Effect of silencing lncRNATUG1 on rapamycin-induced inhibition of endothelial cell proliferation and migration

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Abstract. Angiogenesis refers to the formation of new blood vessels from existing blood vessels. The proliferation and migration of endothelial cells serves a key function in this process. Previous research has demonstrated that rapamycin suppresses endothelial cell proliferation and migration, as well as angiogenesis. However, the mechanism by which rapamycin inhibits the proliferation and migration of endothelial cells remains unclear. Long noncoding RNAs (lncRNAs) serve a key function in the regulation of endothelial cell function. The aim of the current study was to investigate whether lncRNA taurine upregulated 1 (lncRNATUG1) is involved in rapamycin-induced inhibition of proliferation and migration in human umbilical vein endothelial cells (HUVECs). Reverse transcription quantitative polymerase chain reaction results indicated that the expression of lncRNATUG1 was upregulated in HUVECs that had been cultured with rapamycin. Subsequently, HUVECs were transfected with siRNAs and CCK-8 assays were performed to detect cell proliferation; additionally, flow cytometry was employed to detect cell apoptosis, and wound healing assays were performed to investigate cell migration. The results demonstrated that rapamycin suppressed the proliferation and migration of HUVECs, and promoted the apoptosis of HUVECs. In addition, rapamycin downregulated the expression of vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-2 and MMP-9 in HUVECs. However, silencing of lncRNATUG1 was revealed to attenuate rapamycin-induced inhibition of cellular proliferation and migration of HUVECs, as well as upregulating the expression of VEGF, MMP2 and MMP-9. These results suggested that lncRNATUG1 regulates rapamycin-induced inhibition of endothelial cell proliferation and migration. Therefore, lncRNATUG1 may serve a key function in rapamycin-induced inhibition of endothelial cell proliferation and migration.

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Introduction

Endothelial cells are an important feature of blood vessels, and dysfunction of vascular endothelial cell proliferation and migration may result in the development of various vascular diseases, including atherosclerosis, as well as neointimal proliferation associated with vein graft failure and in-stent restenosis. Therefore, the proliferation and migration of vascular endothelial cells serves a key function in vascular diseases, and an understanding of the associated underlying mechanisms is of critical importance.

Long non-coding RNAs (lncRNAs) are defined as transcripts of >200 nucleotides in length that do not encode for proteins. Initially, lncRNAs were considered to be a transcription 'noise', and to not have biological function (1). However, numerous studies have suggested that lncRNAs serve key functions in the regulation of cell development, differentiation, proliferation, migration and apoptosis, as well as other endothelial cell functions and the pathogenesis of cardiovascular diseases (2-7). At present, a number of studies have indicated that certain lncRNAs are involved in the development of cardiovascular disease via the regulation of endothelial cell proliferation, migration and apoptosis. For example, Michalik *et al* (8) demonstrated that lncRNA metastasis-associated lung adenocarcinoma transcript-1 (lncRNAMALAT1) is involved in the regulation of endothelial cell function and vascular growth. In diabetic rats, the downregulation of lncRNAMALAT1 has been revealed to inhibit cardiac myocyte apoptosis and attenuate left ventricular function (9), as well as suppress the proliferation and migration of retinal endothelial cells, and attenuate retinal vascular injury inflammation and function (10). Tao *et al* (11) demonstrated that downregulation of lncRNAH19 inhibits the proliferation of cardiac fibroblasts. Pan (12) revealed that lncRNAH19 regulates the proliferation and apoptosis of human umbilical vein endothelial cells

(HUVECs) and vascular smooth muscle cells (VSMCs) via modulation of the mitogen-activated protein kinase and nuclear factor-κB signaling pathways, which subsequently regulate atherosclerosis formation. Ballantyne *et al* (13) demonstrated that smooth muscle enriched lncRNA is involved in regulating the proliferation of VSMCs and is highly expressed in atherosclerotic plaques. Furthermore, Qiu *et al* (14) revealed that silencing the expression of lncRNA maternally expressed gene 3 (lncRNAMEG3) promotes endothelial cell proliferation and angiogenesis; and upregulation of the expression of lncRNAMEG3 suppresses angiogenesis and the cell cycle in endothelial cells. These results indicate that lncRNAs serve key functions in the regulation of endothelial cell function.

Taurine upregulated 1 (TUG1), a 7.1-kb lncRNA, was first identified in the retinal cells of newborn mice and has been demonstrated to serve a key function in retinal development (15). Previous studies have revealed that lncRNATUG1 is involved in the development of tumors via regulation of tumor cell proliferation and migration. Furthermore, TUG1 is highly expressed in vascular endothelial cells (16,17). Yin *et al* (6) demonstrated that lncRNATUG1 is highly expressed in the mouse pancreas and downregulates the expression of TUG1, which subsequently affects the apoptosis and insulin secretion of pancreatic β cells, thus suggesting that lncRNATUG1 may represent a new target for the treatment of diabetes. However, little is known about the association between TUG1 and vascular diseases. Therefore, investigation into the role of TUG1 is important with regards to vascular endothelial cells and vascular diseases.

