to the heart and abnormal myocardial energy metabolism [1]. Myocardial ischemia and reperfusion (I/R) injury eventually contribute to myocardial cells damage, such as oxidative stress, inflammation, and apoptosis [2]. Myocardial I/R injury may sometimes be caused by various treatments for acute coronary artery diseases, such as stenting to restore myocardial blood flow, direct coronary intervention, and thrombolysis [3]. Although considerable progress has been made in the development of therapeutic strategies for myocardial I/R injury, the complexities of pathogenesis may limit the success of its therapeutic effects [4]. Therefore, it is imperative to further understand the underlying mechanism and search for effective therapeutic targets for myocardial I/R injury.

Recently, microRNAs (miRNAs), small non-coding RNAs, have been reported to play a significant role in regulating oncogenesis *via* targeting the gene expressions and pathways related to cancer [5]. The reports suggest that miRNAs are involved in immune response, inflammation reaction, infection, and cell metabolism, growth, and migration [6, 7]. Several studies have focused on the potential of miRNAs in various diseases such as cancers, autoimmune diseases, neurodegenerative diseases, and cardiovascular diseases [8]. Therefore, understanding the underlying mechanisms of miRNAs in myocardial I/R injury would contribute to search for an effective treatment for myocardial I/R injury.

Table 1. Primer sequences for specific genes

Gene	Primer sequence
Caspase-3 forward	5'-TGTCATCTCGCTCTGGTACG-3'
Caspase-3 reverse	5'-AAATGACCCCTTCATCACCA-3'
Beclin-1 forward	5'-GAGGGATGGAAGGGTCTAAG-3'
Beclin-1 reverse	5'-GCCTGGGCTGTGGTAAGT-3'
LC3II forward	5'-GATGTCCGACTTATTCGAGAGC -3'
LC3II reverse	5'-TTGAGCTGTAAGCGCCTTCTA -3'
Bcl-2 forward	5'-CTGGTGGACAACATCGCTCTG-3'
Bcl-2 reverse	5'-GGTCTGCTGACCTCACTTGTG-3'
ATG4D forward	5'-TCTATGCTGGAGACAGGAAGG -3'
ATG4D reverse	5'-CCAAGGTGAACATGGGGTAC -3'
GAPDH forward	5'-GAAGGTGAAGGTCGGAGTC-3'
GAPDH reverse	5'-GAAGATGGTGATGGGATTTC-3'

ATG4D, autophagy-related 4D; GAPDH, glyceraldehyde-3-phosphate dehydrogenas

It has been shown that miR-139-5p exerts the anti-cancer effect in various type of cancers, such as hepatocellular carcinoma, breast cancer, and colorectal cancer [9], and it is considered as a promising biomarker for cancer diagnostics [10]. MiR-139-5p has been proven to be associated with cell proliferation, apoptosis, invasion, and metastasis [11]. Notably, down-regulated miR-139-5p has been found to be associated with acute human left ventricular ischemia [12]. However, the role and mechanism of miR-139-5p in myocardial I/R injury are still unclear.

In the present study, cardiomyocyte hypoxia/reoxygenation (H/R) model was deployed to investigate the effect and underlying mechanism of miR-139-5p in myocardial I/R injury. The level of miR-139-5p was detected in cardiomyocyte with H/R, and the effects of miR-139-5p or aberrant expression of its target gene autophagy-related 4D (ATG4D) were evaluated on cell viability, cell apoptosis, and cell autophagy, as well as the expression levels of proteins related to adenosine 5'-monophosphate-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR)/uncoordinated 51-like kinase 1 (ULK1) signaling pathway.

Materials and methods

Cell culture and treatment

The H9c2 cardiomyocytes (Cell Bank of Chinese Academy of Sciences, Shanghai, China) were maintained in Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (1:1, Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS). To induce cell H/R model, H9c2 cardiomyocytes cell cultures were maintained in serum-free DMEM under 5% CO_2 for 12 h at 37°C, followed by 4-h incubation in 1% O_2/94% N_2 /5% CO_2 at 37°C. Thereafter, H9c2 cardiomyocytes were cultured for 3 h in normal condition (DMEM containing 10% FBS, 37°C, 5% CO_2). In order to evaluate the role of miR-139-5p in H/R-treated cells, the H9c2 cells were transfected with mimic NC, miR-139-5p mimic, inhibitor NC, miR-139-5p inhibitor, pcDNA3.1, pcDNA-ATG4D, siNC, or si-ATG4D using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Cell proliferation assays

The H9c2 cardiomyocytes (5×10^3 cells/well) were grown in 96-well plates and subjected to the above-mentioned treatments for 48 h. Cell Counting Kit-8 (CCK-8, Dojindo Laboratory, Kumamoto, Japan) was used to detect the cell viability. Cells were treated with 10% WST-8 for 4 h at 37°C, and the absorbance at 450 nm was measured using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Apoptosis assay

Cell apoptosis was detected by Annexin V-FITC/PI Apoptosis Detection kit (Beijing Biosea Biotechnology, Beijing, China). The treated cells were harvested by centrifugation (1,500 rpm, 6 min), and resuspended in 1X binding buffer. In turn, FITC-Annexin V and PI were added into resuspended cells and incubated for 15 min in the dark at 25°C. Finally, the cells were detected using flow cytometer (BD, San Diego, CA, USA).

Dual luciferase reporter assay

The wildtype (WT) 3'UTR fragment of ATG4D that can bind to miR-139-5p or mutant 3'UTR fragment was amplified from the genomic DNA and subsequently cloned into the pGL3 expression vector. The H9c2 cardiomyocytes were cotransfected with miR-139-5p mimic and luciferase reporter plasmid vector harboring wild-type or mutant ATG4D for 48 h using Lipofectamine 2000. The Dual Luciferase Assay kit (Promega) was used to measure the luciferase activity.

