Long non-coding RNA TUG1/microRNA-29/PTEN axis in ischemia-reperfusion in a rat model

Zhiquan Xu

Rheumatology and Immunology, Hainan Women and Children's Medical Center

Xiaoyan Huang

Metabolism and Endocrinology, Hainan Women and Children's Medical Center

Qiuyu Lin

Hainan Maternal and Children 's Medical Center

Wei Xiang (✉ Profxiangw1230@163.com)

Hainan Medical University <https://orcid.org/0000-0002-4784-0846>

Research

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Abstract

Objective: Long non-coding RNA (lncRNA) taurine upregulated gene 1 (TUG1) is increased under ischemia. This study intended to identify the potential competing endogenous RNA network involving TUG1 in renal ischemia-reperfusion (I/R).

Methods: A rat model of acute renal injury induced by I/R was established, and the differentially expressed genes were analyzed by microarray. The levels of blood urea nitrogen (BUN), serum creatine (SCr), methylenedioxypheptamine (MDA) and superoxide dismutase (SOD) in serum of rats were measured. HE staining evaluated the pathological damage of renal tissues, western blot analysis detect the levels of apoptosis- and autophagy-related proteins, immunofluorescence staining detected LC3 fluorescence intensity, and transmission electron microscope observed autophagosomes. Pull-down assay and dual luciferase reporter gene assay were used to verify the targeting relationship among TUG1, miR-29 and PTEN. The effects of TUG1 on biological behaviors of renal tubular cells were evaluated by simulating the acute renal injury induced by I/R *in vitro*.

Results: In vivo, the levels of BUN, SCr and MDA in serum of I/R-treated rats were increased, SOD level and autophagosomes were reduced, tubule epithelial cells were necrotic, and TUG1 was upregulated in renal tissues of I/R-treated rats, which were reversed by TUG1 knockdown. Autophagy inhibition attenuated the protective effect of TUG1 knockdown on I/R-treated rats. TUG1 could competitively bind to miR-29 to promote PTEN expression. In vitro, low expression of TUG1 promoted proliferation and autophagy of renal tubular cells and inhibited apoptosis.

Conclusion: TUG1 knockdown promotes autophagy and improves acute renal injury in I/R-treated rats by binding to miR-29 to silence PTEN.

Introduction

Ischemia/reperfusion (I/R) injury is a process when a blood supply returns after hypoxia to tissue, causing ischemia and induces a cascade of events related to oxidative damage and dysfunction, which is responsible for most cardiovascular diseases worldwide [1]. Serious clinical manifestations such as acute heart failure, myocardial infarction, cerebral and gastrointestinal disorder, systemic inflammatory response and multiple organ dysfunction are critical medical conditions of I/R injury [2]. What is worse, clinical symptoms are often subtle at first, making it impossible to identify the exact time of the onset of ischemia; if it is diagnosed within 24 hours after symptoms appear, the survival rate of acute ischemia is about 50%, but this rate decreases to 30% or lower in case of delayed diagnosis [3]. I/R injury is the main negative factor influencing the outcome after kidney transplantation and is associated with organ rejection [1]. Post-ischemic AKI is characterized by decreased glomerular filtration rate and high renal vascular resistance with endothelial activation and dysfunction, a process of critical importance that is followed by a reduction in microvascular blood flow mainly affecting the renal outer medulla [4]. I/R injury of the kidney is a leading cause of acute kidney injury, and it may result in worsening or even loss

of organ function and is also a common and unavoidable phenomenon in kidney transplantation [5]. Tubular cells are critical targets of I/R injury in renal transplantation [6]. If we want to define biomarkers or develop targeted therapeutic interventions, it is urgent to comprehend the mechanism of renal tubular cells on I/R response.

