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RESEARCH ARTICLE

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Downregulation of tripartite motif protein 11 attenuates cardiomyocyte apoptosis after ischemia/reperfusion injury via DUSP1-JNK1/2

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Abstract

Currently, the prevention of ischemic diseases such as myocardial infarction associated with ischemia/reperfusion (I/R) injury remains to be a challenge. Thus, this study was designed to explore the effects of tripartite motif protein 11 (TRIM11) on cardiomyocytes I/R injury and its underlying mechanism. Cardiomyocytes AC16 were used to establish an I/R injury cell model. After TRIM11 downregulation in I/R cells, cell proliferation (0, 12, 24, and 48 h) and apoptosis at 48 h as well as the related molecular changes in oxidative stress-related pathways was detected. Further, after the treatment of TRIM11 overexpression, SP600125, or DUSP1 overexpression, cell proliferation, apoptosis, and related genes were detected again. As per our findings, it was determined that TRIM11 was highly expressed in the cardiomyocytes AC16 after I/R injury. Downregulation of TRIM11 was determined to have significantly reduced I/R-induced proliferation suppression and apoptosis. Besides, I/R-activated c-Jun N-terminal kinase (JNK) signaling and cleaved caspase 3 and Bax expression were significantly inhibited by TRIM11 downregulation. In addition, the overexpression of TRIM11 significantly promoted apoptosis in AC16 cells, and JNK1/2 inhibition and DUSP1 overexpression potently counteracted the induction of TRIM11 overexpression in AC16 cells. These suggested that the downregulation of TRIM11 attenuates apoptosis in AC16 cells after I/R injury probably through the DUSP1-JNK1/2 pathways.

KEYWORDS
apoptosis, caspase 3, ischemia/reperfusion injury, JNK1/2 pathways, TRIM11

1 | INTRODUCTION

Acute myocardial infarction (MI) refers to myocardial ischemic necrosis which is mainly caused by severe coronary stenosis and myocardial insufficiency due to coronary atherosclerosis or coronary embolism, inflammation, sputum, and coronary occlusion (Hausenloy et al., 2016; Heusch & Gersh, 2016; Rentrop & Feit, 2015). During the rescue and treatment of ischemic diseases, medical scientists have gradually discovered that myocardial reperfusion therapy, rather than ischemia, is the main cause of tissue damage. Currently, myocardial reperfusion therapy has been identified to be essential; however, studies have shown that reperfusion can induce cardiomyocyte necrosis, apoptosis, mitochondrial dysfunction, increased lipid peroxides, large amounts of free radicals, and the inhibition of myocardial function such as malignant

arrhythmia, decreased left ventricular contractility, and decreased intraventricular pressure (Elahi et al., 2009; Yang et al., 2012). Thus, identifying novel therapeutic approaches that reduce myocardial ischemia/reperfusion (I/R) injury is deemed of great importance.

Tripartite motif proteins (TRIMs), characterized by three unique structural domains, including a RING finger, a B-box, and a coiled-coil, have been reported to be involved in a variety of cellular processes such as development and oncogenesis (Crawford et al., 2018; Hatakeyama, 2017; Petrera & Meroni, 2012; Tocchini & Ciosk, 2015). There are multiple TRIMs found to have a function in I/R injury. For example, TRIM72, also known as MG53, can ameliorate I/R injury in various organs such as the heart, lung, kidney, and brain (Duann et al., 2015; Jia et al., 2014; Liu et al., 2015; Yao et al., 2016). The knockdown of TRIM47 can attenuate cerebral I/R injury by inhibiting apoptosis and inflammation (Hao et al., 2019), whereas a deficiency in TRIM8 can relieve hepatic I/R injury. Tao et al. (2019) TRIM11 is a novel humanin (HN)-interacting protein, and it has been found that TRIM11 binds to HN and can downregulate HN levels. Niikura et al. (2003) HN, which originated from the mitochondrial genome, is a newly discovered 24-amino acid polypeptide that provides cardioprotection against cardiac I/R injury (Muzumdar et al., 2010; Thummasorn et al., 2016).

Mitogen-activated protein kinases (MAPKs) are described as a group of evolutionarily conserved serine-threonine protein kinases. They are activated by a series of extracellular stimuli and mediate signals from the cell membrane to the nucleus, regulating a number of physiological activities such as inflammation, apoptosis, canceration, invasion, and metastasis of tumor cells. The activation of MAPKs has been identified as a consequence of the early events following I/R injury. Toledo-Pereyra et al. (2004) Studies have related extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), which are members of the MAPK superfamily, to I/R injury (Lai et al., 2004; Wu et al., 2013). For example, tyrosol can prevent I/R-induced cardiac injury through the mitigation of reactive oxygen species and the inactivation of ERK and JNK signaling (Sun et al., 2015).

Threonine-tyrosine dual-specificity protein phosphatase (DUSP), also known as MAPK phosphatase, can dephosphorylate and inactivate MAPKs, ERK, p38, and JNK in a context-dependent manner (Johnson & Lapadat, 2002; Sabathié et al., 1975). DUSP1, which is a member of DUSP, is an antiapoptotic phosphatase and is found abundantly in tissues, especially the heart, liver, and lungs (Kwak et al., 1994). Studies have revealed that DUSP1 can promote cancer progression by suppressing cancer cell apoptosis, and the inhibition of DUSP1 may be a neoadjuvant therapy for cancer via the enhancement of apoptosis (Candas & Li, 2015; Li et al., 2015; Wainstein & Seger, 2016; Wang et al., 2006). In cardiac I/R, the antiapoptotic effect of DUSP1 has been found to unexpectedly improve cardiac I/R injury (Zhang et al., 2018). In addition, DUSP1 can alleviate cardiac I/R injury through the JNK pathways. Jin et al. (2018) However, whether TRIM11 can regulate the apoptosis of cardiomyocytes after I/R injury by regulating DUSP1 expression remains to be unknown.