Quantitative reverse transcription (qRT) PCR

After various treatments, total RNA was extracted using Trizol (Invitrogen) and complementary DNA (cDNA) synthesis was carried out using microRNA specific primers (Invitrogen). The PCR primers for miR-139-5p and U6 were commercially obtained from Applied Biosystems (Foster City, CA, USA). The primersfor Caspase-3, Bcl-2, Beclin-1, LC3II, ATG-4D, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are shown in **Table 1**. The PCR conditions were set as follows: 95°C for 10 min, 40 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 15 s. U6 or GAPDH was used as the reference genes and the relative gene expression level was calculated using comparative threshold (*C*t) cycle method ($2^{-\Delta\Delta}$*C*t). All the experiments were performed in triplicate.

Western blotting

The total cellular protein was extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology, China) and concentration was measured using the BCA Protein Quantitative Assay (Beyotime Institute of Biotechnology). Total 50 *μg* protein sample (per lane) was separated on an SDS-PAGE gel, blotted onto PVDF membranes, and blocked in 5% nonfat milk for 1 h. The membranes were probed with mouse anti-Caspase-3, Bcl-2, Beclin-1, LC3II/LC3I, ATG4D, AMPK, phospho-AMPK (p-AMPK), p-Raptor, Raptor, mTOR, p-mTOR, and ULK1 polyclonal antibodies (1:500, Santa Cruz) and mouse anti-GAPDH monoclonal antibody (1:2000, Sigma) overnight at 4°C, respectively. After three washes with PBS, the membranes were incubated with appropriate IgG (H+L)-HRP (1:5000, Santa Cruz) second antibody for 2 h at room temperature. Ultimately, the proteins were detected with Enhanced chemiluminescence (Millipore, Bedford, MA, USA).

Statistical analysis

Statistical analysis was carried out using statistical analysis software (SPSS 19.0, SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± S.D. and analyzed by one-way analysis of variance (ANOVA). A *P*-value of < 0.05 was considered to indicate a statistically significant result.

Results

Effect of H9c2 cardiomyocytes with H/R treatment on cell viability, apoptosis, autophagy, and miR-139-5p level

After treatment of H/R, the cell viability was significantly reduced, and the ratio of cell apoptosis was noticeably increased as compared to the untreated cells (all $P < 0.05$, **Figure 1A**, **1B**). The expression levels of apoptosis-related proteins such as cleaved-Caspase-3 and Bcl-2 were analyzed. The increased levels of cleaved-Caspase-3 but reduced levels of Bcl-2 were recorded in H/R-treated cells against the untreated cells (all $P < 0.05$, **Figure 1C**). Meanwhile, the expression levels of autophagy-related proteins, including Beclin-1 and LC3II/LC3I, were remarkably higher in H/R-treated cells than the untreated ones (all $P < 0.05$, **Figure 1C**). In addition, this study also revealed that H/R treatment significantly inhibited miR-139-5p level compared with untreated cells (all $P < 0.01$, **Figure 1D**).

Effects of miR-139-5p aberrant expression on cell apoptosis and autophagy in H/R-treated H9c2 cardiomyocytes

As shown in **Figure 2A**, the miR-139-5p level was significantly increased in cells with miR-139-5p mimic compared to the mimic control ($P < 0.01$), and the transfection of miR-139-5p inhibitor significantly inhibited the miR-4262 level compared to that of inhibitor control ($P < 0.01$). The results revealed that the ratio of cell apoptosis was substantially increased in the cells treated with H/R and miR-139-5p mimic as compared to the H/R + mimic control treated cells ($P < 0.05$, **Figure 2B**). The expression of cleaved-Caspase-3 was significantly increased, but on the contrary, the Bcl-2 level was noticeably suppressed in the cells with H/R + miR-139-5p mimic compared with H/R + mimic control treated cells ($P < 0.05$, **Figure 2C**). In addition, the expression levels of Beclin-1 and LC3II/LC3I were remarkably lower in the cells treated with H/R and miR-139-5p than those in H/R + mimic control treated cells ($P < 0.01$, **Figure 2C**). Consistently, the cells treated with the H/R and miR-139-5p inhibitor exhibited the opposite effects on cell apoptosis and autophagy (all $P < 0.05$, **Figure 2B**, **2C**).

