

lncRNA TUG1 Promotes Cell Proliferation, Migration, and Invasion in Hepatocellular Carcinoma via Regulating miR-29c-3p/*COL1A1* Axis

This article was published in the following Dove Press journal:
Cancer Management and Research

Wei Zhao^{1,*}
Xue Jiang^{2,*}
Shuxia Yang²

¹Department of Functional Examination, The Sixth People's Hospital of Qingdao, Qingdao City, Shandong Province 266033, People's Republic of China;

²Health Examination Center, The Sixth People's Hospital of Qingdao, Qingdao City, Shandong Province 266033, People's Republic of China

*These authors contributed equally to this work

Background: Taurine upregulated gene 1 (TUG1) has been recognized as a novel oncogenic gene. The current study was established to explore the function and regulatory mechanism of TUG1 in hepatocellular carcinoma (HCC).

Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression of TUG1, miR-29c-3p, and *COL1A1* in tissues and cell lines. MTT assay, wound-healing and transwell assay were utilized for the detection of cell viability, migration and invasion, respectively. The interactions between miR-29c-3p and TUG1/*COL1A1* were predicted by starBase v2.0 (<http://starbase.sysu.edu.cn/>) and verified by the dual-luciferase reporter or RNA immunoprecipitation assay. Western blot assay was performed to determine the protein levels of *COL1A1*, cyclin D1, E-cadherin, N-cadherin, Bcl-2, and Bax.

Results: Dramatically increased expression of TUG1 was noticed in HCC tissues and cell lines. TUG1 knockdown restrained the proliferation, migration, and invasion, and promoted the apoptosis of HCC cells. TUG1 targeted miR-29c-3p and inhibited miR-29c-3p expression. Overexpression of miR-29c-3p inhibited the proliferation, migration and invasion of HCC cells. MiR-29c-3p directly targeted *COL1A1* and down-regulated *COL1A1* expression. In addition, downregulation of miR-29c-3p and upregulation of *COL1A1* both reversed the effects of TUG1 knockdown on the proliferation, apoptosis, migration, and invasion of HCC cells.

Conclusion: TUG1 could promote the proliferation, migration and invasion of HCC cells through regulating miR-29c-3p/*COL1A1* axis. This novel finding might provide a latent target for the treatment of HCC.

Keywords: TUG1, miR-29c-3p, miR-29c-3p/*COL1A1*, hepatocellular carcinoma

Introduction

As a common lethal malignant neoplasm, hepatocellular carcinoma (HCC) ranks the third cancer-related mortality all over the world.¹ Although great progress has been made in the treatment of HCC using hepatic resection, liver transplantation, and radiofrequency ablation in recent years, the long-term prognosis remains disappointing and the 5-year overall survival is only about 30%.² Therefore, it is urgent to have a deep understanding of the potential mechanisms involving the occurrence and development of HCC, and develop novel therapeutic targets for HCC.³

Long non-coding RNAs (lncRNAs) are a kind of RNAs with no protein-coding ability at more than 200 nucleotides in length.⁴ Some lncRNAs have been proved by previous studies to play a critical role in tumorigenesis and metastasis of HCC,

Correspondence: Shuxia Yang
Tel +86-532-81636889
Email yangshuxia268@163.com

such as DBH-AS1,⁵ MCM3AP-AS1⁶ and CARL.⁷ lncRNAs may act as competitive endogenous RNA (CeRNA) through sponging miRNAs, thus regulating miRNA expression in cells.⁸ MiRNAs are an abundant class of highly conserved small non-coding RNAs (ncRNAs) at approximately 20–25 nucleotides in length. MiRNAs can regulate gene expression post-transcriptionally via precisely interacting with the 3'-untranslated regions of mRNAs.⁹ Intensive researches have proved that miRNAs have inhibitory effects in a variety of malignancies.^{10–12} Some miRNAs are implicated in the occurrence, growth, and metastasis of HCC, which are considered as prognostic factors for HCC,¹³ such as miR-449a,¹⁴ miR-1296,¹⁵ miR-300,¹⁶ and miR-874.¹⁷ Recently, upregulation of miR-29c-3p has been reported to reverse the epithelial–mesenchymal transition (EMT) and inhibit metastasis of gallbladder carcinoma.¹⁸ Additionally, miR-29c-3p restrains the progression of HCC via LATS1-related Hippo and DNMT3B signaling pathways in vivo.¹⁹ However, the interaction between TUG1 and miR-29c-3p has not been clarified in HCC.

