Hyperoside protects cardiomyocytes against hypoxia-induced injury via upregulation of microRNA-138

SIYI HE[1], XIAOQIANG YIN[1,2], FAN WU[1], SHAOJIE ZENG[3], FENG GAO[1], MEI XIN[1], JIAN WANG[1], JIE CHEN[1], LE ZHANG[4] and JINBAO ZHANG[1]

[1]Department of Cardiovascular Surgery, General Hospital of Western Theater Command, Chengdu, Sichuan 610083; [2]Department of Graduate Student, North Sichuan Medical College, Nanchong, Sichuan 637199; [3]Medical Team, Unit 95437, People's Liberation Army, Nanchong, Sichuan 637100; [4]National Drug Clinical Trial Institution, Second Affiliated Hospital, Army Medical University, Chongqing 400037, P.R. China

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Abstract. Following hypoxia, cardiomyocytes are susceptible to damage, against which microRNA (miR)-138 may act protectively. Hyperoside (Hyp) is a Chinese herbal medicine with multiple biological functions that serve an important role in cardiovascular disease. The aim of the present study was to investigate the role of Hyp in hypoxic cardiomyocytes and its effect on miR-138. A hypoxia model was established in both H9C2 cells and C57BL/6 mice, which were stimulated by Hyp. The expression levels of miR-138 were increased in the hypoxic myocardium in the presence of Hyp at concentrations of >50 μmol/l *in vivo* and >50 mg/kg *in vitro*. Using Cell Counting Kit-8 and 5-ethynyl-2'-deoxyuridine assays, it was observed that Hyp improved hypoxia-induced impairment of cell proliferation. Cell apoptosis was evaluated by flow cytometry and a TUNEL assay. The number of apoptotic cells in the Hyp group was lower than that in the control group. As markers of myocardial injury, the levels of lactate dehydrogenase, creatine kinase-myocardial band isoenzyme and malondialdehyde were decreased in the Hyp group compared with the control group, whereas the levels of superoxide dismutase were increased. A marked decrease in the levels of cleaved caspase-3 and cleaved poly(ADP) ribose polymerase and a marked increase in expression levels of Bcl-2 were observed in the presence of Hyp. However, miR-138 inhibition by antagomir attenuated the protective effects of Hyp. Furthermore, Hyp treatment was associated with marked downregulation of mixed lineage kinase 3 and lipocalin-2, but not pyruvate dehydrogenase kinase 1, in hypoxic H9C2 cells. These findings demonstrated that Hyp may be beneficial for myocardial cell survival and may alleviate hypoxic injury via upregulation of miR-138, thereby representing a promising potential strategy for clinical cardioprotection.

Correspondence to: Dr Jinbao Zhang, Department of Cardiovascular Surgery, General Hospital of Western Theater Command, 270 Rongdu Avenue, Jinniu, Chengdu, Sichuan 610083, P.R. China
E-mail: jinbaozhang001@163.com

Dr Le Zhang, National Drug Clinical Trial Institution, Second Affiliated Hospital, Army Medical University, 183 Xinqiao Main Street, Chongqing 400037, P.R. China
E-mail: zhanglexq@163.com

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Introduction

A number of conditions cause cardiomyocyte damage due to lack of oxygen, such as coronary and cyanotic congenital heart disease, high altitude and cardiopulmonary bypass. The heart is one of the organs most sensitive to lack of oxygen (1). Following hypoxia, cardiomyocytes are susceptible to necrosis and apoptosis via multiple signaling pathways, thus leading to cardiac dysfunction and poor clinical prognosis (2). Therefore, effective strategies to protect cardiomyocytes against hypoxia-induced injury are required.

Traditional Chinese medicine has attracted increasing attention in cardiovascular medicine research due to its potential ability to target a number of signaling pathways (3,4). Quercetin -3-O-β-D-galactopyranoside, also known as hyperoside (Hyp), is a flavonoid glycoside extracted from *Hypericum* plants and may exert cytoprotective effects, including antioxidant effects, decreasing calcium overload and inhibiting apoptosis (5). In an ischemia/reperfusion-mediated acute myocardial infarction model, administration of Hyp improved heart contractility and decreased the area of infarcted myocardium via activation of the extracellular signal-regulated kinase 1/2 signaling pathway (6), and has been identified to be associated with high levels of ATP, as well as decreased oxidative stress (7). In addition, Hyp has been demonstrated to attenuate hypoxia/reoxygenation-induced apoptosis in cardiomyocytes by suppressing Bcl-2 interacting protein 3 expression (8). However, the role of Hyp in the hypoxic heart and its potential underlying regulatory mechanism have yet to be fully elucidated.