Emerging studies have implicated a fundamental role for non-coding RNAs, such as microRNAs (miRs), and more recently long non-coding RNAs (lncRNAs) in acute I/R injury [7, 8]. lncRNA taurine upregulated gene 1 (TUG1) is essential for retinal development in the developing mouse eye, and it is abnormally regulated in tumorigenesis, either as a potential tumor suppressor or oncogene [9]. TUG1 has been identified to protect mouse livers against cold-induced liver damage in liver transplantation via inhibiting apoptosis and inflammation [10]. In addition, TUG1 protects renal tubular epithelial cells from lipopolysaccharide-induced damage by regulating miR-223 [11]. It has been documented that hypoxia treatment significantly increases expression and overexpression of TUG1 aggravates hypoxia-induced injury in H9c2 cells [12]. lncRNA TUG1 may function as a competing endogenous RNA (ceRNA) for miR-145 to upregulate aquaporin-4 to induce cell damage, possibly providing a new target in cerebral I/R injury [13]. In light of these references, we hypothesize there is an underlying ceRNA network involving TUG1 in renal I/R injury. Therefore, we carried out in vivo and in vitro experiments to figure out the protective roles of TUG1 in renal I/R injury through which pathway.

Materials And Methods

Ethics statement

This study was ratified and supervised by the ethics committee of Hainan Medical University. We made significant efforts to minimize animals used and their suffering.

Establishment of renal I/R model

After adaptive feeding for 3 days, the rats had free access to food and water and randomized to sham group (n = 10, rats received surgical procedures without renal arterial clamping); I/R group (n = 10, rats underwent renal ischemia for 45 minutes followed by 24-hour reperfusion); negative control (NC) group (n = 10, intravenous injection of 20 µg empty vectors for 15 minutes before renal artery clamping); knockdown TUG1 (ko-TUG1) group (n = 10, injected with 20 µg TUG1 [14] for 15 minutes after removal of renal artery clamp), ko-TUG1 + 3-MA group (n = 10, injected with 20 µg TUG1 and 3-MA (30 mg/kg, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) [15] for 15 minutes after removal of renal artery clamp).

Rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital (50 mg/kg). Then the right kidney of each rat was removed and the left renal artery was exposed and clamped for 45 minutes. Subsequently, the clamp was removed and the kidney was observed for 4–5 minutes to assure reperfusion was established successfully. After 24 hours of reperfusion, the left kidney of each rat was removed and rats were euthanized. The serum of 10 rats in each group was applied for detecting serum

index, the kidney tissues of 3 rats was used for tissue homogenate, and the kidney tissues of 6 rats was used for tissue section detection.

Microarray analysis

Total RNA of kidney tissues in rats from the sham and I/R groups was extracted by a TRIzol kit (Invitrogen Inc., Carlsbad, CA, USA). Double strand cDNA was synthesized by SuperScript Double-stranded cDNA synthesis kit (Invitrogen), and labeled and hybridized to an lncRNA expression microarray (12 × 135K, Arraystar Inc., Rockville, MD, USA). After hybridization and washing, processed slides were scanned by an Axon GenePix 4000B scanner (Molecular Devices Inc., Sunnyvale, CA, USA). Raw data were extracted as pair files using NimbleScan software (Version 2.5; Roche). The threshold for up- and downregulated genes was set as fold change ≥ 1.5 and p value ≤ 0.05 . All the above works were completed by Shanghai Sensichip Hightech Co., Ltd. (Shanghai, China). Hierarchical cluster analysis was done by Shanghai Novel Bioinformatics Company (Shanghai, China).

Detection of renal function

Peripheral blood samples obtained from each group were centrifuged at 3000 r/min for 10 minutes. Then 500 μ L serum was taken for determination of serum creatinine (SCr) and blood urea nitrogen (BUN) using an automatic biochemical analyzer (Roche Diagnostics, Indianapolis, Indiana, USA) with an AU2700 Analyzer (Olympus, Tokyo, Japan).

Detection of contents of renal malondialdehyde (MDA) and superoxide dismutase (SOD)

The MDA content in the rat kidneys was measured using an MDA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) and the thiobarbituric acid method [16]. The renal SOD content in rats was detected using a SOD kit (Nanjing Jiancheng Bioengineering Institute) [17].