Rapamycin is a potent immunosuppressive agent, which is able to inhibit the proliferation and migration of endothelial cells and angiogenesis. Rosner *et al* (18) identified that rapamycin inhibits human in-stent restenosis by inhibiting the proliferation and migration of vascular smooth muscle cells. Kawatsu *et al* (19) identified that rapamycin inhibits autologous vein graft restenosis by impeding venous neointimal hyperplasia. Although treatment with rapamycin reduces the incidence of postoperative restenosis by inhibiting the proliferation and migration of vascular smooth muscle cells, it increases the incidence of thrombosis. However, although certain drugs may be used to treat the occurrence of thrombus, they may be non-responsive and exhibit an efficacy that is below expectations (20-23). To date, the mechanism by which rapamycin inhibits the proliferation and migration of endothelial cells has been studied (24); however, the underlying mechanisms remain unclear. Recent research has revealed that lncRNAs serve key functions in the regulation of cell proliferation and migration. Li *et al* (25) reported that the lncRNA HOX transcript antisense intergenic RNA regulates the AKT/mammalian target of rapamycin (mTOR) signaling pathway, which subsequently enhances osteosarcoma cell proliferation and metastasis. Wang *et al* (26) demonstrated that lncRNA colorectal neoplasia differentially expressed promotes the proliferation and invasion of glioma cells by regulating the mTOR pathway. Matsumoto *et al* (27) demonstrated that the polypeptide encoded by LINC00961 serves a key function in regulating mTOR complex 1 activity. These results indicate that lncRNAs may be involved in regulating the function of endothelial cells treated with rapamycin. Therefore, it is important to determine the underlying molecular mechanism associated with inhibition of the proliferation and migration of rapamycin-treated vascular endothelial cells. The current study aimed to investigate the effect of rapamycin on the proliferation, migration and apoptosis of endothelial cells, as well as to investigate the association of lncRNATUG1 with these processes.

Materials and methods

Cell culture. Human umbilical vein endothelial cells (HUVECs; cat. no. CRL-1730) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). HUVECs were cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.) and 10 ng/ml vascular endothelial growth factor (VEGF; PeproTech, Inc., Rocky Hill, NJ, USA) at 37˚C in 5% CO_2. All experiments were performed according to the manufacturer's protocol.

Small-interfering RNA (siRNA) and cell transfection. siRNA specifically targeting TUG1 (siTUG1: siTUG1-1, 5'-GCUUGG CUUCUAUUCUGAAUCCUUU-3'; siTUG1-2, 5'-CAGCUG UUACCAUUCAACUUCUUAA-3') and negative control siRNA (siNC, 5'-UUCUCCGAACGUGUCACGUTT-3') were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). Once HUVECs reached 50-70% confluence, they were seeded in 6-well plates and transfected with 100 nM siTUG1 and siNC using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. At 24 h post-transfection, DMEM and 100 ng/ml rapamycin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) were added. Subsequently, the cells were harvested for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and western blot analysis.

RT-qPCR. Total RNA was extracted from cells using RNAiso Plus reagent (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocol. cDNA was synthesized from the extracted RNA using the Fast Quant RT kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's protocol. qPCR was performed using a Super Real PreMix Plus kit (Tiangen Biotech Co., Ltd.) and the ABI 7500 qPCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions used for qPCR were as follows: 95˚C for 5 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. The sequences of primers used for qPCR were as follows: TUG1 forward, 5'-TCCAGACCC TCAGTGCAAAC-3' and reverse, 5'-TGTTGTGGTGTATGT GGGCA-3'; VEGF forward, 5'-CTACCTCCACCATGCCAA GT-3' and reverse, 5'-GCAGTAGCTGCGCTGATAGA-3'; matrix metalloproteinase (MMP)-2 forward, 5'-TGATGGCAT CGCTCAGATCC-3' and reverse, 5'-GGCCTCGTATACCGC ATCAA-3'; MMP-9 forward, 5'-GTCATCCAGTTTGGTGTC GC-3' and reverse, 5'-GGACCACAACTCGTCATCGT-3'; B-cell lymphoma 2 (Bcl-2) forward, 5'-TGTGTGTGGAGA GCGTCAAC-3' and reverse, 5'-GGGCCGTACAGTTCCACA AA-3'; Caspase3 forward, 5'-ATGGAAGCGAATCAATGG AC-3' and reverse, 5'-GCTGCATCGACATCTGTACC-3'; and GAPDH forward, 5'-TCTCTGCTCCTCCTGTTCGA-3' and

reverse, 5'-GCGCCCAATACGACCAAATC-3'. GAPDH was used as an internal control. The relative gene expression levels were determined using the $2^{-\Delta\Delta Cq}$ method (28).