MicroRNAs (miRNAs/miRs) are a type of endogenous small RNA 18-25 nucleotides in length that serve key roles in numerous pathophysiological cell processes, such as

apoptosis, proliferation and inflammation (9). miR-138 has been demonstrated to have notable cardioprotective properties. Our previous study demonstrated that miR-138 is upregulated in chronically hypoxic cardiomyocytes (10). By inhibiting the expression levels of mixed lineage kinase 3 (MLK3) and the phosphorylation of its downstream signaling targets, miR-138 effectively alleviates apoptosis. Similar anti-apoptotic effects of miR-138 on hypoxic cardiomyocytes are achieved via targeting of lipocalin-2 (Lcn2) (11). Pyruvate dehydrogenase kinase 1 (PDK1) has also been demonstrated to be a target protein of miR-138 (11). By inhibiting glycolysis and promoting mitochondrial respiration, increased miR-138 levels improve myocardial viability under prolonged hypoxia (12).

In the present study, the effects of Hyp on the expression levels of miR-138 in hypoxic cardiomyocytes were investigated. miR-138 antagomir transfection was performed to assess the effect of Hyp on hypoxia-induced myocardial injury and its underlying mechanism. The present study aimed to identify a potential therapeutic strategy for chronic heart disease.

Materials and methods

Cell culture and treatment. The embryonic rat cardiomyoblast H9C2 cell line was obtained from American Type Culture Collection and cultured in DMEM with 10% FBS (both Gibco; Thermo Fisher Scientific, Inc.). In the normoxia group, cells were incubated in an atmosphere of 5% CO_2 and 95% air at 37˚C. In the hypoxia group, a Forma™ Series II 3131 incubator (Thermo Fisher Scientific, Inc.) was used to maintain O_2 at 1% by N_2 displacement, and cells were incubated at 37˚C for 24 h. Hyp (molecular weight, 464.38; purity, ≥97%) was purchased from Sigma-Aldrich (Merck KGaA). At the beginning of hypoxia exposure, Hyp was added at various concentrations (1, 10, 50 and 100 μmol/l). Cells in the control group were cultured without the administration of Hyp.

Animal treatment. Furthermore, a total of 100 6-week-old male C57BL/6 mice (weight, 20±2 g) were purchased from Vital River Laboratory Animal Technology Corporation. Overall, a total of 90 mice were included in the present study, as 10 mice were scarified after improper establishment of the experimental model during the process of tail vein injection. Mice were randomly divided into various groups (n=9 per group): i) Control group; ii) 10 mg/kg group; iii) 50 mg/kg group; iv) 100 mg/kg group; v) 200 mg/kg group; vi) normoxia group; vii) hypoxia control group; viii) hypoxia Hyp group; ix) hypoxia Hyp + antagomir group; and x) hypoxia Hyp + antagomir-NC group. All animals were fed with free access to standard chow and tap water under specific pathogen-free conditions with a 12-h light/dark cycle at 22±1˚C and 45-55% relative humidity. In the hypoxia group, mice were housed in a normobaric hypoxic chamber at an oxygen concentration of 10% for 1 week. Hyp (10 mg/ml in dimethyl sulfoxide) was administrated via oral gavage at various doses (10, 50, 100 and 200 mg/kg) daily, while mice in control group were housed without the addition of Hyp. In the normoxia group, animals were maintained in a normobaric chamber at an oxygen concentration of 21% for 1 week. All animal procedures were approved by the Animal Care and Use Committee of the General Hospital of Western Theater Command and Army Medical University (Chengdu, China), and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, 2011; National Institutes of Health) (13).

Reverse transcription-quantitative PCR (RT-qPCR) analysis. Total RNA of myocardial tissues was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RNA was reverse transcribed to cDNA using the PrimeScript RT reagent kit (Takara Bio, Inc.) according to the manufacturer's instructions. Stem-loop primers of miR-138 (cat. no. MQPSCM001-1) and internal control U6 (cat. no. MQPS0000002-1-100) were synthesized by Guangzhou RiboBio Co., Ltd. PCR was performed using the TB Green™ Premix Ex Taq™ GC kit (Takara Bio, Inc.) on the Applied Biosystems® 7500 Sequence Detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. The thermocycling conditions were as follows: Initial denaturation for 30 sec at 95˚C, followed by 40 cycles of 5 sec at 95˚C and 34 sec at 60˚C. Each reaction was run in triplicate. The relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (14).

miRNA antagomir transfection. miR-138 antagomir, a single-stranded RNA for silencing of the expression of miR-138, was synthesized by Genepharm, Inc. Antagomir and matched negative control (NC) were dissolved in DEPC H_2O at 20 μM. H9C2 cells were transfected with miR-138 antagomir (5'-CGGCCCUGAUUCACAACACCAGCU-3') or NC (5'-CAGUACUUUUGUGUAGUACAA-3') at a concentration of 50 nmol/l using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, and incubated at 37˚C for 48 h. C57BL/6 mice were injected with miR-138 antagomir or NC (10 mg/kg) via the tail vein once every 2 days for 2 weeks. Cells and myocardial tissues were collected to detect the expression of miR-138 using RT-qPCR. Following successful establishment of the miR-138 silencing model, cells and mice were stimulated with Hyp and hypoxia as aforementioned.

