BMC Cardiovascular Disorders

RESEARCH

Open Access

HACE1 protects against myocardial ischemia–reperfusion injury via inhibition of mitochondrial fission in mice

Bang-Xia Liu[1,2], Juan Zheng[3], Zhan-Wei Tang[4], Lei Gao[5,6], Meng Wang[7], Ying Sun[4], Chen Chen[8] and Heng-Chen Yao[4*]

Abstract

Background HECT domain and Ankyrin repeat Containing E3 ubiquitin-protein ligase 1 (HACE1) has been found to be associated with mitochondrial protection. Mitochondrial damage is a critical contributor to myocardial ischemia–reperfusion injury (I/RI). However, little is known about the role of HACE1 in the pathogenesis of myocardial I/RI.

Methods Male C57BL6 mice with HACE1 knockout (KO) were subjected to 30 min of ischemia via ligation of the left anterior descending artery, followed by 0, 2, 6, or 24 h of reperfusion. The mice were evaluated for myocardial histopathological injury, serum troponin I (cTnI) levels, oxidative stress injury, apoptosis and cardiac function. Prior to ischemia, Mdivi-1(1.2 mg/kg) or vehicle was administered.

Results The study revealed that increased expression of HACE1 was associated with myocardial ischemia/reperfusion injury (I/RI), and that knockout of HACE1 resulted in more severe myocardial damage and cardiac dysfunction during I/R($P < 0.05$). The HACE1 knockout group exhibited higher levels of malondialdehyde (MDA), greater mitochondrial fission, and dissipation of mitochondrial membrane potential (MMP), leading to more apoptosis and severe cardiac dysfunction compared to the wild-type I/R group($P < 0.05$). On the other hand, HACE1 knockout further reduced superoxide dismutase (SOD) activity in the myocardium($P < 0.05$), further supporting the findings. However, the adverse effects were almost completely eliminated by pharmacological blockade of the dynamin-related protein 1 (Drp1) inhibitor, Mdivi-1, which inhibits mitochondrial fission during cardiac I/R($P < 0.05$).

Conclusion Collectively, our data show that myocardial I/RI is associated with HACE1 downregulation and Drp1 activation, causing cardiomyocytes to undergo cell death. Therefore, HACE1 could be a promising therapeutic target for the treatment of myocardial I/RI.

Keywords HACE1, Ischemia/reperfusion, Drp1, Mitochondrial fission, Apoptosis

*Correspondence:
Heng-Chen Yao
yaohc66@126.com
Full list of author information is available at the end of the article

Introduction

Myocardial infarction (MI) is currently recognized as the leading cause of mortality worldwide. Although timely restoration of blood flow through intervention or thrombolytic treatment has improved, it can lead to myocardial ischemia–reperfusion injury (I/RI) [1], offsetting the benefits of revascularization [2, 3], and the extent of I/RI determines the prognosis of patients with acute MI [3, 4]. Investigating the molecular mechanisms underlying I/RI is crucial to develop therapeutic approaches.

Mitochondria are essential organelles in cardiomyocytes, producing ATP necessary for myocardial contraction and survival [5]. Recent research has highlighted the importance of mitochondrial dynamics, as fission and fusion events continuously occur and may impact cell fate during myocardial I/RI [6, 7]. Improper mitochondrial fission has been associated with larger infarction size and worse cardiac function, while inhibiting fission has been found to improve cardiac function and reduce myocardial damage following I/RI [8–10]. Dynamin-related protein 1 (Drp1) and its receptors, such as mitochondrial fission factor (Mff) and mitochondrial fission one protein (Fis1), primarily regulate mitochondrial fission [11]. In cardiac I/RI models, Drp1 activation triggers mitochondrial dysfunction and fission, while inhibiting Drp1 reduces infarct size and mitochondrial fission [12]. Overall, these findings highlight the critical role of Drp1-mediated mitochondrial fission in myocardial I/RI.

HACE1 is a protein that plays a crucial role in oxidative stress and is known to act as a tumor suppressor [13–15]. It is expressed in various tissues, including the heart, brain, and kidney [16, 17]. Studies on zebrafish have shown that HACE1 affects cardiac development through ROS-dependent mechanisms [18]. In patients with heart failure, HACE1 expression was found to be up-regulated, while HACE1 inactivation increased heart failure and mortality in mice under hemodynamic stress [19, 20]. In the cell hypoxia-reoxygenation model, HACE1 has been found to protect against myocardial damage by modulating the Keap1/Nrf2 pathway during I/R [21]. Furthermore, HACE1 is required for mitochondrial protection [22], and its inactivation triggers improper mitochondrial fission [19]. However, little research has investigated its role in cardiac I/RI. In the present study, HACE1 knockout mice were used to explore the function of HACE1-Drp1during myocardial I/RI.

Materials and methods

Construction of HACE1 knockout mice

The Nanjing Biomedical Research Institute of Nanjing University in Jiangsu Province used CRISPR/Cas9-mediated genome engineering to create HACE1 knockout mice. The HACE1-201 transcript (ENSMUST00000037044.12) was targeted in this study. The HACE1 gene is composed of 24 exons, with the start codon (ATG) located in exon 1 and the stop codon (TAA) in exon 24. Cas9 mRNA and sgRNA were co-injected into zygotes to induce a double strand break (DSB) in intron 1–2 and intron 4–5 through direct Cas9 endonuclease cleavage. The DSBs were repaired by non-homologous end joining (NHEJ), resulting in the disruption of the HACE1 gene. The HACE1-KO positive mice were identified by PCR genotyping and subsequent sequence analysis.

Animal feeding and experimental design

Male C57BL/6 J HACE1 knockout mice used in this study were purchased from Nanjing University's laboratory, while wild-type (WT) mice were obtained from Shandong Jinan Lu Kang Co., Ltd. The animals were cared for in accordance with the revised guidelines of the National Institutes of Health (1996 revision, page 85–2) and were housed in an SPF-level animal facility with controlled temperature (22 ± 1 °C), humidity (55 ± 5%), and a 12-h light/dark cycle. The mice used in the experiment were aged 8–10 weeks and weighed 22-26 g.

Based on the preliminary experimental design, the mice were randomly divided into several groups (n = 6 in each group): WT-Sham, WT-IR, KO-Sham, KO-IR, KO-IR-DMSO (80 mg/kg, administered intravenously 15 min before ischemia), and KO-IR-Mdivi-1 (1.2 mg/kg, administered intravenously 15 min before ischemia). Mdivi-1, a Drp1-specific inhibitor, was used in the study. Following previous experiments [23], the mice were randomly assigned to receive either vehicle (0.1 mL of 0.1% dimethyl sulfoxide) or Mdivi-1 (1.2 mg/kg) intravenously 15 min before myocardial ischemia.

Construction of the cardiac I/R model in mice

All animal experiments were performed under the guidelines of the "Guide for the care and use of laboratory animals" published by National Institutes of Health (revised 2011), and were approved by the Institutional Review Board of Liaocheng People's Hospital (Liaocheng, China). The mouse I/R model was created using a previously reported method [24]. Mice were anesthetized with pentobarbital sodium and a skin incision was made along the lower edge of the pectoralis major muscle. The LAD of the left coronary artery was occluded for 30 min followed by reperfusion for 0 h, 2 h, 6 h, 24 h. Sham-operated mice underwent the same procedure without LAD ligation of cardiac I/R model. After the surgery, whole blood was collected from the heart and residual blood was flushed with isotonic saline. The heart was then excised and fixed with buffered 4% paraformaldehyde for subsequent molecular and pathological testing.

Detection of myocardial infarction markers

Blood samples (0.5–0.8 mL) were obtained from the apex of the heart and centrifuged at 1,500 × g for 10 min at 4 °C. The obtained serum was immediately stored at -80 °C until future analysis. The serum levels of cardiac troponin I (cTnI) were determined using ELISA kits (Cloudy clone, JL46089-48 T).