Histological examination

Kidney tissues were fixed in 10% buffered formalin, embedded in paraffin and cut at 4- μ m. After deparaffinization and rehydration, sections were stained with hematoxylin and eosin (HE) for the quantitative evaluation of renal injury score. A histologic score of tissue damage was estimated by two external pathologists according to the following criteria: tubular dilatation, cast deposition, brush border loss and necrosis in 25 randomly chosen non-overlapping fields. Lesions were graded on a scale from 0 (normal) to 5 (extensive damaged).

TUNEL assay

TUNEL assay was performed with an In situ cell death detection kit (fluorescein, Roche). Paraffined sections were deparaffinized, sectioned at 4- μ m, treated with 20 mg/mL proteinase K, and treated with 3% hydrogen peroxide. Subsequently, the sections were treated with a mixture of nucleotides and TdT enzyme at 37 °C for 1 hour, and then treated with the converter conjugated with horseradish peroxidase at 37 °C for 30 minutes. Under the fluorescence microscope (Carl Zeiss, Jena, Germany), cells with green

stained nuclei were regarded as TUNEL-positive and expressed as the percentage of total cells. Finally, TUNEL-positive cells were counted in 10 randomly selected fields ($\times 200$) in a blinded manner.

Electronic microscopy

The renal samples at 1 mm^3 were fixed with 2.5% glutaraldehyde overnight at $4\text{ }^{\circ}\text{C}$, and treated with osmium tetroxide, embedded and sliced. Autophagosome ultrastructure was observed under the transmission electron microscope (Olympus).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA of renal tissues or tubular cells from each group of rats was extracted by TRIzol reagent (Invitrogen). After the concentration and purity of RNA were determined, cDNA was synthesized by a reverse transcription kit, amplified by PCR instrument, and the expression of each primer in (Table 1) was detected by a SYBR PCR Master Mix kit (Bio-Rad, Inc., Hercules, CA, USA), with U6 or β -actin as an internal reference. All primers used in the experiment were designed by Primer 3Plus website and synthesized by Genewiz Biotechnology Co., Ltd. (Suzhou, Jiangsu, China). The experiment was repeated 3 times to calculate the average CT value, and the concentration of each sample was calculated according to $2^{-\Delta\text{CT}}$ to detect the relevant RNA level in cells and tissues.

Table 1
Primer sequences for RT-qPCR

Primer	Sequence (5'→3')
TUG1	F: GCTATTGGTATGGCTGGCCT
	R: AAGGAGAGAAATGGACGCGG
CHCHD4P4	F: TGACCCACCTCTTCTTTGG
	R: AGACATTA ACTGGAACCGTCC
AU015836	F: GCCTCCCAGCCATTAGGTTT
	R: GCCACCGTGTAGAGGTCAAA
MALAT1	F: CAGCAGCAGACAGGATTCCA
	R: ATTGCCGACCTCACGGATT
HIF1A-AS1	F: TGGATGCCACATGCATTATGA
	R: AGCAAGGGCTGTTCCATGTT
PVT1	F: GGGGAATAACGCTGGTGGAA
	R: CCCATGGACATCCAAGCTGT
PTEN	F: GTGCAGATAATGACAAG
	R: GATTTGACGGCTCCTCT
β-actin	F: GTCATTCCAAATATGAGAGATGCGT
	R: GCTATCACCTCCCCTGTGTG
miR-29	F: TGCCAGGAGCTGGTGATTTCCT
	R: ACGGGCGTACAGAGGATCCCC
U6	F: AACGCTTCACGAATTTGCGT
	R: GGTGTACTCTTGGGGAACCAG
Note: RT-qPCR, reverse transcription quantitative polymerase chain reaction; TUG1, taurine upregulated gene 1; PTEN, phosphatase and tensin homolog; miR-29, microRNA-29; F, forward; R, reverse.	

Western blot analysis

The renal tissues were homogenized centrifuged at 12,000 g for 10 minutes at 4 °C. Then proteins were collected and quantified using a bicinchoninic acid kit (Thermo Fisher Scientific, Rockford, IL, USA), and then separated by electrophoresis and transferred onto nitrocellulose membranes. The blots were blocked with 5% non-fat dry milk in tris-buffered saline-tween (TBST) at 37 °C for 2 hours, followed by incubation with primary antibodies at (Table 2) 4 °C overnight. After TBST washes, the membranes were incubated

for 1 hour with horseradish-peroxidase conjugated secondary antibody. After TBST washes, protein bands were detected by an enhanced chemiluminescence system and visualized using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA). Target protein/glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as relative protein expression.

