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LncRNA TUG1 aggravates cardiomyocyte apoptosis and myocardial ischemia/reperfusion injury

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Summary. Cardiomyocyte apoptosis is a fundamental pathogenic factor leading to myocardial ischemia/reperfusion (MI/R) injury. The long non-coding RNA (lncRNA) TUG1 regulates apoptosis in various cell types. We report here that TUG1 expression is induced in mouse heart following MI/R injury as well as in cardiomyocytes subjected to simulated ischemia/reperfusion (SI/R) *in vitro*. Clinically, TUG1 expression is also elevated in plasma from patients with acute myocardial infarction (AMI), which implies its potential application as a disease biomarker. Functionally, TUG1 overexpression promotes, and its knockdown reduces SI/R-induced lactate dehydrogenase (LDH) release and caspase-3 activity in cardiomyocytes *in vitro*, illustrating that TUG1 exacerbates SI/R-induced apoptosis. Furthermore, *in vivo*, TUG1 aggravates MI/R injury in a mouse model, and subsequent observations show concurrent increased apoptosis of cardiomyocytes. Hence, this study unveils a clinical relevance and functional role of TUG1 in MI/R injury, and also implicates that targeting TUG1 may have therapeutic effects in treating MI/R injury.

Key words: LncRNA, TUG1, Myocardial ischemia/reperfusion injury, Cardiomyocyte, Apoptosis

Introduction

Acute myocardial infarction (MI) is a life-threatening cardiovascular disease with high mortality and morbidity in the world (Boon and Dimmeler, 2015). Currently, timely and effective reperfusion remains a mainstay therapy for MI (Eltzschig and Eckle, 2011). However, despite its efficacy in reducing ischemic injury and limiting MI size, reperfusion induces cell death of cardiomyocytes, also referred to as myocardial reperfusion injury (Hausenloy and Yellon, 2013). To protect the heart against myocardial ischemia/reperfusion (MI/R) injury, new therapeutic strategies are urgently needed to prevent cardiomyocyte death (Hausenloy et al., 2017). In recent years, a growing body of evidence has established that cardiomyocyte apoptosis is the hallmark of MI and a major cause of cardiomyocyte death that underlies the pathological process of MI/R injury, which leads to irreversible impairment of myocardial function and heart failure (Prech et al., 2010; Konstantinidis et al., 2012; Zhao et al., 2018). Therefore, targeting the molecular mechanisms of cardiomyocyte apoptosis holds great potential to provide novel treatments for MI/R injury. In addition, to assess the severity of MI/R injury in patients, cardiac magnetic resonance imaging (MRI) is a frequently used modality (Carrick et al., 2016). However, cardiac MRI is a limited technique in terms of accessibility and availability of specialists (Ong et al., 2018). Hence, there is also a need for identifying ideal biomarkers that could be used to improve the diagnosis and prognosis of MI/R injury.

More recently, emerging studies have implicated a critical role for the long non-coding RNAs (IncRNAs), a novel class of RNA molecules that do not code proteins, in the pathology of MI/R injury (Vausort et al., 2014; Guo et al., 2017; Ong et al., 2018). For instance, inhibition of some lncRNAs, such as Mirt1, MALAT1, RNA-ZFAS1 and KCNQ1OT1, was reported to attenuate MI/R injury in experimental animal models (Li et al., 2017b,c; Wu et al., 2017; Hu et al., 2018). On the other hand, the aberrant upregulation of some IncRNAs was found in the murine heart with MI/R injury and also in peripheral blood cells from patients with MI (Liu et al., 2014; Vausort et al., 2014). Moreover, it has been reported that the circulating lncRNA HOTAIR is an essential mediator of MI (Gao et al., 2017), and that some lncRNAs may be useful for early diagnosis of MI, such as the circulating lncRNA UCA1, ZFAS1 and CDR1AS (Yan et al., 2016; Zhang et al., 2016b). These observations suggest that lncRNAs may serve both as

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potential therapeutic targets and biomarkers for MI.

The lncRNA taurine upregulated gene 1 (TUG1) is thought of as a pivotal oncogenic lncRNA participating in apoptosis regulation in a variety of human cancer cells (Zhang et al., 2013; Huang et al., 2015; Li et al., 2016a,b). TUG1 was also shown to play a role in hypoxia induced myocardial cell injury *in vitro* (Wu et al., 2018). Moreover, TUG1 can act as a ceRNA to bind to miRNAs like miR-132 (Su et al., 2020), miR-421 (Chen et al., 2019) and miR-29b (Zou et al., 2019), which are associated with MI/R injury. Yet, to our knowledge, whether TUG1 has a direct connection to MI/R injury and the underlying mechanisms or not have not been investigated. In this study, we report its upregulated expression in mouse model of MI/R injury and in plasma from patients with ischemic cardiomyopathy. We also show a beneficial effect of TUG1 knockdown on attenuating MI/R injury, which is associated with reduced cardiomyocyte apoptosis. Hence, TUG1 may function as a novel regulator in the pathology of MI/R injury.

Materials and methods

Mouse MI/R injury model

MI/R injury was established in mice as described previously (Pei et al., 2015). In brief, C57BL/6 mice aged 10-12 weeks were anesthetized with 2% isoflurane and a left thoracic incision was made. Myocardial infarction was developed by placing a 6-0 silk suture slipknot around the left anterior descending coronary artery. After 30 min of myocardial ischemia, the slipknot was released to allow myocardium reperfusion for different time periods. Sham-operated mice underwent the same surgical procedures without tied suture. Nine mice were included in each group. All animal experiments were taken in China-Japan Friendship Hospital and conducted in accordance with the protocols approved by the Animal Care and Use Committee of China-Japan Friendship Hospital (approval number: 190202).

Isolation and culture of ventricular cardiomyocytes

Ventricular cardiomyocytes were isolated from C57/BL6 mice as described in a previous study (Chouchani et al., 2013). Briefly, mice were killed by cervical dislocation under anesthesia using pentobarbital sodium (50 mg/kg body weight), and hearts were excised and immersed in 4 mL of digestion buffer and teased apart by gentle pipetting. After centrifugation, heart cells were cultured in M199 culture medium supplemented with 25 μmol/L blebbistatin, 9.9 μmol/L BDM and 100 IU/mL penicillin/streptomycin.

Simulated ischemia/reperfusion in vitro

The culture medium of ventricular cardiomyocytes was replaced by Hanks' balanced salt solution, and then placed in a hypoxia incubator chamber (1% O_2, 5% CO_2, 94% N_2) (STEMCELL Technologies). After 12 h of hypoxia, cells were cultured in normal culture medium for additional time periods in a normal CO_2 incubator for reperfusion.

Plasma samples from AMI patients

Fifteen AMI patients hospitalized at China-Japan Friendship Hospital were recruited in this study. AMI was diagnosed based on guidelines of the European Society of Cardiology and American College of Cardiology (Apple et al., 2002). Meanwhile, eighteen healthy donors were recruited as negative controls. Written informed consent was obtained from each subject prior to blood sampling. The study protocol was approved by the Ethics Committee of Affiliated Hospital of Guangdong Medical University and was conducted in accordance with the declaration of Helsinki and German Federal Guidelines. To obtain plasma and the following TUG1 detection, whole blood samples (5 mL) were collected from each subject, and then centrifuged at 1000 g for 5 min. The plasma was transferred into RNase-free tubes for total RNA extraction.

qRT-PCR analysis

Total RNA was extracted from ventricular cardiomyocytes or plasma samples using miRcute miRNA Isolation kit (TIANGEN), and then reversely transcribed by MulV reverse transcriptase (Transgen) according to the manufacturer's instructions. qRT-PCR was conducted via SYBR Green qPCR Master Mix (ThermoFisher Scientific) and a Real-Time PCR system (Applied Biosystems 7500). The sequences of primers are listed as follows: human TUG1 forward 5'-TAGCAGTTCCCCAATCCTTG-3', human TUG1 reverse 5'-CACAAATTCCCATCATTCCC-3'; mouse TUG1 forward 5'-CATAGTATCATCTTCGGGTTAC-3', mouse TUG1 reverse 5'-CACAAAATGCATGTAG GTTC-3'; human 18S forward 5'-GGCCCTGTAAT TGGAATGAGTC-3', human 18S reverse 5'-CCAAGATCCAACTACGAGCTT-3'; mouse β-Actin forward 5'-AGCCATGTACGTAGCCATCC-3', mouse β-Actin reverse 5'-CTCAGCTGTGGTGGTGAA-3'. The relative expression of TUG1 was calculated with the comparative cycle threshold (CT) ($2^{-\Delta\Delta CT}$) method. Mouse β-Actin and human 18S were used as reference controls to normalize the data of cellular and serum samples, respectively.