TUG1 is recognized as a transcript in microarray screening, which is upregulated developing mouse retinal cells during taurine treatment.²⁰ Accumulating evidence has indicated that the dysregulation of lncRNA TUG1 affects cell apoptosis and proliferation in various human cancers. For instance, knockdown of TUG1 restrains cell proliferation and accelerates cell apoptosis in osteosarcoma,²¹ over-expression of TUG1 shows facilitation effects on cell colony formation, migration, and invasion in colorectal cancer²² and in esophageal squamous cell carcinoma.²³ Nonetheless, the biological role of TUG1 in HCC remains unclear.

Collagen type 1 alpha 1 (*COL1A1*) is a regulator of hepatic fibrosis.²⁴ It has been revealed that *COL1A1* is related to the overall survival and disease-free survival of patients with gastric cancer.^{25,26} In addition, *COL1A1* acts as a latent prognostic biomarker in various kinds of malignant tumors, such as gastric cancer,^{25,26} colorectal cancer,²⁷ and HCC.²⁸ However, the potential role of *COL1A1* in HCC remains to be elucidated.

In the current study, TUG1 was identified to be upregulated in HCC tissues and cell lines. TUG1 knockdown showed inhibitory effects on cell proliferation, migration and invasion. Additionally, miR-29c-3p was identified as a target of TUG1. Similarly, *COL1A1* was verified to be a target gene of miR-29c-3p. Besides, TUG1 regulated *COL1A1* expression by sponging miR-29c-3p. Taken

together, our study may bring a latent therapeutic target for HCC.

Materials and Methods

Clinical Tissues

Fifty-one patients with HCC were enrolled in this study between March 2016 and June 2018. HCC was pathologically diagnosed. HCC tissues and paired adjacent non-tumor tissues were obtained by hepatectomy. All samples were preserved in liquid nitrogen prior to further experiments. This study was approved by the Ethics Committee of our hospital and informed consent was obtained from each patient.

Cell Lines

Normal hepatic cell line (L-02) and HCC cell lines (SMMC-7721, HeP3B, HepG2 and sk-Hep-1) were gained from American Type Culture Collection (ATCC, USA). The above cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA) at 37°C and 5% CO₂.

Cell Transfection

siRNA targeting TUG1 (si-TUG1-1 and si-TUG1-2), siRNA-*COL1A1* (si-*COL1A1*) and their negative control (si-NC); miR-29c-3p mimics/inhibitor and their negative control (miR-NC); overexpression-TUG1 (pcDNA-TUG1), overexpression-*COL1A1* (pcDNA-*COL1A1*) and their negative control (pcDNA-NC) were purchased from GenePharma (Shanghai, China). Lipofectamine 2000 reagent (Invitrogen, Carlsbad, Calif, USA) was used to transfect the above agents into HCC cells.

qRT-PCR

MiRNeasy Mini Kit (Qiagen) was used for the extraction of total RNA from tissue samples and cells. Reverse transcription was carried out using the PrimeScript™ RT Master Mix (Takara, Dalian, China). The expression levels of target mRNAs and miRNAs were detected in an ABI 7500 PCR system (Applied Biosystems) using the SYBR premix ESx Taq II (Takara Bio Inc.). The PCR amplification condition was 95°C for 5 min, followed by 40 cycles of 95°C for 10 s and 60°C for 20 s. GAPDH and U6 were taken as the internal controls. 2^{-ΔΔCt} method was adopted for the analyses of relative expression of target genes.

MTT Assay

The transfected HeP3B and sk-Hep-1 cells were cultured for 0, 1, 2, 3 or 4 days at 37°C, respectively. Twenty-microliter MTT (5 mg/mL) was then added into cells at different time points. After 4 h of incubation, 150 μ L DMSO was added in cells. The absorbance value at 450 nm was measured by a microplate spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA)

Wound-Healing Assay

Cell migration ability was measured using the scratch assay. HeP3B and sk-Hep-1 cells were planted into 6-well plates at a density of 1×10^6 cells/well and cultured until 90% confluence. A micropipette tip was then used to make a perpendicular scratch in the middle of each well. After incubation for 24 h, images were taken and wound-healing rate was assessed by ImageJ.

Transwell Assay

The invasion ability was measured using the transwell assay. Briefly, the upper chamber (Corning, Cambridge, MA, USA) was planted with HeP3B and sk-Hep-1 cells suspended in 200 μ L serum-free DMEM. The lower chamber was added with DMEM containing 10% FBS. After 24 h of incubation, cells that invaded through the matrigel membrane were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 30 min. The stained cells were then observed and counted under a light microscope (Olympus BX53, Tokyo, Japan) at three random fields.