Here, we constructed a model of myocardial I/R and explored the role ofTRIM11 in I/R injury, as well as its underlying mechanism. It was found that the downregulation of TRIM11 inhibited apoptosis after myocardial I/R injury through the DUSP1-JNK1/2 pathways, thus alleviating cardiac I/R injury.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Human cardiomyocytes AC16 were purchased from Suzhou Beina Chuanglian Biotechnology Co., LTD (BNCC337712). In a 37°C incubator with 5% CO_2, AC16 cells were cultured in DMEM medium containing 10% fetal bovine serum (16000-044, CIBCO) and 1% double antibiotic (100×, P1400, Solarbio).

2.2 | TRIM11 overexpression or downregulation and DUSP1 overexpression

According to TRIM11 (NM_145214.2) sequences, the primers were designed: TRIM11-F: 5′-CGGAATTCATGGCCGCCCCCGAC-3′ (EcoR I), TRIM11-R: 5′-CGGGATCCTCACTGGGGAGCCAGGGTG-3′ (BamH I). The coding sequences of TRIM11 containing the restriction site were amplified by Jinweizhi Gene Company following the primers. The TRIM11 coding sequence was inserted into the pLVX-Puro vector (Clontech) through EcoR I and BamH I restriction sites to construct a pLVX-Puro-TRIM11 plasmid (oeTRIM11). PLVX-Puro-TRIM11 was mixed with psPAX2 and pMD2G packaging plasmids (Addgene) to transfect 293T cells, and the virus was harvested 48 h later. Lenti-X GoStix (Clontech) was used to determine the virus titer, AC16 cells were infected according to multiplicity of infection (MOI) = 10.

TRIM11 interference sequences (as shown in Table 1) were cloned to pLKO.1-puro plasmids to silence TRIM11 (shTRIM11-1/-2/-3).

According to DUSP1 (NM_004417.4) sequences, the primers were designed: DUSP1-F: 5′-CGGGATCCATGGTCATGGAAGTGGGCAC-3′ (BamH I), DUSP1-R: 5′-CGGAATTCTCAGCAGCTGGGAGAGGTC-3′ (EcoR I). The coding sequences of DUSP1 containing the restriction site were amplified by Jinweizhi Gene Company following the primers. The DUSP1 coding sequence was inserted into the pCMV-Tag2B vector (Addgene) through BamH I and EcoR I restriction sites to construct a pCMV-Tag2B-DUSP1 plasmid (oeDUSP1). pCMV-Tag2B-DUSP1 plasmid was mixed with psPAX2 and pMD2G packaging plasmids (Addgene) to transfect 293T cells, and the virus was harvested 48 h later. Lenti-X GoStix (Clontech) was used to determine the virus titer, AC16 cells were infected according to MOI = 10.

TABLE 1 TRIM11 interference sequences

Name	Sequences
TRIM11 site 1 (672–690)	GGAGAAGTCACTGGAGCAT
TRIM11 site 2 (708–726)	GGATGCGTTGCTGTTCCAA
TRIM11 site 3 (746–764)	GCGTCTTGTGGCAGAAGAT

2.3 | Cell transfection

When in the logarithmic growth phase, AC16 cells and I/R injury cells were trypsinized and counted for 1×10^6 cells/ml suspension; then, 2 ml of suspension was inoculated into six-well plates for overnight culture at 37°C in a 5% CO_2 incubator. When the cells grow to 60%–70% confluency, I/R injury cells were transfected with shTRIM11-1/-2/-3 lentivirus (1.5 μg) by Lipo2000. AC16 cells were then transfected with oeTRIM11 or oeDUSP1 lentivirus. After 6 h of transfection, the serum-free transfer solution was replaced by the complete medium for the 48-hour culture.

2.4 | Establishment of myocardial I/R cell model

Ischemia was stimulated in cardiomyocytes AC16 for 3 h by incubating them with serum-free DMEM/F-12 medium in an incubator containing 1% O_2 and 5% CO_2 at 37°C. Next, reperfusion was carried out under normoxic conditions (21% O_2, 5% CO_2, and 74% N_2) using DMEM/F-12 medium containing 10% fetal bovine serum for 6, 12, and 24 h. Finally, the I/R injury model for 24 h was selected for subsequent experiments.

3 | LACTIC DEHYDROGENASE (LDH) ACTIVITY DETECTION

The levels of LDH in cells were measured using the LDH (A020-2) Kit (Nanjing, Jiancheng Biotechnology Research Institute) according to the manufacturers' instructions. Assays were performed in triplicate, and the mean values of each sample were calculated manually.