Evaluation of myocardial infarction size

To assess myocardial infarction size, 2% TTC and 1% Evans blue staining were used. Prior to staining, 2% TTC was placed at room temperature for 1 h. After 6 h of reperfusion, 1% Evans blue was retrogradely injected above the ascending aorta root. Once the non-infarcted area was fully stained blue, the hearts were washed with PBS and cut into 4–5 slices. The sections were then stained with 2% TTC for 20 min at 37 °C, and images were taken with a Nikon D90 digital camera. The infarct and non-infarct areas were determined using ImageJ software, and the infarct size was modified to be expressed as the ratio of infarct area to left ventricular area (IA/LVA).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

Apoptosis was detected using a TUNEL assay (Beyotime Biotechnology, C1089) performed in situ. Sections were deparaffinized, rehydrated, treated with proteinase K (20 μg/mL in 10 mM Tris–HCl, pH 7.6), and then incubated with a labelling solution containing terminal deoxynucleotidyl transferase, its buffer, and fluorescein d UTP. After washing with PBS, apoptosis was evaluated in six randomly selected fields per section in the ischemic zone. The images were captured and digitized using image analysis software.

H&E and Masson trichrome& immunohistochemistry

Myocardial tissue from mice was fixed in 10% neutral-buffered formalin for 24 h at room temperature and embedded in paraffin. 5 μm sections were cut using a Tissue-Tek® DRS™ 2000 from Sakura Finetek Japan Co., Ltd. and stained with H&E and Masson trichrome to analyze tissue morphology and fibrosis. A digital microscope (Nikon Corporation, Tokyo, Japan) and ImageJ software were used to measure the results. Immunohistochemistry with anti-Bax (Abcam, ab32503) was performed on 4 μm sections of the paraffin-embedded heart specimens. Positive staining areas were calculated in 10 random high-power fields (×20 or×40) using an Olympus BX-51 light microscope (Olympus America Inc, Melville, NY, USA) after counterstaining with hematoxylin.

Mitochondrial extraction and mitochondrial membrane potential (MMP)

Mitochondria were extracted from the myocardial tissue using the Beyotime Biotechnology kit (C3606), following the manufacturer's instructions, after the surgery. The mitochondrial membrane potential (MMP) was measured using the JC-1 Kit (Beyotime Biotechnology, C2006), and fluorescence images were captured with an Olympus DX51 fluorescence microscope in order to determine the change in MMP. The ratio of red to green fluorescence intensity was used to analyze the results, which were further processed using ImageJ software.

Transmission Electron Microscope (TEM)

Samples were obtained from the affected area of the heart after 6 h of reperfusion and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for 30 min. The tissue was cut into small pieces, returned to the fixing solution for 2 h at room temperature, and then left overnight at 4 °C after fixing in 4% glutaraldehyde. Semi-thin sections were cut, stained with 1% azure II in 1% sodium borate, and imaged using TEM. Ultrathin sections, 80 nm thick, were cut using LKB-NOVA, stained with uranium acetate and lead citrate, and observed using a Hitachi H-7500 TEM at 60 kV.

Determination of MDA and SOD levels in myocardium

The hearts of the mice were harvested and washed with normal saline after the I/R protocol. The ischemic heart tissue (0.5 g) was then homogenized at 0-4 °C. Next, the homogenate was subjected to centrifugation at 1,200×g for 30 min at 4 °C. The resulting supernatant was collected and stored at -80 °C.MDA and SOD activity levels were measured using Biotechnology kits (S0131S for MDA and S0101S for SOD) according to the manufacturer's instructions.

Echocardiography

In this study, cardiac function was assessed using echocardiography 28 days after I/R using a previously described protocol [25]. Mice were sedated with 1% isoflurane and echocardiographic images were recorded using a Visual Sonics Vevo770 with a 2100 transducer. M-mode images were obtained when the heart rate was maintained at 450–500 bpm. Left ventricular ejection fraction (LVEF), left ventricular end-systolic dimension (LVESD), left ventricular end-diastolic dimension (LVEDD), interventricular septum thickness (IVS), and left ventricular posterior wall thickness (LVPW) were measured. LVEF was calculated using the formula: LVEF (%) = 100[(LVEDD^3-LVESDs)^3)/

$LVEDD^3$]. Three experienced technicians performed the measurements, and all values were averaged from three consecutive cardiac cycles. The technicians were unaware of the identities of the animal groups.

Western blot

In this experiment, cytoplasmic and mitochondrial proteins were extracted using specific protein extraction kits (Beyotime Biotechnology, P0027 and C3606), and their concentrations were determined using the BCA protein assay kit (Biotechnology, P0012S) to ensure equivalence. Briefly, protein samples were diluted to appropriate concentrations, and a standard curve was prepared using known concentrations of bovine serum albumin (BSA). The absorbance was measured at 562 nm, and the total protein concentration was calculated based on the standard curve. The samples were mixed with 5×loading buffer and boiled, then loaded onto an SDS-PAGE gel and transferred to a PVDF membrane (Millipore). The membrane was blocked with 5% milk containing 0.05% Tween20 and incubated with primary antibodies overnight at 4 °C, including Drp1 (Abcam, #ab184427), Fis1 (Abcam, #ab189864), Mff (Abcam, #ab129075), Bcl2 (Abcam, #ab194583), Bax (Abcam, #ab32503), and Caspase3 (Cell Signaling Technology, CST9662s) and Cleaved Caspase3 (Cell Signaling Technology, #9664 s), Cytochrome c(Cyt c) (Biotechnology, #AC909), and VDAC1 (Biotechnology, #AF1027). The membrane was then incubated with a horseradish peroxidase-labeled anti-mouse or anti-rabbit secondary antibody (Cell Signaling Technology, #58802S, #5127S), and antibody binding was detected using an enhanced chemiluminescence (ECL) reagent (Beyotime Biotechnology, P0018S). β-actin (Abcam, #ab8226) and VDAC1 (Beyotime Biotechnology, #AF1027) were used as internal references for total or cytoplasmic protein and mitochondrial protein, respectively. The results were analyzed using ImageJ software.

Quantitative Real-Time Polymerase Chain Reaction (qPCR)

RNA was extracted from ischemic myocardium using RNeasy Minikit (74,106, Qiagen), and mRNA expression was quantified using SYBR Green (DRR420A, Takara) on an ABI Prism Sequence Detection system after generating cDNA through Takara (perfect real time RR037A, Takara) reverse transcription. The expression levels were normalized to GAPDH.

Primer sequences were designed as follows:

Mice Hace1 (F-primer 5'-GGCGTTGTGCGTGAA TGGTTTG', R-primer 5'-GTTGCTGTTGGGCTGGAA AGTTG'),

Mice Drp1 (F-primer 5'-GAGAACTACCTTCCGCTG TATCGC', R-primer 5'-CACCATCTCCAATTCCAC CACCTG'),

Mice Mff (F-primer 5'-GAAGAGCAGAGCCGAGCA GTTG', R-primer 5'-ACCACGCAACACAGGTATGGA ATC'),

Mice Fis1 (F-primer 5'-TTGAATATGCCTGGTGCC TGGTTC', R-primer 5'-CATAGTCCCGCTGTTCCTCTT TGC'),

Mice Bax (F-primer 5'-CGTGAGCGGCTGCTTGTC TG', R-primer 5'-ATGGTGAGCGAGGCGGTGAG'),

Mice Bcl2 (F-primer 5'-AGCCCAATGCCCTCCAGA GC', R-primer 5'-TCTCAAGCCTTCACGCAAGTTCAG'),

Mice GAPDH (F-primer 5'-GGTTGTCTCCTGCGA CTTCA', R-primer 5'-TGGTCCAGGGTTTCTTACTCC').

Statistical analysis

All statistical analyses were conducted using GraphPad Prism 8 software (IBM Corp., Armonk, NY, USA). Comparisons among groups were performed with One-way ANOVA. When a significant F value was obtained, means were compared by use of Tukey test. HACE1 levels were evaluated by the Student t test between WT and KO group. All data are expressed as mean ± standard deviation. Statistical significance was considered when $P < 0.05$.

Results

HACE1 expression was up-regulated by cardiac I/R

The study evaluated the impact of I/R on the expression of HACE1 in the heart using Western blot analysis. Results showed that HACE1 expression significantly increased after 6 h of reperfusion compared to the control group *(See Supplemental* Fig. 1A–B). To investigate the role of HACE1 in myocardial I/R, an I/R mouse model was constructed, and HACE1 expression was

(See figure on next page.)