Cell proliferation assay. A total of ~1x10^5 HUVECs were seeded into 96-well plates and subsequently incubated for 24 h at 37˚C in 5% CO_2. Once the cells reached a confluence of 70%, they were transfected with siTUG1-1 and siNC using Lipofectamine™ 2000, according to the manufacturer's protocol. At 24 h post-transfection, DMEM with or without 100 ng/ml rapamycin was added to the cells and subsequently 10 μl of CCK-8 reagent (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to each well at 24, 48 and 72 h time intervals. The cells were then incubated at 37˚C for 4 h. The absorbance was measured at 450 nm with an EnVision® Multi-Mode Plate Reader (PerkinElmer, Inc., Waltham, MA, USA).

Cell apoptosis analysis. Cell apoptosis was determined using an Annexin V-FITC/PI Apoptosis kit [MultiSciences (Lianke) Biotech Co., Ltd, Hangzhou, China]. Then, the cells were harvested by trypsinization without EDTA via centrifugation at 300 x g for 5 min at 4˚C. Cells (1x10^6 cells/ml) were then washed twice with cold PBS and subsequently incubated with 500 μl binding buffer containing 5 μl Annexin V-FITC and 10 μl PI staining solution for 15 min at room temperature in the dark. Apoptosis was then determined using a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). Results were analyzed using FlowJo 7.6.5 software (FlowJo, LLC, Ashland, OR USA).

Wound healing assay to evaluate cell migration. When cells reached a confluence of 90%, 24 h post-transfection, the monolayer of cells was scraped from each of the six wells using a sterile 200 μl pipette tip across the diameter of the wells to form an artificial wound, and the plates were subsequently washed twice with PBS. Following this, serum-free medium with/without 100 ng/ml rapamycin was added to the cells, which were then cultured at 37˚C and 5% CO_2 for 24 h. An IX51-A21PH inverted microscope (Olympus Corporation, Tokyo, Japan) was used to capture images.

Western blot analysis. Total protein was extracted from the cells using RIPA lysis buffer [MultiSciences (Lianke) Biotech Co., Ltd.] supplemented with 1 nM PMSF. Following lysis for 30 min, total protein was isolated via centrifugation at 13,201 x g for 20 min at 4˚C. Protein content was then determined using a BCA Protein Assay kit (Beyotime Institute of Biotechnology, Shanghai, China). A total of ~20 μg of protein was separated by 10% SDS-PAGE and subsequently transferred onto polyvinylidene difluoride membranes. Membranes were blocked using 5% skimmed milk for 2 h at room temperature. The membranes were then incubated with anti-VEGF (cat. no. ab46154; 1:2,000; Abcam, Cambridge, UK), anti-MMP2 (cat. no. ab37150; 1:2,000; Abcam); anti-MMP9 (cat. no. 3852), anti-Bcl-2 (cat. no. 2870), anti-Caspase3 (cat. no. 9662) and anti-GAPDH (cat. no. 5174; all 1:1,000; all from Cell Signaling Technology, Inc., Danvers, MA, USA) overnight at 4˚C. Following this, membranes were incubated with a horseradish peroxidase-labeled secondary antibody (cat. no. 7074; 1:5,000; Cell Signaling Technology, Inc.) for 2 h at room temperature. Finally, protein bands were detected using an enhanced chemiluminescence kit (EMD Millipore, Billerica, MA, USA) and a western blotting detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). GAPDH was used as the reference protein. Relative protein content was determined using ImageJ software version 1.48v (National Institutes of Health, Bethesda, MD, USA).

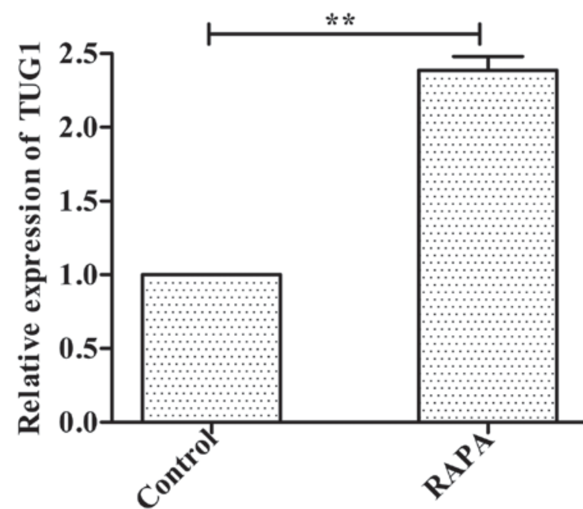

Figure 1. Expression of TUG1 is upregulated in human umbilical vein endothelial cells incubated with RAPA. **P<0.01. TUG1, taurine upregulated 1 (non-protein coding); RAPA, rapamycin.