Western blotting. Antibodies against cleaved caspase-3 (1:1,000; cat. no. AF7022), cleaved poly(ADP)-ribose polymerase (PARP; 1:1,000; cat. no. AF7023), Bcl-2 (1:1,000; cat. no. AF6139) and the internal control GAPDH (1:2,000; cat. no. AF7021) were obtained from Affinity Biosciences. Antibodies against Lcn2 (1:1,000; cat. no. ab216462) and PDK1 (1:1,000; cat. no. ab202468) were obtained from Abcam. The anti-MLK3 antibody (1:1,000; cat. no. sc-166639) was obtained from Santa Cruz Biotechnology, Inc. H9C2 cells and heart tissues were lysed in SDS lysis buffer (Beyotime Institute of Biotechnology) containing protease inhibitor. Protein concentration was detected using a BCA protein assay. Equivalent amounts of proteins (30-100 μg per lane) were loaded and separated via SDS-PAGE on 6-15% gels. After being transferred to PVDF membranes (Roche Diagnostics), the samples were blocked with 5% non-fat milk powder at room temperature for 2 h, and then incubated with primary antibodies at 4˚C overnight. Anti-rabbit (cat. no. SE134) or anti-mouse (cat. no. SE131) secondary antibodies were purchased from Beijing Solarbio

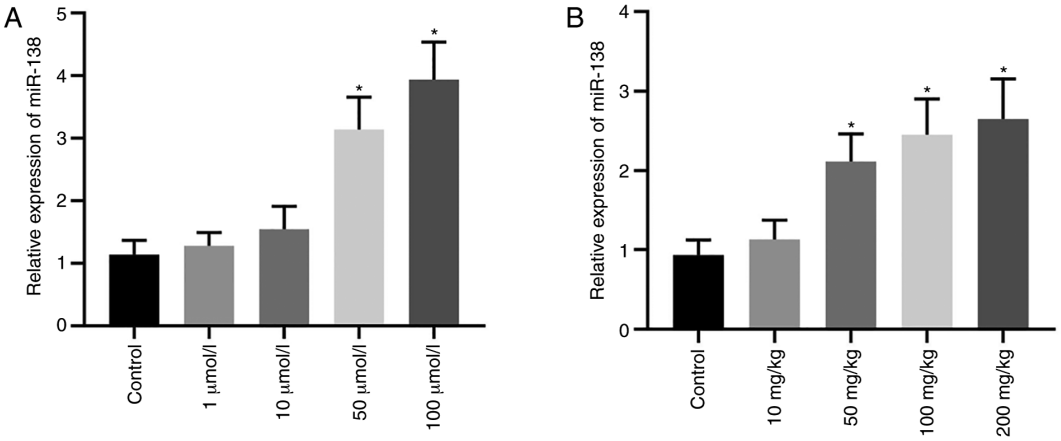

Figure 1. Hyp upregulates the expression levels of miR-138 in hypoxic conditions. (A) Following exposure to hypoxia for 24 h, expression levels of miR-138 in H9C2 cells were increased by Hyp treatment at 50 and 100 μmol/l. (B) C57BL/6 mice were maintained in a hypoxic chamber for 1 week. Hyp (50, 100 and 200 mg/kg) increased the expression levels of miR-138. Data are presented as the mean ± SD (n=9). *P<0.05. Hyp, hyperoside; miR-138, microRNA-138.

Science & Technology Co., Ltd., and diluted in TBS with 0.1% Tween-20 (1:5,000). Subsequently, secondary antibodies were added at room temperature for 1 h and the blots were detected using an ECL kit (Affinity Biosciences). Image-Pro Plus 6.0 software (Media Cybernetics, Inc.) was used for densitometry.

Cell survival assay. Cell viability was evaluated using a Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology). H9C2 cells were seeded in a 96-well plate ($4x10^4$ cells per well) and treated with CCK-8 reagent. After incubation at 37˚C for 2 h, the absorbance was measured at 450 nm. Additionally, a 5-ethynyl-2'-deoxyuridine (EdU) kit (Guangzhou RiboBio Co., Ltd.) was used to assess the cell proliferation ability. EdU labeling medium was added and nuclei were stained with Hoechst 33342 at room temperature for 30 min, according to the manufacturer's protocols. EdU-positive cells were observed under a fluorescence microscope.

Cell apoptosis assay. The ratio of apoptotic H9C2 cells was determined using Annexin V/FITC and PI (BD Biosciences). Cells were harvested and suspended in binding buffer, and then stained with Annexin V for 15 min and PI for 5 min at room temperature in dark conditions. A flow cytometer (FACSCalibur; BD Biosciences) was used to analyze cell apoptosis. Data was calculated using FlowJo software v10.5 (FlowJo LLC). For heart tissue samples from C57BL/6 mice, the apoptosis ratio was determined via a TUNEL assay. The specimens were first fixed with 4% paraformaldehyde at room temperature overnight, and then sliced horizontally at a thickness of 5 μm. Following embedding, deparaffinization and rehydration, the sections were stained at 37˚C for 1 h with the *in situ* Cell Death Detection kit (cat. no. 11684817910; Roche Diagnostics) according to the manufacturer's protocol. Subsequently, nuclei were stained with hematoxylin at room temperature for 10 sec. Sections were sealed with neutral resin. TUNEL-positive cells were visualized using a light microscope in six randomly selected fields (magnification, x400) and are presented as the percentage of total cells counted.