Fig. 1 Effects of HACE1 knockout on the heart after ischemia–reperfusion. **A** Hearts after reperfusion were harvested and stained with TTC + Evans Blue. **B** The infarct size was expressed as the ratio of infarct area to left ventricular area (IA/LVA). **C** The level of serum cTnI in each group. **D** HE and representative photomicrographs of immunohistochemical staining of Bax in myocardial tissue sections(×400). **E** Quantification of mean optical density (AOD)for immunohistochemistry. **F** One-step TUNEL detection (×400) and Masson trichrome staining (×200). **G** Comparison of apoptotic rates by TUNEL analysis. **H** The fibrosis area was expressed as a percentage within each group. **I** HACE1 knockout aggravated cardiac dysfunction following myocardial ischemia–reperfusion. The representative M-mode echocardiograms were recorded. **J**, **K**, **L**, **M** and **N** presented parameters of cardiac function, including LVEF (%), LVFS (%), LVESD (cm), LVPW (cm)and IVS (cm) in each group after myocardial ischemia–reperfusion measured by echocardiography. O The oxidant factor MDA was analyzed via ELISA. P) The antioxidant factor SOD was detected via ELISA. All numerical data were presented as the mean ± SD ($n = 6$, in each group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

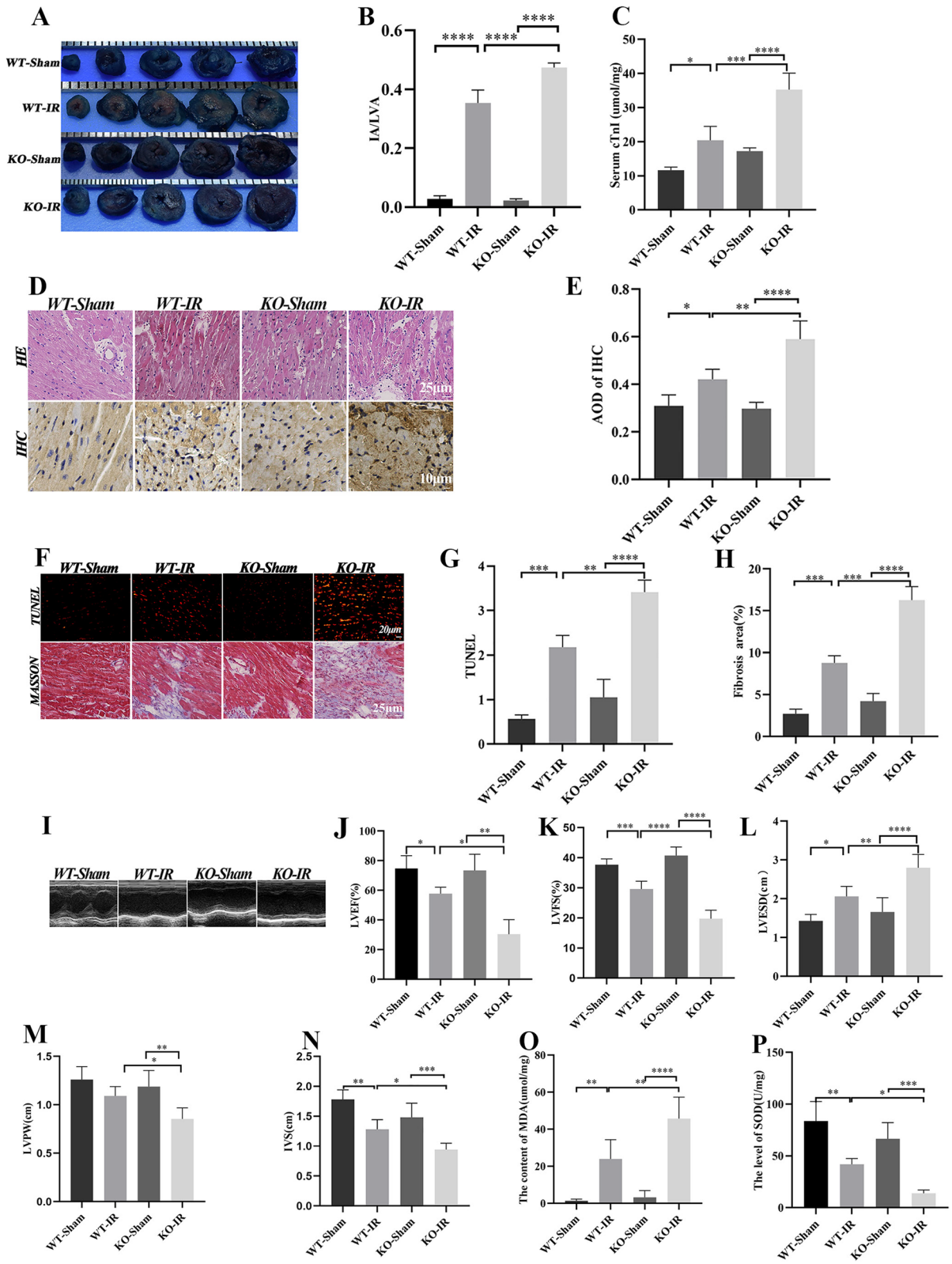

Fig. 1 (See legend on previous page.)

evaluated in both wild-type (WT) and HACE1 knockout (KO) groups. Results showed that HACE1 mRNA and protein expression significantly decreased in the KO group, confirming successful model construction (All $P<0.05$) *(See Supplemental* Fig. 1C–D). Finally, the role of HACE1 in myocardial I/R was further explored in both WT and HACE1-KO mice for 30 min and 6 h.

HACE1 deficiency was responsible for myocardial I/RI

Figure 1A and B show that HACE1-KO mice had a larger infarction size (IS) after I/R than the WT group ($P<0.05$) (Table 1). The level of cTnI presented in Fig. 1C was significantly higher in the HACE1-KO IR group ($P<0.05$ (Table 1). Additionally, HACE1-KO IR mice showed more severe bleeding, inflammation, and apoptosis than the WT-IR group (Fig. 1D and E). One-step TUNEL and Masson staining analyses revealed that HACE-KO mice had more TUNEL-positive cells and fibrosis area than the WT-IR group (Fig. 1F-H) (All $P<0.05$). Moreover, HACE1 loss was closely associated with heart function after cardiac I/R injury, as evidenced by the significant decline in LVEF and LVFS in HACE1-KO mice compared to the WT group (Fig. 1I-K) (All $P<0.05$). Figure 1L showed that LVESD was significantly larger in HACE1-KO mice compared to the WT group ($P<0.05$), while LVEDD did not differ significantly among groups (Table 1and *See Supplemental* Fig. 2A). Additionally, LVPW and IVS showed a more significant decrease in HACE1-KO mice than in the WT-IR group (Fig. 1M and N) ($P<0.05$). In addition, as we all know, HACE1 is an oxidative stress gene. Therefore, the content of MDA and the activity of SOD were measured after I/R. Compared with the Sham group, I/R increased MDA content and reduced SOD activity, and these results were exacerbated by HACE1 deficiency (Fig. 1O and P) (All $P<0.05$) (Table 1).