Statistical analysis. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Data are presented as the mean ± standard deviation. Each experiment was repeated ≥3 times and differences between two groups were statistically analyzed using a Student's t-test. Differences among groups were statistically analyzed using one-way analysis of variance followed by a Tukey's post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of lncRNATUG1 is upregulated in HUVECs incubated with rapamycin. Following incubation of HUVECs with 100 ng/ml rapamycin for 24 h, the RT-qPCR results indicated that expression of lncRNATUG1 was significantly upregulated compared with the control group (P<0.01; Fig. 1).

Rapamycin induces cell apoptosis in HUVECs. In order to investigate the effect of rapamycin on cell apoptosis, HUVECs were treated with 100 ng/ml rapamycin for 24 h. The results indicated that rapamycin significantly increased HUVEC apoptosis (P<0.01; Fig. 2A). Additionally, Bcl-2 expression was decreased, whereas the expression of Caspase3 was increased, following rapamycin treatment (P<0.01; Fig. 2B and C).

Rapamycin inhibits cell proliferation and migration in HUVECs. The effects of rapamycin on the proliferation and

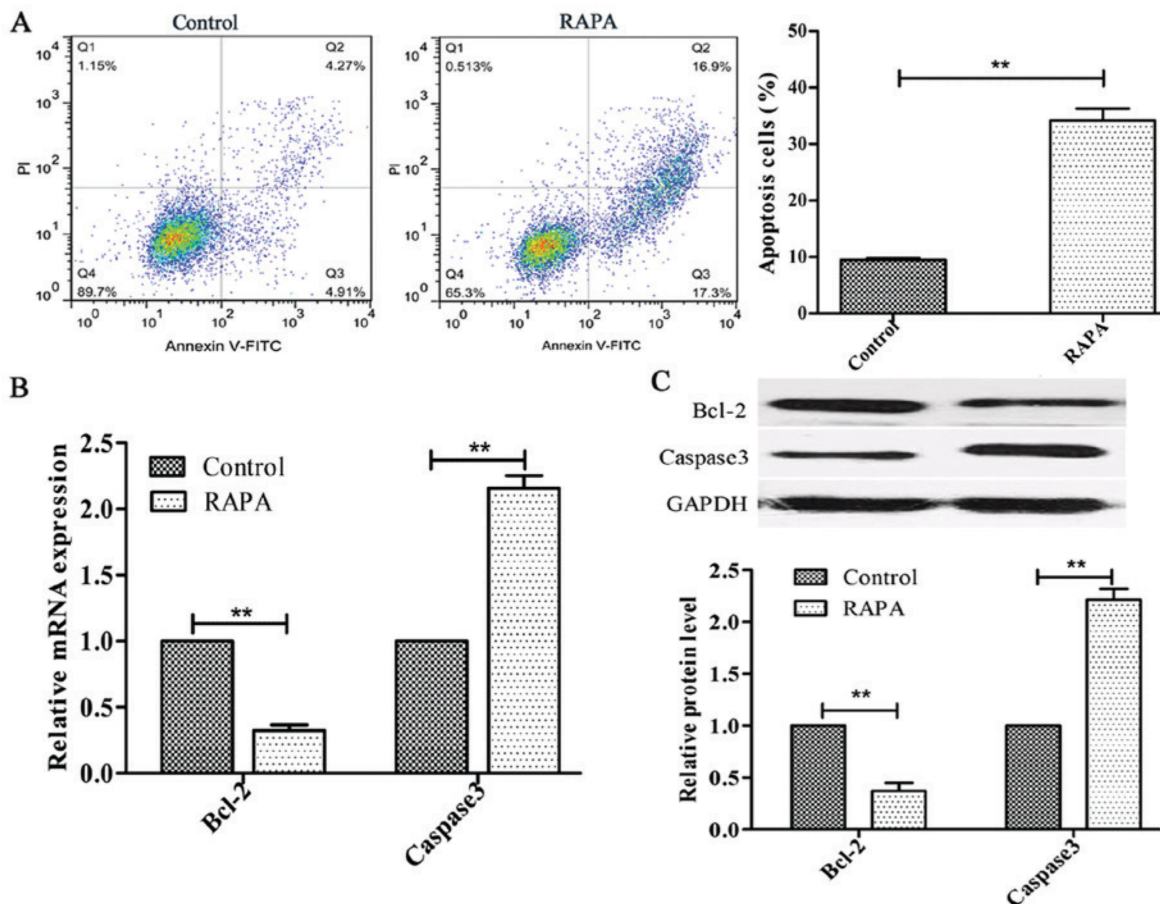

Figure 2. RAPA significantly increases the apoptosis rate of human umbilical vein endothelial cells. (A) Apoptosis rate of HUVECs. Expression of apoptosis-associated proteins was evaluated at the (B) mRNA and (C) protein level. **P<0.01. RAPA, rapamycin; Bcl-2, B-cell lymphoma 2.

migration of HUVECs were investigated using CCK-8 and cell scratch assays, respectively. The results indicated that rapamycin inhibited cell proliferation (P<0.01; Fig. 3A) and migration (Fig. 3B). In addition, the levels of VEGF, MMP-2 and MMP-9 were significantly decreased following treatment with rapamycin (P<0.01; Fig. 3C and D).