Measurement of myocardial injury markers. After being fed under normoxic or hypoxic conditions for 1 week, mice were sacrificed by cervical dislocation. Immediately, blood samples from the right ventricle were collected by cardiac puncture, and were centrifuged within 30 min to obtain the supernatant serum. The serum was subsequently examined for lactate dehydrogenase (LDH; cat. no. A020) and creatine kinase-MB isoenzyme (CK-MB; cat. no. H197) using ELISA kits (Nanjing Jiancheng Bioengineering Institute). Heart tissue was also quickly obtained after sacrifice and stored in ice-cold PBS, and then homogenized within 30 min. The supernatant was separated at a speed of 1,000 x g for 15 min at 4˚C for the detection of malondialdehyde (MDA; cat. no. A003) and superoxide dismutase (SOD; cat. no. A001) (both from Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocols.

Statistical analysis. All data are presented as the mean ± SD. SPSS v22.0 software (IBM Corp.) was used to perform statistical analysis. Comparisons between two groups were analyzed using an unpaired t-test. The differences among multiple groups were compared by one-way ANOVA followed by Tukey's post hoc test. Each experiment was repeated ≥3 times. All P-values were two-tailed. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of Hyp on the expression levels of miR-138 in hypoxic cardiomyocytes. H9C2 cells were stimulated by Hyp at various concentrations under hypoxic conditions. After 24 h, the expression levels of miR-138 were significantly increased compared with the control group when the concentration of Hyp was 50 and 100 μmol/l (Fig. 1A). Additionally, in C57BL/6 mice fed under hypoxic conditions for 1 week, the expression levels of miR-138 in heart tissues were significantly increased compared with the control group when Hyp was administered at 50, 100 and 200 mg/kg (Fig. 1B). These data demonstrated that Hyp treatment upregulated the expression levels of miR-138.