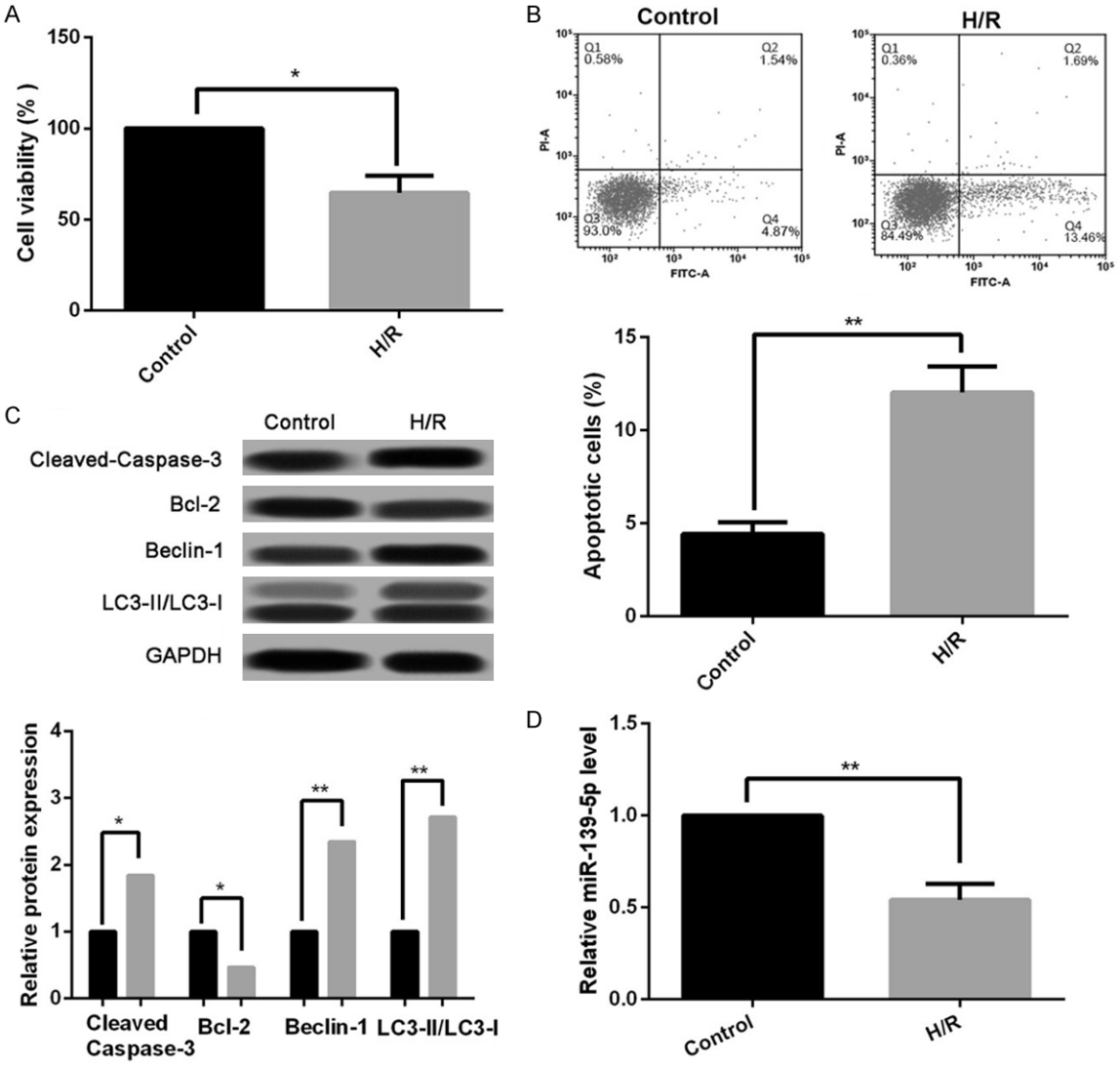


Figure 1. H/R induces hypoxia injury in H9c2 cells. A. The cell viability in untreated cells and H/R-treated cells using CCK-8; B. The cell apoptosis in untreated cells and H/R-treated cells using Annexin V-FITC/PI Apoptosis Detection kit; C. The expression of apoptosis-related proteins, such as cleaved-Caspase-3 and Bcl-2 as well as autophagy-related proteins, including Beclin-1 and LC3II/LC3I, in untreated cells and H/R-treated cells using the western blotting; D. The level of miR-139-5p in untreated cells and H/R-treated cells using quantitative reverse transcription PCR. *P < 0.05, and **P < 0.01. H/R, hypoxia/reoxygenation.

Confirmation of the targeting effects of miR-139-5p

Sequence analysis revealed that ATG4D was a potential target gene of miR-4262 (**Figure 3A**). H/R treatment significantly increased the expression level of ATG4D compared with untreated cells (P < 0.05, **Figure 3B**). Besides, the luciferase reporter assay also showed that miR139-5p mimic significantly inhibited the luciferase activity in WT cells (P < 0.05) but not that of the mutant ATG4D compared with untreated cells (**Figure 3C**). In addition, compared to the cells with respective controls, miR-139-5p mimic or inhibitor significantly up or down regulated the expression of ATG4D (P < 0.01, **Figure 3C**).

Effects of aberrant expression of ATG4D on cell apoptosis and autophagy in H/R-treated H9c2 cardiomyocytes

As shown in **Figure 4A**, the protein level of ATG4D was significantly higher in cells with pcDNA-ATG4D than those with pcDNA (P < 0.001), and the transfection of si-ATG4D re-