RNA-Binding Protein

Immunoprecipitation (RIP) Assay

The binding capacity between TUG1 and miR-29c-3p gene was tested using a Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Bedford, MA, USA). Sk-Hep-1 and HeP3B cells were lysed by RIP buffer and incubated with magnetic beads conjugated with human anti-Ago2 antibody or negative control mouse IgG. Co-precipitated RNAs were purified and further used for qRT-PCR analysis.

Dual-Luciferase Reporter (DLR) Assay

Cells grown in 24-well plates were co-transfected with miR-29c-3p mimics/miR-NC and luciferase reporter vectors TUG1-WT/TUG1-Mut using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA). A Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA)

was used to test the luciferase activity after the co-transfection for 48 h.

Western Blot

Total protein was extracted from cells using RIPA lysis buffer. The proteins were separated by 10% SDS-PAGE and then transferred to PVDF membranes. The membrane was incubated with primary antibodies COL1A1 (1:1000, Abcam, Cambridge, UK), cyclin D1 (1:1000, Abcam), Bax (1:1000, Abcam), Bcl-2 (1:1000, Abcam), E-cadherin (1:1000, Abcam), N-cadherin (1:1000, Abcam), and β -actin (1:2000, Abcam) at 4°C overnight. The membrane was then incubated with donkey anti-rabbit IgG (1:10,000; Abcam) for another 1 h. The Western blot images were visualized using an ECL reagent (Millipore, Billerica, MA, USA). β -actin was used as an internal control.

Statistical Analysis

Data were expressed as the mean \pm SD and analyzed using SPSS 20.0 (SPSS; Chicago, USA). *t*-test was used for the comparison between two groups. The differences among more than two groups were analyzed by one-way ANOVA, followed by Tukey's multiple comparisons test. Pearson correlation coefficient (*r*) was adapted for correlation analysis. *P* < 0.05 presented statistically significant.

Results

The Expression of TUG1 Was Increased in HCC Tissues and Cell Lines

The TUG1 expression in 51 paired HCC tissues and the corresponding adjacent non-cancerous tissues was detected by qRT-PCR. Notably higher expression of TUG1 was found in HCC tissues than that in adjacent non-cancerous tissues (*P* < 0.001) (Figure 1A). HCC patients were then divided into high-expression (\geq median expression) group and low-expression (<median expression) groups. Subsequently, the correlation between TUG1 expression and clinicopathological parameters were further analyzed. The expression of TUG1 was positively associated with the tumor stage (Table 1, *P* = 0.005), but not associated with the age, gender, tumor diameter, and resection degree. Besides, HCC patients at TNM stage III/IV had a significantly higher TUG1 expression than those at TNM stage I/II (*P* < 0.01) (Figure 1B). Next, the expression of TUG1 was assessed in HCC cell lines. A remarkable upregulation of TUG1 was discovered in HCC cell lines (sk-Hep-1, HeP3B, HepG2 and SMMC-7721) compared to

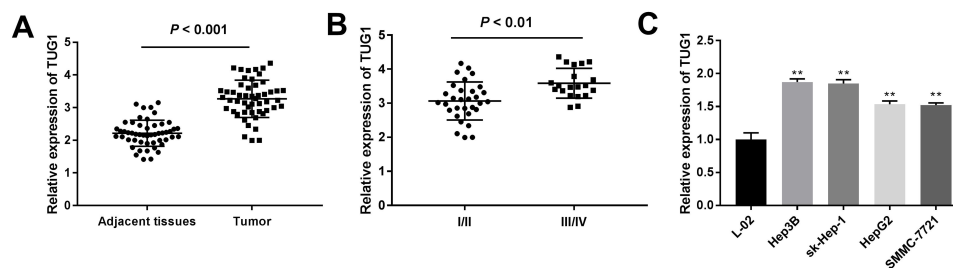


Figure 1 The expression of taurine upregulated gene 1 (TUG1) in primary hepatocellular carcinoma (HCC) tissues and cell lines was detected by quantitative reverse-transcription PCR (qRT-PCR). **(A)** The expression of TUG1 in 51 paired HCC tissues and matched adjacent non-tumor tissues was examined by qRT-PCR. $P < 0.001$. **(B)** Relative expression of TUG1 in HCC patients at tumor-node-metastasis (TNM) stage I/II and TNM stage III/IV. $P < 0.01$ **(C)** The expression of TUG1 in four HCC cell lines (sk-Hep-1, Hep3B, HepG2 and SMMC-7721) and normal human liver cell line L-02 was assessed by qRT-PCR. $**P < 0.01$ vs the L-02 cells group. Data were represented as mean \pm standard deviation (SD) of at least two independent experiments.

that in L-02 cells ($P < 0.01$) (Figure 1C). Sk-Hep-1 and Hep3B cells were selected for subsequent experiments due to relatively high expression of TUG1. The above data suggested that TUG1 was remarkably overexpressed in HCC tissues and cell lines, and the upregulation of TUG1 was correlated with the tumor stage.