Table 2
Antibodies used in experiment

Antibody	Information	Dilution rate
Bax	ab32503, ABcam	1/1000
Bcl-2	ab182858, ABcam	1/2000
p62	ab91526, ABcam	2 µg/mL
Becline-1	ab207612, ABcam	1/2000
LC3II	ab48394, ABcam	2 µg/mL
LC3I	ab51520, ABcam	2 µg/mL
PTEN	ab32199, ABcam	1/10000
GAPDH	ab181602, ABcam	1/10000
Note: Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X; LC3, light chain 3; PTEN, phosphatase and tensin homolog; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.		

RNA pull-down

TCMK-1 cells were transfected with biotinylated miR, and were collected after 48-hour transfection. The cell lysates were incubated with M-280 streptavidin magnetic beads (Invitrogen). The bound RNAs were purified using TRIzol reagent (Invitrogen) for further RT-qPCR analysis.

Dual luciferase reporter gene assay

TUG1 and phosphatase and tensin homolog (PTEN) 3'untranslated region (3'UTR) sequences containing miR-29 binding site were synthesized respectively, followed by construction of TUG1 and PTEN 3'UTR wild type (WT) plasmids. Then the constructed plasmids were respectively transfected with NC and miR-29 plasmids into 293T cell (ATCC, Manassas, Virginia, USA). After 48 hours of transfection, cells were collected and lysed. Luciferase activity was detected by a luciferase detection kit (BioVision, SanFrancisco, CA, USA) and Glomax20/20 luminometer (Madison, Wisconsin, USA).

Cell culture and grouping

TCMK-1 cells purchased from ATCC (CCL-139™) were cultured in Dulbecco modified Eagle medium (HyClone, Logan, UT, USA) with 10% heat-inactivated fetal bovine serum (FBS, HyClone). For hypoxia treatment, TCMK-1 cells were incubated in Forma Series II Water Jacketed CO₂ incubator (Thermo)

containing 94% N₂ and 5% CO₂ to maintain the oxygen concentration at 1%. During reoxygenation incubation, cells were removed to a normoxic chamber (21% O₂).

Cells were allocated into blank group (in the normoxic chamber at indicated time points), hypoxia/reoxygenation (H/R) group (cells were treated with H/R), NC group (after H/R treatment, cells were treated with empty vectors), and si-TUG1 group (after H/R treatment, cells were treated with si-TUG1, at 50 nM) [18]. All transfections were done with HiPerFect transfection reagent (QIAGEN, Valencia, CA, USA).

Cell counting kit-8 (CCK-8)

Cell viability was detected by a CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) according to the instructions. The absorbance at a wavelength of 450 nm was measured using a microplate reader. The percentage of living cells was calculated by the ratio of absorbance of the H/R group to that of the blank group.

Flow cytometry

Cells were planted into 6-well plates at 5×10^5 /mL cells/well and 1 mL/well. After cell adherence for 24 hours, cells were stimulated by hypoxia and H/R in refreshed medium. Cells in each group were collected into flow tubes, and each tube was added with 5 uL fluorescein isothiocyanate-labeled Annexin-V buffer and 100 uL $1 \times$ loading buffer, followed by 30-minute incubation without light exposure. Cell apoptosis was detected by a flow cytometer (Beijing YourHope Medical Equipment Co., Ltd., Beijing, China).

Statistical analysis

Statistical analysis was conducted by SPSS 21.0 (IBM Corp. Armonk, NY, USA). All the data were in normality distribution checked by the Kolmogorov-Smirnov test. Measurement data were expressed as mean $\pm$ standard deviation. The *t* test processed comparisons between two groups, while one-way or two-way analysis of variance (ANOVA) processed comparisons among multiple groups, and Tukey's multiple comparisons test or Sidak's multiple comparisons test was used for post-hoc test. The Kaplan-Meier method was used to draw the survival curve. The *p* value was obtained by a two-tailed test and *p* < 0.05 indicated a statistical difference.