Expression levels of lncRNATUG1 in HUVECs are decreased following transfection. In order to downregulate the expression of lncRNATUG1, lncRNATUG1 siRNAs were transfected into HUVECs. The results of RT-qPCR analysis indicated that siTUG1-1 and siTUG1-2 significantly inhibited the expression of lncRNATUG1 in HUVECs (P<0.01; Fig. 4A); however, siTUG1-1 demonstrated a greater knockdown efficiency compared with siTUG1-2. Therefore, siTUG1-1 was selected for use in subsequent experiments.

Silencing of lncRNATUG1 attenuates rapamycin-induced apoptosis in HUVECs. To investigate whether silencing of lncRNATUG1 could affect rapamycin-induced apoptosis in HUVECs, transfected HUVECs were treated with 100 ng/ml rapamycin for 24 h. The results of flow cytometry analysis demonstrated that downregulation of lncRNATUG1 expression significantly suppressed rapamycin-induced apoptosis in HUVECs compared with the negative control (P<0.01; Fig. 4B). In addition, the expression of Bcl-2 was revealed to be significantly upregulated, whereas the expression of Caspase3 was revealed to be downregulated (P<0.01; Fig. 4C and D;) following downregulation of lncRNATUG1 compared with the negative control. These results suggested that downregulation of lncRNATUG1 inhibits rapamycin-induced cell apoptosis.

Silencing of lncRNATUG1 attenuates rapamycin-induced inhibition of HUVEC proliferation and migration. To investigate the effect of silencing lncRNATUG1 expression on the rapamycin-induced inhibition of HUVEC proliferative ability, a CCK-8 assay was performed. Following transfection, cells were treated with 100 ng/ml rapamycin for 24, 48 and 72 h. The results indicated that suppression of lncRNATUG1 expression promoted the proliferation of HUVECs compared with the negative control (P<0.01; Fig. 5A). Additionally, subsequent wound healing analysis indicated that downregulation of lncRNATUG1 expression increased the migration ability of HUVECs compared with the negative control (Fig. 5B). Furthermore, the results of RT-qPCR and western blot analyses indicated that silencing of lncRNATUG1 significantly upregulated the expression levels of MMP-2 (P<0.01), MMP-9 (P<0.05 for mRNA expression, P<0.01 for protein expression) and VEGF (P<0.01 for mRNA expression, P<0.05 for protein expression; Fig. 5C and D) compared with the negative control. These results suggested that lncRNATUG1 may regulate rapamycin-induced inhibition of HUVEC proliferation and migration.