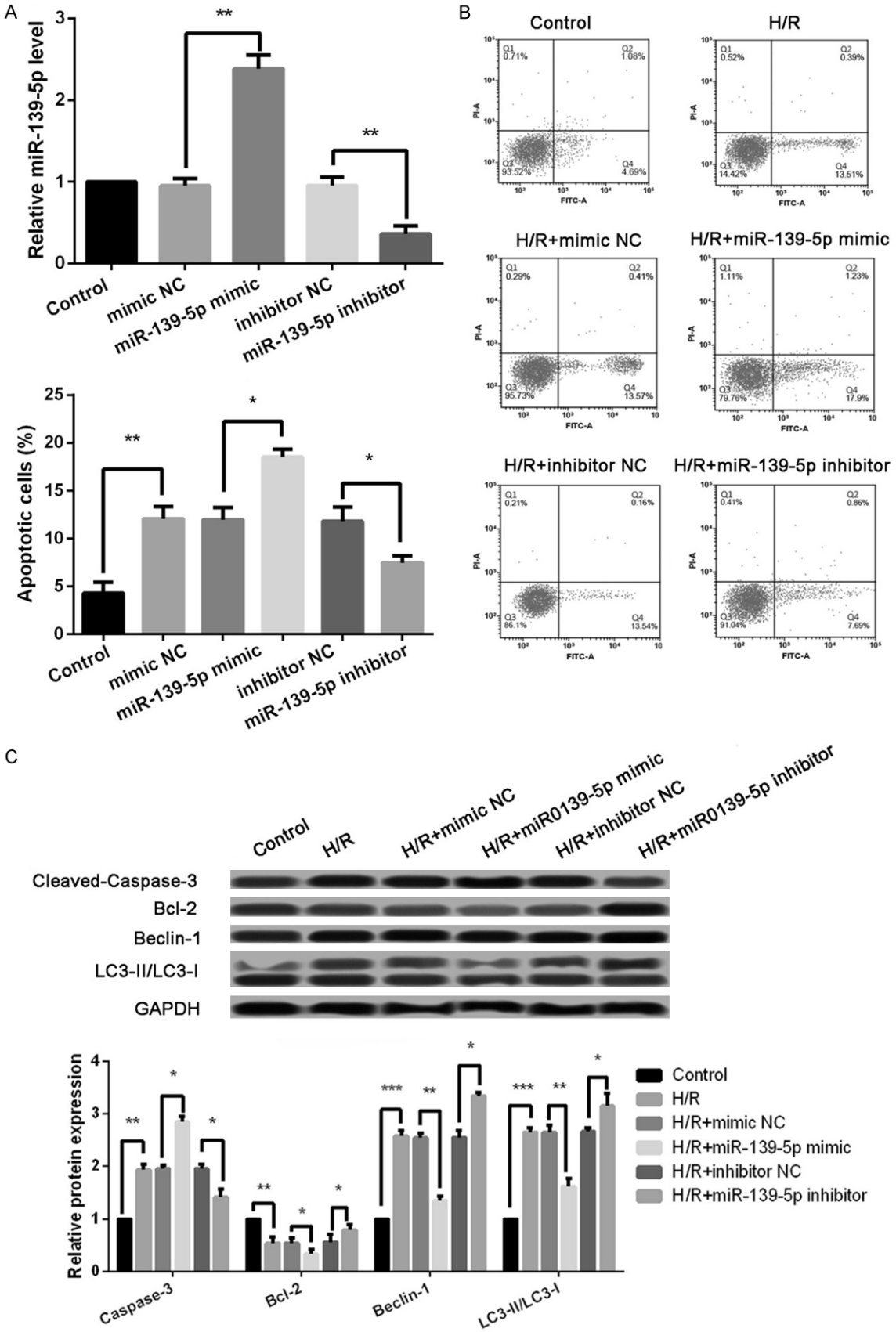


Figure 2. Upregulated miR-139-5p increases cell apoptosis and decreases cell autophagy in H/R -treated H9c2 cells. A. The miR-139-5p level in untreated cells, cells with mimic NC, miR-139-5p mimic, inhibitor NC, and miR-

139-5p inhibitor by quantitative reverse transcription PCR; B. The cell apoptosis in untreated cells, cells with mimic NC, miR-139-5p mimic, inhibitor NC, and miR-139-5p inhibitor using Annexin V-FITC/PI Apoptosis Detection kit; C. The expression of apoptosis-related proteins, such as cleaved-Caspase-3 and Bcl-2 as well as autophagy-related proteins, including Beclin-1 and LC3II/LC3I, in untreated cells, cells with mimic NC, miR-139-5p mimic, inhibitor NC, and miR-139-5p inhibitor using the western blotting. *P < 0.05, **P < 0.01, and ***P < 0.001. H/R, hypoxia/reoxygenation.