3.1 | Cell proliferation assay

When in the logarithmic growth phase, I/R-injured-AC16 cells after 48 h of transfection were trypsinized and counted for 5×10^4 cells/ml suspension; then, each well of 96-well plates was seeded with 100 μl of suspension for overnight culture at 37°C. Groups were then divided to be treated with shNC (negative control) and shTRIM11-1/-2/-3, while AC16 cells without treatment served as controls. At a volume ratio of 1:10, Cell Counting Kit 8 (CCK-8, SAB, CP002) was mixed with serum-free DMEM medium; then, at 0, 12, 24, and 48 h of culture, 100 μl of the mixture was added to each well and incubated for 1 h. The optical density at 450 nm indicating cell proliferation was measured on a microplate reader (DNM-9602, Perlong).

3.2 | Cell apoptosis assay

Annexin V-fluorescein isothiocyanate (FITC) cell apoptosis detection kit (C1063, Beyotime) was used for apoptosis detection. Briefly, the cells (1×10^6) were gently resuspended in 195 μl of Annexin V-FITC binding solution and incubated for 15 min with 5 μl Annexin V-FITC at 4°C in the dark. Similarly, at 4°C in the dark, the cells were incubated with 5 μl of propidium iodide (PI) staining solution for 5 min. Cells without treatment of either Annexin V-FITC or PI were served as negative controls. Flow cytometry (FCM) experiments were performed immediately after cell pretreatment, and the percentage of apoptosis was evaluated using BD Accuri™ C6 (Version 1.0.264.21; BD Biosciences).

3.3 | Real-time polymerase chain reaction (RT-PCR) assay

After the treatment of the I/R injury (6, 12, 24 h) or transfection (shTRIM11-1/-2/-3, oeTRIM11, and oeDUSP1), AC16 cells were collected, and then TRIzol reagent (1596-026, Invitrogen) was used to extract the total RNAs. Following quantification, Reverse Transcription Kit (#K1622, Fermentas) was used to reverse-transcribe RNA into cDNA. With an SYBR Green PCR Kit (Thermo Fisher Scientific, Inc.), RT-PCR, taking cDNA as templates, was conducted on the ABI 7300 Real-Time PCR system (ABI-7300, Applied Biosystems) according to the procedures: 95°C, 10 min (95°C, 15 s; 60°C, 45 s) × 40. Using the $2^{-\triangle\triangle C_q}$ method, the mRNA expression of TRIM11 and DUSP1, relative to GAPDH, was calculated. Livak and Schmittgen (2001) All primers are listed in Table 2.

4 | CO-IMMUNOPRECIPITATION ASSAY (CO-IP)

Cell lysates were extracted using IP-lysis buffer (20 mM Tris-HCl Ph 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, and protease inhibitors). Anti-TRIM11 antibody (10851-1-AP, Proteintech) and anti-DUSP1 antibody (#48625, CST) were first, followed by the addition of Protein A/G PLUS-Agarose beads (50% slurry) to the precleared lysates for overnight incubation at 4°C. Rabbit IgG (Santa Cruz Biotechnology, sc-2027) was used as a control. The beads were washed, resuspended in loading buffer (10% SDS, 0.2 m Tris pH 6.8, 50% glycerol, 0.1% (w/v) bromophenol blue (5×), 1.42 m 2-mercaptoethanol), and incubated for 30 min at 37°C before being boiled for 10 min. Finally, the antibodies of anti-TRIM11 (Ab111694, Abcam) and anti-DUSP1 (#35217, Cell Signaling Technology) were used for western blot analysis to analyze the interaction between TRIM11 and DUSP1.

TABLE 2 Primer sequences

Name	Primers
TRIM11	Primer F: 5′-GCCTTCTGTGGCGACGAG-3′
	Primer R: 5′-GCATCCTGCATCTGCTTCC-3′
DUSP1	Primer F: 5′-ACTGCCGCTCCTTCTTCG-3′
	Primer R: 5′-GCTCGTCCAGCAACACCAC-3′
GAPDH	Primer F: 5′-AATCCCATCACCATCTTC-3′
	Primer R: 5′-AGGCTGTTGTCATACTTC-3′

4.1 | Western blot analysis analysis

After extracting the total proteins using RIPA buffer (containing protease and phosphatase inhibitor, R0010, Solarbio), the proteins were quantified using a BCA Protein Quantitation Kit (PICPI23223, Thermo), resolved by 10% SDS-PAGE electrophoresis, transferred to polyvinylidene fluoride membranes (HATF00010, Millipore), blocked in skimmed milk, and then incubated with primary and secondary antibodies. The protein bands were exposed on an ECL imaging system (Tanon 5200, Tanon) after being developed using a chemiluminescent reagent (WBKLS0100, Millipore). The protein levels, relative to GAPDH, were calculated using ImageJ version 1.47v. Anti-TRIM11 (Ab111694), anti-cleaved caspase 3 (Ab2302), anti-JNK1/2 (Ab179461), anti-p-JNK1/2 (Ab124956), p38 (1:1000, Ab59461, Abcam), and p-p38 (1:1000, Ab4822, Abcam) from Abcam (Shanghai, China) and anti-ERK1/2 (#9102), anti-p-ERK1/2 (#9101), anti-DUSP1 (#35217), and anti-GAPDH (#5174) antibodies from Cell Signaling Technology were used.

4.2 | Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software, Inc.). With at least three independent experiments, continuous data were presented as mean ± standard deviation (SD). Significance among multiple groups was analyzed using a one-way analysis of variance with Tukey's posttest. Values of $p < .05$ were considered to be statistically significant.