Cell transfection

Ventricular cardiomyocytes were seeded in 6-well plates and transfected with plasmids of pcDNA3.1 (Invitrogen) or pcDNA-TUG1 (2 μg/μl) through Lipofectamine 2000 Transfection Reagent (ThermoFisher Scientific) according to the

manufacturer's instructions. The transfection of siRNA targeting negative control (siNC) or TUG1 (siTUG1) was performed by Lipofectamine RNAiMAX (ThermoFisher Scientific). The final concentration of siRNA was 20 nM. The overexpression and knockdown of TUG1 was confirmed by qRT-PCR analysis at 2 or 3 days after transfection.

Caspase-3 activity detection

Caspase-3 activity in ventricular cardiomyocytes cultured *in vitro* or isolated from mouse heart was detected using Ac-DEVD-AFC Caspase-3 Fluorogenic Substrates from a fluorometric assay kit (BD Pharmingen, 556574) based on the manufacturer's instructions. Briefly, supernatants were collected by the lysis of ventricular cardiomyocytes on ice. The protein concentration of samples was quantified by BCA method. Samples containing 50 μg proteins were mixed with the assay buffer for enzymatic reaction. The fluorescence of 7-amino-4-trifluoromethyl-coumarin (AFC) was measured at 505 nm by SpectraMax Plus 384 Microplate Reader (Molecular Devices). Each sample was assayed in triplicate. Results were expressed as nmol AFC/h/mg protein.

TUG1 in vivo knockdown and overexpression

The *in vivo* knockdown of cardiac TUG1 using siRNA transfection was performed as previously described (Zhao et al., 2018). In brief, equal amounts of TUG1 siRNA (siTUG1, Invitrogen) or non-specific control siRNA (siNC, Invitrogen) (1 µg/µl) were pre-mixed with *in vivo*-jetPEI delivery reagent (Polyplus, 201-50G) and then delivered by separate intra-myocardial injections using a 32-gauge needle (Sigma-Aldrich). The adenoviruses expressing mouse TUG1 were constructed using the Adeno-X adenoviral system 3 (Takara). The adenoviruses expressing scramble sequence were used as negative control. The intra-myocardial delivery of 2×10^{11} m.o.i. adenoviruses into mice was performed as described previously (Koeppen et al., 2018). The knockdown and overexpression efficiency was assessed by qRT-PCR analysis at the end point of animal experiments.

TTC staining and CK release measurement

Following MI/R injury, the heart was removed and horizontally cut into 5-6 slices, and then incubated with 2,3,5-triphenyl tetrazolium chloride (TTC) (Sigma-Aldrich, 1%) for 30 min at room temperature. The stained slices of 9 mice in each group were used to analyze the percentage of infarct area (IA) by Image J software. For measuring CK release, the blood samples were collected via tail veins of mice following MI/R injury. Samples were assayed in triplicate for each mouse, and each group included 9 mice. Myocyte injury was measured by assessing the level of serum CK using a Creatine Kinase Assay Kit (BioAssay Systems (ECPK-100) according to the manufacturer's instructions. CK activity (U/L) was measured at 340 nm in 96-well plates.

Immunoblot analysis

Proteins extracted from ventricular cardiomyocytes of mouse heart were separated by SDS-PAGE gels, and then transferred onto PVDF membranes (Millipore), which were incubated with primary antibodies against Bax (Santa Cruz, 1:500 dilution) and Bcl-2 (Abcam, 1:1000 dilution) followed by the incubation of HRP-conjugated secondary antibodies (Santa Cruz, 1:5000 dilution). Mouse β-Actin (Santa Cruz, 1:2000 dilution) was detected as a loading control for each well. After repeated rinsing, protein blots were developed by using SuperSignal Chemiluminescent HRP Substrates (ThermoFisher Scientific), and imaged by Amersham Imager 600 (GE Healthcare). The quantification of protein blots was analyzed by Image J software.

LDH release measurement and TUNEL assay

LDH release was measured as described previously (Cheng et al., 2015). LDH release was defined as LDH activity in culture medium divided by that in cell lysates. LDH activity was determined using Lactate Dehydrogenase Activity Assay Kit (Sigma-Aldrich) following the manufacturer's instructions. Each treatment was assayed in triplicate, and the percentage of LDH release was calculated. The apoptosis of cardiomyocytes on heart slices from mice following MI/R injury was detected by TUNEL staining as described in a previous study (Zhang et al., 2015). Briefly, heart slices were incubated in the dark with reagents from an In Situ Cell Death Detection Kit (Roche) for visualizing apoptotic cardiomyocytes and further stained with DAPI for detecting nuclei. Images were photographed under a BX51 fluorescence microscope (Olympus). The total number of nuclei (blue) and TUNEL-positive nuclei (green) from at least 3 random slides of each mouse was counted individually by Image J software. Nine mice were included in each group. The index of apoptosis was calculated as the number of TUNEL-positive nuclei/total number of nuclei ×100.

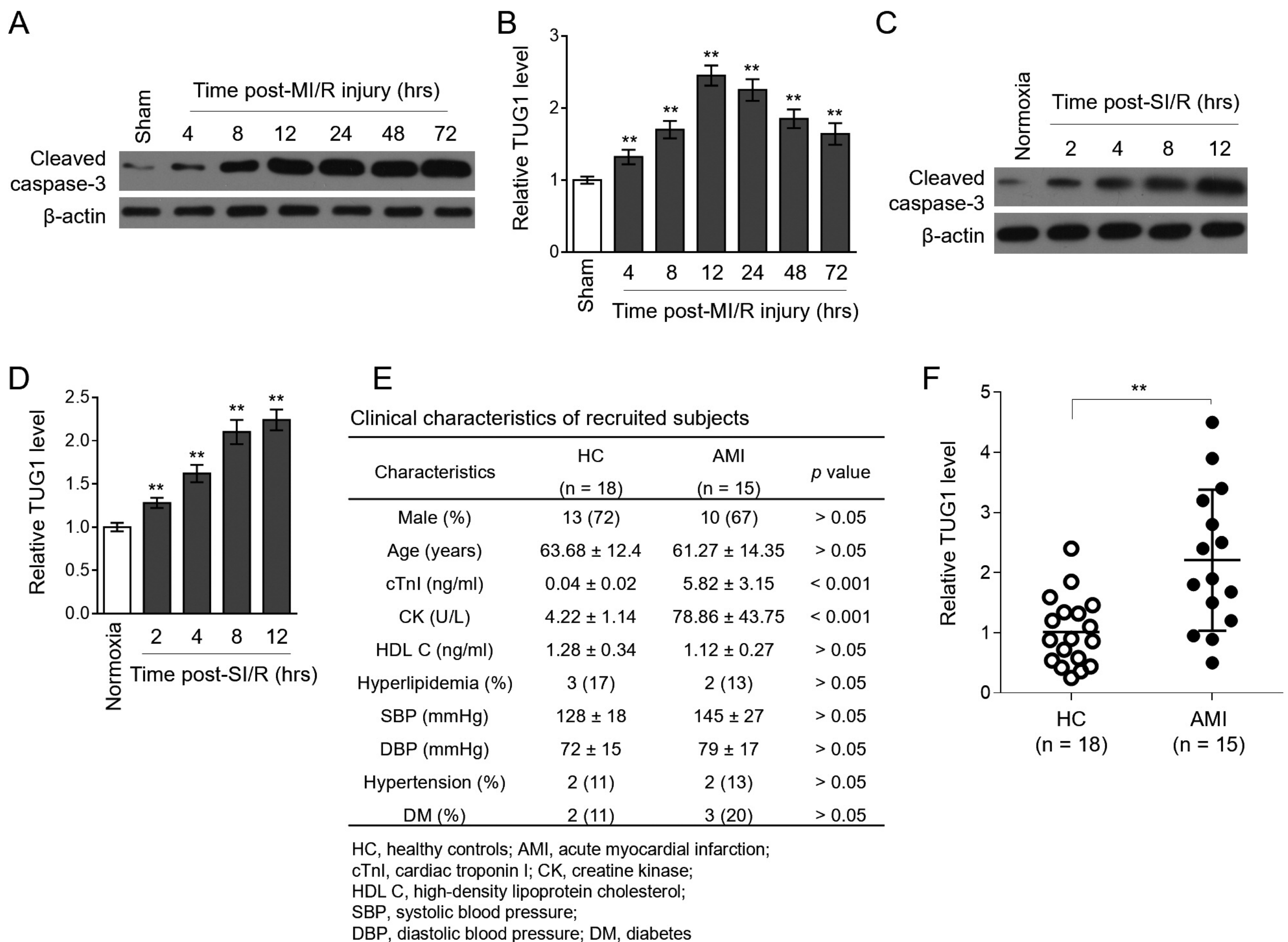
Statistical analysis

All data were presented as the mean ± standard deviation (SD). Statistical analysis between two sets of data was performed by two-tailed Student's t-test, while data among more than two groups were compared using one-way ANOVA. A P value less than 0.05 was defined as statistically significant.