TUG1 Knockdown Suppressed the Proliferation, Migration, and Invasion of HCC Cells

The biological function of TUG1 in HCC cells was further explored. TUG1 knockdown was conducted by using specific siRNAs (si-TUG1-1/si-TUG1-2), and the silence

efficiency was determined by qRT-PCR. Both si-TUG1-1 and si-TUG1-2 significantly decreased the expression of TUG1 in HCC cells ($P < 0.01$) (Figure 2A). MTT assay revealed that si-TUG1-1 caused a significant decrease in cell viability ($P < 0.01$) (Figure 2B). Furthermore, si-TUG1-1 group showed decreased wound-healing rate and number of invasive cells compared with si-NC group ($P < 0.01$) (Figure 2C and D). These results indicated that TUG1 could aggravate the proliferation, migration and invasion of HCC cells.

miR-29c-3p Was a TUG1

To elucidate the latent mechanism of TUG1 in HCC cells, the target miRNAs were predicted using the starBase v2.0 (<http://starbase.sysu.edu.cn/>). A predicted binding site between TUG1 and miR-29c-3p was discovered (Figure 3A). Then, the regulatory role of TUG1 on miR-29c-3p was explored. As a result, TUG1 knockdown significantly upregulate miR-29c-3p expression ($P < 0.01$) (Figure 3B). Meanwhile, RIP assay implied that both TUG1 and miR-29c-3p were immunoprecipitated by anti-Ago2 ($P < 0.01$) (Figure 3C). DLR assay illustrated an obviously reduced luciferase activity of TUG1-WT in miR-29c-3p mimics group compared with that in miR-NC group ($P < 0.01$) (Figure 3D). No notable difference was detected in the luciferase activity of TUG1-Mut between miR-29c-3p mimics and miR-NC groups (Figure 3D). Subsequently, significantly lower expression of miR-29c-3p was observed in HCC tissues than adjacent tissues ($P < 0.001$) (Figure 3E). Besides, correlation analysis revealed a negative correlation between miR-29c-3p and TUG1 expression ($r = -0.4735$; $P = 0.0004$) (Figure 3F). Compared with the L-02 cell line, obviously decreased expression of miR-29c-3p was shown in HCC cell lines ($P < 0.01$) (Figure 3G). These results suggested that TUG1 directly targeted and negatively regulated miR-29c-3p.

Table 1 Clinicopathological Characteristics of the HCC Patients

Characteristics	n	TUG1 Expression		P value
		Low (n=25)	High (n=26)	
Age				0.886
<50 years	25	12	13	
≥ 50 years	26	13	13	
Gender				0.877
Male	28	14	14	
Females	23	11	12	
Diameter				0.867
<5cm	21	10	11	
≥ 5 cm	30	15	15	
Resection degree				0.877
Total resection	28	14	14	
Subtotal resection	23	11	12	
WHO Grade				0.005*
I+II	31	20	11	
III+IV	20	5	15	

Note: * $P = 0.005$.

