lncRNA TUG1 protects intestinal epithelial cells from damage induced by high glucose and high fat via AMPK/SIRT1

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Received March 24, 2021; Accepted August 12, 2021

DOI: 10.3892/mmr.2022.12655

Abstract. The incidence of obesity and type 2 diabetes mellitus (T2DM) is increasing year by year and shows a trend towards younger age groups worldwide. It has become a disease that endangers the health of individuals all over the world. Among numerous weight loss surgeries, sleeve gastrectomy (SG) has become one of the most common surgical strategies for the treatment of T2DM. However, SG-mediated alterations to the molecular mechanism of metabolism require further investigation. Thus, reverse transcription-quantitative PCR was used to detect the expression levels of long non-coding (lnc)RNA taurine-upregulated gene 1 (TUG1), Sirtuin 1 (SIRT1), AMP-activated protein kinase (AMPK) and uncoupling protein 2 (UCP2) in the serum of T2DM patients, as well as in HIEC-6 and SW480 cells following treatment with high glucose and high fat (HGHF). Protein expression was detected by western blotting. Cell Counting Kit-8 assays were performed to analyze cell viability, and flow cytometry and a TUNEL assay was performed to evaluate cell apoptosis. The secretion of ILs in the culture medium was detected by conducting ELISAs. The results showed that lncRNA TUG1 and UCP2 expression was upregulated, SIRT1 and AMPK expression levels were decreased by SG. Under HGHF conditions, HIEC-6 and SW480 cell viability was inhibited, apoptosis was promoted, TUG1 expression was downregulated, and SIRT1 and AMPK expression levels were upregulated. The secretory levels of IL-1β, IL-6 and IL-8 were increased, whereas the secretion of IL-10 was decreased under HGHF conditions. lncRNA TUG1 overexpression significantly reversed the effects of HGHF on cell viability, apoptosis and SIRT1, AMPK, UCP2 and Bcl-2 expression levels. Together, the findings of the present study demonstrated that lncRNA TUG1 alleviated the damage induced by HGHF in intestinal epithelial cells by downregulating SIRT1 and AMPK expression, and upregulating UCP2 expression. Thus, the lncRNA TUG1/AMPK/SIRT1/UCP2 axis may serve an important role in the treatment of T2DM.

Introduction

Obesity and type 2 diabetes mellitus (T2DM) have become diseases that endanger the health of individuals all over the world, especially in China. In 2018, 10% of the global population was obese and China ranks first in the world in terms of obesity (1). T2DM was declared a global public health emergency as early as 2015, and in 2017, the number of T2DM patients reached 425 million; China also has many patients with T2DM, and 90% of these patients have obesity combined with T2DM (2). Obesity is closely associated with T2DM. The incidence rate of obesity combined with T2DM increases year by year (3). Compared with traditional drug therapy and diet regulation, surgical weight loss surgery improves the remission rate of T2DM in obese patients, reduces the weight of obese patients and has fewer postoperative complications (4). Due to the rapid development of metabolic surgery, weight loss surgery has become the main treatment strategy for obesity and T2DM (5).

Patients with T2DM often suffer from a high glucose and high fat environment, leading to damage of myocardial cells, podocytes and other tissue cells, which affects cell proliferation, morphology and apoptosis (6-8). Long non-coding (lnc)RNAs have transcripts of >200 nt and are widely distributed in the nucleus and cytoplasm. They cannot encode proteins, but can regulate gene expression (9). It has been found that lncRNAs are widely involved in physiological and pathological processes, serving an important role in the occurrence and development of a variety of tumors (10). A study showed that lncRNA transcribed ultraconserved element 338 was overexpressed in lung cancer and its expression might be associated with the prognosis of lung cancer (11). Another study indicated that lncRNA nuclear enriched abundant transcript 1 activated Wnt signaling to promote colorectal cancer

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Key words: sleeve gastrectomy, obesity, type 2 diabetes, long non-coding RNA taurine-upregulated gene 1

progression and metastasis (12). In addition to tumor diseases, lncRNAs also serve a regulatory role in a number of other diseases, including metabolic diseases. It has been found that lncRNA glycolysis-associated lncRNA of colorectal cancer (GLCC1) affects carbohydrate metabolism by regulating c-Myc and further promoting cell proliferation (13). Another study found that lncRNA breast cancer anti-estrogen resistance 4 (BCAR4) coordinates Hippo and Hedgehog signaling to enhance the transcription of glycolysis activators HK2 and PFKFB3, then affects glycolysis via the YAP-breast cancer anti-estrogen resistance 4-glycolysis axis (14). Therefore, it was hypothesized that an lncRNA may also be involved in the regulation of T2DM, serving a role in mediating high glucose and high fat-induced alterations in cells.

lncRNA taurine upregulated gene 1 (TUG1) was initially identified and confirmed in the related research of retinal development (15). TUG1 displays abnormal expression and function in bladder cancer, glioma and other tumors (16,17). A previous study showed that lncRNA TUG1 expression was obviously upregulated in patients with CHD, and metformin activated the AMPK/mTOR pathway by regulating lncRNA TUG1 to promote autophagy and inhibit atherosclerosis (18). Based on the aforementioned studies, the present study detected alterations in lncRNA TUG1 expression levels in blood samples from patients with T2DM before and after sleeve gastrectomy (SG), and detected the effect of lncRNA TUG1 on the viability of intestinal epithelial cells under high glucose and high fat conditions, as well as the possible signaling pathway involved in these regulatory roles.