Table 1 Effect of HACE1 lack on IS, cTnI, MDA, SOD and cardiac function under I/R

Variable	WT-Sham	WT-IR	KO-Sham	KO-IR
IA/LVA	0.03 ± 0.01	0.37 ± 0.03[a]	0.03 ± 0.005	0.48 ± 0.02[bc]
cTnI(μmol/mg)	10.44 ± 1.95	20.44 ± 3.50[a]	23.76 ± 3.97	38.34 ± 4.94[bc]
MDA(μmol/mg)	1.39 ± 0.81	23.95 ± 9.29[a]	2.04 ± 0.98	47.75 ± 12.92[bc]
SOD(U/mg)	83.7 ± 16.29	41.96 ± 4.78[a]	66.51 ± 13.57	13.97 ± 2.67[bc]
LVEF(%)	78.73 ± 5.580	57.82 ± 3.86[a]	74.22 ± 5.38	31.38 ± 6.10[bc]
LVFS(%)	37.67 ± 1.73	29.62 ± 2.31[a]	40.72 ± 2.53	19.71 ± 2.54[bc]
LVESD(cm)	1.83 ± 0.40	2.24 ± 0.36[a]	1.81 ± 0.55	2.69 ± 0.34[bc]
LVEDD(cm)	3.19 ± 0.42	3.34 ± 0.47	2.85 ± 0.33	3.45 ± 0.21
LVPW(cm)	1.26 ± 0.12	1.09 ± 0.09	1.19 ± 0.15	0.85 ± 0.10[bc]
IVS(cm)	1.78 ± 0.14	1.18 ± 0.13[a]	1.48 ± 0.21	1.02 ± 0.09[c]

[*] Data are presented as the mean ± standard deviation. [a]$P<0.05$, vs. WT-Sham; [b]$P<0.05$, vs WT-IR; [c]$P<0.05$, vs. KO-Sham; *IR* Ischemia reperfusion, *IS* Infarct size, *cTnI* cardiac troponin I, *MDA* Malondialdehyde, *SOD* Superoxide dismutase, *LVEF* Left ventricular ejection fraction, *LVFS* Left ventricular shortening, *LVESD* Left ventricular end-systolic dimension, *LVPW* Left ventricular posterior wall thickness, *IVS* Interventricular septum

In summary, these findings illustrate that HACE1 deficiency may aggravate myocardial damage triggered by cardiac I/R and contribute to oxidative stress to a certain extent. In the subsequent experiments, this study aimed to investigate the mechanisms underlying the phenotypic changes associated with HACE1 deficiency under cardiac I/RI.

HACE1 deficiency aggravates I/R mediated mitochondrial fission and apoptosis

To investigate the effect of HACE1 on mitochondrial fission, the researchers used transmission electron microscopy (TEM) to examine cardiac mitochondrial ultrastructure. The results indicated that mitochondrial injury, such as swelling, disorganization, and reduction or disappearance of the crista, induced by I/R was worsened by HACE1 KO (Fig. 2A). Mitochondrial membrane potential (MMP), as a sign of early apoptosis was detected. As shown in Fig. 2B and C, I/R led to a collapse of MMP, as presented by a decreasing ratio of red-to-green fluorescence intensity. Unfortunately, HACE1 KO further rescued MMP, suggesting the protective role of HACE1 on I/R-insulted mitochondria. At the molecular level, the transcription *(See Supplemental* Fig. 2B–D*)* and protein expression of mitochondrial fission factors, Drp1, Mff, and Fis1 (Fig. 2E-H) (All $P<0.05$) were upregulated in the I/R group compared to the Sham group, and this effect was further exacerbated by HACE1 KO.

In addition, the results found that I/RI increased the expression levels of pro-apoptotic proteins, such as Cytocyt c, Bax, Caspase-3, and Cleaved caspase-3, which were further worsened by HACE1 KO (Fig. 2H-L). Similar results were observed in the mRNA expression of Bax in HACE1-KO mice (All $P<0.05$) *(See Supplemental* Fig. 2E*).* However, the expression of Bcl2, an anti-apoptotic factor, was significantly reduced by I/R at both the protein and transcriptional levels *(See Supplemental* Fig. 2F*).* Additionally, HACE1 knockout further decreased Bcl2 expression (Fig. 2M) (All $P<0.05$). Overall, our data support the notion that HACE1 deficiency promotes I/R-mediated cardiomyocyte injury, at least in part, by activating myocardial mitochondrial apoptosis.

HACE1 deficiency participates in I/R induced myocardial damage and mitochondrial apoptosis via promoting mitochondrial fission

Mice were given Mdivi-1, a specific inhibitor of Drp1, 15 min before inducing the I/R model to confirm the role of HACE1 deficiency-induced mitochondrial fission

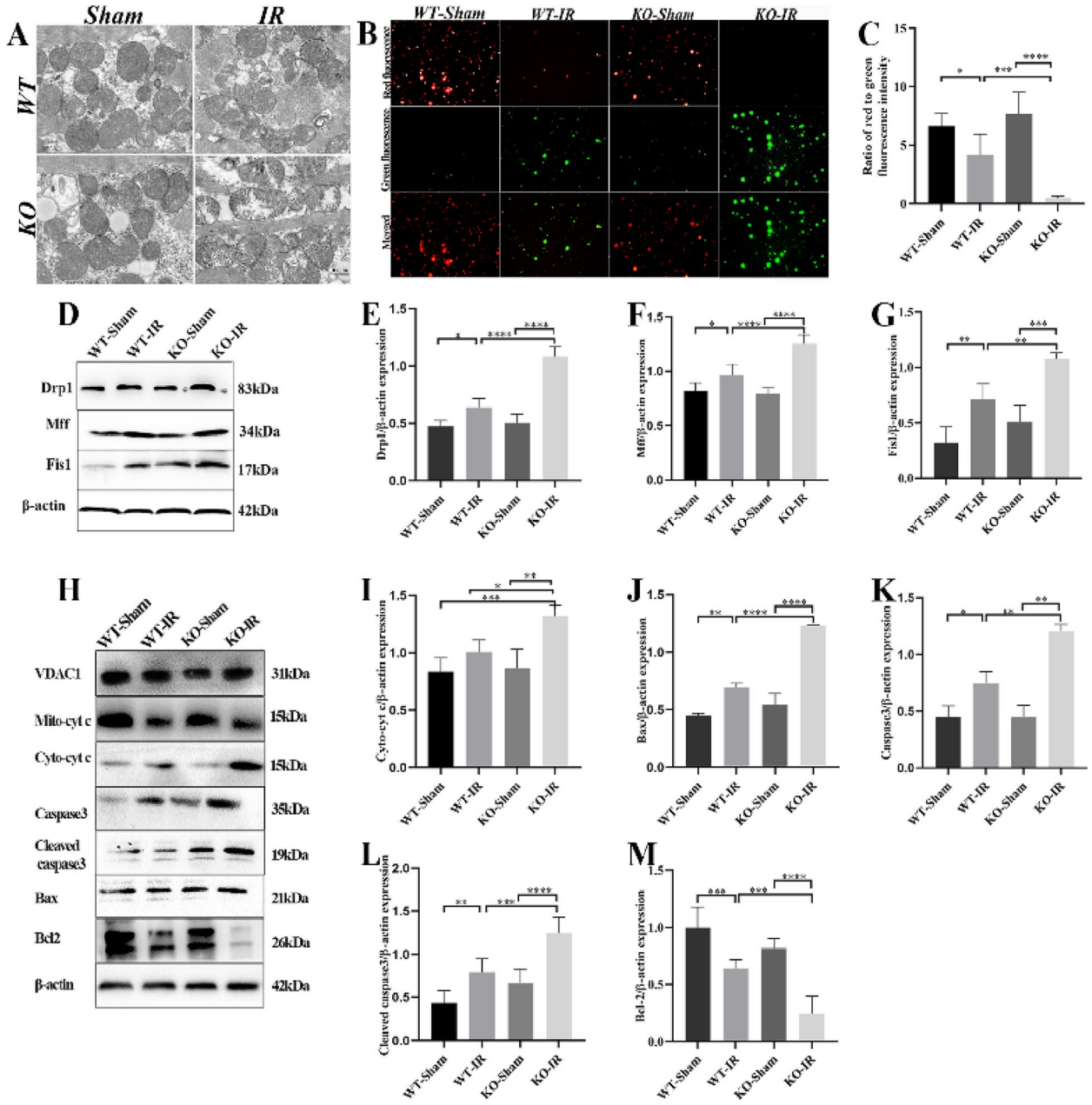

Fig. 2 HACE1 knockout aggravates ischemia–reperfusion (I/R) mediated mitochondrial fission and apoptosis. **A** Transmission Electron Microscope (TEM) was used to observe the alterations in mitochondrial ultrastructure after I/R injury. **B**, **C** Mitochondrial potential was measured via JC-1 staining and analyzed by image J. (Magnification = 400 ×). **D**-**G** Western blots were utilized to validate the expression of Drp1, Mff, and Fis1 under I/R. H-M) illustrated expression of cytoplasmic cytochrome c (Cyto-cytc), Bax, Caspase-3, Cleaved-caspase3 and Bcl2 via western blot assay. All numerical data were presented as the mean ± SD ($n = 6$, in each group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

in myocardial damage. Mdivi-1 pretreatment significantly reduced IS (Fig. 3A and B) and Serum cTnI content (Fig. 3C) (Table 2), while also decreasing bleeding, inflammation, and apoptosis compared to the DMSO group (All $P < 0.05$) (Fig. 3D and E). Moreover, TUNEL and Masson analysis showed fewer TUNEL-positive cells and fibrosis in Mdivi-1 pretreated mice (Fig. 3F-H). Further, Mdivi-1 pretreatment also reversed LVEF, LVFS and LVESD, as well as LVPW and IVS (All $P < 0.05$) (Fig. 3I-N), while LVEDD did not differ significantly among groups (Table 2 *and See Supplemental* Fig. 2I). Additionally, Mdivi-1 increased SOD activity and decreased MDA