5 | RESULTS

5.1 | TRIM11 was highly expressed in the myocardial I/R cell model

After treatments for 6, 12, and 24 h, LDH activity was detected to ensure that the myocardial I/R cell model was successfully established. As shown in Figure 1a, LDH activity in AC16 cells after I/R injury was significantly increased in a time-dependent manner. TRIM11 expression was detected by RT-PCR and western blot. As shown in Figures 1b,c, both mRNA and protein expressions of TRIM11 were significantly increased in AC16 cells after I/R injury in a time-dependent manner. Thus, the 24 h of treatment duration was selected for subsequent experiments.

5.2 | Downregulation of TRIM11 significantly inhibited the apoptosis in AC16 cells after I/R injury

After lentivirus injection, the efficiency of TRIM11 interference was determined, and the results showed that both TRIM11 mRNA (Figure 2a) and protein (Figure 2b) expressions were significantly downregulated in AC16 cells. After I/R injury, cell proliferation was significantly reduced (Figure 2c), whereas apoptosis was significantly enhanced (Figure 2d). Currently, apoptosis-related proteins Bax and cleaved caspase 3 were identified to have significantly increased in myocardial cells after I/R injury, whereas Bcl-2 significantly decreased (Figure 2e). Furthermore, the protein levels of phosphorylated-ERK1/2 (p-ERK1/2), p-JNK1/2, and p-p38 were significantly increased in myocardial cells after I/R injury (Figure 2e). In addition, the downregulation of TRIM11 potently inhibited I/R injury-induced apoptosis and JNK1/2 activation in AC16 cells, whereas I/R injury-activated ERK1/2 and p38 did not change significantly after TRIM11 downregulation.

5.3 | TRIM11-regulated apoptosis in AC16 cells probably through the JNK1/2 pathway

Next, we explored the role of JNK1/2 pathway in TRIM11 regulating apoptosis in AC cells. The efficiency of TRIM11 overexpression was determined (Figure 3a,b). We found that the upregulation of TRIM11 significantly promoted apoptosis in AC16 cells accompanied by increased cleaved caspase 3 and Bax, decreased Bcl-2, and increased p-JNK1/2 level. Furthermore, JNK1/2 inhibitors could potently reverse the induction of TRIM11 overexpression (Figure 3c,d).

5.4 | The interaction between TRIM11 and DUSP1 in AC16 cells

Further, we have found that the overexpression of TRIM11significantly decreased DUSP1 protein, whereas DUSP1 mRNA remains unchanged (Figure 4a,b). Furthermore, Co-IP showed that TRIM11 interacted with DUSP1 (Figure 4c), and the overexpression of TRIM11 significantly promoted DUSP1 ubiquitination (Figure 4d). Consistent with this, TRIM11 overexpression was noted to significantly decrease DUSP1 protein, and MG132 (proteasome inhibitor) potently counteracted the effect of TRIM11 overexpression (Figure 4e).

5.5 | TRIM11-regulated apoptosis in AC16 cells probably through the modulation of DUSP1 expression

The efficiency of DUSP1 overexpression was determined (Figure 5a,b). We found that the overexpression of DUSP1 significantly decreased TRIM11-induced cleaved caspase-3, Bax, and p-JNK1/2, and apoptosis in myocardial cells, whereas it increased Bcl-2 (Figure 5c,d).

6 | DISCUSSION

Currently, the prevention of ischemic diseases such as MI associated with I/R remains a challenge. TRIM11 is mainly found to play a key role in cell processes of various human cancers,

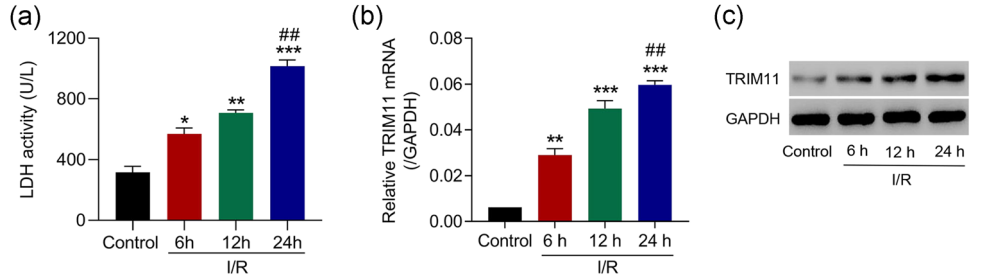

FIGURE 1 TRIM11 was highly expressed in the myocardial I/R cell model. After the myocardial I/R cell model was established for 6, 12, and 24 h, (a) the LDH activity and the mRNA (b) and (c) protein expression of TRIM11 were detected. *$p < .05$, **$p < .01$, ***$p < .001$ versus. control, ##$p < .01$ versus I/R (12 h). Control, AC16 cells