Materials and methods

Cell culture and treatment. The human normal intestinal epithelial cell line (HIEC-6) and human colorectal adenocarcinoma epithelial cell line (SW480) were obtained from American Type Culture Collection. Cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C with 5% CO_2. The cell injury model was induced using DMEM supplemented with 50 mmol/l glucose and 500 mmol/l saturated free fatty acid (palmitate; Sigma-Aldrich; Merck KGaA) for 48 h at 37˚C. The control group was treated with 30 mmol/l mannitol for 48 h at 37˚C. lncRNA TUG1 was overexpressed using a TUG1 lentiviral vector (LV; LV-TUG1) and a lentiviral negative control (NC; LV-NC) obtained from Shanghai GeneChem Co., Ltd. After cell counting, cells ($1.5x10^5$ cells/well) were inoculated into a 6-well plate and divided into the LV-NC and LV-TUG1 groups, followed by culture in an incubator at 37˚C. After 12 h of cell inoculation and the number of cells reached $2x10^5$ in each well, the empty vector (LV-NC) and the lentiviral vector (Shanghai GeneChem Co., Ltd., China) overexpressing lncRNA TUG1 (LV-TUG1) were transfected into the LV-NC and LV-TUG1 groups, respectively, followed by culture in an incubator at 37˚C. After 48 h post-transfection, cells were collected by trypsin digestion and TUG1 expression was detected by reverse transcription-quantitative PCR (RT-qPCR) to verify the transfection efficiency of the lentiviral vector.

T2DM patients. A total of 50 T2DM patients (26 female patients and 24 male patients; age range, 32-71 years; average age, 45.7±4.6 years) were selected in the present study. Patients in the study were diagnosed with T2DM and treated at the First Affiliated Hospital of Jiamusi University (Jiamusi, China) between March 2017 and March 2019. The following inclusion criteria were used: i) Presented with T2DM for the first time with no complications; ii) follow-up for >1 year; and iii) underwent SG after the diagnosis of T2DM. The basis for selecting patients was polyuria, fatigue, weight loss or polydipsia, 2 h post-load glucose ≥11.1 mmol/l following 75 g, oral glucose uptake and random plasma glucose ≥11.1 mmol/l. All patients has 10 ml peripheral blood sample from venous blood at the elbow and the 1 week prior to SG, 6 months after surgery and 12 months after surgery. All blood samples were stored at -80˚C after adding anticoagulants. The present study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Jiamusi University (approval no. 2019018) and all the patients signed informed consent.

RT-qPCR. Total RNA was extracted from HIEC-6 cells, SW480 cells and blood samples using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.), then reverse transcribed into cDNA using the PrimeScript RT reagent kit with gDNA Eraser (Takara Bio, Inc.) according to the manufacturer's protocol. qPCR was performed using SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Inc.). The thermocycling conditions were: 1 min 10 sec at 95˚C and then 39 cycles of 12 sec at 95˚C and 30 sec at 59.5˚C. The relative expression of mRNA was normalized to the internal reference gene β-actin and calculated using $2^{-\Delta\Delta Ct}$ method (19). The primers used for qPCR were synthesized by Guangzhou RiboBio Co., Ltd. The primer sequences were as follows: TUG1 forward, 5'-CTGGACCTGGAACCCCAAAG-3' and reverse, 5'-GGTAGTGCTTGCTCAGTCGT-3'; AMPK forward, 5'-GGTAGTGCTTGCTCAGTCGT-3' and reverse, 5'-GGTAGTGCTTGCTCAGTCGT-3'; SIRT1 forward, 5'-AAGATGACGTCTTATCCTCT-3' and reverse, 5'-GCTTCATTAATTGCCTCTTG-3'; UCP2 forward, 5'-GCTGGTGGTGGTCGGAGAT-3' and reverse, 5'-TGAAGTGGCAAGGGAGGT-3'; β-actin forward, 5'-AAGATGACGTCTTATCCTCT-3' and reverse, 5'-GCTTCATTAATTGCCTCTTG-3'.

Cell counting kit-8 (CCK-8) assay. HIEC-6 and SW480 cells challenged with high glucose and high fat and/or transfected with LV-TUG1 were seeded into a 96-well plate at $1x10^4$ in each well and incubated for 12 h. Cell viability was analyzed using the CCK-8 kit (Dojindo Molecular Technologies, Inc.) according to the manufacturer's protocol. CCK-8 (10 μl) were added to each well for incubation at 37˚C with 5% CO_2 for 1 h. A microplate reader at 450 nm wavelength was used for detecting optical density values.

TUNEL assay. To detect cell apoptosis, a TUNEL detection kit (Roche Diagnostics) was used according to the manufacturer's protocol. Treated cells were cultured overnight and then washing twice with PBS and collected on the slide, fixed with 4% paraformaldehyde for 15 min at 25˚C and permeabilized in 0.25% Triton X-100 for 20 min. The cells were incubated in terminal deoxynucleotidyl transferase (TdT) reaction cocktail for 45 min at 37˚C, followed by treatment with Click-iT reaction cocktail. The nucleus of HIEC-6 and SW480 were stained