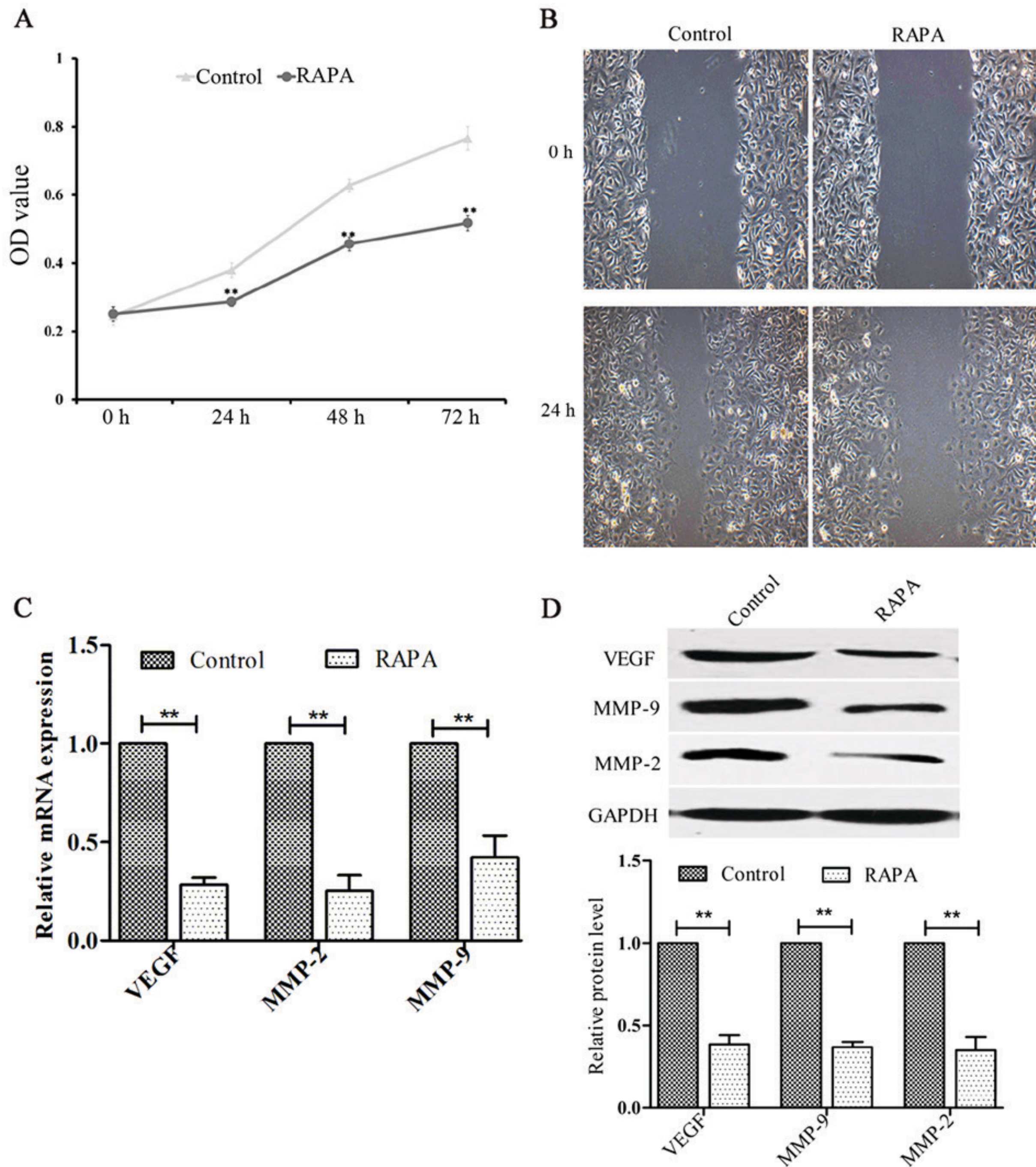

Figure 3. RAPA significantly decreases the (A) proliferation and (B) migration of human umbilical vein endothelial cells. Magnification, x100. VEGF, MMP-2 and MMP-9 expression levels were significantly decreased following treatment with RAPA at the (C) mRNA and (D) protein level. **P<0.01 vs. Control group. RAPA, rapamycin; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; OD, optical density.

Discussion

In the current study, it was demonstrated that rapamycin suppresses the proliferation and migration of HUVECs, and enhances the apoptosis of HUVECs, via the upregulation of lncRNATUG1. In addition, the expression levels of VEGF, MMP-2 and MMP-9 were indicated to be downregulated following treatment with rapamycin. The results of the present study identified that silencing lncRNATUG1 expression decreased the apoptosis of HUVECs and promoted the proliferation and migration of HUVECs treated with rapamycin; whereas the expression levels of VEGF, MMP-2 and MMP-9 were upregulated following the silencing of lncRNATUG1 expression. Furthermore, the results of the present study revealed that the effect of rapamycin on the proliferation, migration and apoptosis of HUVECs may be associated with the upregulation of lncRNATUG1 expression. Therefore, lncRNATUG1 may represent a novel target for the treatment of vascular disease.

It has been well established that the proliferation and migration of vascular endothelial cells serves a key function in angiogenesis (29,30). Previous studies have identified that