Results

TUG1 is upregulated in mouse cardiomyocytes following MI/R injury and in plasma from AMI patients

To seek a possible role of TUG1 involved in MI/R injury, we initially examined its expression in ventricular cardiomyocytes isolated from hearts of mice which underwent MI/R injury developed by the left coronary artery (LCA) occlusion and reperfusion (Lai et al., 2010). The MI/R injury imposed on ventricular cardiomyocytes was reflected by the increased expression of cleaved caspase-3, a typical apoptosis marker (Fig. 1A). Next, qRT-PCR analysis showed that compared to that in sham-operated mice, TUG1 level was markedly increased in ventricular cardiomyocytes following MI/R injury, which peaked at 12 h, and then gradually declined, but still remained significantly higher thereafter (Fig. 1B). This indicates that TUG1 expression in ventricular cardiomyocytes is induced in response to MI/R injury, implying a potential association between TUG1 induction and the pathology of MI/R injury. We then checked whether this phenomenon could be reproduced in primary ventricular cardiomyocytes subjected to simulated ischemia/reperfusion (SI/R), an *in vitro* MI/R injury



Clinical characteristics of recruited subjects

Characteristics	HC (n = 18)	AMI (n = 15)	*p* value
Male (%)	13 (72)	10 (67)	> 0.05
Age (years)	63.68 ± 12.4	61.27 ± 14.35	> 0.05
cTnI (ng/ml)	0.04 ± 0.02	5.82 ± 3.15	< 0.001
CK (U/L)	4.22 ± 1.14	78.86 ± 43.75	< 0.001
HDL C (ng/ml)	1.28 ± 0.34	1.12 ± 0.27	> 0.05
Hyperlipidemia (%)	3 (17)	2 (13)	> 0.05
SBP (mmHg)	128 ± 18	145 ± 27	> 0.05
DBP (mmHg)	72 ± 15	79 ± 17	> 0.05
Hypertension (%)	2 (11)	2 (13)	> 0.05
DM (%)	2 (11)	3 (20)	> 0.05

HC, healthy controls; AMI, acute myocardial infarction; cTnI, cardiac troponin I; CK, creatine kinase; HDL C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes

Fig. 1. TUG1 expression is induced in mouse cardiomyocytes with MI/R injury and also elevated in plasma from patients with AMI. **A, B.** Western blotting analysis of cleaved caspase-3 **(A)** and qRT-PCR analysis of TUG1 expression **(B)** in ventricular cardiomyocytes isolated from hearts of mice at indicated different time periods following MI/R injury. Those isolated from hearts of mice subjected to sham operation were used as treatment controls. The level of mouse β-Actin was used as a loading and reference control. All results are expressed as relative to sham. Each group includes 9 mice. **C, D.** Western blotting analysis of cleaved caspase-3 **(C)** and qRT-PCR analysis of TUG1 expression **(D)** in primary ventricular cardiomyocytes cultured in normoxic or SI/R conditions for different time periods as indicated. All results were normalized to β-Actin and expressed as relative to normoxia group. Data are mean ± SD (n=3). Statistical analysis was performed by one-way ANOVA test. **, P<0.01. **E, F.** Clinical characteristics **(E)** and qRT-PCR analysis of TUG1 expression in plasma samples **(F)** of healthy controls (HC, n=18) or AMI patients (AMI, n=15). Plasma parameters of cTnI and CK showing significant differences are highlighted in bold. The expression of 18S was used as an internal control. Results are expressed as relative to HC group. Statistical analysis was performed by Student's t-test. **, P<0.01.

model (Gonzalez-Reyes et al., 2015). Analogue to apoptosis induction in mouse ventricular cardiomyocytes following MI/R injury (Fig. 1A), evident apoptosis was also detected in primary ventricular cardiomyocytes upon exposure to SI/R (Fig. 1C), indicating that this inferior culture condition induces cellular injury in cardiomyocytes. Moreover, remarkably, we found that similar to MI/R injury, SI/R treatment also induced a significant TUG1 upregulation in primary ventricular cardiomyocytes as compared to those cultured under a normoxic condition (Fig. 1D). These *in vivo* and *in vitro* observations clearly show that TUG1 is induced in cardiomyocytes following I/R injury.

Lately, the expression profile of IncRNAs was found to be altered in plasma samples and heart tissue from patients with AMI (Ounzain et al., 2014; Yan et al., 2016), which suggests a potential application of IncRNAs, particularly circulating lncRNAs, as promosing biomarkers for disease diagnosis. The aberrant upregulation of TUG1 observed in experimental MI/R injury prompted us to ask whether it has clinical relevance to AMI. To test this idea, we compared its expression in plasma samples collected from AMI patients and healthy controls (HC). As shown in Fig. 1E, significant differences were found in plasma cardiac troponin I (cTnI) and creatine kinase (CK), two well-established biomarkers of myocardial injury (Apple et al., 2003), between AMI and HC subjects. Then, qRT-PCR results showed that TUG1 expression was significantly elevated in plasma samples from AMI patients as relative to that in HC (Fig. 1F). Therefore, the elevated level of circulating TUG1 in plasma of AMI patients reveals its potential clinical relevance to AMI pathology.

TUG1 promotes SI/R-induced cardiomyocyte apoptosis in vitro

TUG1 is implicated in the regulation of cancer cell apoptosis, although both pro- and anti-apoptotic activities were found in different cell types (Zhang et al., 2013, 2016a; Huang et al., 2015; Li et al., 2016a). In non-cancerous cells, two studies have shown that TUG1 promotes the apoptosis of neurons under ischemia (Chen et al., 2017) and H9c2 cardiomyocytes under hypoxia (Wu et al., 2018). These reports suggest that TUG1 may function as a regulator of apoptosis in broad range of cell types. Since it's well-established that cardiomyocyte apoptosis is pivotal for MI/R injury, to assess its functional role involved in MI/R injury, we therefore investigated whether TUG1 affects the apoptosis of cardiomyocytes under pathological conditions. To test this, we transiently overexpressed TUG1 in primary ventricular cardiomyocytes stimulated by SI/R. Firstly, the overexpression of TUG1 was confirmed by qRT-