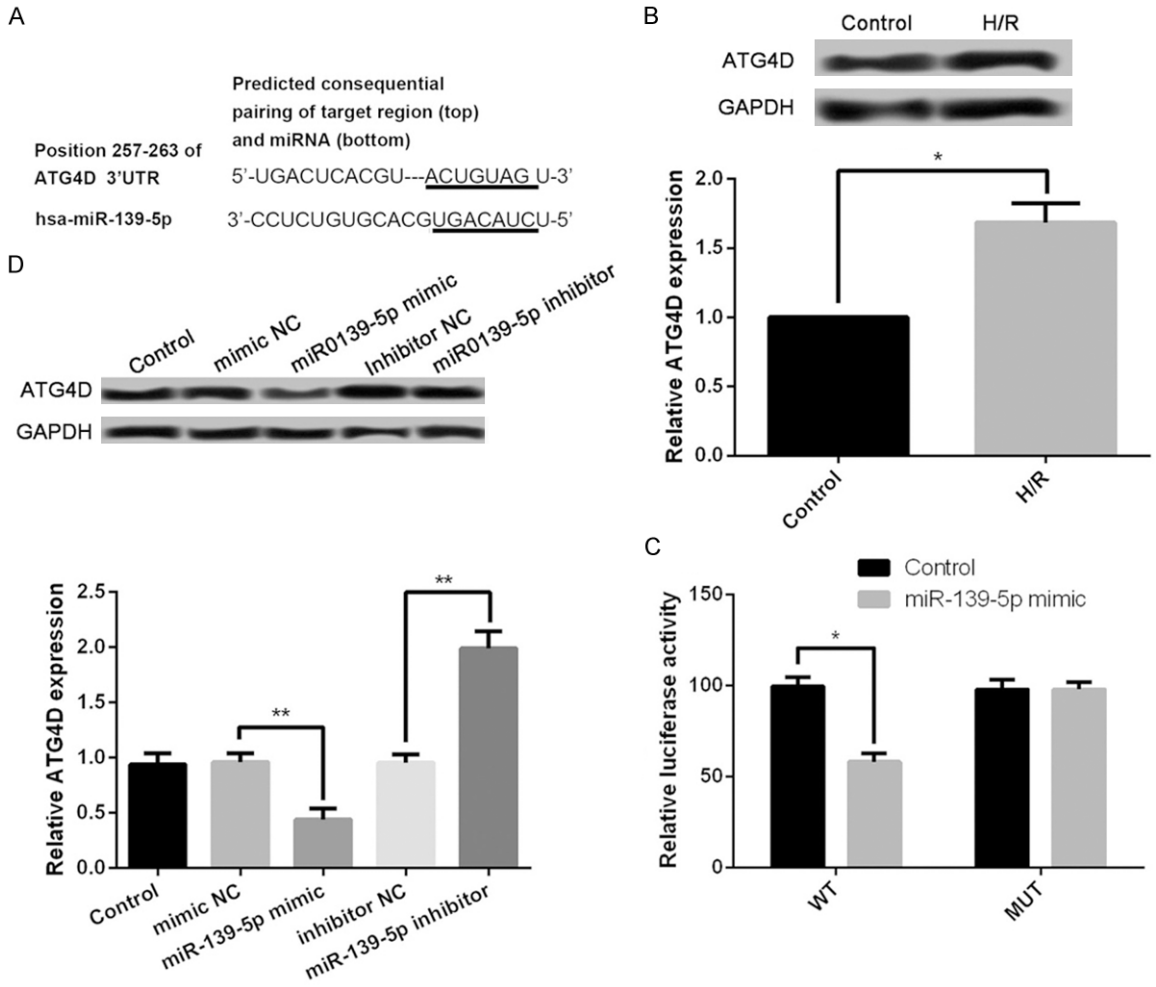
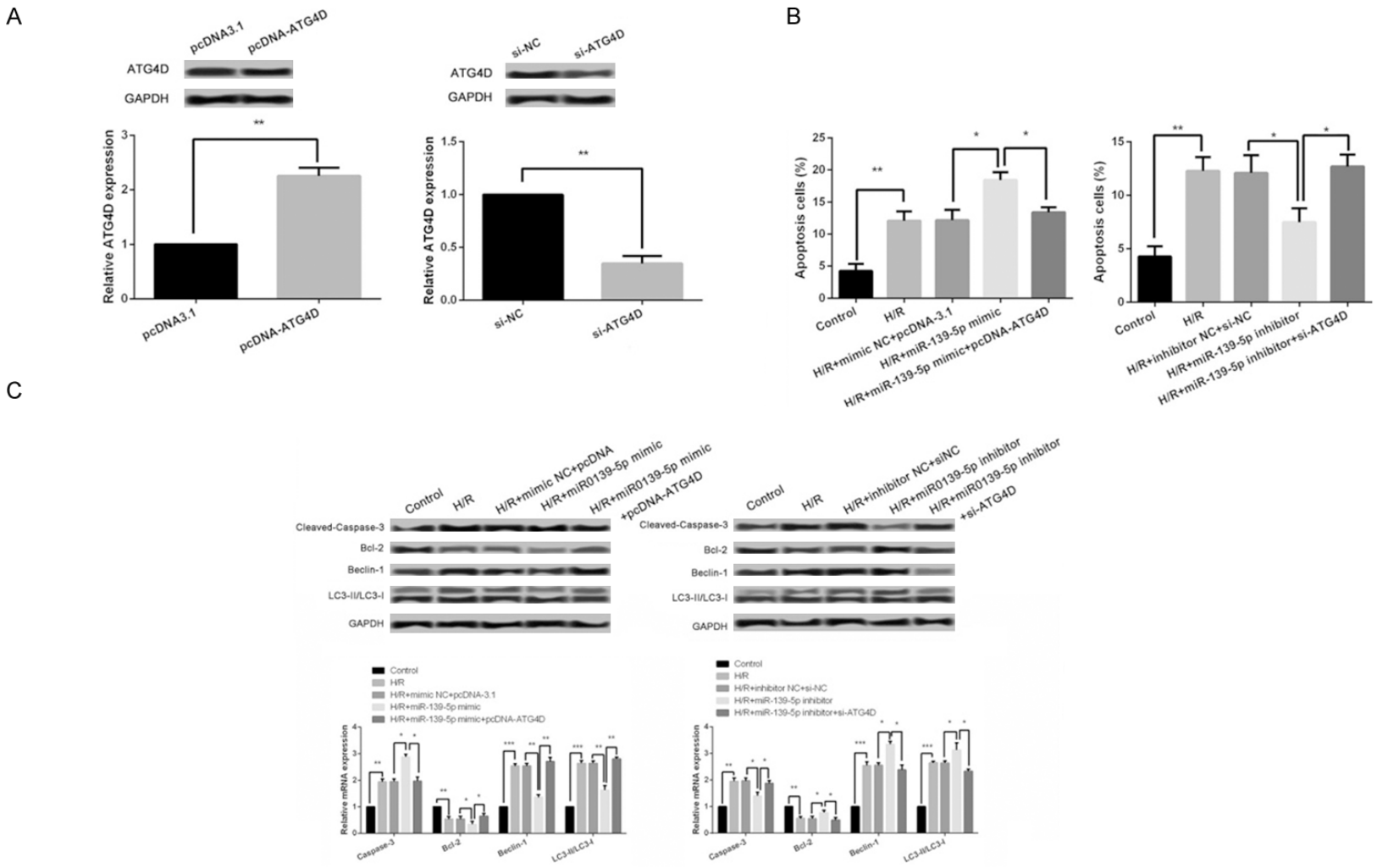
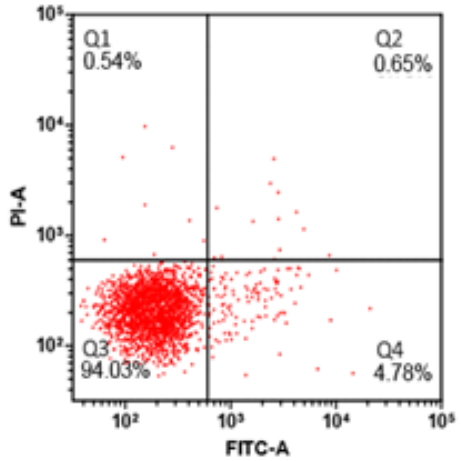


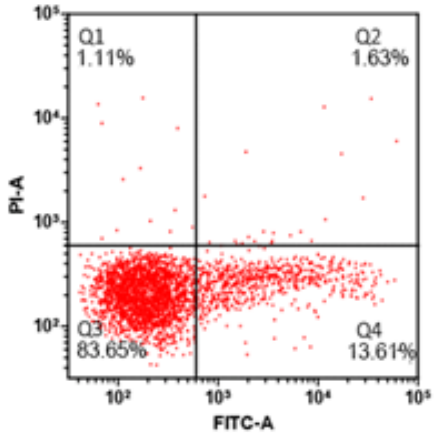
Figure 3. ATG4D as a potential target gene of miR-139-5p. A. Prediction of the target gene of miR-139-5p by sequence analysis; B. The level of ATG4D in untreated cells and H/R-treated cells using the western blotting; C. Luciferase activity in cells with wild type (WT) and mutant SIRT1 using the luciferase reporter assay; D. ATG4D level in untreated cells, cells with mimic NC, miR-139-5p mimic, inhibitor NC, and miR-139-5p inhibitor by the western blotting. *P < 0.05, and **P < 0.01. H/R, hypoxia/reoxygenation; ATG4D, autophagy-related 4D.