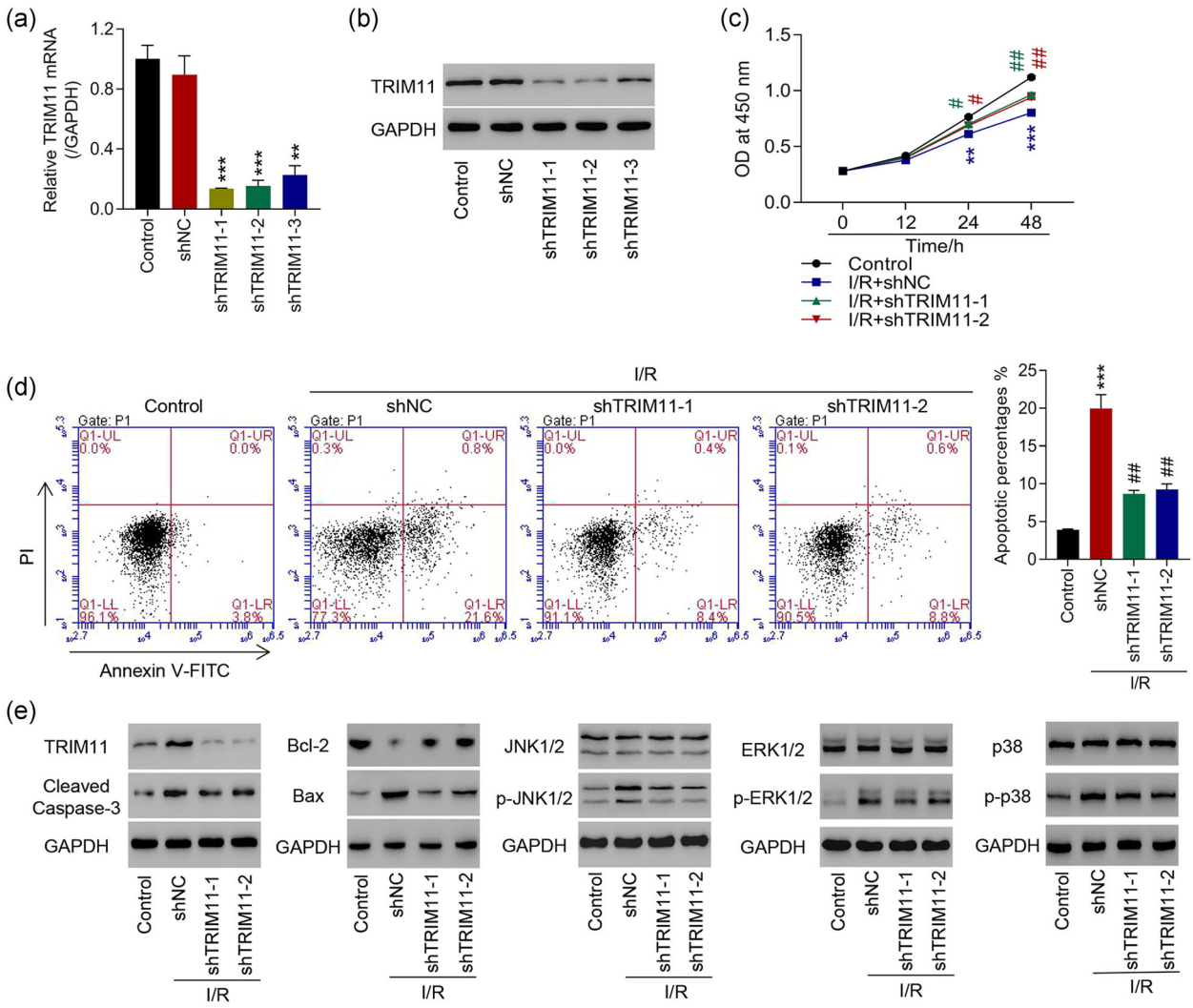

FIGURE 2 Downregulation of TRIM11 significantly inhibited the apoptosis in AC16 cells after I/R injury (a, b). After injection with the shTRIM11-1/-2/-3 lentivirus, the efficiency of TRIM11 downregulation was determined via real-time polymerase chain reaction (a) and western blot analysis (b). **$p < .01$, ***$p < .001$ versus shNC. I/R-injured AC16 cells were infected with shTRIM11-1/-2. (c) The cell proliferation at 0, 12, 24, and 48 h was detected. (d) Apoptosis at 48 h was detected. (e) The protein expressions of TRIM11, cleaved caspase-3, Bcl-2, Bax, JNK1/2, p-JNK1/2, ERK1/2, p-ERK1/2, p38, and p-p38 were detected. **$p < .01$, ***$p < .001$ versus control, #$p < .05$, ##$p < .01$ versus I/R + shNC. Control, AC16 cells; shNC, negative control of TRIM11 interference