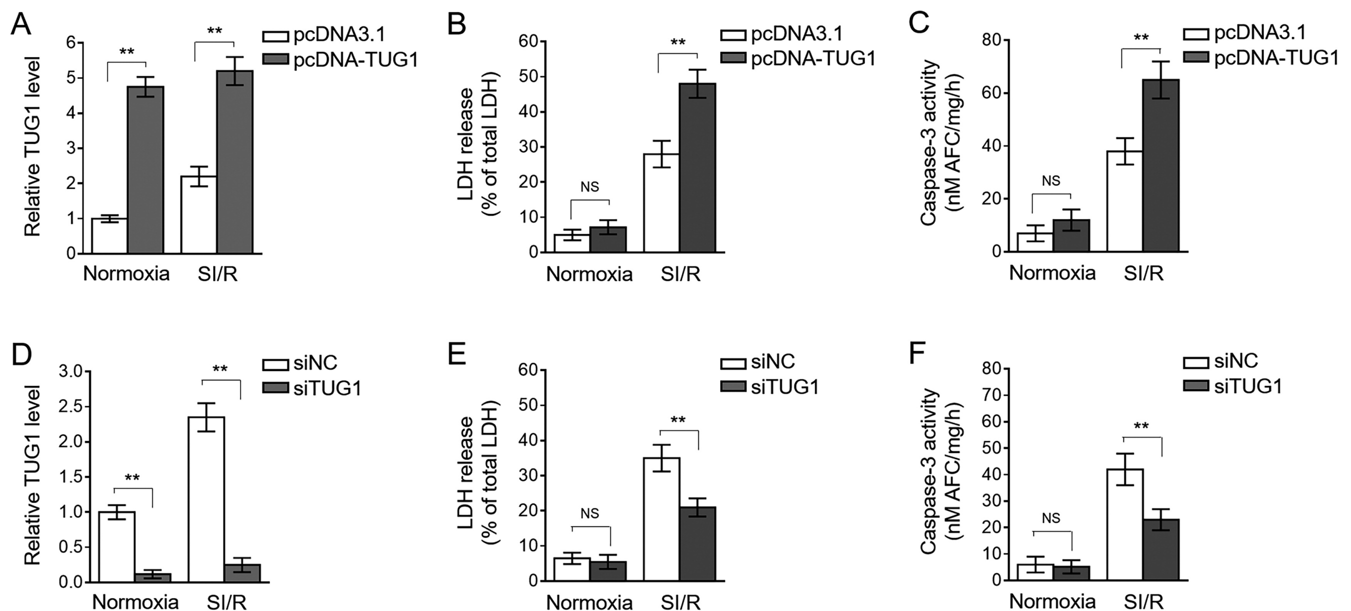


Fig. 2. TUG1 promotes I/R injury in cardiomyocytes *in vitro*. **A-C.** Primary ventricular cardiomyocytes were transfected with pcDNA3.1 vector or pcDNA-TUG1 for 2 days, followed by 12-h culture in normoxic or SI/R condition. **A.** The expression of TUG1 was determined by qRT-PCR analysis. β-Actin was used as a reference control. All results are expressed as relative to normoxia group transfected with pcDNA3.1 vector. **B.** LDH release in each group was determined by LDH release assay. Results are expressed as LDH activity in culture medium divided by that in cell lysates (%). **C.** Caspase-3 activity in each group was measured via a fluorometric method. Results are expressed as nmol AFC/h/mg protein. **D-F.** Primary ventricular cardiomyocytes were transfected with siRNA control or siRNA TUG1 for 2 days, followed by 12-h culture in normoxic or SI/R condition. The measurement of the expression of TUG1 **(D)**, LDH release **(E)**, and caspase-3 activity **(F)** was performed and analyzed as in **A-C**. Data are mean ± SD (n=3). Statistical analysis was performed by Student's t-test. **, P<0.01. NS, not significant.

PCR analysis (Fig. 2A). Consequently, we found that in contrast to normoxia, SI/R drastically induced the levels of both LDH release and caspase-3 activity in cardiomyocytes (Fig. 2B,C, column 1 vs. column 3), indicating the induction of cardiomyocyte apoptosis. Moreover, TUG1 overexpression further increased SI/R-induced LDH release and caspase-3 activity, whereas, no obvious effect was observed under normoxic conditions (Fig. 2B,C). Hence, these data suggest that TUG1 promotes SI/R-induced cardiomyocyte apoptosis. To validate the pro-apoptotic function of TUG1, siRNA transfection was applied to knock down TUG1 expression in cardiomyocytes (Fig. 2D). On the contrary, TUG1 depletion resulted in decreased LDH release and caspase-3 activity induced by SI/R (Fig. 2E,F). Together, these *in vitro* findings reveal that TUG1 functions as a positive regulator of SI/R-cardiomyocyte apoptosis.

TUG1 aggravates MI/R injury in a mouse model

We then asked whether TUG1 knockdown has alleviating effects on MI/R injury *in vivo*. To this end, we depleted cardiac TUG1 expression in MI/R mice through intra-myocardial delivery of siRNA. The efficacy of *in vivo* knockdown was verified by qRT-PCR analysis (Fig. 3A). As analyzed by TTC staining of heart slices, we found that the infarct size following MI/R injury was considerably minimized along with TUG1 depletion (Fig. 3B). In addition, the level of plasma CK release in MI/R mice was also reduced by TUG1 depletion (Fig. 3C). Noteworthily, TUG1 depletion in sham-operated mice did not have obvious effects (Fig. 3B,C). To verify these findings, adenoviruses expressing TUG1 were delivered intra-myocardially into mouse heart to achieve enforced local overexpression (Fig. 3D).

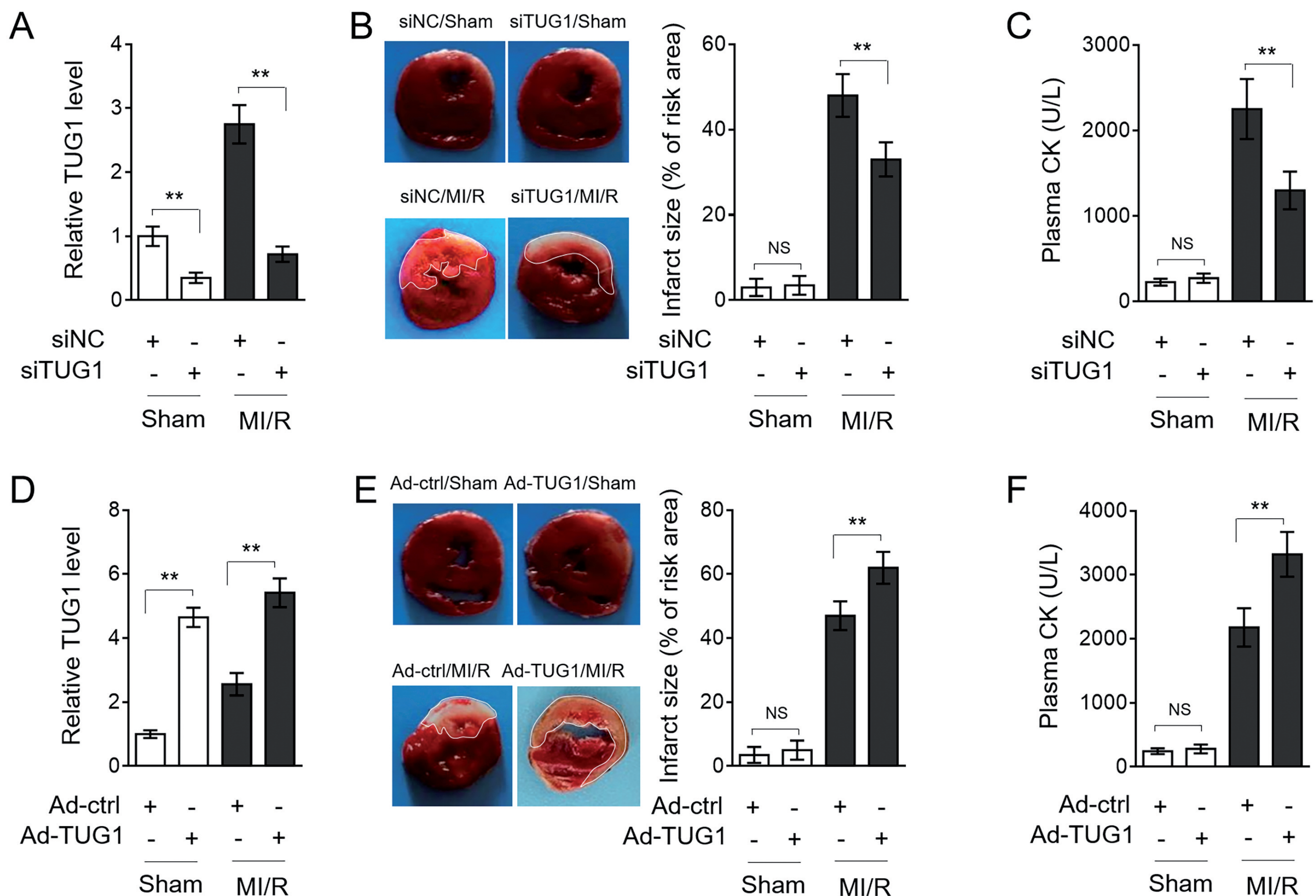


Fig. 3. TUG1 promotes MI/R injury in a mouse model. **A-C.** Mice were intra-myocardially injected with siRNA control or siRNA TUG1. After 72-h, mice were subjected to M/R injury for 24 h. Mice which underwent sham surgery were used as surgical control. Each group includes 9 mice. **A.** The expression of TUG1 in ventricular cardiomyocytes isolated from hearts of mice was determined by qRT-PCR analysis. β-Actin was used as a reference control. All results are expressed as relative to sham. **B.** Quantification analysis of percentage myocardial infarction size relative to risk area. **C.** The level of released CK in plasma samples (U/L). **D-F.** Mice were intra-myocardially delivered with adenoviruses expressing scrambled control (Ad-ctrl) or mouse TUG1 (Ad-TUG1). After 72-h, mice were subjected to M/R injury for 24 h. The expression of TUG1 **(D)**, infarct size **(E)**, and level of released plasma CK **(F)** were analyzed as in **A-C**. Data are mean ± SD (n=9). Statistical analysis was performed by Student's t-test. **, $P<0.01$; NS, not significant.