Results

TUG1 is upregulated in renal I/R-treated rats

To understand the renal injury of rats after I/R treatment, the levels of serum BUN, SCr, MDA and SOD in sham-operated rats and I/R-treated rats. Compared with the sham-operated rats, the levels of BUN, SCr and MDA in the serum of I/R-treated rats were increased, and the levels of SOD was decreased significantly (all *p* < 0.01) (Fig. 1A). Then, HE staining evaluated the pathological damage of renal tissues. The results showed that the structure of renal tubules in sham-operated rats was clear and complete, the

renal tissues in I/R-treated rats were seriously damaged, the epithelial cells of renal tubules were degenerated and necrotic, part of basement membrane was exposed, and the lumen of tubules was dilated (Fig. 1B). In addition, lncRNA microarray analysis was performed in two groups of rats. The results showed multiple differentially expressed lncRNAs in the two groups of rats, while in I/R-treated rats, TUG1 was significantly upregulated ($p < 0.01$) (Fig. 1C). To verify the most significant upregulation of TUG1 in clinical practice, we selected the first six lncRNAs with the most significant difference in microarray analysis for clinical validation. The results of RT-qPCR showed that compared with other lncRNAs, TUG1 was significantly upregulated (all $p < 0.01$) (Fig. 1D).

TUG1 knockdown improves renal IR injury and inhibits cell apoptosis

In the above microarray analysis, we know that lncRNA TUG1 is significantly upregulated in acute renal injury after I/R, so we suspected that TUG1 also had an effect on acute renal injury after I/R. To confirm this conjecture, we injected the knockdown TUG1 into I/R treated rats, and detected the levels of BUN, SCr, MDA and SOD in the serum of rats. The results showed that compared with the NC group, the serum BUN, SCr and MDA levels of rats in the ko-TUG1 group were reduced, while SOD level was significantly elevated (all $p < 0.01$) (Fig. 2A). Then pathological morphology of the rats was evaluated by HE staining, and it revealed that the inflammatory infiltration in the renal tissues was reduced and the pathological morphology was improved after the acute renal I/R injury in the ko-TUG1 group (Fig. 2B). After that, flow cytometry and TUNEL staining detected the apoptosis of renal cells. The results showed that the apoptosis and TUNEL-positive cells in the ko-TUG1 group were notably reduced (both $p < 0.01$) (Fig. 2C/D).

TUG1 knockdown promotes autophagy in rats with acute renal injury induced by I/R

Autophagy plays an important role in acute renal injury [19]. POC can reduce renal damage and epithelial-mesenchymal transition after I/R injury by enhancing autophagy activation [20]. Therefore, we explored the effect of TUG1 on autophagy in acute renal injury induced by I/R. The results of transmission electron microscope observation displayed that compared with the sham group, autophagosomes in renal tissue of rats in I/R group were significantly reduced, but notably increased after the treatment of knocking down TUG1 was (Fig. 3A). In addition, western blot analysis detected levels of autophagy-related proteins. The results showed that relative to the sham group, light chain 3 (LC3)-II/LC3I and Beclin-1 level in the I/R group were significantly decreased, and level of p62 was elevated. After knocking down TUG1, the autophagy-related proteins showed the opposite trends (all $p < 0.01$) (Fig. 3B). Immunofluorescence staining showed that relative to the I/R group, the fluorescence intensity of LC3 was increased after knocking down TUG1.

Inhibition of autophagy attenuates the protective effect of TUG1 knockdown on rats with acute renal injury induced by I/R

To further confirm the protective effect of TUG1 on rats with acute renal injury induced by I/R through autophagy, a functional rescue experiment was carried out by setting up a combination group of autophagy pathway inhibitor (3-mA) and knockdown TUG1, with the ko-TUG1 group as the control. The results showed that compared with the ko-TUG1 group, the serum BUN, SCr and MDA levels of rats in the ko-TUG1 + 3-mA group were significantly increased, while SOD was reduced, apoptosis and TUNEL-positive cells were increased, LC3-II/LC3I and Becline-1 level were reduced, and p62 level was increased (all $p < 0.01$) (Fig. 4A-D).