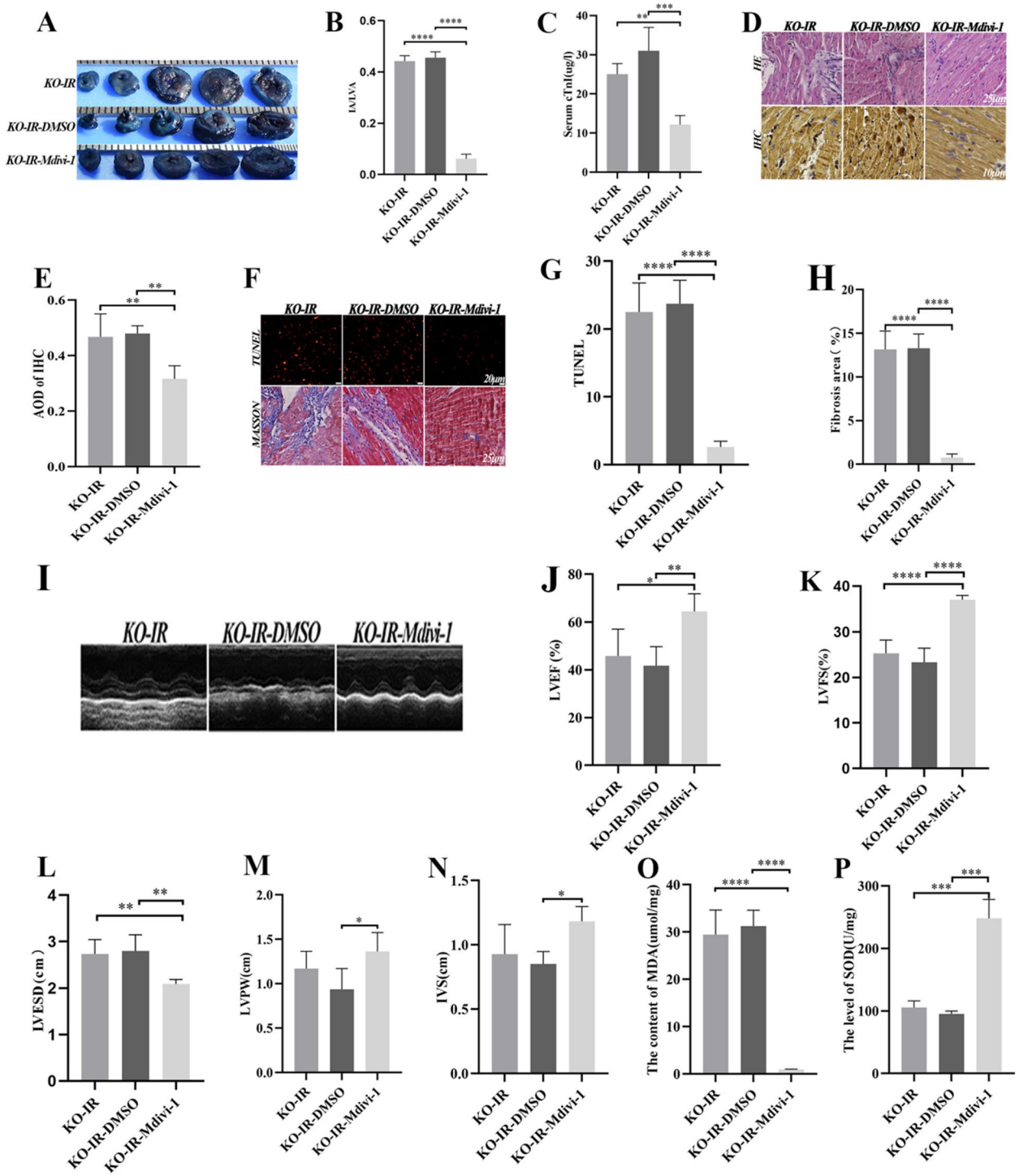

Fig. 3 Mdivi-1 pre-treatment reversed HACE1-deficiency-induced myocardial ischemia–reperfusion injury. **A** Hearts after reperfusion were harvested and stained with TTC + Evans Blue. **B** The infarct size was expressed as the ratio of infarct area to left ventricular area (IA/LVA). **C** The level of serum cTnI in various groups. **D** HE and representative photomicrographs of immunohistochemical staining of Bax in myocardial tissue sections(×400). E) Quantification of mean optical density (AOD)for immunohistochemistry. **F** One-step TUNEL detection (×400) and Masson trichrome staining (×200). **G** Comparison of apoptotic rates by TUNEL analysis. H) The fibrosis area was expressed as a percentage in each group. I) Mitochondrial fission modulated cardiac function following myocardial ischemia reperfusion. The representative M-mode echocardiograms were recorded, and **J**, **K**, **L**, **M**, and **N** present parameters of cardiac function, including LVEF (%), LVFS (%), LVESD (cm), LVPW (cm), and IVS (cm) in each group measured by echocardiography. **O**-**P**. Oxidative factor MDA and antioxidant factor SOD were assessed using the ELISA assay. All numerical data were presented as the mean ± SD ($n = 6$/group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 2 Effect of Mdivi-1 pretreatment on IS, cTnI, MDA, SOD and cardiac function after I/R

Variable	KO-IR	KO-IR-DMSO	KO-IR-Mdivi-1
IS	0.48 ± 0.02	0.49 ± 0.02	0.07 ± 0.02[ab]
cTnI(μmol/mg)	25.43 ± 2.98	31.08 ± 6.35	19.80 ± 2.64[ab]
MDA(μmol/mg)	29.40 ± 5.35	31.26 ± 3.49	0.79 ± 0.13[ab]
SOD(U/mg)	105.12 ± 10.94	95.04 ± 4.62	247.69 ± 30.26[ab]
LVEF(%)	45.58 ± 11.35	41.70 ± 8.08	65.63 ± 5.98[ab]
LVFS(%)	22.27 ± 6.72	22.27 ± 3.38	36.85 ± 1.04[ab]
LVESD(cm)	2.74 ± 0.32	2.94 ± 0.51	2.09 ± 0.10[ab]
LVEDD(cm)	3.31 ± 0.11	3.81 ± 0.60	3.35 ± 0.16
LVPW(cm)	1.17 ± 0.20	0.93 ± 0.24	1.36 ± 0.21[b]
IVS(cm)	0.92 ± 0.23	0.85 ± 0.10	1.18 ± 0.12[b]

Data are presented as the mean ± standard deviation

IR Ischemia reperfsion, *IS* Infarct size, *cTnI* cardiac troponin I, *MDA* Malondialdehyde, *SOD* Superoxide dismutase, *LVEF* Left ventricular ejection fraction, *LVFS* Left ventricular shortening, *LVESD* Left ventricular end-systolic dimension, *LVPW* Left ventricular posterior wall thickness, *IVS* Interventricular septum

[a] $P < 0.01$, vs. KO-IR; [b] $P < 0.01$, vs DMSO

content, further supporting its protective effects (All $P < 0.05$) (Fig. 3O and P) (Table 2). These results suggest that Mdivi-1 pretreatment attenuated myocardial damage mediated by HACE1 deficiency by suppressing mitochondrial fission during I/R.

In addition, the study demonstrated that Mdivi-1 reversed the MMP collapse and inhibited Cyt c release into the cytoplasm caused by HACE1 deficiency under I/RI. (All $P < 0.05$) (Fig. 4A-D). At both the transcriptional and or protein levels, Mdivi-1 also down-regulated pro-apoptotic proteins (Bax, Caspase3, and Cleaved Caspase3) and up-regulated anti-apoptotic protein Bcl2, leading to a decrease in apoptosis (All $P < 0.05$) (Fig. 4E-K). These findings suggest that HACE1 deficiency induces cardiac damage and mitochondrial apoptosis during cardiac I/RI via the activation of mitochondrial fission.