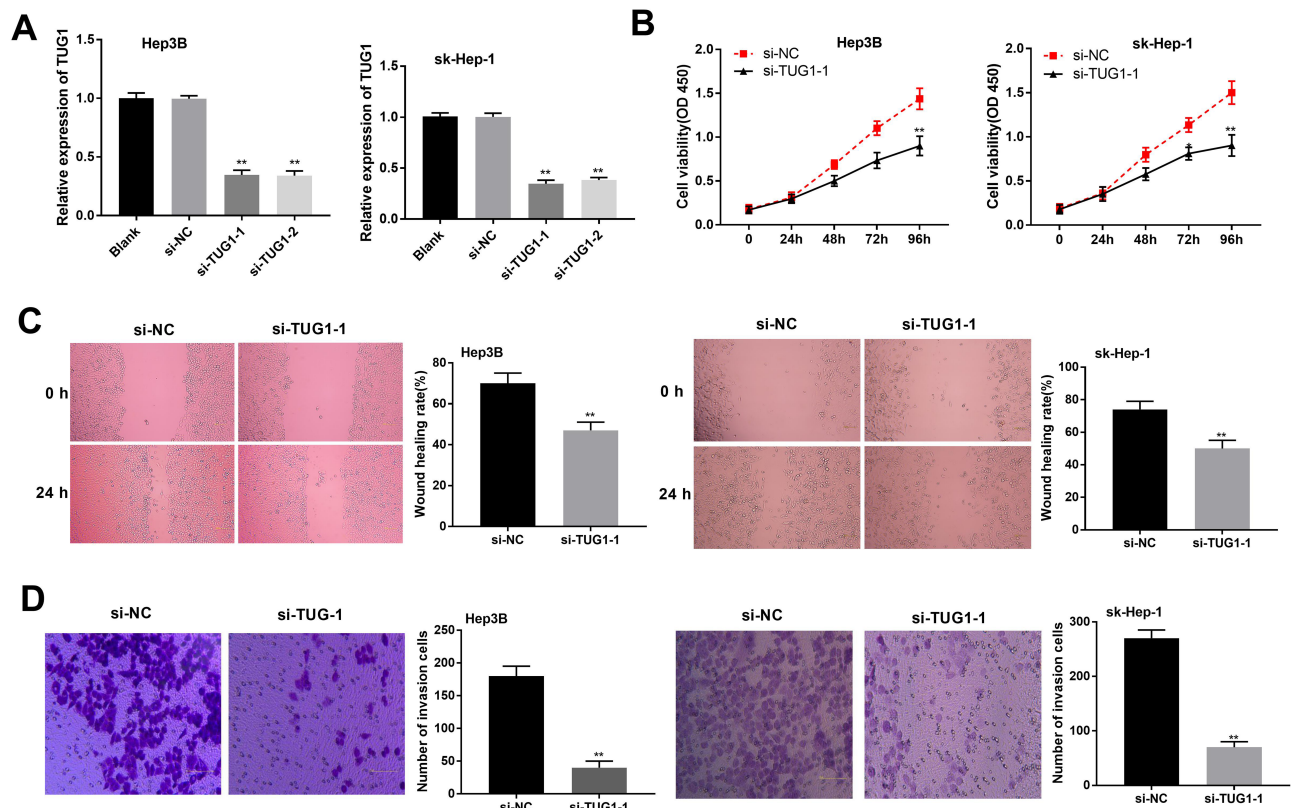


Figure 2 Taurine upregulated gene 1 (TUG1) knockdown inhibited the proliferation, migration and invasion of hepatocellular carcinoma (HCC) cells. Hep3B and sk-Hep-1 cells were transfected with si-TUG-1, si-TUG-2 or si-NC. **(A)** qRT-PCR was used to detect the expression of TUG1. **(B)** 3-(4,5-Dimethyl-2-Thiazolyl)-2, 5-Diphenyl-2-H-Tetrazolium Bromide (MTT) assay was applied to measure cell proliferation at 0, 24, 48, 72 and 96 h in transfected Hep3B and sk-Hep-1 cells. **(C)** Wound-healing assay was conducted to evaluate the migration of transfected Hep3B and sk-Hep-1 cells. **(D)** Transwell invasion assay was used to detect the invasion of transfected Hep3B and sk-Hep-1 cells. ** $P < 0.01$ vs the si-NC group. Scale bar = 100 μ m, $\times 400$ magnification. Data were represented as mean \pm standard deviation (SD) of at least two independent experiments.

miR-29c-3p Restrained the Proliferation, Migration, and Invasion of HCC Cells

The expression of miR-29c-3p was measured in HCC cells transfected with miR-29c-3p mimics/inhibitors. MiR-29c-3p mimics group showed a strikingly upregulation of miR-29c-3p, while miR-29c-3p inhibitor group exhibited an obvious downregulated expression ($P < 0.01$) (Figure 4A). MTT assay implied that miR-29c-3p mimics significantly decreased the cell viability ($P < 0.01$) (Figure 4B). Moreover, wound healing and transwell assays indicated that the transfection of miR-29c-3p mimics resulted in decreased wound-healing rate and number of invasion cells ($P < 0.01$) (Figure 4C and D). The above results indicated that miR-29c-3p had inhibition effects on cell proliferation, migration, and invasion in HCC.

COL1A1 Was a Target Gene of miR-29c-3p

TargetScan software was used to predict the target genes of miR-29c-3p. Figure 5A shows a putative binding site between miR-29c-3p and COL1A1. DLR assay showed that the

luciferase activity of COL1A1-WT was obviously decreased in the miR-29c-3p mimics + pcDNA-NC group ($P < 0.01$). Overexpression of TUG1 restored the suppressed luciferase activity caused by miR-29c-3p mimics ($P < 0.05$) (Figure 5B). Additionally, HCC tissues had an evidently increased mRNA expression of COL1A1 compared with adjacent tissues ($P < 0.001$) (Figure 5C). There was a negative correlation between COL1A1 and miR-29c-3p expression ($r = -0.4811$, $P < 0.0004$) (Figure 5D), and a positive correlation between TUG1 and COL1A1 expression ($r = 0.3853$, $P < 0.0052$) (Figure 5E). Finally, the mRNA expression of COL1A1 was obviously increased in HCC cell lines in comparison to the L-02 cell line ($P < 0.01$) (Figure 5F). Together, we proved that COL1A1 was a target gene of miR-29c-3p.