TUG1 competitively binds to miR-29 to promote PTEN expression

To find out whether there is a ceRNA network in acute renal I/R injury, we predicted that there is a targeting relationship between TUG1 and multiple miRs through bioinformatics website <http://starbase.sysu.edu.cn/index.php>. miR-29a overexpression can protect against I/R injury [21]. As expected, we found a binding site between TUG1 and miR-29 by pull-down assay (Fig. 5A), and we predicted that there are multiple target genes in miR-29a, including PTEN. We further verified the relationship among TUG1, miR-29 and PTEN. Through the verification of dual luciferase reporter gene assay, the luciferase activity of TUG1 (WT) and miR-29 was decreased significantly, while the luciferase activity of PTEN (WT) and miR-29 were decreased (Fig. 5B). Compared with the sham group, miR-29 expression in the I/R group was reduced while PTEN levels were elevated; compared with the NC group, miR-29 expression in the ko-TUG1 group was significantly elevated while PTEN levels were downregulated (all $p < 0.05$) (Fig. 5C).

Low expression of TUG1 promotes proliferation and autophagy of TCMK-1 cells and inhibits apoptosis

To confirm the effect of TUG1 *in vitro*, we simulated the I/R injury of renal tubular cells (TCMK-1) by H/R, and measured the activity of TCMK-1 cells in each group by CCK-8 method. It showed that the activity of TCMK-1 cells in the H/R group was lower than that in the blank group, and that in the si-TUG1 group was higher than that in the NC group (Fig. 6A). Flow cytometry and TUNEL staining evaluated the apoptosis of TCMK-1 cells. Compared with the blank group, the apoptosis rate of TCMK-1 cells in the H/R group was significantly enhanced; compared with the NC group, the apoptosis rate in the si-TUG1 group was decreased (Fig. 6B-C). In addition, western blot analysis detected the levels of autophagy-related proteins, and immunofluorescence staining detected the fluorescence intensity of LC3. Compared with the blank group, the autophagy of TCMK-1 cells in the H/R group was inhibited; compared with the NC group, the autophagy of TCMK-1 cells in the si-TUG1 group was promoted (Fig. 6D-E). The levels of miR-29 and PTEN were detected. The results showed that compared with the blank group, miR-29 expression in

TCMK-1 cells was reduced, and PTEN levels in the H/R group were significantly elevated; compared with the NC group, miR-29 expression in TCMK-1 cells was upregulated, and PTEN levels in the si-TUG1 group were significantly decreased (Fig. 6F-G) (all $p < 0.01$).

Discussion

Renal ischemia, the most common cause of acute kidney injury (AKI) is related to adverse outcome and high mortality, and it is estimated that AKI occurs in about 1 of 5 hospitalizations and is associated with a more than 4-fold increased likelihood of death [22, 23]. Therefore, it is of prime urgency and importance to search for effective approaches for I/R injury, especially for renal I/R injury. It has been identified that lncRNA TUG1 was significantly overexpressed in oxygen-glucose deprivation/reperfusion (OGD/R)-induced myocardial HL-1 cells [24]. The microarray analysis and RT-qPCR also validated TUG1 upregulation in renal tissues of I/R-treated rats. We further verified that TUG1 knockdown could promote autophagy and improved acute renal injury in I/R-treated rats by binding to miR-29 to silence PTEN.