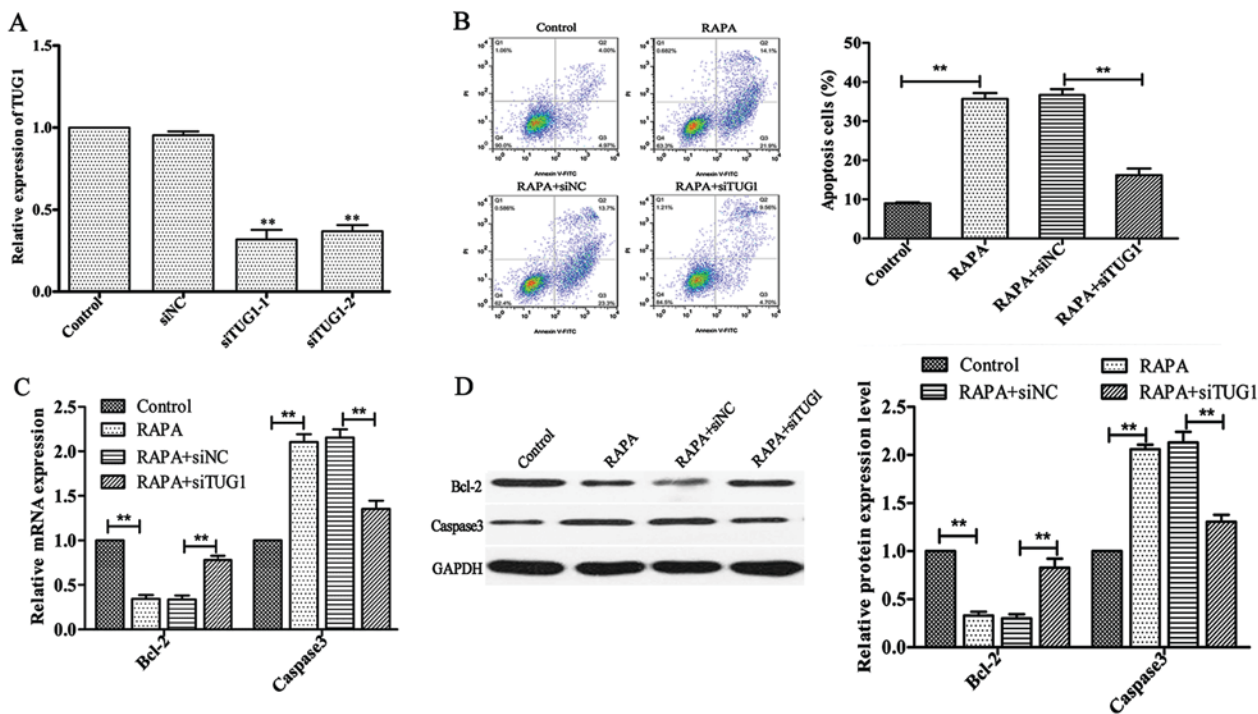

Figure 4. Silencing of TUG1 significantly attenuates RAPA-induced apoptosis of HUVECs. (A) TUG1 expression following HUVEC transfection with siTUG1 and siNC. (B) Apoptosis of HUVECs. Expression of apoptosis-associated proteins following TUG1 silencing was evaluated at the (C) mRNA and (D) protein level. **P<0.01 vs. Control group. RAPA, rapamycin; HUVEC, human umbilical vein endothelial cell; si, small interfering RNA; TUG1, taurine upregulated 1 (non-protein coding); NC, negative control; Bcl-2, B-cell lymphoma 2.

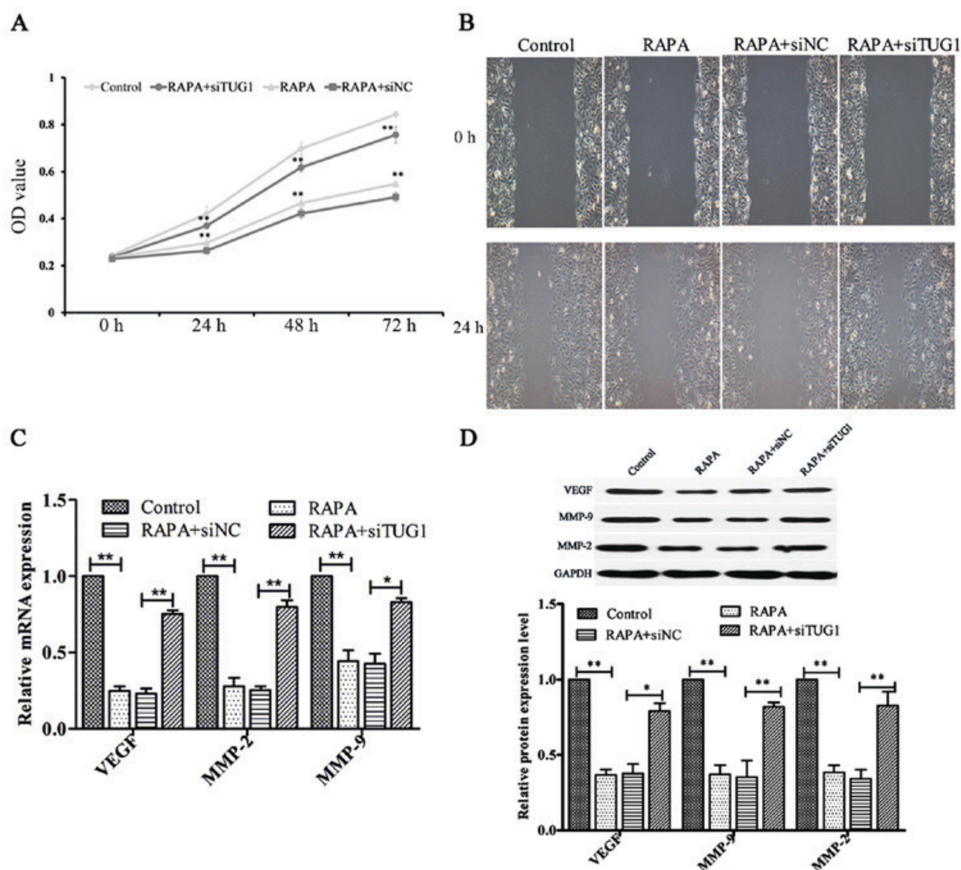

Figure 5. Silencing of TUG1 attenuates rapamycin-induced inhibition of (A) proliferation and (B) migration of human umbilical vein endothelial cells. Magnification, x100. VEGF, MMP-2 and MMP-9 expression levels were evaluated at the (C) mRNA and (D) protein level. *P<0.05 and **P<0.01 vs. the siNC group. RAPA, rapamycin; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; OD, optical density; si, small interfering RNA; TUG1, taurine upregulated 1 (non-protein coding); NC, negative control.