markably reduced the ATG4D level compared with the transfection of si-NC (P < 0.01). The results revealed that compared to the cells with H/R and miR-139-5p mimic, the ratio of cell apoptosis was significantly reduced in cells with the treatments of H/R, miR-139-5p mimic and pcDNA-ATG4D (P < 0.05, **Figure 4B**); whereas as compared to the cells with H/R and miR-139-5p inhibitor, the ratio of cell apoptosis was dramatically increased in cells with the treatments of H/R, miR-139-5p inhibitor and si-ATG4D (P < 0.05, **Figure 4B**). The expression of cleaved-Caspase-3 was significantly inhibited, and the levels of Bcl-2, Beclin-1 and LC3II/LC3I were notably increased in the cells with the treatments of H/R, miR-139-5p mimic and pcDNA-ATG4D compared with H/R + miR-139-5p mimic treated cells (P < 0.05, **Figure 4C**); while the expression levels of these proteins were contrasting in the cells cotreated with H/R,

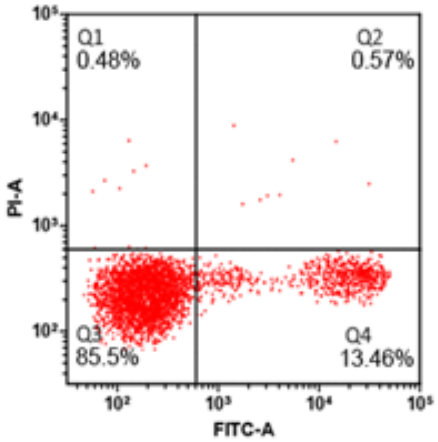

A
pcDNA3.1
pcDNA-ATG4D
ATG4D
GAPDH
Relative ATG4D expression
si-NC
si-ATG4D
B
Apoptosis cells (%)
Control
H/R
H/R+mimic NC+pcDNA-3.1
H/R+miR-139-5p mimic
H/R+miR-139-5p mimic+pcDNA-ATG4D
H/R+inhibitor NC+si-NC
H/R+miR-139-5p inhibitor
H/R+miR-139-5p inhibitor+si-ATG4D
C
Cleaved-Caspase-3
Bcl-2
Beclin-1
LC3-II/LC3-I
Relative mRNA expression
Caspase-3
Bcl-2
Beclin-1
LC3-II/LC3-I