Initially, we carried out microarray analysis to screen out the differentially expressed lncRNAs. Among these lncRNAs, TUG1 was significantly upregulated in I/R-treated rats. It was also reported that TUG1 was upregulated in the brain of middle cerebral artery occlusion (MCAO) and OGD/R-treated SH-SY5Y cells, indicating the therapeutic potential of TUG1 in I/R [25]. In addition, we found levels of BUN, SCr and MDA in the serum of I/R-treated rats were increased, and the level of SOD was decreased significantly, which were reversed after knocking down TUG1. The urine volume, BUN, SCr and MDA concentrations, LC3II/LC3I, and autophagosomes were significantly elevated 24 hours after renal I/R, while p62 and SOD concentrations were decreased, which were consistent with our results [17]. BUN and SCr in pediatric patients with AKI were decreased in parallel during hospitalization in all age groups [26]. BUN and SCr are both considered specific markers to measure kidney function and pathological injuries after I/R [27]. SOD could limit oxidative stress and renal I/R injury and is considered as the most relevant molecule against I/R-induced changes [28]. Significantly enhanced myocardial SOD activity and reduced MDA level were beneficial for I/R-treated rats [29]. Thus, our study supported the demonstration that TUG1 knockdown may be beneficial for the treatment of renal I/R injury.

After knocking down TUG1, autophagosomes in renal tissues of rats was notably increased, LC3-II/LC3I and Beclin-1 level were increased, and level of p62 was reduced. Autophagy is responsible for damaged organelles, and provides energy for cell renewal and internal environment stability, and loss of autophagy is associated with I/R injury [30]. LC3 and Beclin-1 are specific markers to monitor autophagy, and the amount of LC3-II is closely correlated with autophagosomes [31, 32]. A study supported the notion that the upregulation of autophagy was associated with a 45% reduction in infarct size [33]. TUG1 knockdown reduced the infarction area and cell apoptosis in MCAO model mice, thus effectively protecting against brain I/R injury [13]. Taken together, knockdown TUG1 could alleviate renal I/R injury by promoting autophagy.

Furthermore, we verified that TUG1 competitively binds to miR-29 to promote PTEN expression. I/R-induced TUG1 bound to miR-132-3p to activate histone deacetylase 3 and then provoked intracellular reactive oxygen species (ROS) accumulation, and worsened the injury of acute myocardial infarction [34]. The miR sponge function of TUG1 was supported by the interaction between TUG1 and miR-145 using bioinformatic analysis and by that after knockdown of TUG1, miR-145 level was significantly upregulated [13]. miR-29a mimic protects against cell injury and mitochondrial dysfunction after ischemia-like stresses in vitro, and increasing miR-29a expression might be a novel option for protection against I/R injury [21]. A close association between TUG1 and miR-29b was verified in inhibiting apoptosis and inflammation in lipopolysaccharide-treated H9c2 cells [35]. The directing targeting relationship between miR-29a and PTEN was previously found in osteosarcoma cells, in which miR-29 served as a tumor promoter, in osteosarcoma progression and metastasis by targeting PTEN [36].

Apoptosis is a process of programmed cell death that is activated under hypoxic stress in ischemic injury and during the production of ROS in reperfusion injury [37]. In the present study, low expression of TUG1 promoted proliferation and autophagy of TCMK-1 cells and inhibited apoptosis. Activated autophagy notably reduced renal tissue damage and tubular cell apoptosis in I/R and H/R models [15]. Similarly, TUG1 was upregulated in ischemic heart and cardiomyocytes and knockdown of TUG1 inhibited cardiomyocyte apoptosis and markedly ameliorated impaired cardiac function of myocardial infarction mice by upregulating miR-9 expression [38].

Conclusion

To sum up, TUG1 knockdown could promote autophagy and improved acute renal injury in I/R-treated rats by binding to miR-29 to silence PTEN. In addition, studies conducted to date on animal models warrant further investigation and development because many underlying mechanisms remain unknown and need to be clarified. Additional clinical trials are warranted to justify this approach for lncRNA TUG1 target therapy in the future.

Declarations

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Not applicable.

Availability of data and materials

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

Authors' contributions

ZQX is the guarantor of integrity of the entire study and contributed to the concepts and design of this study; XYH contributed to the definition of intellectual content and literature research of this study; ZQX and XYH contributed to the experimental studies; QYL and WX contributed to the data acquisition and analysis; ZQX and QYL took charge of the manuscript preparation; WX contributed to the manuscript review. All authors read and approved the final manuscript.