Discussion

In this study, we report for the first time that HACE1 expression is upregulated following I/R injury, especially after 6 h of reperfusion, and that HACE1 deficiency exacerbates myocardial I/R injury and impairs cardiac function. However, inhibition of mitochondrial fission with Mdivi-1 reverses cardiac I/R injury and improves cardiac function. These results indicate a critical pathological role of HACE1-mediated mitochondrial fission in myocardial I/R injury. These findings suggest that targeting HACE1 inhibition may represent a promising and novel therapeutic strategy for myocardial I/R injury.

HACE1 is a well-known gene for oxidative stress and tumor suppression, containing a HECT domain and Ankyrin repeat [14, 16, 26]. It is widely expressed in various tissues, including the heart, brain, and kidney [18]. Previous studies have demonstrated that HACE1 inactivation can cause cardiac defects in zebrafish via ROS-dependent mechanisms, and HACE1 loss can aggravate cardiac dysfunction and mortality in mice [27, 28]. Furthermore, in patients with heart failure, HACE1 expression is significantly elevated [20]. Also, a recent study in vitro found that HACE1 overexpression can mitigate myocardial damage by modulating the Keap1/Nrf2 pathway during I/R. However, this present investigation demonstrates, for the first time, that HACE1 KO worsens myocardial damage and heart dysfunction in vivo under I/RI conditions. As we all know, heart function is significantly affected by myocardial fibrosis, and it serves as an early indication of the onset of clinical heart failure [29]. Similarly, our study found that the lack of HACE1 increased myocardial fibrosis and led to more cardiomyocyte apoptosis, resulting in higher levels of cTnI, a marker of acute myocardial injury, compared to the WT-IR group after 28 days of I/RI. These results indicate that HACE1 has a crucial protective role in myocardial I/RI. However, the mechanisms underlying the myocardial damage caused by HACE1 in this context are still unclear.

Multiple studies have consistently demonstrated that myocardial I/R is a significant contributor to the generation of ROS [30–32]. In addition to their signaling function, ROS can cause damage to cells by oxidizing cellular components, such as lipids, proteins, and DNA, leading to cellular dysfunction and death [33]. Furthermore, ROS-mediated cardiomyocyte necrosis and apoptosis have been identified as major determinants of IS [34], which is closely related to all-cause mortality and rehospitalization for heart failure within the following year [35]. Notably, this study demonstrates that HACE1 deficiency further reduces superoxide dismutase (SOD) activity and augments malondialdehyde (MDA) content triggered by I/R. Additionally, several studies performed in mice and cell models have proposed that HACE1 inactivation promotes oxidative stress damage [13, 14, 17]. However, HACE1 overexpression reduces myocardial damage by inhibiting oxidative stress under I/R conditions [21]. Therefore, the findings of our study reaffirm that HACE1 could exert its cardioprotective effects by suppressing oxidative stress in the context of I/R.

During cardiac I/R, mitochondrial fission and fragmentation are commonly observed, as reported in previous studies [36–38]. Improper mitochondrial fission can exacerbate cell damage by promoting the release of Cyt c from mitochondria into the cytoplasm, which is accompanied by the collapse of mitochondrial membrane potential (MMP) [11, 37]. Inhibition of mitochondrial fission has been shown to reduce cell apoptosis after I/R

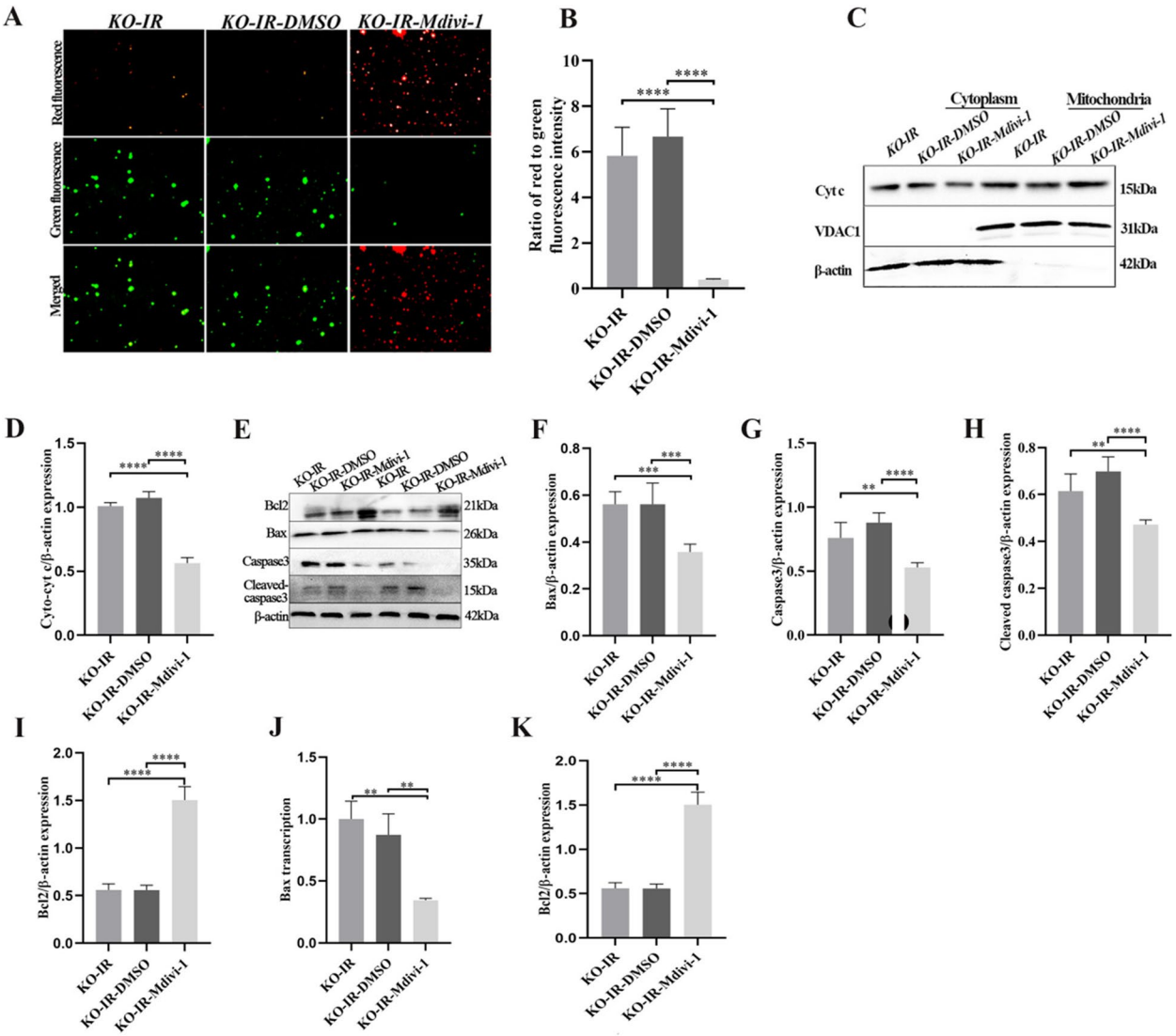

Fig. 4 Drp1-dependent mitochondrial fission was required for HACE1-mediated damage to cardiomyocyte mitochondria. **A**-**B** The mitochondrial membrane potential was assessed using JC-1 staining and analyzed with ImageJ software. **C**-**D** The expression of cytoplasmic cytochrome c (Cyto c) was assessed using Western blot and analyzed with ImageJ software. **E**-**I** Expression levels of Bax, Caspase3,Cleaved caspase3 and Bcl2 were evaluated by western blot and analyzed using image J. **J**-**K** qPCR was utilized to determine the mRNA expression levels of Bax and Bcl2 in various groups.All numerical data were presented as the mean ± SD ($n = 6$, in each group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

injury [23, 38]. Similarly, our study showed that HACE1 loss increases mitochondrial damage and fission, leading to myocardial cell death during I/RI. Further investigation revealed that pretreatment with Mdivi-1, an inhibitor of Drp1, reverses myocardial cell damage in HACE1-deficient mice under I/RI. These findings, combined with previous results, suggest that mitochondrial fission may be a critical event in the execution of myocardial I/R injury. Additionally, previous investigations also have been shown that HACE1 deficiency disrupts mitochondrial homeostasis [22]. Thus, our data suggest that HACE1 loss may aggravate myocardial injury by activating mitochondrial fission under I/RI.