Contrary to TUG1 knockdown, its overexpression instead resulted in an increase in infarct size (Fig. 3E) and plasma CK release (Fig. 3F) following MI/R injury. Therefore, these lines of evidence from an animal model prove that TUG1 functions to aggravate MI/R injury.

TUG1 promotes cardiomyocyte apoptosis following MI/R injury

Finally, to establish a potential mechanistic link between TUG1-promoted cardiomyocyte apoptosis *in vitro* (Fig. 2) and MI/R injury *in vivo* (Fig. 3), we determined its effect on cardiomyocyte apoptosis in the hearts of MI/R mice. Consistent with the above-mentioned results, TUNEL assay on the heart slices showed that TUG1 knockdown reduced the number of apoptotic cardiomyocytes following MI/R injury (Fig. 4A). In support of this finding, the ratio of Bax/Bcl-2 (Fig. 4B) in the heart of MI/R mice was also decreased when TUG1 was depleted. Furthermore, keeping in

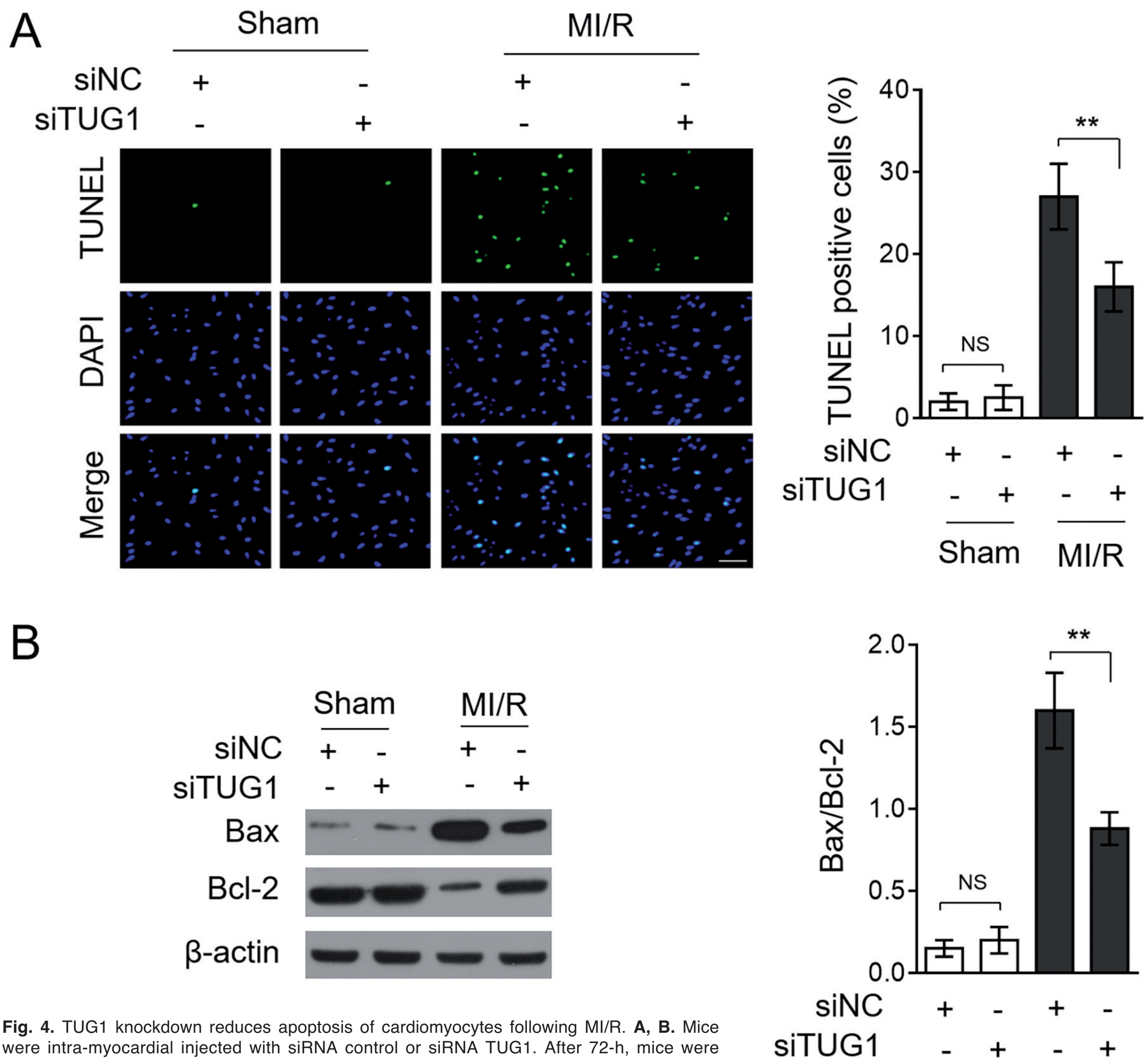


Fig. 4. TUG1 knockdown reduces apoptosis of cardiomyocytes following MI/R. **A, B.** Mice were intra-myocardial injected with siRNA control or siRNA TUG1. After 72-h, mice were subjected to M/R injury for 24 h. Mice which underwent sham surgery were used as surgical control. Each group includes 9 mice. **A.** The representative of TUNEL staining of heart slices is shown. Green, TUNEL positive cells; Blue, nuclei. The quantification analysis of percentage of TUNEL positive cells is shown on the right. **B.** The protein expression of Bax and Bcl-2 in ventricular cardiomyocytes isolated from hearts of mice was determined by Western blotting analysis. β-Actin was used as a loading control. The quantification analysis of the ratio of Bax to Bcl-2 is shown right. Data are mean ± SD (n=9). Statistical analysis was performed by Student's t-test. **, P<0.01; NS, not significant.

concert with these results, TUG1 overexpression increased cardiomyocyte apoptosis (Fig. 5A,B). Altogether, given the fundamental role of cardiomyocyte apoptosis in MI/R injury, we suppose that TUG1-aggravated MI/R injury in a mouse model could be prominently attributed to its effect on promoting cardiomyocyte apoptosis.

Discussion

MI/R injury is thought of as cardiomyocyte death and consequent functional deterioration that occur