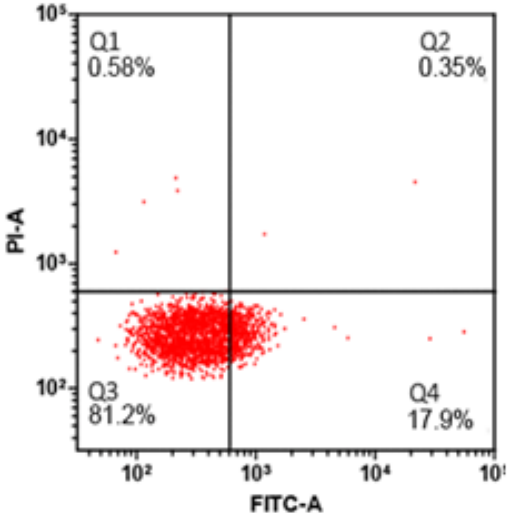
Control



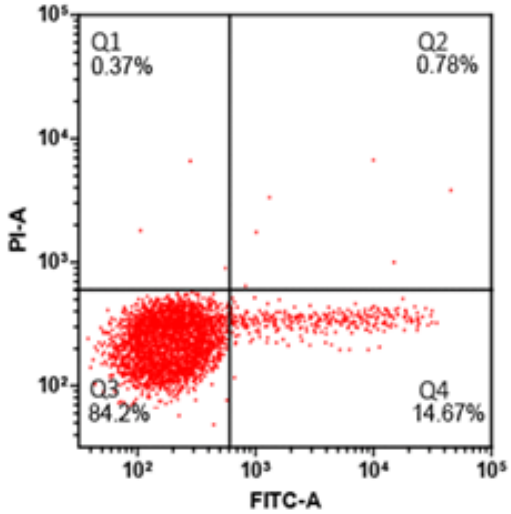
H/R



H/R+ mimic NC
+pcDNA3.1



H/R+ miR-139-5p
mimic



H/R+ miR-139-5p mimic
+pcDNA-ATG4D

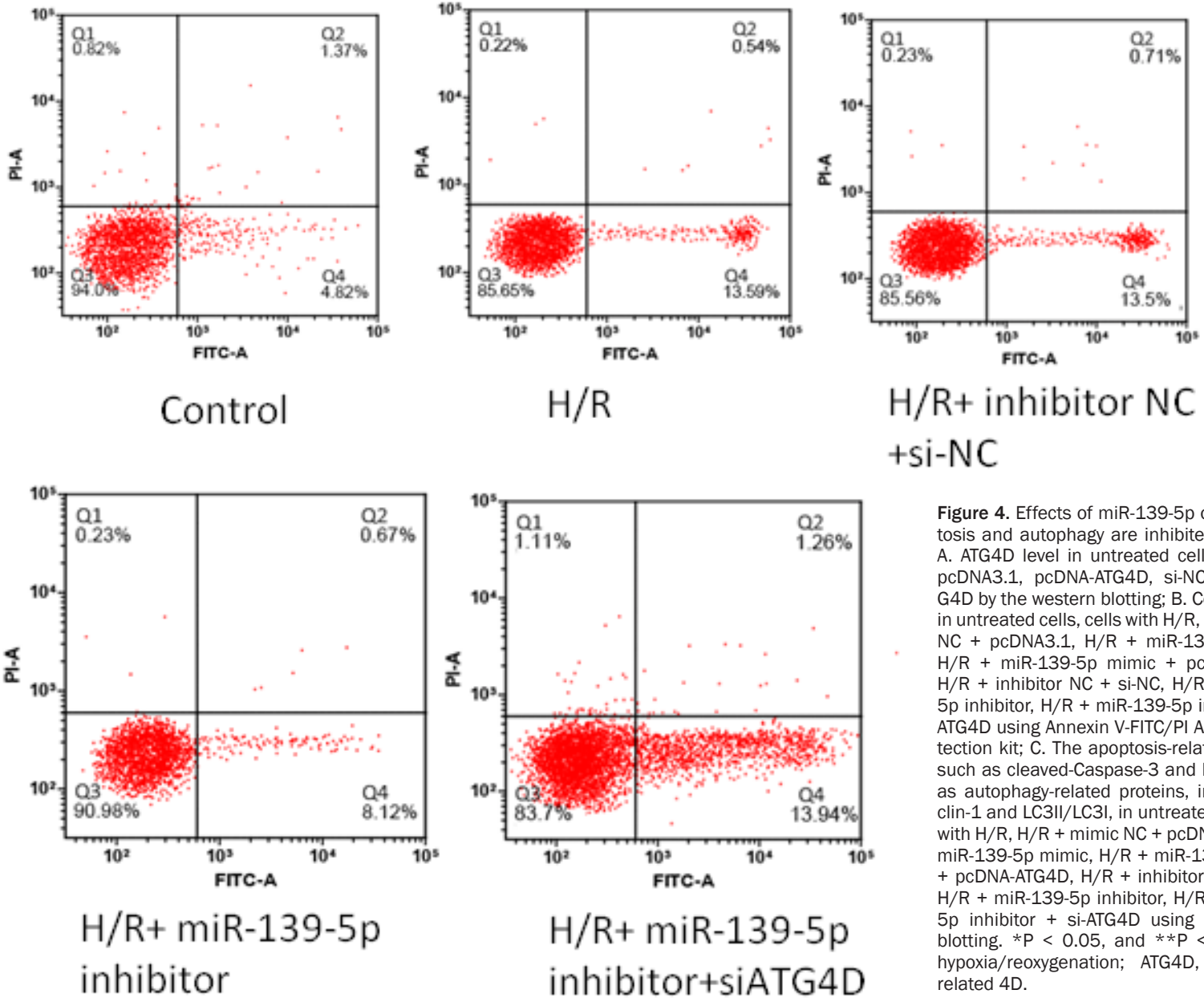


Figure 4. Effects of miR-139-5p on cell apoptosis and autophagy are inhibited by ATG4D. A. ATG4D level in untreated cells, cells with pcDNA3.1, pcDNA-ATG4D, si-NC, and si-ATG4D by the western blotting; B. Cell apoptosis in untreated cells, cells with H/R, H/R + mimic NC + pcDNA3.1, H/R + miR-139-5p mimic, H/R + miR-139-5p mimic + pcDNA-ATG4D, H/R + inhibitor NC + si-NC, H/R + miR-139-5p inhibitor, H/R + miR-139-5p inhibitor + si-ATG4D using Annexin V-FITC/PI Apoptosis Detection kit; C. The apoptosis-related proteins, such as cleaved-Caspase-3 and Bcl-2 as well as autophagy-related proteins, including Beclin-1 and LC3II/LC3I, in untreated cells, cells with H/R, H/R + mimic NC + pcDNA3.1, H/R + miR-139-5p mimic, H/R + miR-139-5p mimic + pcDNA-ATG4D, H/R + inhibitor NC + si-NC, H/R + miR-139-5p inhibitor, H/R + miR-139-5p inhibitor + si-ATG4D using the western blotting. *P < 0.05, and **P < 0.01. H/R, hypoxia/reoxygenation; ATG4D, autophagy-related 4D.

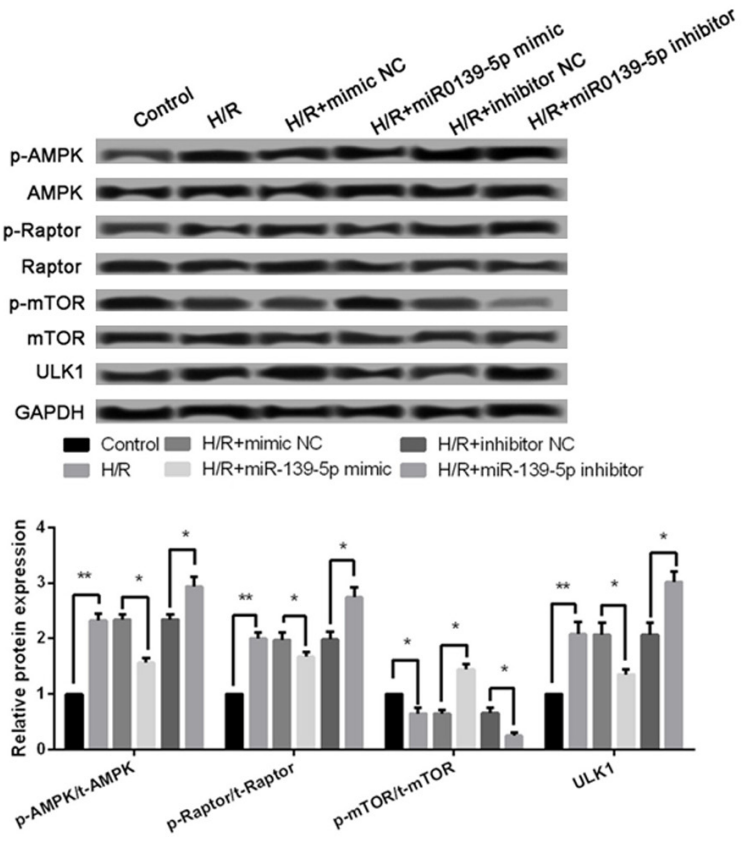


Figure 5. Upregulated miR-139-5p inhibits the AMPK/mTOR/ULK1 signaling pathway in H/R-treated H9c2 cells. Expression levels of p-AMPK, p-Raptor, p-mTOR, and ULK1 in untreated cells, cells with mimic NC, miR-139-5p mimic, inhibitor NC, and miR-139-5p inhibitor using the western blotting. *P < 0.05, and **P < 0.01. H/R, hypoxia/reoxygenation; AMPK, adenosine 5'-monophosphate-activated protein kinas; mTOR, mammalian target of rapamycin; ULK1, uncoordinated 51-like kinase.

miR-139-5p inhibitor, and si-ATG4D than those in the cells with H/R + miR-139-5p inhibitor (P < 0.05, **Figure 4C**).