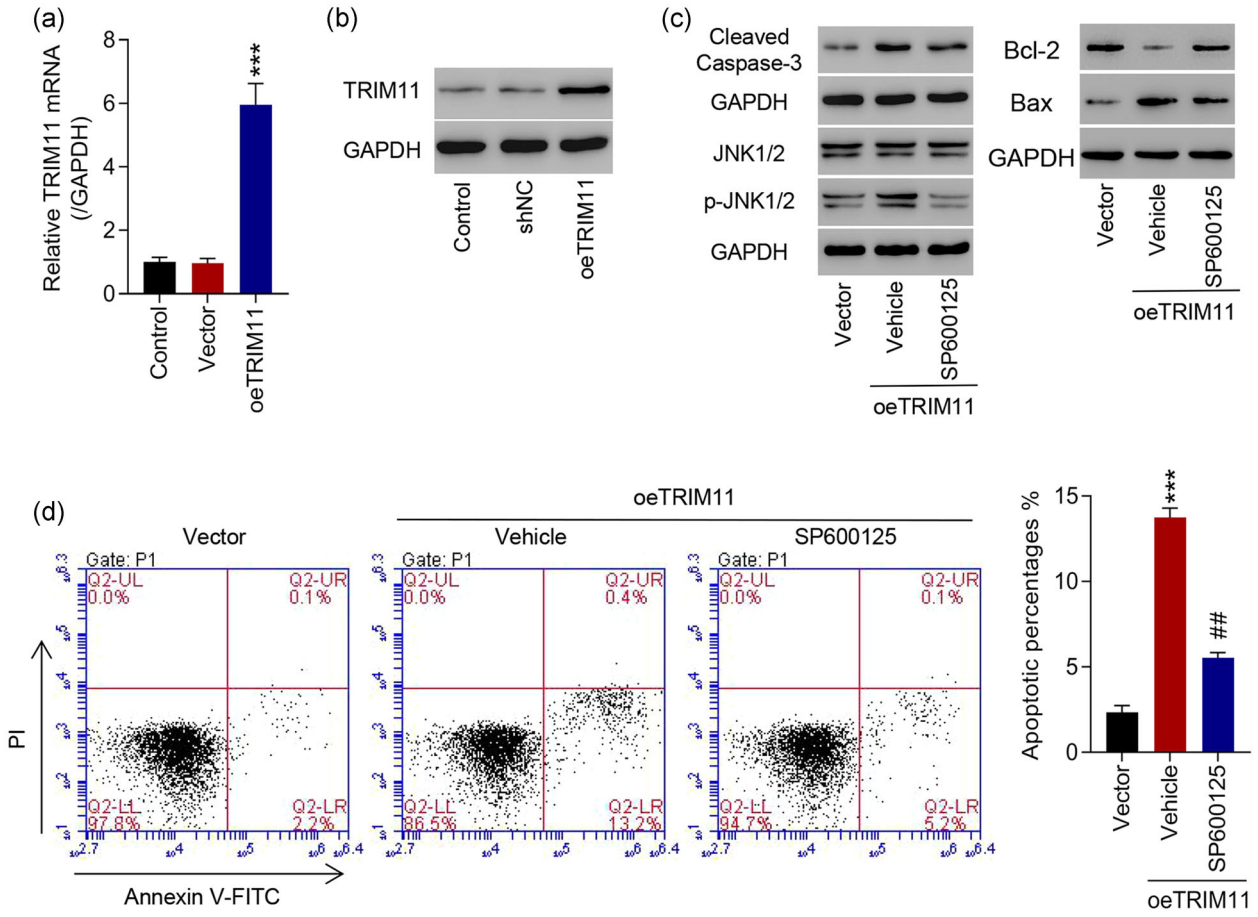

FIGURE 3 TRIM11-regulated apoptosis in AC16 cells probably through the JNK1/2 pathway (a, b) After injection with the oeTRIM11 lentivirus, the efficiency of TRIM11 overexpression was determined using real-time polymerase chain reaction (a) and western blot analysis (b). ***$p < .001$ versus vector. AC16 cells were treated with the oeTRIM11 lentivirus or SP600125 (JNK1/2 inhibitor, 1 μM). (c) The protein levels of cleaved caspase-3, Bcl-2, Bax, JNK1/2, p-JNK1/2 were detected. (d) Apoptosis at 48 h was detected. ***$p < .001$ versus vector, ##$p < .01$ versus oeTRIM11 + vector. Control, AC16 cells; vector, negative control of TRIM11 overexpression

including cell proliferation, apoptosis, and metastasis (Di et al., 2013; Yin et al., 2016; Zhang et al., 2017). In this study, we have found that TRIM11 was highly expressed in AC16 cells after I/R injury. The downregulation of TRIM11 significantly promoted cell proliferation and inhibited apoptosis in I/R-injured AC16 cells, together with decreased cleaved caspase 3 and Bax, and increased Bcl-2, whereas TRIM11 overexpression promoted apoptosis in AC16 cells. Caspase 3, a cysteine protease, has been reported to regulate apoptosis and act function by its activated form, cleaved caspase 3 (Brentnall et al., 2013). It is reported that TRIM47 can regulate apoptosis and inflammation by modulating caspase 3 (Hao et al., 2019). These findings suggested that the downregulation of TRIM11 may attenuate I/R injury in AC16 cells by inhibiting apoptosis.

We have also investigated the potential molecular mechanism of TRIM11 in regulating I/R-induced apoptosis in AC16 cells. MAPKs are suggested to be important regulators of apoptosis in response to myocardial I/R (Kobayashi et al., 2006). Two members of the MAPK family, that is, ERK and JNK, are often activated immediately in organs with I/R injury and play an important role in inducing apoptosis after I/R injury (Armstrong, 2004; Baines & Molkentin, 2005). For instance, the activation of the JNK signaling cascade promotes apoptosis (Chen, 2012; Dhanasekaran & Reddy, 2008), whereas ERK activation contributes to cell injury prevention (Kunduzova et al., 2002). Moreover, by activating the AMPK/ERK pathways, trimetazidine has a protective effect on the heart after I/R injury (Liu et al., 2016). Here we found that the downregulation of TRIM11 significantly inhibited the I/R-induced activation of the JNK1/2 signaling pathway. Additionally, the inhibition of JNK1/2 potently counteracted the induction of TRIM11 overexpression in AC16 cells. A previous study demonstrated that TRIM8 deficiency is capable of alleviating apoptosis after hepatic I/R in vivo by inhibiting TAKI-p38/JNK signaling pathway activation and also during cerebral I/R injury (Tao et al., 2019). Besides, in a variety of human diseases, the TRIM11-mediated ERK/JNK signaling pathway has been found to participate in cellular processes such as cell proliferation and apoptosis (Dai et al., 2019; Wang