TUG1 Acted as a ceRNA to Modulate HCC Progression via miR-29c-3p/ COL1A1 Axis

Western blot showed that the protein expression of COL1A1 was significantly decreased by the transfection

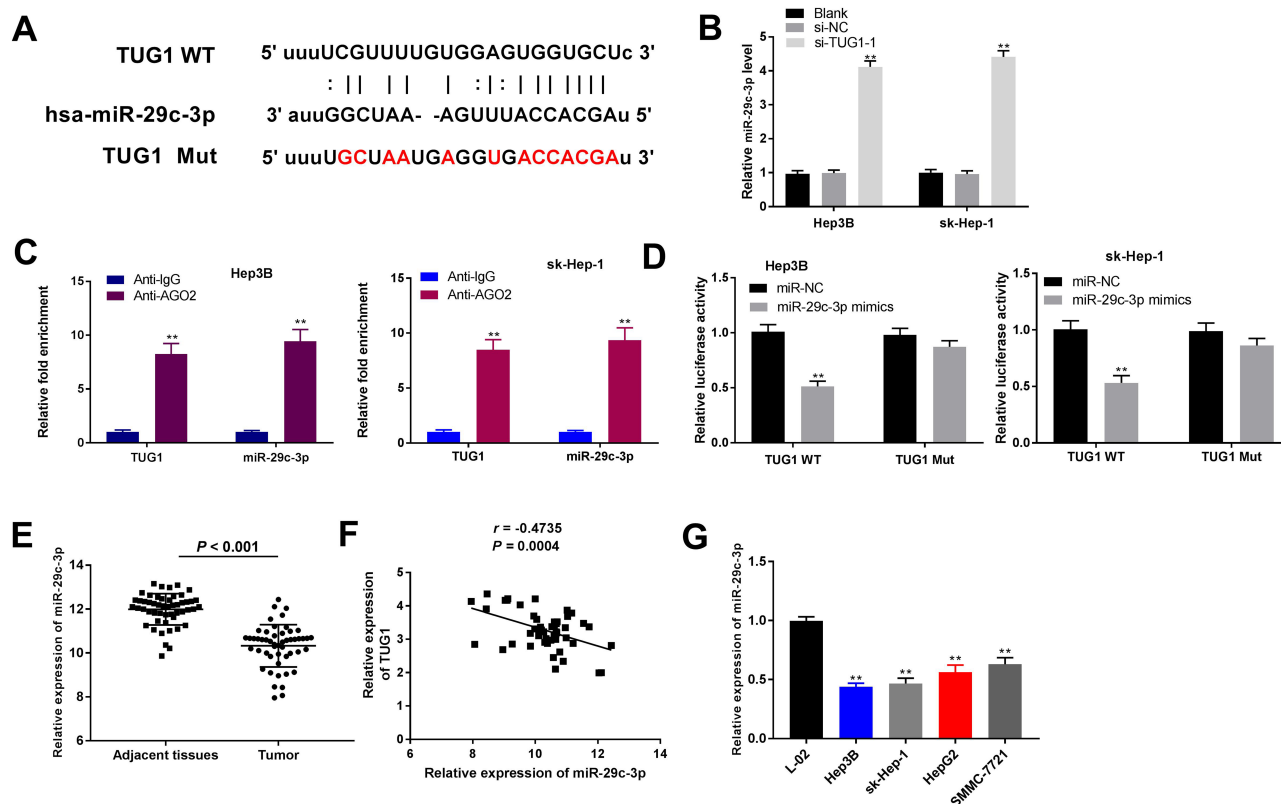


Figure 3 MicroRNA (MiR)-29c-3p was a target gene of taurine upregulated gene 1 (TUG1) in hepatocellular carcinoma (HCC). **(A)** Bioinformatics analysis showed the predicted binding site between TUG1 and miR-29c-3p. **(B)** Relative expression of miR-29c-3p in Hep3B and sk-Hep-1 cells transfected with si-NC or si-TUG1-1 was measured by quantitative reverse-transcription PCR (qRT-PCR). $**P < 0.01$ vs the si-NC group. **(C, D)** RNA-binding protein immunoprecipitation (RIP) and dual-luciferase reporter (DLR) assays were used to verify the targeting relationship between the TUG1 and miR-29c-3p. **C**, $**P < 0.01$ vs the anti-IgG group; **D**, $**P < 0.01$ vs the miR-NC group. **(E)** The expression of miR-29c-3p in 51 paired HCC tissues and matched adjacent non-tumor tissues was examined by qRT-PCR. $**P < 0.001$. **(F)** Correlation analysis between the expression of miR-29c-3p and TUG1. $P = 0.0004$, $r = -0.4735$. **(G)** The expression of miR-29c-3p in four HCC cell lines (sk-Hep-1, Hep3B, HepG2 and SMMC-7721) and normal human liver cell line L-02 was assessed by qRT-PCR. $**P < 0.01$ vs the L-02 cells group. Data were represented as mean \pm standard deviation (SD) of at least two independent experiments.