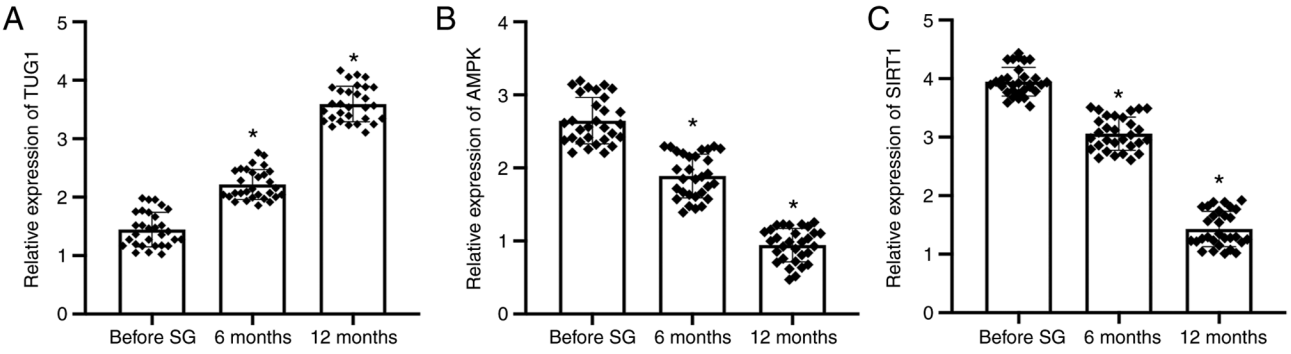

Figure 1. Expression of lncRNA TUG1, AMPK and SIRT1 in blood. Expression of (A) lncRNA TUG1, (B) AMPK and (C) SIRT1 in the blood of patients with type 2 diabetes mellitus at different times as measured by reverse transcription-quantitative PCR. Data are presented as the mean ± standard deviation. *P<0.05 vs. before SG. lncRNA, long non-coding RNA; TUG1, taurine-upregulated gene 1; AMPK, AMP-activated protein kinase; SIRT1, Sirtuin 1; SG, sleeve gastrectomy; 6 month, 6 months following SG; 12 month, 12 months following SG.

with DAPI (0.5 μg/ml) at room temperature for 15 min and then observed under a fluorescence microscope (five fields were selected at a magnification of x40).

LDH assay. Treated cells were seeded in 96-well plates ($4x10^3$ cells/well) and incubated for 48 h at 37˚C. Cell damage was detected by the LDH Cytotoxicity Assay kit (Beyotime Institute of Biotechnology). Briefly, LDH release regent (150 μl) was added to the 96-well plate following removal of the supernatant. The cells were incubated at 37˚C for 1 h with 5% CO_2. The absorbance at 490 nm was detected with Microplate Reader (Bio-Rad Laboratories, Inc.).

Flow cytometry assay. Flow cytometry was performed to further assess the apoptotic rate. HIEC-6 or SW480 cells were plated into 6-well plates ($6x10^5$ cells/well). At 48 h treatment with high glucose and high fat-containing medium, pre-cooled PBS was used to wash cells three times. Subsequently, the cells were analyzed using the Annexin V-APC Apoptosis Detection kit (Beyotime Institute of Biotechnology) following the manufacturer's protocol. The apoptotic rate including early apoptosis and late apoptosis was assessed using a flow cytometer (FACSCanto II; BD Biosciences) by measuring the percentage of Annexin V^+ and PI^- cells. The data was analyzed using FlowJo software (version 7.2.4; FlowJo LLC).

Western blot analysis. Cells on the walls of the culture dish were washed three times with PBS. Subsequently, total proteins were harvested with RIPA buffer (Beyotime Institute of Biotechnology) and quantified using a bicinchoninic acid assay (Beyotime Institute of Biotechnology). Proteins (30 μg/lane) were separated via 10% SDS-PAGE and transferred onto PVDF membranes. Following blocking with 5% skimmed milk for 1 h at room temperature, the membranes were incubated overnight at 4˚C with the following primary antibodies: Anti-SIRT1 (cat. no. ab189494; Abcam; 1:1,000), anti-Bcl-2 (cat. no. ab32124; Abcam; 1:1,000), anti-AMPK (cat. no. ab32047; Abcam; 1:1,000), anti-UCP2 (cat. no. ab97931; Abcam; 1:1,000) and anti-β-actin (cat. no. ab8226; Abcam; 1:8,000). The membranes were incubated with horseradish peroxidase-conjugated second antibody (goat anti-rabbit IgG, 1:2,000, goat anti-mouse IgG, 1:2,000, TransGen Biotech Co., Ltd.) for 1 h at room temperature. The proteins were determined by immunoblotting analysis using an ECL immunoblotting kit (Millipore, Sigma). Each protein expression was normalized to β-actin. Densitometry was performed using ImageJ software (version 1.38X; National Institutes of Health).

ELISA. Briefly, cells were inoculated ($2x10^5$ cells/well) into a 6-well plate and cultured overnight with 1 ml/well DMEM supplemented with 10% FBS at 37˚C. After 24 h of culture, cells were divided into several groups and treated with DMEM containing concentrations of high glucose and high fat without FBS. The IL-1β (cat. no. P1305), IL-6 (cat. no. P1330), IL-8 (cat. no. P1640) and IL-10 (cat. no. P1528) levels in culture medium were determined using ELISA kits (Beyotime Institute of Biotechnology) according to the manufacturer's protocols.

Statistical analysis. Statistical analysis was performed using a paired t-test or one-way ANOVA followed by Tukey's post hoc test with SPSS 17.0 statistical software (SPSS, Inc.). Each experiment was performed in triplicate. Data are presented as the mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Results

The expression of lncRNA TUG1, AMPK and SIRT1 is affected by SG. Peripheral blood samples were obtained from T2DM patients at 1 week before surgery, 6 months after surgery and 12 months after surgery. RT-qPCR was used to measure the mRNA expression levels of TUG1, SIRT1 and AMPK. The results confirmed that following SG, the expression of lncRNA TUG1 was significantly increased, reaching a maximum at 12 months post-SG surgery, compared with that of the before SG samples (Fig. 1A). The expression levels of AMPK and SIRT1 were significantly downregulated after SG compared with those before SG, reaching the lowest levels at 12 months among the three group (Fig. 1B and C).