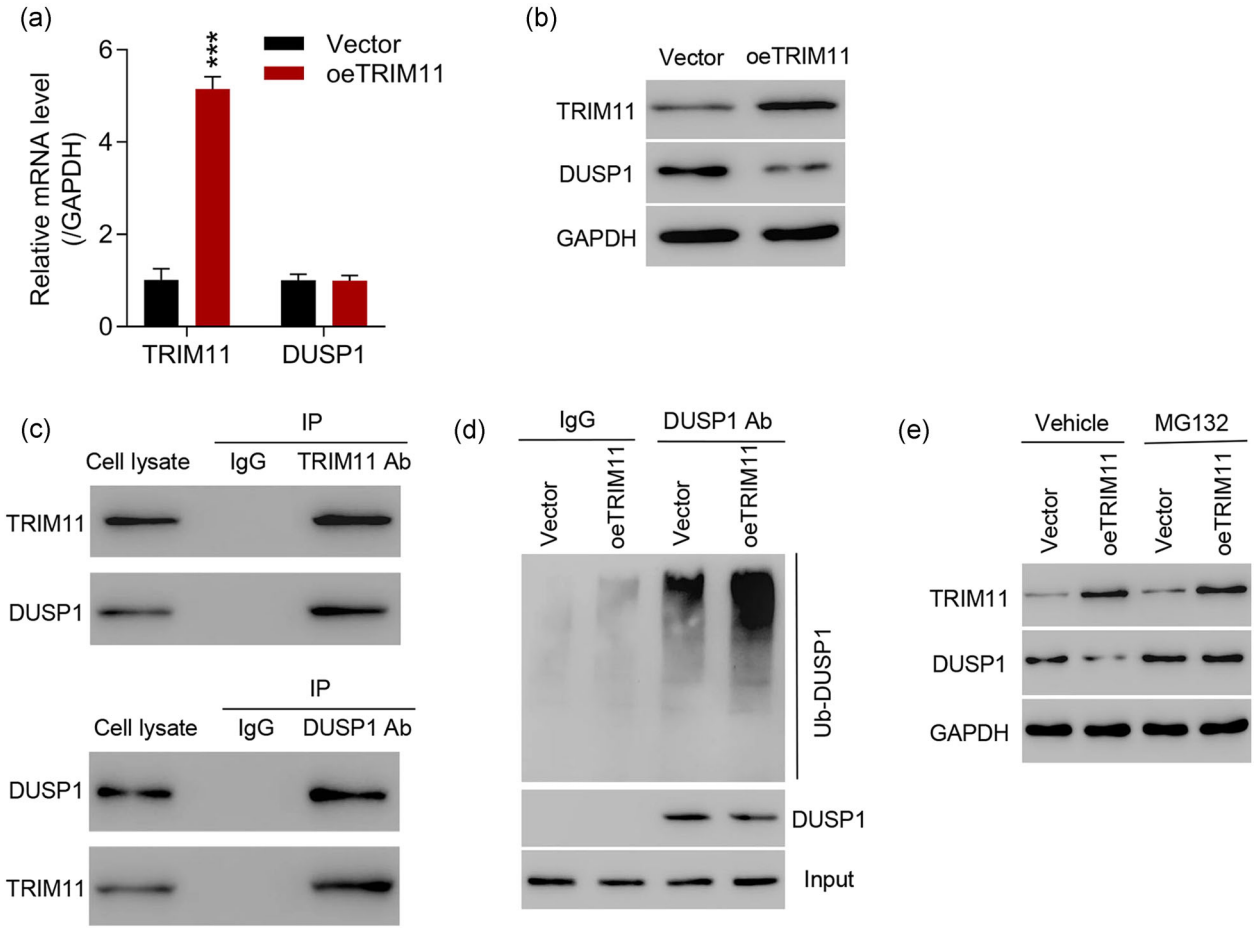

FIGURE 4 The interaction between TRIM11 and DUSP1 in AC16 cells (a, b). After injection with the oeTRIM11 lentivirus, the mRNA (a) and protein (b) expressions of TRIM11 and DUSP1 were detected. ***$p < .001$ versus vector. (c) Co-immunoprecipitation assay of the interaction between TRIM11 and DUSP1. (d) DUSP1 ubiquitination assay. (e) After oeTRIM11 or MG132 (10 μmol/L) treatment, the protein levels of TRIM11 and DUSP1 were detected

et al., 2016). In line with these reports, it can be inferred that TRIM11 downregulation has suppressed apoptosis in AC16 cells after I/R injury probably by inhibiting the activation of the JNK1/2 pathways.

Further, we found that DUSP1 overexpression significantly inhibited TRIM11-induced apoptosis in AC16 cells, and its downstream JNK1/2 pathways were inactivated. It has been reported that the low expression of DUSP1 can impede the protective effect of NaHS on myocardial I/R injury (Ren et al., 2020). DUSP1 can alleviate cardiac I/R injury by suppressing mitochondrial fission and mitophagy via the JNK pathways (Jin et al., 2018). Besides, USP49 inhibits I/R-induced cell viability suppression and apoptosis in AC16 cells via the DUSP1-JNK1/2 pathways (Zhang et al., 2019). Altogether, we speculated that TRIM11-regulated I/R-induced apoptosis probably through the DUSP1-JNK1/2 pathways.