of miR-29c-3p mimics in Hep3B and sk-Hep-1 cells ($P < 0.01$). Overexpression of TUG1 significantly reversed the decreased protein expression of COL1A1 induced by miR-29c-3p mimics ($P < 0.05$) (Figure 6A). In order to reveal the regulatory role of *COL1A1* in HCC cells, si-*COL1A1* was used to silence *COL1A1* in Hep3B cells. qRT-PCR showed that the mRNA expression of *COL1A1* was significantly decreased by the transfection of si-*COL1A1* ($P < 0.01$) (Figure 6B). The transfection of si-*COL1A1* significantly suppressed the viability, migration and invasion of Hep3B cells ($P < 0.01$) (Figure 6C–E). The transfection of si-*COL1A1* also significantly decreased the expression of Cyclin D1, Bcl-2, and N-cadherin, and increased the expression of E-cadherin and Bax in Hep3B cells ($P < 0.01$) (Figure 6F). Si-TUG1-1 exhibited consistent results with si-*COL1A1* in Hep3B cells ($P < 0.01$) (Figure 6C–F). Interestingly, downregulation of miR-29c-3p and upregulation of *COL1A1* both reversed the suppressive effects of

TUG1-1 knockdown on the viability, migration and invasion of Hep3B cells ($P < 0.05$) (Figure 6C–E). In addition, the suppressive effects of TUG1-1 knockdown on the expression of Cyclin D1, Bcl-2, and N-cadherin, and the promoting effects of TUG1-1 knockdown on the expression of E-cadherin and Bax were significantly reversed by miR-29c-3p downregulation and *COL1A1* upregulation ($P < 0.05$) (Figure 6F). All these results indicated that silencing of TUG1 may inhibit the progression of HCC via regulating miR-29c-3p/*COL1A1* axis.

Discussion

Since distance migration and drug resistance are two important obstacles to the prognosis of HCC, it is urgent to understand the molecular mechanisms and to find new therapeutic targets for HCC. Previous studies have validated that abnormal expression of lncRNAs is linked to the tumorigenesis and metastasis of cancer.^{5,29} Overexpression of the lncRNAs