High glucose and high fat induce a high apoptotic rate and low viability. The effects of high glucose and high fat on the apoptosis and viability of HIEC-6 and SW480 cells were explored. The CCK-8 assay was performed to measure cell viability, and the apoptotic rate was detected by performing lactate

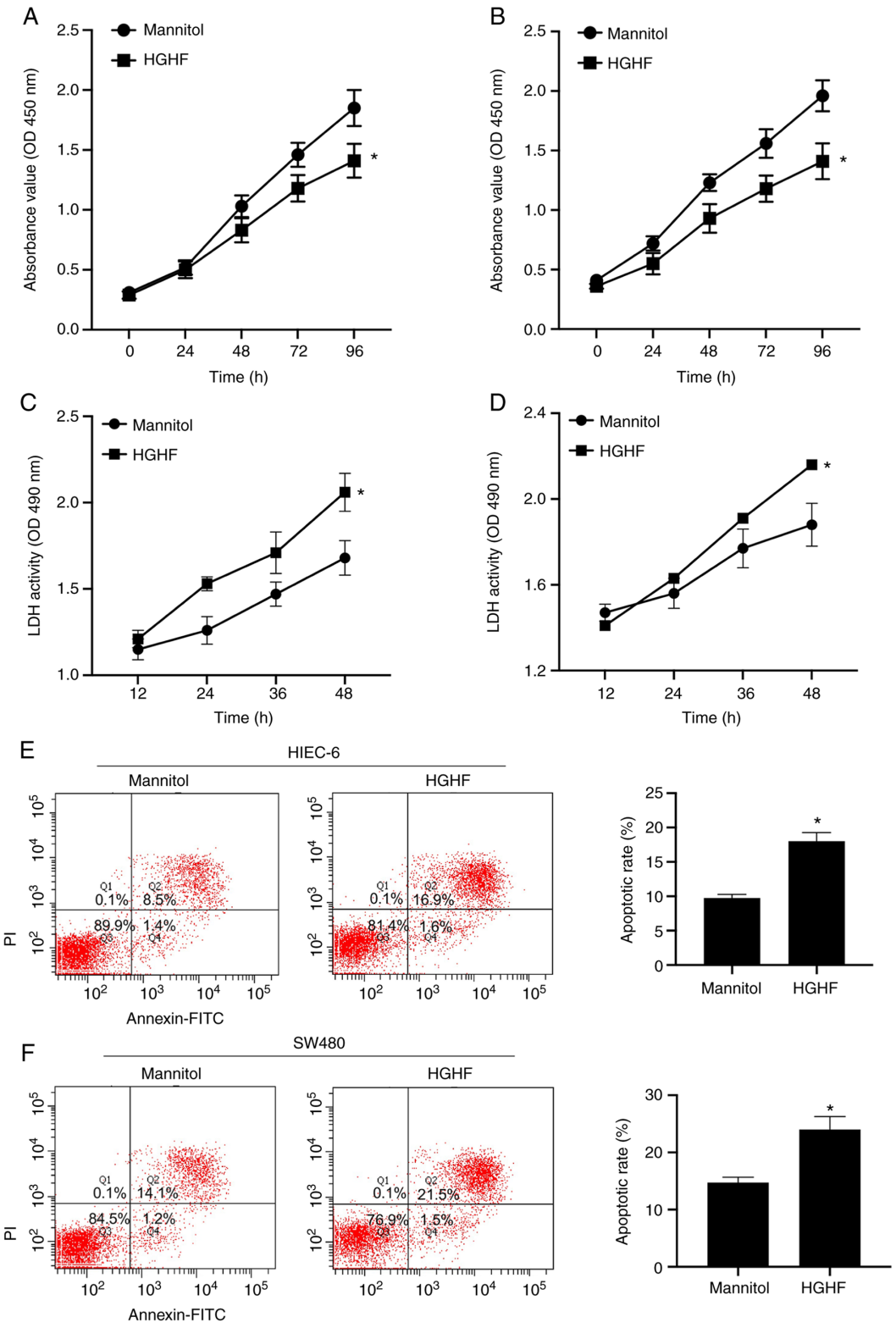

Figure 2. HGHF culture promotes cell apoptosis and inhibits cell viability. HGHF inhibited the viability of (A) HIEC-6 and (B) SW480 cells. LDH secretion by (C) HIEC-6 and (D) SW480 cells was increased by HGHF. HGHF promoted the apoptotic rate of (E) HIEC-6 and (F) SW480 cells. Data are presented as the mean ± standard deviation. *P<0.05 vs. mannitol. HGHF, high glucose and high fat; LDH, lactate dehydrogenase; OD, optical density.

dehydrogenase (LDH) cytotoxicity and flow cytometry assays. As expected, compared with the mannitol group, under high glucose and high fat conditions, the viability of HIEC-6 and SW480 cells was significantly inhibited (Fig. 2A and B), the release of LDH was significantly increased (Fig. 2C and D) and the apoptotic rate was significantly increased (Fig. 2E and F).

High glucose and high fat induce downregulation of lncRNA TUG1 and UCP2, and upregulation of AMPK and SIRT1. Following the detection of alterations in the apoptosis and viability of HIEC-6 and SW480 cells, it was hypothesized that the expression of lncRNA TUG1 and glucose metabolism-associated proteins was also affected. The RT-qPCR