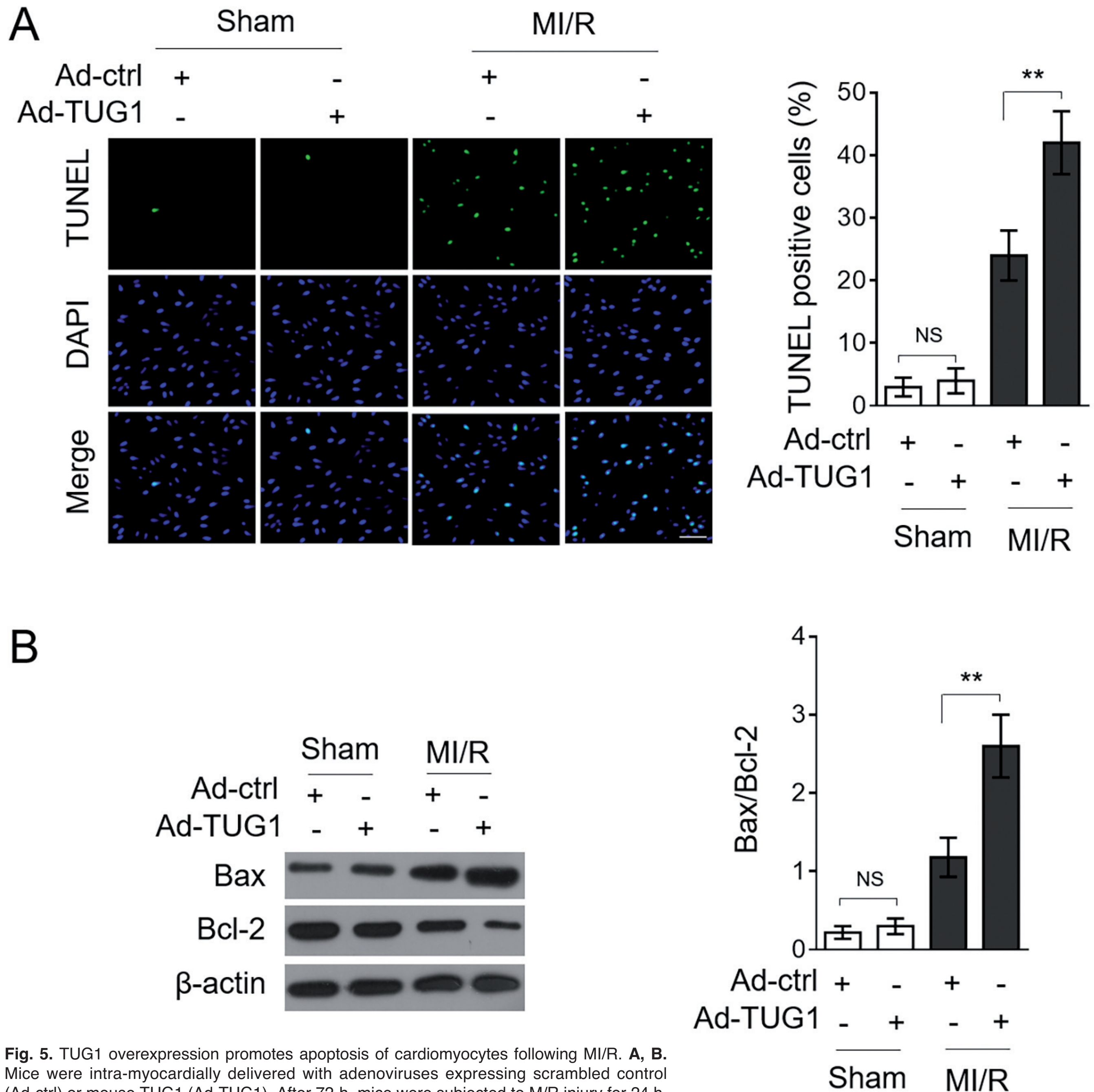


Fig. 5. TUG1 overexpression promotes apoptosis of cardiomyocytes following MI/R. **A, B.** Mice were intra-myocardially delivered with adenoviruses expressing scrambled control (Ad-ctrl) or mouse TUG1 (Ad-TUG1). After 72-h, mice were subjected to M/R injury for 24 h. **A.** The representative of TUNEL staining of heart slices is shown. Green, TUNEL positive cells; Blue, nuclei. The quantification analysis of percentage of TUNEL positive cells is shown on the right. **B.** The protein expression of Bax and Bcl-2 in ventricular cardiomyocytes isolated from hearts of mice was determined by Western blotting analysis. β-Actin was used as a loading control. The quantification analysis of the ratio of Bax to Bcl-2 is shown right. Data are mean ± SD (n=9). Statistical analysis was performed by Student's t-test. **, P<0.01; NS, not significant.

during the process of reperfusion therapy, which is originally intended to restore the occluded blood flow and salvage ischemia-induced cardiomyocyte death in AMI patients (Moens et al., 2005; Yellon and Hausenloy, 2007). The pathogenesis leading to cardiomyocyte death during MI/R injury is very complex and several mechanisms have been revealed to play an important role, such as inflammation, excessive reactive oxygen species (ROS) production, intracellular calcium overload and mitochondrial dysfunction, etc, (Nakamura et al., 2016; Lesnefsky et al., 2017; Zhai et al., 2017; Wang et al., 2018). As is well known, in addition to cell death via necrosis, apoptosis is another pivotal element for causing the progressive loss of cardiomyocytes following MI/R injury (Kajstura et al., 1996). Therefore, targeting cardiomyocyte apoptosis presumably holds great promise to enhance the effectiveness of reperfusion therapy by reducing reperfusion injury, for which, however, no effective treatment is available at present. In recent years, the aberrant expression and dysfunction of several lncRNAs have been found in patients and animal models with MI/R injury, which open new avenues to search for promising biomarkers and therapeutic targets for this pathological condition (Vausort et al., 2014; Ong et al., 2018). Prompted by the clues that lncRNA TUG1 participates in the regulation of cell apoptosis, our current study was designed to explore the regulation and function of TUG1 involved in MI/R injury.

Initially, by taking advantage of *in vivo* and *in vitro* models, we noticed that the expression of TUG1 was induced in ventricular cardiomyocytes following MI/R injury. In addition, of certain clinical relevance, we also found that in the plasma samples of AMI patients, TUG1 expression was significantly higher than that in healthy donors. Furthermore, in functional studies via the manipulation of TUG1 expression, we discovered that TUG1 promoted SI/R-induced cardiomyocyte apoptosis *in vitro*, and more importantly, that TUG1 aggravated MI/R injury in a mouse model, including augmented infarct size and increased cardiomyocyte injury. Meanwhile, in this mouse model, cardiomyocyte apoptosis was also enhanced by TUG1. Thus, as far as we know, this is the first time the association between TUG1 and MI/R injury has been unveiled. Moreover, we also uncover the unprecedented pro-apoptotic function of TUG1 in cardiomyocytes in response to MI/R injury, which constitutes a critical mechanism that underlies its detrimental role in exaggerating MI/R injury.

It was previously reported that TUG1 expression was upregulated in H9c2 cells and vascular smooth muscle cells (VSMCs) cultured under hypoxic conditions (Li et al., 2018; Wu et al., 2018). In our study, the expression of TUG1 was also found to be induced upon MI/R injury, suggesting that its transcription may be activated during this process. Given the possibility that TUG1 might be a hypoxia-inducible lncRNA, we suspect it is very likely that TUG1 expression is transcriptionally induced in cardiomyocytes during the ischemia phase, which is then aggravated following reperfusion, although the specific underlying mechanisms are still unknown at present. It also has been demonstrated that TUG1 expression is induced by Notch1 in glioma cells (Katsushima et al., 2016), and that it is a direct transcriptional target of p53 in non-small cell lung carcinoma (NSCLC) cells (Zhang et al., 2014). Notch1 inhibition displays cardioprotection in MI/R injury (Pei et al., 2013), and p53 is upregulated during the reperfusion phase (Yano et al., 2018), indicating their involvement in MI/R injury. Therefore, it is also possible that Notch1 and p53 are associated with TUG1 induction in response to MI/R injury. Further study efforts are needed to uncover the specific mechanisms.

Furthermore, we found an elevation of TUG1 level in serum samples from AMI patients, which we suppose is secreted from the injured heart, implying that TUG1 expression may also be induced in the heart in AMI patients. The upregulation of TUG1 level in serum of AMI patients implicates that it may be used as a biomarker for disease diagnosis or prognosis. However, it should be noted that the sample size recruited in this study is very limited, future studies with larger sample size are definitely required to consolidate the aberrant expression of TUG1 in the plasma of AMI patients. Another caution is that its upregulation was also found in the serum of patients with atherosclerosis (Li et al., 2018). For an ideal biomarker for AMI, in addition to good accessibility, other traits such as high sensitivity, specificity and predictability should also be required (Li et al., 2012). Therefore, more issues should be addressed prior to considering TUG1 as a fitting candidate for AMI, including whether serum TUG1 level is correlated with AMI severity and whether it is affected by other complications associated with AMI.

The overexpression of TUG1 has been shown to aggravate hypoxia-induced apoptosis in H9c2 cells (Wu et al., 2018). We found that TUG1 promoted SI/R-cardiomyocyte apoptosis, and reversely, its knockdown reduced cardiomyocyte apoptosis in mice with MI/R injury, therefore providing a new example for supporting a pro-apoptotic function of TUG1. The increased release of LDH and CK upon TUG1 overexpression might be attributed to cardiomyocyte cell death promoted by TUG1, since these two enzymes can be released to the outside of the cells, and therefore be detected in culture medium or blood. These observations are in line with the alleviated MI/R injury we observed in mouse model, since cardiomyocyte apoptosis plays a vital role in mediating myocardial infarction and injury. Nonetheless, how exactly TUG1 promotes cardiomyocyte apoptosis following MI/R injury at the molecular level is unclear based on our available data. Several targets of TUG1 have been shown to exert its pro-apoptotic function, such as miR 145 5p/Binp3 (Wu et al., 2018), miR-9/Bcl2l11 (Chen et al., 2017), miR-421/caspase-3 (Li et al., 2017a) and miR-9a-5p/KLF5 (Yang et al., 2019). It would be interesting to test whether these targets are associated with TUG1 function under our experimental

scenarios. It should be noted that apoptosis is not the sole type of cell death involved in MI/R injury, others like necrosis and ferroptosis also play important roles (McCully et al., 2004; Yan et al., 2020). Further studies are required to explore whether these types of cell death participate in TUG1-related cardiomyocyte apoptosis and MI/R injury. In any case, the protection of TUG1 knockdown against cardiomyocyte apoptosis and MI/R injury in an animal model suggests that it deserves in-depth studies in the future.