Patient consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate

All experimental protocols were recommended and got approval from the Hainan Women and Children's Medical Center(Approval number:SYXK琼2018-0015). The experimental process strictly followed the approved protocol. Significant efforts were made to minimize animal numbers and suffering.

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Figures

Figure 1

TUG1 is upregulated in renal I/R-treated rats. A, levels of BUN and SCr in serum of rats were detected by automatic biochemical instrument, and levels of MDA and SOD in serum of rats were detected by kits (n = 10); B, HE staining evaluated the pathological damage of renal tissues ($\times 200$) (n = 6); C, lncRNA microarray analyzed the differentially expressed genes in sham-operated rats and I/R-treated rats (n = 3); D, RT-qPCR detected the mRNA expression of differentially expressed genes in sham-operated rats and I/R-treated rats (n = 3). Data in panel B were analyzed by the t test, and data in panels A and D were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test. **p < 0.01.

Figure 2

TUG1 knockdown improves renal IR injury and inhibits cell apoptosis. A, levels of BUN and SCr in serum of rats were detected by automatic biochemical instrument, and levels of MDA and SOD in serum of rats were detected by kits (n = 10); B, HE staining evaluated the pathological damage of renal tissues ($\times 200$) (n = 6); C, western blot analysis detected the levels of apoptotic proteins (n = 3); D, TUNEL assay measured the TUNEL-positive cells in renal tissues of rats in each group (n = 6). Data in panel B were analyzed by one-way ANOVA, and data in panels A and C were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test. **p < 0.01.

Figure 3

TUG1 knockdown promotes autophagy in rats with acute renal injury induced by I/R. A, the number of autophagosomes ($\times 5000$) ($n = 6$) was observed by transmission electron microscope; B, western blot analysis detected the levels of autophagy-related proteins ($n = 3$); C, Immunofluorescence staining detected the fluorescence intensity of LC3 ($\times 400$) ($n = 6$). Data in panel B were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test. $**p < 0.01$.

Figure 4

Inhibition of autophagy pathway attenuates the protective effect of TUG1 knockdown on rats with acute renal injury induced by I/R. A, levels of BUN and SCr in serum of rats were detected by automatic biochemical instrument, and levels of MDA and SOD in serum of rats were detected by kits (n = 10); B, HE staining evaluated the pathological damage of renal tissues ($\times 200$) (n = 6); C, TUNEL assay measured the TUNEL-positive cells in renal tissues of rats in each group ($\times 200$) (n = 6); D, western blot analysis detected the levels of autophagy-related proteins (n = 3). Data in panels A and D were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons test; and data in panels B and C were analyzed by the t test. **p < 0.01.

Figure 5

TUG1 competitively binds to miR-29 to promote PTEN expression. A, pull-down assay confirm a binding site between TUG1 and miR-29; B, dual luciferase reporter gene assay verified a binding site between TUG1 and miR-29, and a binding site between PTEN and miR-29; C, RT-qPCR detected the mRNA expression of PTEN and miR-29 in kidney tissues; D, western blot analysis detected protein level of PTEN. Replicates = 3. Data in panel B were analyzed by two-way ANOVA, data in panels B and C were analyzed by one-way ANOVA, followed by Tukey's multiple comparisons test. **p < 0.01.

Figure 6

Low expression of TUG1 promotes proliferation and autophagy of renal tubular cells and inhibits apoptosis. A, CCK-8 method detected cell viability in each group; B, flow cytometry detected cell apoptosis in each group; C, TUNEL assay measured the TUNEL-positive cells in renal tissues of rats in each group ($\times 200$); D, western blot analysis detected the levels of autophagy-related proteins; E, immunofluorescence staining detected the fluorescence intensity of autophagy-related proteins; F, RT-qPCR detected the mRNA expression of PTEN and miR-29 in kidney tissues; G, western blot analysis detected protein level of PTEN. Data in panel D were analyzed by two-way ANOVA, data in panels A, B, C, F and G were analyzed by one-way ANOVA, followed by Tukey's multiple comparisons test. $**p < 0.01$.