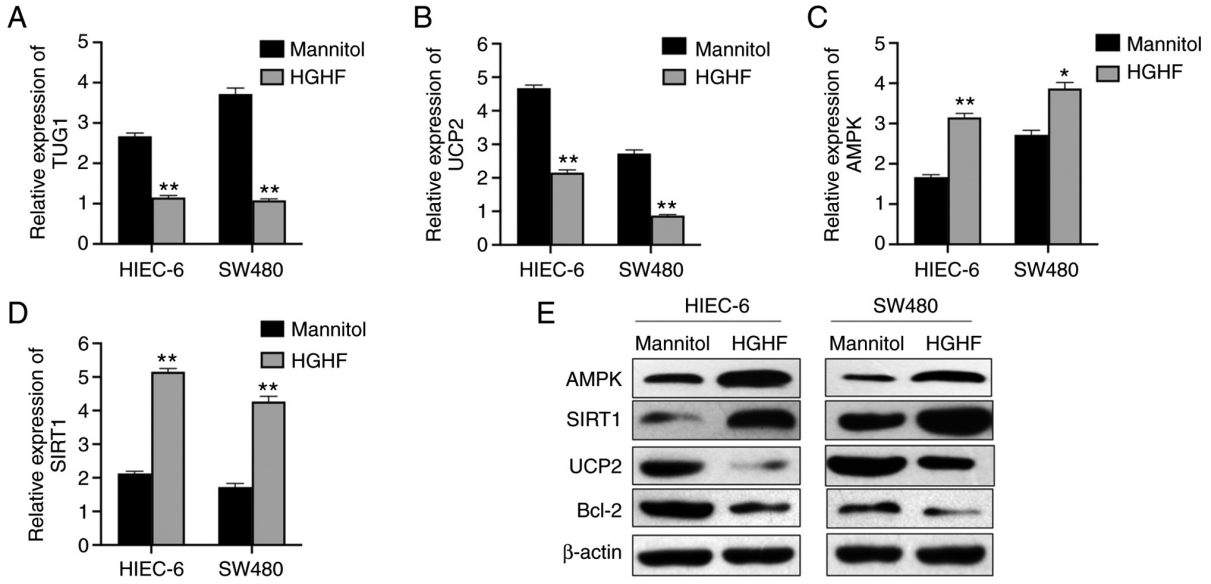

Figure 3. HGHF inhibits the expression of lncRNA TUG1 and UCP2, and promotes the expression of AMPK and SIRT1. (A) Expression of lncRNA TUG1 in HIEC-6 and SW480 cells as measured by reverse transcription-quantitative PCR. HGHF decreased the expression of (B) UCP2, and increased the expression of (C) AMPK and (D) SIRT1. (E) HGHF-mediated alterations to the protein expression levels of AMPK, SIRT1, UCP2 and Bcl-2 were determined by western blotting. Data are presented as the mean ± standard deviation. *P<0.05 and **P<0.01 vs. mannitol. HGHF, high glucose and high fat; lncRNA, long non-coding RNA; TUG1, taurine-upregulated gene 1; UCP2, uncoupling protein 2; AMPK, AMP-activated protein kinase; SIRT1, Sirtuin 1.

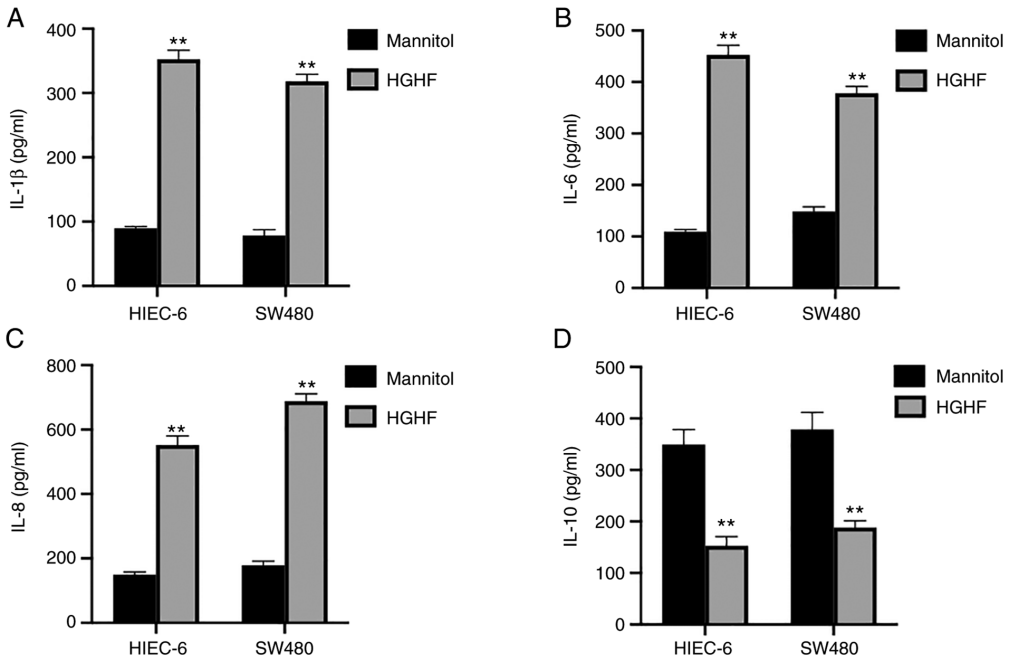

Figure 4. Effects of HGHF on the levels of IL-1β, IL-6, IL-8 and IL-10 in HIEC-6 and SW480 cells. Levels of (A) IL-1β, (B) IL-6, (C) IL-8 and (D) IL-10 in HIEC-6 and SW480 cells as determined by performing ELISAs. Data are presented as the mean ± standard deviation. **P<0.01 vs. mannitol. HGHF, high glucose and high fat.

results confirmed that lncRNA TUG1 and UCP2 were significantly downregulated (Fig. 3A and B), whereas AMPK and SIRT1 were significantly upregulated under high glucose and high fat conditions compared with those in the mannitol group (Fig. 3C and D). The western blotting results showed markedly lower protein expression levels of UCP2 and Bcl-2, and higher protein expression levels of SIRT1 and AMPK following high glucose and high fat treatment compared with those following mannitol treatment (Fig. 3E).