Conclusions

Overall, according to our findings, we propose that TUG1 might be regarded as a potential biomarker for AMI, and that targeting TUG1 expression in the heart may have potential benefits in decreasing cardiomyocyte apoptosis and attenuating MI/R injury in clinical practice.

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References

Apple F.S., Wu A.H. and Jaffe A.S. (2002). European Society of Cardiology and American College of Cardiology guidelines for redefinition of myocardial infarction: How to use existing assays clinically and for clinical trials. Am. Heart J. 144, 981-986.

Apple F.S., Quist H.E., Doyle P.J., Otto A.P. and Murakami M.M. (2003). Plasma 99th percentile reference limits for cardiac troponin and creatine kinase mb mass for use with european society of cardiology/american college of cardiology consensus recommendations. Clin. Chem. 49, 1331-1336.

Boon R.A. and Dimmeler S. (2015). MicroRNAs in myocardial infarction. Nat. Rev. Cardiol. 12, 135-142.

Carrick D., Haig C., Rauhalammi S., Ahmed N., Mordi I., McEntegart M., Petrie M.C., Eteiba H., Hood S., Watkins S., Lindsay M., Mahrous A., Ford I., Tzemos N., Sattar N., Welsh P., Radjenovic A., Oldroyd K.G. and Berry C. (2016). Prognostic significance of infarct core pathology revealed by quantitative non-contrast in comparison with contrast cardiac magnetic resonance imaging in reperfused st-elevation myocardial infarction survivors. Eur. Heart J. 37, 1044-1059.

Chen J., Yu W., Ruan Z. and Wang S. (2019). TUG1/miR-421/PINK1: A potential mechanism for treating myocardial ischemia-reperfusion injury. Int. J. Cardiol. 292, 197.

Chen S., Wang M., Yang H., Mao L., He Q., Jin H., Ye Z.M., Luo X.Y., Xia Y.P. and Hu B. (2017). LncRNA TUG1 sponges microRNA-9 to promote neurons apoptosis by up-regulated Bcl2l11 under ischemia. Biochem. Biophys. Res. Commun. 485, 167-173.

Cheng L., Jin Z., Zhao R., Ren K., Deng C. and Yu S. (2015). Resveratrol attenuates inflammation and oxidative stress induced by myocardial ischemia-reperfusion injury: Role of Nrf2/ARE pathway. Int. J. Clin. Exp. Med. 8, 10420-10428.

Chouchani E.T., Methner C., Nadtochiy S.M., Logan A., Pell V.R., Ding S., James A.M., Cocheme H.M., Reinhold J., Lilley K.S., Partridge L., Fearnley I.M., Robinson A.J., Hartley R.C., Smith R.A., Krieg T., Brookes P.S. and Murphy M.P. (2013). Cardioprotection by S-nitrosation of a cysteine switch on mitochondrial complex I. Nat. Med. 19, 753-759.

Eltzschig H.K. and Eckle T. (2011). Ischemia and reperfusion-from mechanism to translation. Nat. Med. 17, 1391-1401.

Gao L., Liu Y., Guo S., Yao R., Wu L., Xiao L., Wang Z., Liu Y. and Zhang Y. (2017). Circulating long noncoding RNA HOTAIR is an essential mediator of acute myocardial infarction. Cell Physiol. Biochem. 44, 1497-1508.

Gonzalez-Reyes A., Menaouar A., Yip D., Danalache B., Plante E., Noiseux N., Gutkowska J. and Jankowski M. (2015). Molecular mechanisms underlying oxytocin-induced cardiomyocyte protection from simulated ischemia-reperfusion. Mol. Cell Endocrinol. 412, 170-181.

Guo Y., Luo F., Liu Q. and Xu D. (2017). Regulatory non-coding RNAs in acute myocardial infarction. J. Cell Mol. Med. 21, 1013-1023.

Hausenloy D.J. and Yellon D.M. (2013). Myocardial ischemia-reperfusion injury: A neglected therapeutic target. J. Clin. Invest. 123, 92-100.

Hausenloy D.J., Garcia-Dorado D., Botker H.E., Davidson S.M., Downey J., Engel F.B., Jennings R., Lecour S., Leor J., Madonna R., Ovize M., Perrino C., Prunier F., Schulz R., Sluijter J.P.G., Van Laake L.W., Vinten-Johansen J., Yellon D.M., Ytrehus K., Heusch G. and Ferdinandy P. (2017). Novel targets and future strategies for acute cardioprotection: Position paper of the european society of cardiology working group on cellular biology of the heart. Cardiovasc. Res. 113, 564-585.

Hu H., Wu J., Li D., Zhou J., Yu H. and Ma L. (2018). Knockdown of lncRNA MALAT1 attenuates acute myocardial infarction through miR-320-Pten axis. Biomed. Pharmacother. 106, 738-746.

Huang M.D., Chen W.M., Qi F.Z., Sun M., Xu T.P., Ma P. and Shu Y.Q. (2015). Long non-coding RNA TUG1 is up-regulated in hepatocellular carcinoma and promotes cell growth and apoptosis by epigenetically silencing of KLF2. Mol. Cancer 14, 165.

Kajstura J., Cheng W., Reiss K., Clark W.A., Sonnenblick E.H., Krajewski S., Reed J.C., Olivetti G. and Anversa P. (1996). Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. Lab. Invest. 74, 86-107.

Katsushima K., Natsume A., Ohka F., Shinjo K., Hatanaka A., Ichimura N., Sato S., Takahashi S., Kimura H., Totoki Y., Shibata T., Naito M., Kim H.J., Miyata K., Kataoka K. and Kondo Y. (2016). Targeting the notch-regulated non-coding RNA TUG1 for glioma treatment. Nat. Commun. 7, 13616.

Koeppen M., Lee J.W., Seo S.W., Brodsky K.S., Kreth S., Yang I.V., Buttrick P.M., Eckle T. and Eltzschig H.K. (2018). Hypoxia-inducible factor 2-alpha-dependent induction of amphiregulin dampens myocardial ischemia-reperfusion injury. Nat. Commun. 9, 816.

Konstantinidis K., Whelan R.S. and Kitsis R.N. (2012). Mechanisms of cell death in heart disease. Arterioscler. Thromb. Vasc. Biol. 32, 1552-1562.

Lai R.C., Arslan F., Lee M.M., Sze N.S., Choo A., Chen T.S., Salto-Tellez M., Timmers L., Lee C.N., El Oakley R.M., Pasterkamp G., de Kleijn D.P. and Lim S.K. (2010). Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Res. 4, 214-222.

Lesnefsky E.J., Chen Q., Tandler B. and Hoppel C.L. (2017). Mitochondrial dysfunction and myocardial ischemia-reperfusion: Implications for novel therapies. Annu. Rev. Pharmacol. Toxicol. 57, 535-565.

Li C., Pei F., Zhu X., Duan D.D. and Zeng C. (2012). Circulating microRNAs as novel and sensitive biomarkers of acute myocardial infarction. Clin. Biochem. 45, 727-732.

Li J., Zhang M., An G. and Ma Q. (2016a). LncRNA TUG1 acts as a tumor suppressor in human glioma by promoting cell apoptosis. Exp. Biol. Med. (Maywood) 241, 644-649.

Li Z., Shen J., Chan M.T. and Wu W.K. (2016b). TUG1: A pivotal oncogenic long non-coding RNA of human cancers. Cell Prolif. 49, 471-475.

Li G., Song H., Chen L., Yang W., Nan K. and Lu P. (2017a). TUG1 promotes lens epithelial cell apoptosis by regulating miR-421/caspase-3 axis in age-related cataract. Exp. Cell Res. 356, 20-27.

Li X., Zhou J. and Huang K. (2017b). Inhibition of the lncRNA miRt1 attenuates acute myocardial infarction by suppressing NF-kappaB activation. Cell Physiol. Biochem. 42, 1153-1164.

Li X., Dai Y., Yan S., Shi Y., Han B., Li J., Cha L. and Mu J. (2017c). Down-regulation of lncRNA KCNQ1OT1 protects against myocardial ischemia/reperfusion injury following acute myocardial infarction. Biochem. Biophys. Res. Commun. 491, 1026-1033.