Limitation

This study has limitations that should be acknowledged, such as its use of animal models which may not be directly applicable to humans. Future studies are needed to confirm the clinical relevance of the results in humans. Additionally, the study did not investigate the potential effects of HACE1 deficiency on other cellular processes involved in cardiac I/R injury. Further research should explore these potential mechanisms to gain a comprehensive understanding of the role of HACE1 in cardiac I/R injury. Lastly, while the study suggests that inhibition of mitochondrial fission may be a potential therapeutic strategy for I/R-induced myocardial damage, the safety

and efficacy of Mdivi-1, the specific inhibitor of Drp1, needs further evaluation before considering its use in clinical practice. Overall, while this study provides valuable insights, further research is needed to confirm and extend these findings and identify potential therapeutic strategies for clinical application.

Conclusion

Collectively, our data indicate that HACE1 is critical in I/R-induced cardiac damage. I/R injury elevates HACE1 expression, and HACE1 deficiency activates the Drp1/Cyt c-mediated pathway which induces mitochondrial apoptotic. Extensive mitochondrial fragmentation promotes oxidation stress and increases Cyt c-related mitochondrial apoptosis, thus causing myocardial apoptosis. The findings provide new insights into the development and progression of myocardial I/RI, and may offer a novel therapeutic approach for its treatment.

Abbreviations

HACE1	HECT domain and Ankyrin repeat Containing E3 ubiquitin-protein ligase 1
I/RI	Ischemia-reperfusion injury
KO	Knockout
cTnI	Serum troponin I
MDA	Malondialdehyde
MMP	Mitochondrial membrane potential
ELISA	Enzyme-Linked Immunosorbent Assay
SDS-PAGE	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
SOD	Superoxide dismutase
Drp1	Dynamin-related protein 1
MI	Myocardial infarction
Mff	Mitochondrial fission factor
Fis1	Mitochondrial fission protein 1
IA/LVA	Infarct/ non-infarct areas
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling assay
IHC	Immunohistochemistry
TEM	Transmission Electron Microscope
LVEF	Left ventricular ejection fraction
LVEDD	Left ventricular end-diastolic dimension
LVESD	Left ventricular end-systolic dimension
IVS	Interventricular septum thickness
LVPW	Left ventricular posterior wall thickness
qPCR	Quantitative Real-Time Polymerase Chain Reaction
ANOVA	One-way analysis of variance
Cyt c	Cytochrome c

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12872-024-04445-2.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Acknowledgements

We sincerely thank our supervisor, collaborators, and funding agency for their invaluable support. Special thanks go to our study participants for their time and effort.

Authors' contributions

Hengchen Yao, Lei Gao, and Chen Chen handled the project application. Juan Zheng provided technical support, collected the data, and prepared the supplemental figures. Zhanwei Tang bred the mice, and Ying Sun and Meng Wang handled the reagent ordering. Bangxia Liu performed the experiments, analyzed the data, prepared Figs. 1–4 and Tables I/II, and wrote the manuscript with guidance and feedback from Hengchen Yao. All authors read and approved the final manuscript.

Funding

This research received significant financial support from multiple sources, including the Horizontal Research Project of Shandong University (grant numbers 13450012001901 and 23460012711702), the Natural Science Foundation of Shandong Province (ZR2016HM49), and the Traditional Chinese Medicine Science and Technology Development Plan Project of Shandong Province (No. 2019–0891).

Data availability

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

Declarations

Ethics approval and consent to participate

This project fully protects the rights and interests of mice. All experiments were performed in accordance with relevant guidelines and regulations, and the manuscript report complied with the ARRIVE guidelines for animal experiment reporting [39]. The study has passed the requirements of the Laboratory Animal Ethics Committee of Liaocheng People's Hospital, Liaocheng, China.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Liaocheng People's Hospital, Shandong University, Jinan, China. [2]Department of Cardiology, Heze Mudan People's Hospital, HeZe, Shandong 274000, P.R. China. [3]Department of Joint Laboratory for Translational Medicine Research, Liaocheng People's Hospital, Shandong University, Liaocheng, Shandong 252000, P.R. China. [4]Department of Cardiology, Liaocheng People's Hospital, Shandong University, Liaocheng, Shandong 252000, P.R. China. [5]Department of Nuclear Medicine, Liaocheng People's Hospital, Shandong University, Liaocheng, Shandong 252000, P.R. China. [6]Zhong Yuan Academy of Biological Medicine, Liaocheng People's Hospital, Shandong University, Liaocheng, Shandong 252000, P.R. China. [7]Department of Heart Failure and Cardiac Rehabilitation, Central Hospital affiliated to, Shandong First Medical University, Jinan, Shandong 250000, P.R. China. [8]Department of Urology, Liaocheng People's Hospital, Shandong University, Liaocheng, Shandong 252000, P.R. China.

Received: 13 April 2023 Accepted: 19 December 2024
Published online: 03 February 2025

References

1. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. N Engl J Med. 2007; 357(11):1121–1135.
2. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. J Clin Invest. 2013; 123(1):192–100.
3. Hillis Ld Fau - Lange RA, Lange RA. Myocardial infarction and the open-artery hypothesis. N Engl J Med. 2006;355(323):2475–2477.
4. Andrassy M, Volz HC, Igwe JC, Funke B, Eichberger SN, Kaya Z, Buss S, Autschbach F, Pleger ST, Lukic IK, et al. High-mobility group box-1 in ischemia-reperfusion injury of the heart. Circulation. 2008; 117(25):3216–3226.
5. Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL. Mitochondrial Dysfunction and Myocardial Ischemia-Reperfusion: Implications for Novel Therapies. Annu Rev Pharmacol Toxicol. 2017; 57:535–565.