High glucose and high fat alter the cytokine release from HIEC-6 and SW480. To study the release of cytokines associated with inflammation by HIEC-6 and SW480 cells under high glucose and high fat conditions, ELISAs were performed. The results showed a significant increase in the secretion of IL-1β (Fig. 4A), IL-6 (Fig. 4B) and IL-8 (Fig. 4C), which could promote the inflammatory reaction, but a significant decrease in the secretion of IL-10 (Fig. 4D) under high glucose and high fat conditions compared with those in the mannitol group.

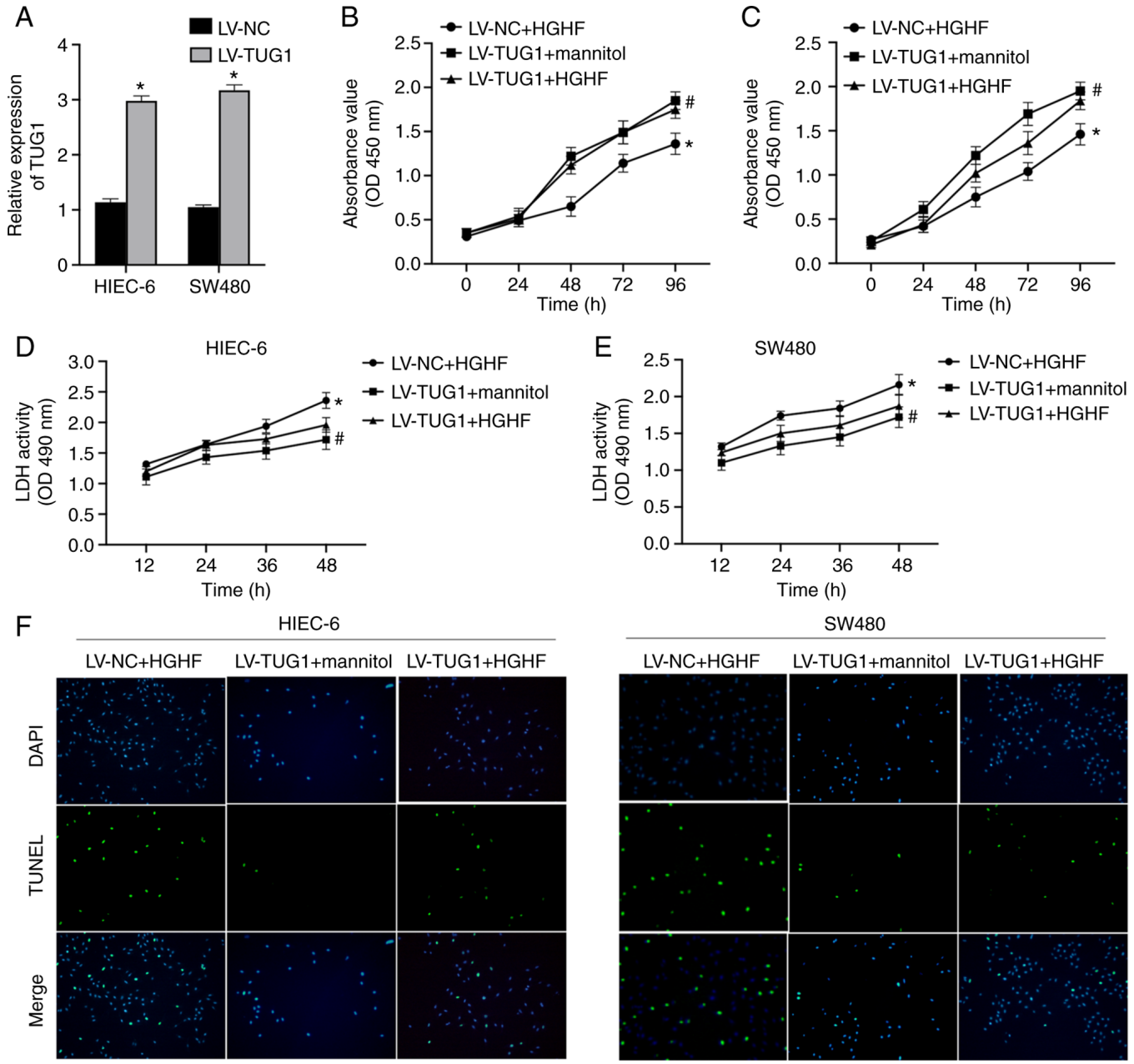

Figure 5. lncRNA TUG1 partly reverses HGHF-induced alterations in cell viability and apoptosis. (A) lncRNA TUG1 was overexpressed by LV-TUG1. Cell Counting Kit-8 assay results showed that LV-TUG1 alleviated HGHF-induced viability inhibition in (B) HIEC-6 and (C) SW480 cells. HGHF-mediated changes in LDH secretion were affected by LV-TUG1 in (D) HIEC-6 and (E) SW480 cells. (F) TUNEL assay results revealed that LV-TUG1 partially reversed HGHF-induced apoptosis promotion in HIEC-6 and SW480 cells (magnification, x40). Data are presented as the mean ± standard deviation. *P<0.05 vs. LV-NC + HGHF, #P<0.05 vs. LV-TUG1 + HGHF. lncRNA, long non-coding RNA; TUG1, taurine-upregulated gene 1; HGHF, high glucose and high fat; LV, lentivirus; NC, negative control; LDH, lactate dehydrogenase; OD, optical density.

lncRNA TUG1 overexpression alleviates the effects of high glucose and high fat on cell viability. To study the effect of lncRNA TUG1 and high glucose and high fat on intestinal epithelial cells, the expression of TUG1 in HIEC-6 and SW480 cells was overexpressed using LV-TUG1. TUG1 overexpression was confirmed by RT-qPCR (Fig. 5A). The treatment groups were as follows: i) LV-NC + high glucose and high fat; ii) LV-TUG1 + high glucose and high fat; and iii) LV-TUG1 + mannitol. TUG1 overexpression significantly enhanced the viability of HIEC-6 and SW480 cells to alleviate high glucose and high fat-induced inhibition of cell viability (Fig. 5B and C). In addition, high glucose and high fat-mediated effects on the secretion of LDH were also ameliorated by TUG1 overexpression (Fig. 5D and E). It was also demonstrated that TUG1 markedly inhibited high glucose and high fat-induced increases in the apoptotic rate of HIEC-6 and SW480 cells (Fig. 5F).