Li F.P., Lin D.Q. and Gao L.Y. (2018). LncRNA TUG1 promotes proliferation of vascular smooth muscle cell and atherosclerosis through regulating miRNA-21/PTEN axis. Eur. Rev. Med. Pharmacol. Sci. 22, 7439-7447.

Liu Y., Li G., Lu H., Li W., Li X., Liu H., Li X., Li T. and Yu B. (2014). Expression profiling and ontology analysis of long noncoding RNAs in post-ischemic heart and their implied roles in ischemia/reperfusion injury. Gene 543, 15-21.

McCully J.D., Wakiyama H., Hsieh Y.J., Jones M. and Levitsky S. (2004). Differential contribution of necrosis and apoptosis in myocardial ischemia-reperfusion injury. Am. J. Physiol. Heart Circ. Physiol. 286, H1923-1935.

Moens A.L., Claeys M.J., Timmermans J.P. and Vrints C.J. (2005). Myocardial ischemia/reperfusion-injury, a clinical view on a complex pathophysiological process. Int. J. Cardiol. 100, 179-190.

Nakamura K., Sano S., Fuster J.J., Kikuchi R., Shimizu I., Ohshima K., Katanasaka Y., Ouchi N. and Walsh K. (2016). Secreted frizzled-related protein 5 diminishes cardiac inflammation and protects the heart from ischemia/reperfusion injury. J. Biol. Chem. 291, 2566-2575.

Ong S.B., Katwadi K., Kwek X.Y., Ismail N.I., Chinda K., Ong S.G. and Hausenloy D.J. (2018). Non-coding rnas as therapeutic targets for preventing myocardial ischemia-reperfusion injury. Expert Opin. Ther. Targets 22, 247-261.

Ounzain S., Pezzuto I., Micheletti R., Burdet F., Sheta R., Nemir M., Gonzales C., Sarre A., Alexanian M., Blow M.J., May D., Johnson R., Dauvillier J., Pennacchio L.A. and Pedrazzini T. (2014). Functional importance of cardiac enhancer-associated noncoding RNAs in heart development and disease. J. Mol. Cell Cardiol. 76, 55-70.

Pei H., Yu Q., Xue Q., Guo Y., Sun L., Hong Z., Han H., Gao E., Qu Y. and Tao L. (2013). Notch1 cardioprotection in myocardial ischemia/reperfusion involves reduction of oxidative/nitrative stress. Basic Res. Cardiol. 108, 373.

Pei H., Song X., Peng C., Tan Y., Li Y., Li X., Ma S., Wang Q., Huang R., Yang D., Li D., Gao E. and Yang Y. (2015). TNF-alpha inhibitor protects against myocardial ischemia/reperfusion injury via notch1-mediated suppression of oxidative/nitrative stress. Free Radic. Biol. Med. 82, 114-121.

Prech M., Marszalek A., Schroder J., Filas V., Lesiak M., Jemielity M., Araszkiewicz A. and Grajek S. (2010). Apoptosis as a mechanism for the elimination of cardiomyocytes after acute myocardial infarction. Am. J. Cardiol. 105, 1240-1245.

Su Q., Liu Y., Lv X.W., Dai R.X., Yang X.H. and Kong B.H. (2020). LncRNA TUG1 mediates ischemic myocardial injury by targeting miR-132-3p/HDAc3 axis. Am. J. Physiol. Heart Circ. Physiol. 318, H332-H344.

Vausort M., Wagner D.R. and Devaux Y. (2014). Long noncoding RNAs in patients with acute myocardial infarction. Circ. Res. 115, 668-677.

Wang R., Yang M., Wang M., Liu X., Xu H., Xu X., Sun G. and Sun X. (2018). Total saponins of Aralia elata (miq) seem alleviate calcium homeostasis imbalance and endoplasmic reticulum stress-related apoptosis induced by myocardial ischemia/reperfusion injury. Cell Physiol. Biochem. 50, 28-40.

Wu T., Wu D., Wu Q., Zou B., Huang X., Cheng X., Wu Y., Hong K., Li P., Yang R., Li Y. and Cheng Y. (2017). Knockdown of long non-coding RNA-ZFAS1 protects cardiomyocytes against acute myocardial infarction via anti-apoptosis by regulating mir-150/crp. J. Biol. Chem. 118, 3281-3289.

Wu Z., Zhao S., Li C. and Liu C. (2018). LncRNA TUG1 serves an important role in hypoxia-induced myocardial cell injury by regulating the miR-145-5-p-Binp3 axis. Mol. Med. Rep. 17, 2422-2430.

Yan H.F., Tuo Q.Z., Yin Q.Z. and Lei P. (2020). The pathological role of ferroptosis in ischemia/reperfusion-related injury. Zool. Res. 41, 220-230.

Yan Y., Zhang B., Liu N., Qi C., Xiao Y., Tian X., Li T. and Liu B. (2016). Circulating long noncoding RNA UCA1 as a novel biomarker of acute myocardial infarction. Biomed. Res. Int. 2016, 8079372.

Yang D., Yu J., Liu H.B., Yan X.Q., Hu J., Yu Y., Guo J., Yuan Y. and Du Z.M. (2019). The long non-coding RNA TUG1-miR-9a-5p axis contributes to ischemic injuries by promoting cardiomyocyte apoptosis via targeting KLF5. Cell Death Dis. 10, 908.

Yano T., Abe K., Tanno M., Miki T., Kuno A., Miura T. and Steenbergen C. (2018). Does p53 inhibition suppress myocardial ischemia-reperfusion injury? J. Cardiovasc. Pharmacol. Ther. 23, 350-357.

Yellon D.M. and Hausenloy D.J. (2007). Myocardial reperfusion injury. N. Engl. J. Med. 357, 1121-1135.

Zhai M., Li B., Duan W., Jing L., Zhang B., Zhang M., Yu L., Liu Z., Yu B., Ren K., Gao E., Yang Y., Liang H., Jin Z. and Yu S. (2017). Melatonin ameliorates myocardial ischemia reperfusion injury through sirt3-dependent regulation of oxidative stress and apoptosis. J. Pineal Res. 63.

Zhang Q., Geng P.L., Yin P., Wang X.L., Jia J.P. and Yao J. (2013). Down-regulation of long non-coding RNA TUG1 inhibits osteosarcoma cell proliferation and promotes apoptosis. Asian Pac. J. Cancer Prev. 14, 2311-2315.

Zhang E.B., Yin D.D., Sun M., Kong R., Liu X.H., You L.H., Han L., Xia R., Wang K.M., Yang J.S., De W., Shu Y.Q. and Wang Z.X. (2014). P53-regulated long non-coding RNA TUG1 affects cell proliferation in human non-small cell lung cancer, partly through epigenetically

regulating HOXB7 expression. Cell Death Dis. 5, e1243.

Zhang Y., Zhao J., Li R., Lau W.B., Yuan Y.X., Liang B., Li R., Gao E.H., Koch W.J., Ma X.L. and Wang Y.J. (2015). Adiporon, the first orally active adiponectin receptor activator, attenuates postischemic myocardial apoptosis through both AMPK-mediated and AMPK-independent signalings. Am. J. Physiol. Endocrinol. Metab. 309, E275-282.

Zhang M., Lu W., Huang Y., Shi J., Wu X., Zhang X., Jiang R., Cai Z. and Wu S. (2016a). Downregulation of the long noncoding RNA TUG1 inhibits the proliferation, migration, invasion and promotes apoptosis of renal cell carcinoma. J. Mol. Histol. 47, 421-428.

Zhang Y., Sun L., Xuan L., Pan Z., Li K., Liu S., Huang Y., Zhao X., Huang L., Wang Z., Hou Y., Li J., Tian Y., Yu J., Han H., Liu Y., Gao F., Zhang Y., Wang S., Du Z., Lu Y. and Yang B. (2016b). Reciprocal changes of circulating long non-coding RNAs ZFAS1 and CDR1AS predict acute myocardial infarction. Sci. Rep. 6, 22384.

Zhao D., Feng P., Sun Y., Qin Z., Zhang Z., Tan Y., Gao E., Lau W.B., Ma X., Yang J., Yu S., Xu X., Yi D. and Yi W. (2018). Cardiac-derived CTRP9 protects against myocardial ischemia/reperfusion injury via calreticulin-dependent inhibition of apoptosis. Cell Death Dis. 9, 723.

Zou X., Wang J., Tang L. and Wen Q. (2019). Lncrna tug1 contributes to cardiac hypertrophy via regulating miR-29b-3p. In Vitro Cell Dev. Biol. Anim. 55, 482-490.

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