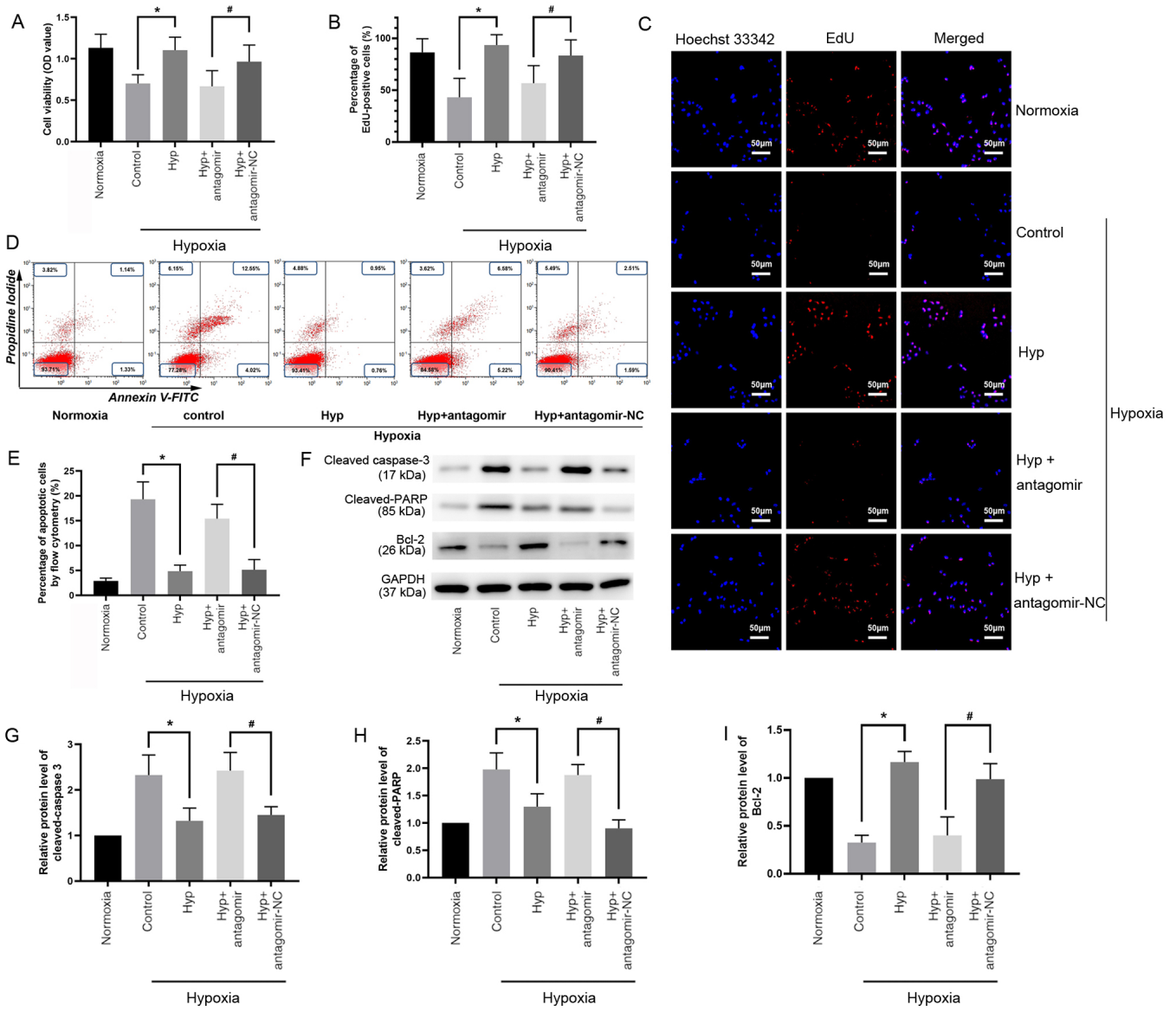

Figure 2. Hyp promotes microRNA-138-mediated H9C2 cell survival under hypoxia. (A) Cell viability was examined using a Cell Counting Kit-8 assay. Absorbance was measured at 450 nm. (B) Capacity of cell proliferation was assessed using an EdU assay. (C) Total cells were stained by Hoechst 33342 (blue). EdU-positive cells were labeled in red fluorescence. The percentage of EdU-positive cells was calculated in the merged image. Scale bar, 50 μm. (D and E) Cell apoptosis was examined by flow cytometry via Annexin V-FITC/PI staining. (F) Western blotting was used to determine the relative expression levels of (G) cleaved caspase-3, (H) cleaved PARP and (I) Bcl-2. GAPDH was used as the internal control. Data are presented as the mean ± SD. *P<0.05. $^{\#}$P<0.05. EdU, 5-ethynyl-2'-deoxyuridine; Hyp, hyperoside; PARP, poly(ADP)-ribose polymerase; NC, negative control; OD, optical density.

Hyp promotes H9C2 cell survival via upregulation of miR-138 expression levels under hypoxic conditions. Since miR-138 serves a notable cardioprotective role (9), it was hypothesized that Hyp promotes cardiomyocyte survival under hypoxic conditions. First, a hypoxic model in H9C2 cells was established to investigate the role of 100 μmol/l Hyp, which had the strongest effect on promoting the expression of miR-138 among the different concentration gradients that were selected. Antagomir was used to silence miR-138 expression. The transfection efficacy is shown in Fig. S1A. A CCK-8 assay demonstrated that cell viability in the Hyp group was 1.5 times higher compared with the control group (Fig. 2A). miR-138 antagomir transfection significantly decreased cell viability compared with antagomir-NC transfection in cells in hypoxic conditions (Fig. 2A). Cells were stained using an EdU assay (Fig. 2C), which revealed that Hyp significantly improved cell proliferation, which had been impaired by hypoxia (Fig. 2B), and the percentage of EdU-positive cells in the Hyp + antagomir group was significantly lower compared with that in the Hyp + antagomir-NC group (Fig. 2B). Cell apoptosis was evaluated by flow cytometry (Fig. 2D). Apoptosis was induced under hypoxia, and this was significantly alleviated by Hyp treatment (Fig. 2E). However, Hyp did not decrease the number of apoptotic cells in the presence of miR-138 antagomir (Fig. 2E). The expression levels of apoptosis-associated proteins were detected by western blotting (Fig. 2F). Downregulated cleaved caspase-3 (Fig. 2G) and cleaved PARP (Fig. 2H), and upregulated Bcl-2 (Fig. 2I) expression was observed in the Hyp group. Compared with the Hyp + antagomir-NC group, Hyp + antagomir significantly increased cleaved caspase-3 (Fig. 2G) and cleaved PARP (Fig. 2H) levels, and decreased Bcl-2 expression levels (Fig. 2I).