TUG1 reverses high glucose and high fat-mediated alterations to the expression levels of AMPK, SIRT1 and UCP2. The gene and protein expression levels in HIEC-6 and SW480 cells were evaluated by RT-qPCR and western blotting, respectively, following treatment with LV-TUG1 and/or high glucose and high fat. At the mRNA level, the high expression

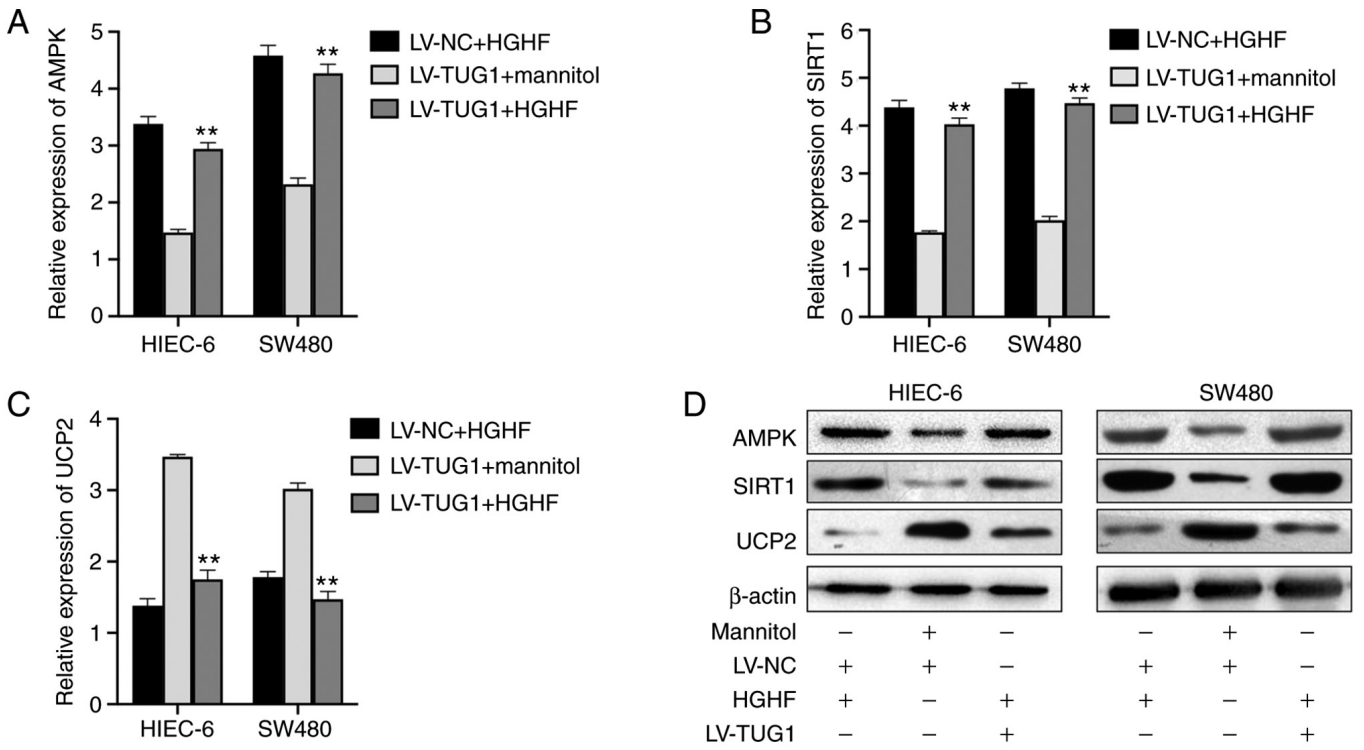

Figure 6. Long non-coding RNA TUG1 partly reverses HGHF-induced alterations to AMPK, SIRT1 and UCP2 expression. Reverse transcription-quantitative PCR showed that HGHF-induced changes in (A) AMPK, (B) SIRT1 and (C) UCP2 expression were reversed to varying degrees by TUG1. (D) Western blotting showed that similar trends were observed for protein expression. Data are presented as the mean ± standard deviation. **P<0.01 vs. LV-NC + HGHF. TUG1, taurine-upregulated gene 1; AMPK, AMP-activated protein kinase; SIRT1, Sirtuin 1; UCP2, uncoupling protein 2; HGHF, high glucose and high fat; LV, lentivirus; NC, negative control.

of AMPK (Fig. 6A) and SIRT1 (Fig. 6B), and the low expression of UCP2 (Fig. 6C) induced by HGHF was partially reversed by LV-TUG1 in HIEC-6 and SW480 cells, the results of LV-TUG1+ Mannitol also confirmed that high glucose and high fat inhibited the changes of expression caused by TUG1. The western blotting results revealed that the increase in UCP2 expression, and decrease in AMPK and SIRT1 expression caused by high glucose and high fat was partly reversed by TUG1 overexpression in HIEC-6 and SW480 cells (Fig. 6D).