lncRNAs are highly expressed in endothelial cells, and are involved in the development of cardiovascular diseases via regulation of the proliferation and migration of endothelial cells. For example, lincRNAp21 regulates the formation of neo-intima, as well as the proliferation and apoptosis of VSMCs, by enhancing the transcriptional activity of P53 and inhibiting the formation of atherosclerosis. Therefore, lincRNA-21 may represent a therapeutic target for atherosclerosis and other cardiovascular diseases (31). Yan *et al* (32) identified that silencing lncRNA myocardial infarction-associated transcript significantly inhibited the proliferation and migration of vascular endothelial cells and suppressed angiogenesis. Zhang *et al* (33) reported that the proliferation of VMSCs was significantly inhibited following treatment with baicalein and that the expression of lncRNAAK021954 was also significantly increased. This suggests that baicalein may inhibit the proliferation of VSMCs via regulation of lncRNAAK021954 expression.

To date, few studies have investigated lncRNATUG1 with regards to vascular disease, having instead predominantly focused on the study of lncRNATUG1 in the context of tumorigenesis. Previous studies have indicated that silencing of lncRNATUG1 affects the proliferation and migration of tumor cells, which subsequently inhibits the formation of tumor blood vessels (34,35). Zhao *et al* (36) indicated that silencing TUG1 with siRNA inhibited proliferation and invasion, and promoted apoptosis, of glioma cells. Zhang *et al* (37) revealed that downregulation of TUG1 inhibited proliferation, migration and invasion, and promoted apoptosis, of renal cell carcinoma. Han *et al* (38) suggested that TUG1 was upregulated in bladder cancer, and silencing TUG1 via siRNA inhibited the proliferation and promoted apoptosis of bladder cancer cell lines. In addition, previous studies have identified that TUG1 is highly expressed in vascular endothelial cells (8,39,40). Therefore, TUG1 may be involved in the regulation of vascular endothelial cell function. Young *et al* (15) demonstrated that lncRNATUG1 serves a key function in the development of the retina, and that silencing of lncRNATUG1 expression promotes retinal cell apoptosis. These studies suggest that TUG1 may serve a function in endothelial cell proliferation and migration, as well as in apoptosis. In the current study, it was revealed that, following treatment with rapamycin, silencing of lncRNATUG1 significantly promoted the proliferation and migration of HUVECs, as well as suppressing the apoptosis of HUVECs. Furthermore, the expression levels of VEGF, MMP-2 and MMP-9 were significantly upregulated following the silencing of lncRNATUG1. The results demonstrated that lncRNATUG1 may serve a key function in endothelial cell function.

It has been widely established that rapamycin inhibits endothelial cell proliferation and migration, and induces apoptosis (41,42). In the current study, the results demonstrated that rapamycin inhibits vascular endothelial cell proliferation and migration, and reduces the expression of VEGF in vascular endothelial cells. Rapamycin has previously been revealed to inhibit neovascularization and the expression levels of VEGF induced by hypoxia (43). Moss *et al* (24) demonstrated that rapamycin regulates endothelial cell migration by regulating the cyclin-dependent kinase inhibitor p27Kip1. In addition, recent studies have identified that lncRNAs affect the proliferation and migration of tumor cells via regulation of the AKT/mTOR signaling pathway (44-46). The results of the present study demonstrated that rapamycin significantly inhibited the proliferation and migration of HUVECs, and enhanced the apoptosis and expression of lncRNATUG1 in HUVECs. However, silencing of TUG1 expression significantly inhibited rapamycin-induced apoptosis and promoted endothelial cell proliferation and migration. In addition, the expression levels of VEGF, MMP-2 and MMP-9 were upregulated in HUVECs treated with rapamycin following silencing of TUG1 expression. These results indicate that lncRNATUG1 affects endothelial cell function and may be involved in the suppression of the proliferation and migration of HUVECs, as well as the promotion of the apoptosis of HUVECs, following treatment with rapamycin.

In conclusion, the results of the present study revealed that rapamycin suppresses the proliferation and migration of HUVECs, and enhances the apoptosis of HUVECs, via the lncRNATUG1 pathway. This suggests that lncRNATUG1 serves a key function in rapamycin-induced inhibition of endothelial cell proliferation and migration, and may represent a novel therapeutic target for the treatment of vascular stenosis. However, the exact regulatory mechanism of lncRNATUG1 requires further study.

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Availability of data and materials

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

Authors' contributions

XG and YY conceived and designed the current study; XG, TZ, XZ and GL performed the experiments and analyzed the data; XG wrote the manuscript; and LD, ZM and JW assisted with performing the experiments and analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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