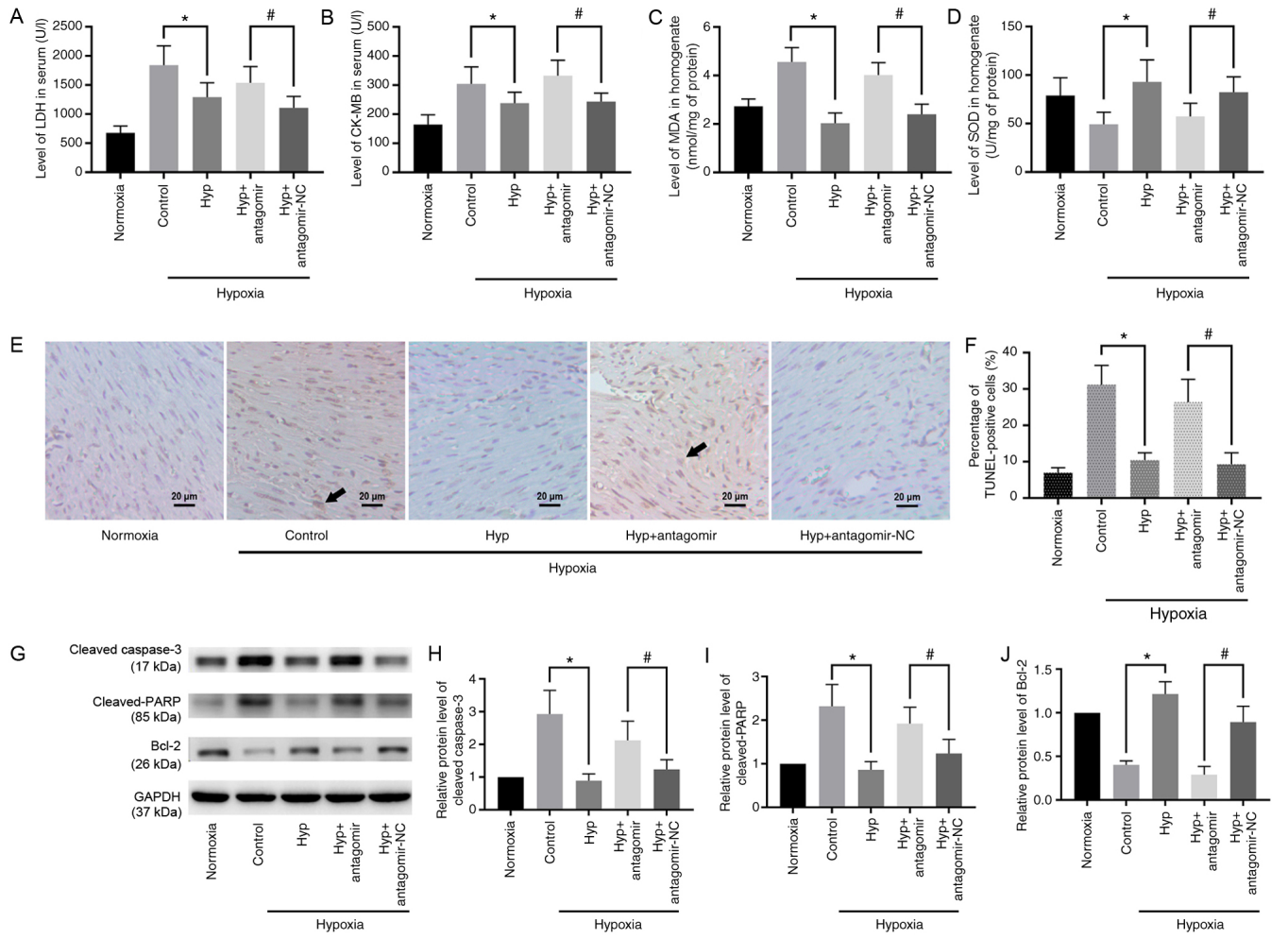

Figure 3. Hyp protects mouse myocardium from hypoxia-induced injury via miR-138. (A) LDH and (B) CK-MB levels in serum, and (C) MDA and (D) SOD levels in myocardial homogenate were detected. (E) Myocardial morphology was assessed using a light microscope. Scale bar, 20 μm. Arrows indicate apoptotic cells. (F) Cell apoptosis was examined using a TUNEL assay. (G) Western blotting revealed the relative expression levels of (H) cleaved caspase-3, (I) cleaved PARP and (J) Bcl-2. Data are presented as the mean ± SD. *P<0.05. $^{\#}$P<0.05. Hyp, hyperoside; miR-138, microRNA-138; LDH, lactate dehydrogenase; CK-MB, creatine kinase-myocardial band; MDA, malondialdehyde; SOD, superoxide dismutase; PARP, poly(ADP)-ribose polymerase; NC, negative control.

These results demonstrated that the protective role of Hyp *in vitro* was mediated via upregulation of miR-138 under hypoxic conditions.

Hyp attenuates hypoxia-induced myocardial injury in mice via miR-138. Subsequently, the effect of Hyp was verified in mice exposed to hypoxia. The dose of Hyp was set as 100 mg/kg, which had the strongest effect on promoting the expression of miR-138 among the different concentration gradients that were selected. Transfection efficacy of miR-138 antagomir is presented in Fig. S1B. Detection of markers of myocardial injury demonstrated that the expression levels of LDH (Fig. 3A), CK-MB (Fig. 3B) and MDA (Fig. 3C) were significantly decreased in the Hyp group compared with the control group, and increased in the Hyp + antagomir group compared with the Hyp + antagomir-NC group. In addition, the levels of SOD exhibited a significant increase in the Hyp group, which was abated in the presence of miR-138 antagomir (Fig. 3D). The morphological changes of the myocardium are shown in Fig. 3E, and apoptotic cells were labeled in a TUNEL assay. Hypoxia-induced apoptosis was significantly suppressed following administration of Hyp (Fig. 3F). This anti-apoptotic effect of Hyp was reversed following inhibition of miR-138 by antagomir (Fig. 3F). The expression levels of apoptosis-associated proteins were detected via western blotting (Fig. 3G). Expression of cleaved caspase-3 (Fig. 3H) and cleaved PARP (Fig. 3I) in the Hyp group were significantly decreased compared with those in the control group, whereas the expression levels of Bcl-2 (Fig. 3J) were significantly upregulated. A significant increase in the levels of cleaved caspase-3 and cleaved PARP, and decreased expression levels of Bcl-2, were observed in the presence of antagomir. Therefore, these findings indicated that Hyp protected the myocardium from hypoxia-induced injury *in vivo* via upregulation of miR-138.