Effects of miR-139-5p on AMPK/mTOR/ULK1 signaling pathway in H/R-treated H9c2 cardiomyocytes

As compared to the untreated cells, the expression levels of p-AMPK, p-Raptor, and ULK1 was significantly enhanced, while the p-mTOR level was inhibited in H/R-treated cells (P < 0.05, **Figure 5**). The co-treatments of H/R and miR-139-5p mimic inhibited the levels of p-AMPK, p-Raptor, and ULK1 but increased that of p-mTOR compared with H/R and mimic NC treated cells (P < 0.05, **Figure 5**). Conversely, miR-139-5p inhibitor posed an opposite role in the expression levels of p-AMPK, p-Raptor, ULK1, and p-mTOR (P < 0.05, **Figure 5**).

Discussion

The current study showed that H/R treatment decreased the cell viability but increased the cell apoptosis and autophagy in H9c2 cardiomyocytes. The expression of miR-139-5p was down regulated in cardiomyocytes with H/R treatment, and over-expressed miR-139-5p significantly promoted cell apoptosis and inhibited cell autophagy induced by H/R; however, its target gene ATG4D was able to reverse the effects of over-expressed miR-139-5p on cell apoptosis and autophagy in H/R-treated cardiomyocytes. Furthermore, H/R treatment enhanced the expression levels of p-AMPK, p-Raptor, and ULK1, and inhibited p-mTOR level, while the upregulated miR-139-5p considerably reversed the expression levels of p-AMPK, p-Raptor, ULK1, and p-mTOR.

Several studies have demonstrated that miRNAs play a vital role in the regulation of myocardial I/R injury, including miR-21, miR-29, miR-92a, and miR-320 [13]. In the current study, the downregulation of miR-139-5p was recorded in H/R-treated cardiomyocytes. Few studies have investigated the roles of miR-139-5p in myocardial I/R injury. In a previous report, it was shown that miR-139-5p could attenuate brain damage induced by hypoxia-ischemia through targeting human growth transformation dependent protein in neonatal rats [14]. Our study exhibited that miR-139-5p increased cleaved-Caspase-3 level and reduced Bcl-2 level in H/R-treated cardiomyocytes. The Bcl-2 protein family, which includes Bcl-2 and Bcl-xL (anti-apoptotic proteins), as well as Bax and Bak (pro-apoptotic proteins), played a regulatory role in cell apoptosis [15]. Under normal conditions, the expressions of Bcl-2 and Bax were balanced. However, under aberrant conditions, the over-expressed Bcl-2 dissociated Bax dimers and promoted the formation of Bax and Bcl-2 complexes, thereby

inhibited cell apoptosis, and upregulated Bax, increased the number of Bax dimers, and consequently promoted cell apoptosis [15]. The activation of Caspase-3 was also considered as an important step in the classical pathway of cell apoptosis [16]. These results suggested that miR-139-5p increased cell apoptosis in H/R-treated cardiomyocytes. An earlier study demonstrated that autophagy is enhanced during cardiac ischemia-reperfusion (I/R). Notably, this study found the reduced levels of autophagy-related proteins, including Beclin-1 and LC3II/LC3I, in H/R and miR-139-5p mimic co-treated cardiomyocytes, suggesting that miR-139-5p might inhibit cell autophagy induced by H/R.

Furthermore, this study confirmed that ATG4D is the potential target gene of miR-139-5p. ATG4D is a member of cysteine aspirate protease ATG4 family and widely expressed in various cellular organizations and organs. A study showed that ATG4D plays a vital role in autophagosome formation [17]. Beclin1, a mammalian ortholog of yeast ATG6, was upregulated during myocardial I/R injury *in vitro* [18]. A study reported that autophagic response to nutrient deficiency could be mediated by Beclin1 *via* regulating Bcl-2 level in cardiac cells [19]. In addition, reactive oxygen species (ROS) were considered to regulate Beclin1 expression and further inhibited ATG4 activity and promoted LC3 lipidation [17]. Bnip-3, as a member of Bcl-2 family, was reported to participate in the upregulation of autophagy in cardiac I/R [20]. All these findings point toward the possible regulatory role of miR-139-5p in cell apoptosis and autophagy by inhibiting ATG4D expression in H/R-treated cardiomyocytes.

In order to further investigate the mechanism of miR-139-5p in myocardial I/R injury, the study was focused on dissecting the AMPK/mTOR/ULK1 signaling pathway. AMPK/mTOR/ULK1 signaling pathway has been proved to be involved in the regulation of processes related to autophagy [21]. The AMPK activation was corroborated and mediated the autophagy during the initial phase of cardiac ischemia [22]. The activated AMPK induced cell autophagy by regulating the phosphorylation of ULK1 directly or indirectly [23]. It has been reported that AMPK induces autophagy through inhibiting the activation of mTOR via phosphorylation of Raptor site and subsequent activation of ULK1 [21]. The present study reported that the upregulated miR-139-5p inhibited the expression of p-AMPK, p-Raptor and ULK1, but increased that of p-mTOR in H/R-treated cardiomyocytes. Therefore, we anticipated that the role of miR-139-5p overexpression in H/R-treated cardiomyocytes might be mediated by the activation of AMPK/mTOR/ULK1 signaling pathway.

The present study postulates that miR-139-5p may protect against myocardial I/R injury by regulating cell apoptosis and cell autophagy through inhibiting the AMPK/mTOR/ULK1 signaling pathway.

Disclosure of conflict of interest

None.

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