Discussion

Obesity is an important problem threatening human health. Excessive obesity causes great stress on human bones, organs and systems, and leads to a variety of chronic diseases, including hypertension and diabetes (20). A previous study (21) found that diabetic patients are also more likely to be obese, which further aggravates the disease. In addition, under the effect of obesity and hyperglycemia, patients are more likely to present with coronary heart disease, stroke, hyperlipidemia and other complications, increasing the risk of disease and mortality (21). Therefore, for T2DM patients with obesity, effective treatment strategies should be employed as soon as possible to intervene and control the weight and blood glucose of patients. SG has a significant effect on the treatment of metabolic syndrome associated with obesity, as well as a positive effect on the complications of T2DM, hypertension and dyslipidemia, and the remission rate of diabetes following SG is as ≤62% (22). However, the underlying molecular mechanisms are not completely understood.

lncRNAs are a type of non-coding RNA that widely exist in plasma, serum and organ tissues. lncRNAs serve an important role in the process of body proliferation and development, immune regulation, cell proliferation, migration, signal transduction, autophagy and inflammation (23-26). A total of 55 differentially expressed lncRNAs were screened from the peripheral blood of six patients with T2DM and 60 healthy subjects by microarray analysis. The top three most differentially expressed lncRNAs were verified again in 60 patients with T2DM and 60 healthy individuals. It was found that these three lncRNAs are associated with fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) (27). In addition, a variety of lncRNAs, including metastasis associated in lung denocarcinoma transcript 1 (MALAT1), maternally expressed gene 3 (MEG3), growth arrest-specific transcript 5 (GAS5), neighbor of BRCA1 gene 2 (NBR2), cyclin-dependent kinase inhibitor 2B antisense RNA 1/antisense non-coding RNA in the INK4 locus (CDKN2BAS1/ANRIL) were identified in the peripheral blood mononuclear cells of patients with T2DM and were positively associated with blood glucose control (28). A previous study found that lncRNAs are involved in the regulation of insulin synthesis and secretion, liver gluconeogenesis and lipid metabolism (29), and adipose tissue glucose uptake through multi-level gene regulation (30), ultimately affecting blood glucose in the human body (31). A previous study found that carbohydrate responsive element binding protein (ChREBP) can coordinate glucose homeostasis by

regulating lncRNA TUG1 transcription in the podocytes in response to increased glucose levels, which also indicates that TUG1 is closely associated with glucose metabolism (32). A study suggests that TUG1 restores high glucose and high fat-treated endothelial progenitor cells function by regulating microRNA (miR)-29c-3p/platelet-derived growth factor-BB (PDGF-BB)/Wnt signaling (33). In addition, the role of lncRNA TUG1 in diabetes also includes inhibiting diastolic dysfunction of diabetic cardiomyopathy by regulating miR-499-5p (34). The aforementioned results indicated that lncRNA TUG1 is closely associated with T2DM, which is of great significance in regulating the dynamic balance of blood glucose in the body. Therefore, the present study hypothesized that SG may affect the expression level of lncRNA TUG1 in the body, which may in turn affect glucose uptake and metabolism, and ultimately alleviate the injury of intestinal epithelial cells induced by high glucose and high fat.

SIRT1 interacts with a number of target proteins involved in metabolism, inflammation, genomic stability and apoptosis. SIRT1 changes the catalytic activity of proteins or serves as an epigenetic signal to change the stability of proteins by removing the acetyl groups of these target proteins (35). The metabolic regulation of SIRT1 includes regulating gluconeogenesis, increasing fatty acid oxidation, decreasing fat production, increasing insulin secretion and regulating autophagy to prolong life (36). SIRT1 activators have been proposed to prevent and counteract metabolic age-related diseases, such as T2DM (37). AMPK is a key regulator of cell energy homeostasis, which regulates cell metabolism through the ratio of AMP/ATP; when the ratio of AMP/ATP decreases, the expression of AMPK increases (38). SIRT1 can deacetylate and activate liver kinase B1 (LKB1) and the activated LKB1 can phosphorylate and activate AMPK (39). A study found that AMPK/SIRT1 can participate in the regulation of glucose metabolism pathway by lncRNA CDKN2B antisense RNA 1, thus affecting cell viability (40). SIRT1 is able to directly bind the UCP2 promoter, repressing its transcription and affecting blood glucose by regulating β-cells (41). The present study hypothesized that lncRNA TUG1 promoted glucose metabolism through the AMPK/SIRT1 pathway following SG, thus affecting the blood glucose level of T2DM patients.

The present study found that high levels of TUG1 were associated with SG in T2DM patients. However, in a high glucose and high fat environment, the expression of TUG1 and the viability of HIEC-6 and SW480 cells was inhibited, whereas apoptosis was promoted. These results suggested that SG surgery may affect blood glucose by altering the expression of lncRNAs in cells and further regulating the downstream genes. In addition, the results of RT-qPCR of blood samples obtained from T2DM patients confirmed that SIRT1 and AMPK expression decreased following SG surgery. Under high glucose and high fat conditions, the trends in expression, cell viability and apoptosis displayed an opposite tendency. Following high glucose and high fat treatment combined with TUG1 overexpression, TUG1 alleviated high glucose and high fat-induced alterations in cell viability and the expression levels of AMPK, SIRT1 and UCP2. In the present study, the research on TUG1-related pathways was not detailed enough and more possible pathways, including glucose metabolism and fat metabolism, need to be explored further. The clinical implications of the present study needs further investigation. In conclusion, these results suggested that AMPK/SIRT1/UCP2 may be one of the pathways altered by SG through modulation of the expression of lncRNA TUG1, which may result in the control of blood glucose in T2DM patients.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Scientific Research of Heilongjiang Health and Family Planning Commission (grant no. 2019-294).

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

WW, XW, YW, SL and DS primarily designed and performed the study. DS, SG, HT and WW analyzed the data. WW and XW confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Jiamusi University (approval no. 2019018). All patients signed informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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