Effect of Hyp on the expression levels of miR-138 target proteins. The expression levels of miR-138 target proteins were detected to elucidate the potential mechanism of action of Hyp (Fig. 4A). Following exposure of H9C2 cells to hypoxia for 24 h, the expression levels of MLK3 and Lcn2 were 22.3 and 38.9%, respectively, of those in the control group (Fig. 4B and C). miR-138 antagomir upregulated the expression levels of these

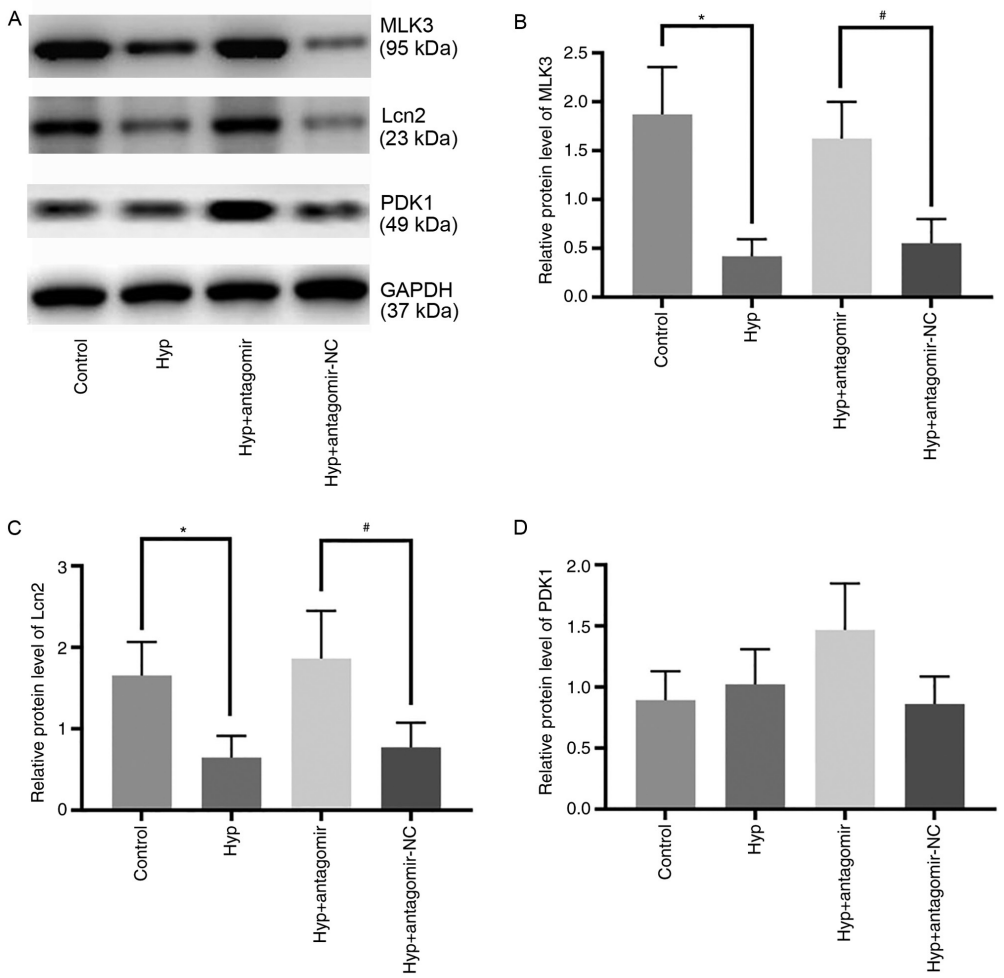

Figure 4. Effect of Hyp on the expression levels of miR-138 target proteins. (A) Expression levels of proteins targeted by miR-138 were assessed by western blotting in H9C2 cells following exposure to hypoxia for 24 h. Densitometric analysis of the relative expression levels of (B) MLK3, (C) Lcn2 and (D) PDK1. GAPDH was used as the internal control. Data are presented as the mean ± SD. *P<0.05. #P<0.05. Hyp, hyperoside; miR-138, microRNA-138; MLK3, mixed lineage kinase 3; Lcn2, lipocalin-2; PDK1, pyruvate dehydrogenase kinase 1.

three target proteins in the Hyp + antagomir group compared with the Hyp + antagomir-NC group (Fig. 4B and C), but there was no significant difference in the expression levels of PDK1 between the Hyp and control groups. These data indicated that Hyp exerted cardioprotective effects by regulating the expression levels of MLK3 and Lcn2 but not PDK1.