6. Westermann B. Mitochondrial fusion and fission in cell life and death. Nat Rev Mol Cell Biol. 2010;11(12):872–884.
7. Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. Science. 2012;337(6098):1062–5.
8. Zepeda R, Kuzmicic J Fau - Parra V, Parra V Fau - Troncoso R, Troncoso R Fau - Pennanen C, Pennanen C Fau - Riquelme JA, Riquelme Ja Fau - Pedrozo Z, Pedrozo Z Fau - Chiong M, Chiong M Fau - Sánchez G, Sánchez G Fau - Lavandero S, Lavandero S. Drp1 loss-of-function reduces cardiomyocyte oxygen dependence protecting the heart from ischemia-reperfusion injury. J Cardiovasc Pharmacol. 2014;63(66):477–487.
9. Sharp WW, Fang Yh Fau - Han M, Han M Fau - Zhang HJ, Zhang Hj Fau - Hong Z, Hong Z Fau - Banathy A, Banathy A Fau - Morrow E, Morrow E Fau - Ryan JJ, Ryan Jj Fau - Archer SL, Archer SL. Dynamin-related protein 1 (Drp1)-mediated diastolic dysfunction in myocardial ischemia-reperfusion injury: therapeutic benefits of Drp1 inhibition to reduce mitochondrial fission. FASEB J. 2014;28(21):316–26.
10. Disatnik MH, Ferreira JC, Campos JC, Gomes KS, Dourado PM, Qi X, Mochly-Rosen D. Acute inhibition of excessive mitochondrial fission after myocardial infarction prevents long-term cardiac dysfunction. J Am Heart Assoc. 2013;2(5):e000461.
11. Vasquez-Trincado C, Garcia-Carvajal I, Pennanen C, Parra V, Hill JA, Rothermel BA, Lavandero S. Mitochondrial dynamics, mitophagy and cardiovascular disease. J Physiol. 2016;594(3):509–25.
12. Wang J, Zhou H. Mitochondrial quality control mechanisms as molecular targets in cardiac ischemia-reperfusion injury. Acta Pharm Sin B. 2020;10(10):1866–79.
13. Rotblat B, Southwell AL, Ehrnhoefer DE, Skotte NH, Metzler M, Franciosi S, Leprivier G, Somasekharan SP, Barokas A, Deng Y, et al. HACE1 reduces oxidative stress and mutant Huntingtin toxicity by promoting the NRF2 response. Proc Natl Acad Sci U S A. 2014;111(118):3032–7.
14. M D, R N, B R, L M, A J, S T, S C-L, B R, L L, A M, et al. Hace1 controls ROS generation of vertebrate Rac1-dependent NADPH oxidase complexes. Nature Commun. 2013;4:2180. https://doi.org/10.1038/ncomms3180.
15. El-Naggar AM, Clarkson PW, Negri GL, Turgu B, Zhang F, Anglesio MS, Sorensen PH. HACE1 is a potential tumor suppressor in osteosarcoma. Cell Death Disease. 2019;10(11):21.
16. Anglesio MS, Evdokimova V, Melnyk N, Zhang L, Fernandez CV, Grundy PE, Leach S, Marra MA, Brooks-Wilson AR, Penninger J, et al. Differential expression of a novel ankyrin containing E3 ubiquitin-protein ligase, Hace1, in sporadic Wilms' tumor versus normal kidney. Human Mol Genetics. 2004;13(18):2061–2074.
17. Kumar B, Roy A, Asha K, Sharma-Walia N, Ansari MA, Chandran B. HACE1, an E3 Ubiquitin Protein Ligase, Mitigates Kaposi's Sarcoma-Associated Herpesvirus Infection-Induced Oxidative Stress by Promoting Nrf2 Activity. J Virol. 2019;93(19):e01812–01818.
18. Razaghi B, Steele SL, Prykhozhij SV, Stoyek MR, Hill JA, Cooper MD, McDonald L, Lin W, Daugaard M, Crapoulet N, et al. hace1 Influences zebrafish cardiac development via ROS-dependent mechanisms. Dev Dynamics. 2018;247(242):289–303.
19. Ugarteburu O, Sanchez-Viles M, Ramos J, Barcos-Rodriguez T, Garrabou G, Garcia-Villoria J, Ribes A, Tort F. Physiopathological Bases of the Disease Caused by HACE1 Mutations: Alterations in Autophagy, Mitophagy and Oxidative Stress Response. J Clin Med. 2020;29(24):913.
20. Zhang L, Chen X, Sharma P, Moon M, Sheftel AD, Dawood F, Nghiem MP, Wu J, Li RK, Gramolini AO, et al. HACE1-dependent protein degradation provides cardiac protection in response to haemodynamic stress. Nat Commun. 2014;15:3430.
21. Chen TY, Zheng SA-O. Hace1 overexpression mitigates myocardial hypoxia/reoxygenation injury via the effects on Keap1/Nrf2 pathway. In Vitro Cell Dev Biol Anim. 2022;58(59):830–9.
22. Ehrnhoefer DE, Southwell AL, Sivasubramanian M, Qiu X, Villanueva EB, Xie Y, Waltl S, Anderson L, Fazeli A, Casal L, et al. HACE1 is essential for astrocyte mitochondrial function and influences Huntington disease phenotypes in vivo. Human Mol Genetics. 2018;27(12):239–253.
23. Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. Circulation. 2010;121(118):2012–2022.
24. Gao E, Lei YH, Shang X, Huang ZM, Zuo L, Boucher M, Fan Q, Chuprun JK, Ma XL, Koch WJ. A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse. Circ Res. 2010;107(112):1445–1453.
25. Gao S, Ho D, Vatner DE, Vatner SF. Echocardiography in Mice. Curr Protoc Mouse Biol. 2011;1:71–83. https://doi.org/10.1002/9780470942390.mo100130.
26. Liu Z, Chen P, Gao H, Gu Y, Yang J, Peng H, Xu X, Wang H, Yang M, Liu X, et al. Ubiquitylation of autophagy receptor Optineurin by HACE1 activates selective autophagy for tumor suppression. Cancer Cell. 2014;26(11):106–120.
27. Razaghi B, Steele SL, Prykhozhij SV, Stoyek MR, Hill JA, Cooper MD, McDonald L, Lin W, Daugaard M, Crapoulet N, et al. hace1 Influences zebrafish cardiac development via ROS-dependent mechanisms. Dev Dynamics. 2018;247(2):289–303.
28. Zhang L, Chen X, Sharma P, Moon M, Sheftel AD, Dawood F, Nghiem MP, Wu J, Li R-K, Gramolini AO, et al. HACE1-dependent protein degradation provides cardiac protection in response to haemodynamic stress. Nat Commun. 2014;5:3430.
29. Wu QQ, Xiao Y, Yuan Y, Ma ZG, Liao HH, Liu C, Zhu JX, Yang Z, Deng W, Tang QZ. Mechanisms contributing to cardiac remodelling. Clin Sci (Lond). 2017;131(118):2319–2345.
30. Jahangiri A, Leifert WR, Kind KL, McMurchie EJ. Dietary fish oil alters cardiomyocyte Ca2+ dynamics and antioxidant status. Free Radical Biol Med. 2006;40(9):1592–602.
31. Yao H, Xie Q, He Q, Zeng L, Long J, Gong Y, Li X, Li X, Liu W, Xu Z, et al. Pretreatment with Panaxatriol Saponin Attenuates Mitochondrial Apoptosis and Oxidative Stress to Facilitate Treatment of Myocardial Ischemia-Reperfusion Injury via the Regulation of Keap1/Nrf2 Activity. Oxid Med Cell Longev. 2022;2022:9626703.
32. Shen Y, Liu X, Shi J, Wu X.: Involvement of Nrf2 in myocardial ischemia and reperfusion injury. Int J Biol Macromol. 2019;125:496–502.
33. Izyumov DS, Domnina LV, Nepryakhina OK, Avetisyan AV, Golyshev SA, Ivanova OY, Korotetskaya MV, Lyamzaev KG, Pletjushkina OY, Popova EN, et al. Mitochondria as source of reactive oxygen species under oxidative stress. Study with novel mitochondria-targeted antioxidants--the "Skulachev-ion" derivatives. Biochemistry (Mosc). 2010;75(2):123–129.
34. Matsui Y, Takagi H Fau - Qu X, Qu X Fau - Abdellatif M, Abdellatif M Fau - Sakoda H, Sakoda H Fau - Asano T, Asano T Fau - Levine B, Levine B Fau - Sadoshima J, Sadoshima J. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ Res. 2007;100(106):914–122.
35. Stone GW, Selker HP, Thiele H, Patel MR, Udelson JE, Ohman EM, Maehara A, Eitel I, Granger CB, Jenkins PL, et al. Relationship Between Infarct Size and Outcomes Following Primary PCI: Patient-Level Analysis From 10 Randomized Trials. J Am Coll Cardiol. 2016;67(14):1674–1683.
36. Zhou H, Wang J, Zhu P, Zhu H, Toan S, Hu S, Ren J, Chen Y. NR4A1 aggravates the cardiac microvascular ischemia reperfusion injury through suppressing FUNDC1-mediated mitophagy and promoting Mff-required mitochondrial fission by CK2α. Basic Res Cardiol. 2018;113(114):123.
37. Jin Q, Li R, Hu N, Xin T, Zhu P, Hu S, Ma S, Zhu H, Ren J, Zhou H. DUSP1 alleviates cardiac ischemia/reperfusion injury by suppressing the Mff-required mitochondrial fission and Bnip3-related mitophagy via the JNK pathways. Redox Biol. 2018;14:576–587.
38. Maneechote C, Palee S, Kerdphoo S, Jaiwongkam T, Chattipakorn SC, Chattipakorn N. Differential temporal inhibition of mitochondrial fission by Mdivi-1 exerts effective cardioprotection in cardiac ischemia/reperfusion injury. Clin Sci (Lond). 2018;132(115):1669–1683.
39. Percie du Sert NA-O, Ahluwalia AA-O, Alam SA-O, Avey MA-O, Baker M, Browne WA-O, Clark AA-O, Cuthill IA-O, Dirnagl UA-O, Emerson M, et al. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 2020; 18(7):e3000411(1545–7885).

Publisher's Note