The findings of our study indicate that TRIM11 plays a key role in cardiac I/R injury; thus, they improve our understanding of the role of TRIM11/DUSP1/JNK1/2 in apoptosis in AC16 after I/R injury. One of the limitations of this study is that the greater part of the study was performed in cell culture experiments. Thus, confirming these findings using in vivo experiments and clinical samples from patients will give us a clearer understanding of the TRIM11/DUSP1/JNK1/2 pathway in cardiac I/R injury. Although further investigations are needed, this study identifies a potential therapeutic approach for cardiac I/R injury.

7 | CONCLUSIONS

In summary, this study indicated that the downregulation of TRIM11 may ameliorate apoptosis in AC16 cells after I/R injury, probably through the DUSP1-JNK1/2 pathways. This indicates that the downregulation of TRIM11 has a cardioprotective effect against I/R injury in cardiomyocytes. Therefore, targeting TRIM11 may open a new perspective for the treatment of the heart after I/R injury.

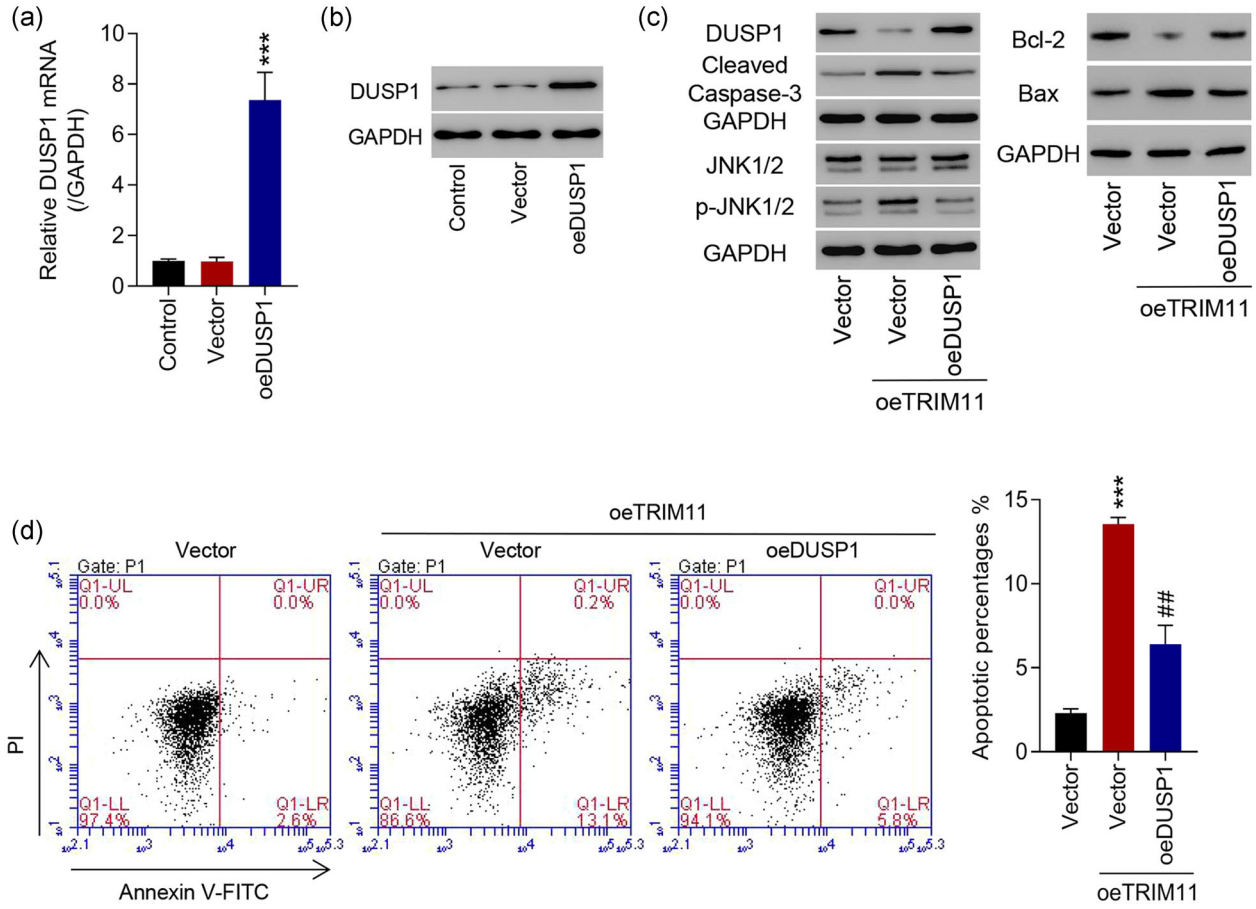

FIGURE 5 TRIM11-regulated apoptosis in AC16 cells probably by modulating DUSP1 expression (a, b). After injection with the oeDUSP1 lentivirus, the mRNA (a) and protein (b) expression of DUSP1 were detected. ***$p < .001$ versus vector. AC16 cells were injected with the oeTRIM11 or oeDUSP1 lentivirus. (c) The protein expressions of cleaved caspase-3, Bcl-2, Bax, JNK1/2, p-JNK1/2 were detected. (d) Apoptosis at 48 h was detected. ***$p < .001$ versus vector; ##$p < .01$ versus oeTRIM11 + vector. Control, AC16 cells; vector, negative control of DUSP1 overexpression

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Lihua Dai conceived and designed the study. Fang He, Zheqian Wu, Yong Wang, Lili Yin, and Shijie Lu performed the experiments. Lihua Dai wrote the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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