Discussion

Hyp is a Chinese herbal medicine with multiple biological properties that serve a key role in a number of diseases (2,3). In an ischemia/reperfusion model, Hyp has been identified to protect the heart (6), kidney (15) and liver (16) against injury by modulating mitochondrial fission, oxidative stress and apoptosis. Hydrogen peroxide induces oxidative stress in granulosa cells, which is effectively suppressed by Hyp via the Sonic hedgehog signaling pathway (17). In addition, Kwon *et al* (18) observed that Hyp decreased apoptosis of dopaminergic neurons via activation of heme oxygenase-1 signaling. Hypoxia is a common condition in numerous cardiovascular events, such as ischemic heart disease, high altitude heart disease and cyanotic congenital heart disease (1). When subjected to hypoxia, cardiomyocytes develop disorders of mitochondria, membrane and lysosomes, leading to the initiation of the caspase cascade and activation of the Bcl-2 family (19). Oxidative stress and apoptosis are key mechanisms underlying hypoxia-induced injury (20) and are primary targets of Hyp (16). In the present study, Hyp was applied to hypoxic cardiomyocytes and its cytoprotective role was verified *in vivo* as well as *in vitro*. The expression of SOD and MDA can partially reflect the level of oxidative stress, which were examined in the present study, thus it was found that hypoxia induced myocardial apoptosis and oxidative stress, which were attenuated by Hyp.

A number of miRNAs serve as downstream factors of Hyp, such as let-7a-5p (21) and miR-27a (22). Our previous study on diabetic nephropathy demonstrated that Hyp suppresses the expression of miR-21 and improves renal function via targeting of matrix metalloproteinase-9 (23). The present study indicated that expression levels of miR-138 were significantly increased in the hypoxic myocardium following addition of Hyp at concentrations of >50 μmol/l *in vivo* and >50 mg/kg *in vitro*. When miR-138 was inhibited by antagomir, the protective role of Hyp was attenuated, suggesting that the effects of Hyp were mediated via miR-138 signaling. Our previous study (9) demonstrated

miR-138 is a key regulator of adaptation to chronic hypoxia in cardiomyocytes. miR-138 serves an anti-apoptotic role by directly affecting the expression levels of caspase and Bcl-2 family members, as well as activation of c-Jun N-terminal kinase and p38 MAPK signaling (9,24,25). Furthermore, miR-138 has been found to exert anti-inflammatory effects by regulating the AKT and NF-κB signaling pathways (26,27), and to maintain mitochondrial homeostasis by targeting hypoxia-inducible factor 1-α (28).

miRNAs act by binding to and degrading target mRNA, and suppress protein translation at the post-transcriptional level (29). Previous data have demonstrated that MLK3 (9), Lcn2 (10) and PDK1 (11) are target proteins of miR-138 in hypoxic cardiomyocytes. In order to elucidate the mechanism of action of Hyp, expression levels of these three targets were assessed. Hyp caused significant downregulation of MLK3 and Lcn2, but not PDK1. MLK3 is an important member of the MAPK family. MLK3 phosphorylates JNK and affects downstream c-Jun signaling, and also serves a key role in cell apoptosis and response to stress (30). Lcn2 is a secreted adipokine of the lipocalin family that binds to and transports small molecules, such as iron and fatty acids (10), and is considered to be a biomarker of metabolic inflammation (31). Absence of Lcn2 induces apoptosis in cardiomyocytes by increasing intracellular iron levels (32). Lcn2 also regulates the innate immune response in the pathogenesis of heart failure (33). PDK1 is an essential enzyme involved in glucose metabolism that promotes gluconeogenesis from pyruvate, lactic acid and alanine (34), and prevents the incorporation of pyruvate in the oxidative phosphorylation process, leading to enhanced anaerobic glycolysis and decreased cellular respiration (11,35). Based on the aforementioned evidence, it was hypothesized that Hyp protects cardiomyocytes from hypoxia-induced injury via regulation of apoptosis and stress response rather than glucose metabolism.

In conclusion, the present study demonstrated that Hyp upregulated miR-138 in hypoxic cardiomyocytes. Hyp treatment promoted cardiomyocyte survival and alleviated hypoxia-induced apoptosis by inhibiting the expression of downstream targets of miR-138, namely MLK3 and Lcn2. These findings may provide promising novel strategies for clinical cardioprotection.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SH wrote the manuscript. SH, XY and JC performed cell experiments. FW, FG and MX performed animal experiments. SZ and JW performed data management and analysis. LZ and JZ designed the study and supervised the research. SH and JZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal procedures were approved by the Animal Care and Use Committee of General Hospital of Western Theater Command and Army Medical University (approval no. 2020ky018; Chengdu, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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