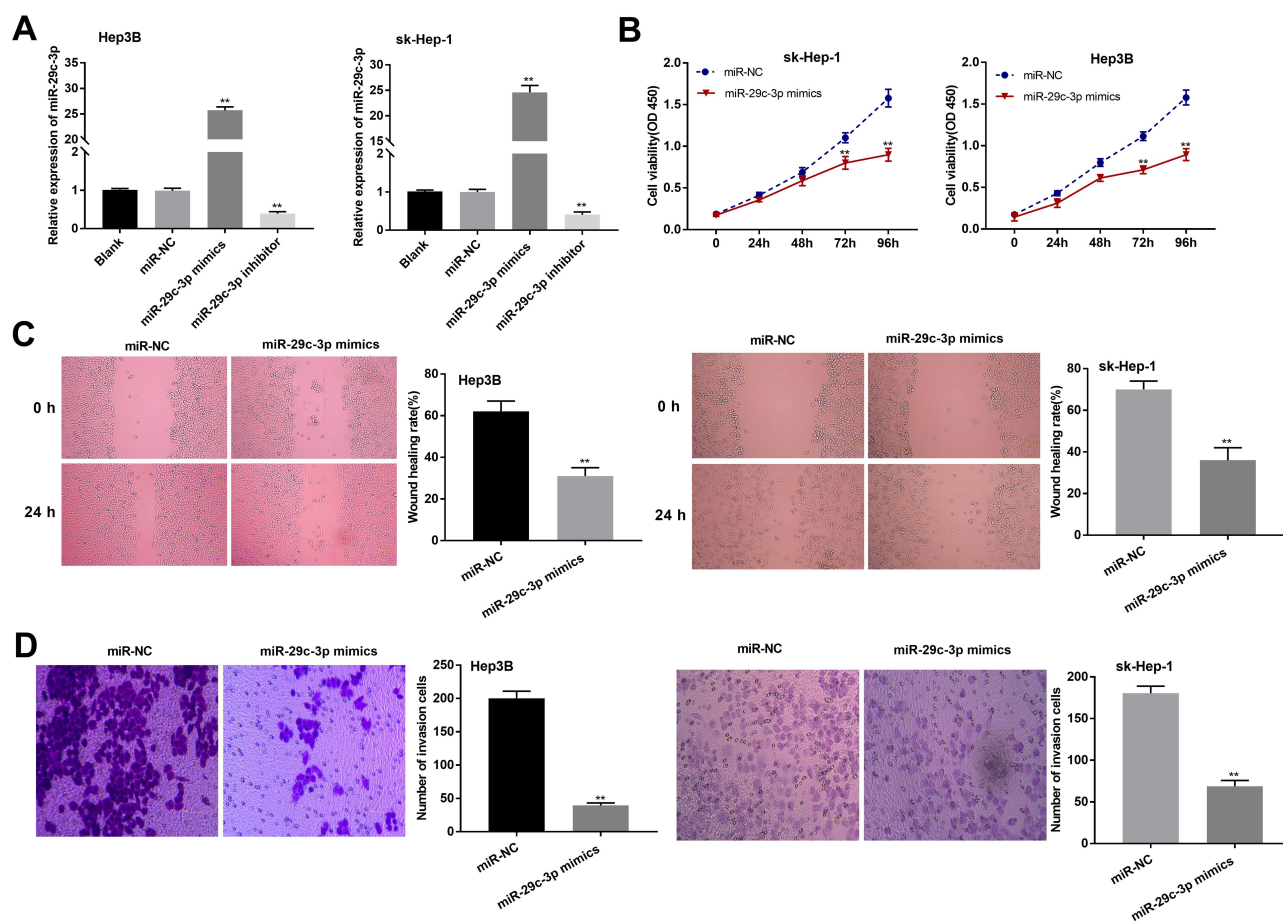


Figure 4 MicroRNA (MiR)-29c-3p inhibited the proliferation, migration and invasion of hepatocellular carcinoma (HCC) cells. Hep3B and sk-Hep-1 cells were transfected with miR-negative control (NC), miR-29c-3p mimics or miR-29c-3p inhibitors. **(A)** Quantitative reverse-transcription PCR (qRT-PCR) was used to detect the expression of miR-29c-3p. **(B)** 3-(4, 5-Dimethyl-2-Thiazolyl)-2, 5-Diphenyl-2-H-Tetrazolium Bromide (MTT) assay was applied to measure cell proliferation at 0, 24, 48, 72 and 96 h in transfected Hep3B and sk-Hep-1 cells. **(C)** Wound-healing assay was conducted to evaluate cell migration of transfected Hep3B and sk-Hep-1 cells. **(D)** Transwell invasion assay was used to detect the invasion of Hep3B and sk-Hep-1 cells. Scale bar = 100 μ m, \times 400 magnification. $**P < 0.01$ vs the miR-NC group. Data were represented as mean \pm standard deviation (SD) of at least two independent experiments.

has been reported to be closely associated with the advanced TNM stages of patients with malignant tumor.^{30,31} In the current study, significantly upregulated expression of lncRNA TUG1 was detected in HCC tissues. The increased expression of TUG1 was also identified to be correlated with the TNM stage of HCC.

Dysregulation of lncRNAs has been frequently detected in various cancers and plays vital role in diverse biological processes, including cell proliferation, apoptosis, metastasis, and metabolism.^{32,33} TUG1 is an important lncRNA that has been identified as an oncogene in various types of cancers. For instance, knockdown of TUG1 suppresses the progress of osteosarcoma.²¹ Overexpression of TUG1 promotes the metastasis of colorectal cancer in vivo²² and promotes the proliferation and migration of esophageal squamous cell carcinoma cells in vitro.²³ A recent study has indicated that TUG1 accelerates cell proliferation, colony formation, and

tumorigenicity of HCC through epigenetically inhibiting Kruppel-like factor 2 transcription,³⁴ suggesting a key regulatory role of TUG1 in HCC. In this study, TUG1 was silenced to investigate the effects of TUG1 dysregulation on HCC cells. As a result, TUG1 knockdown showed a suppressive effect on HCC, which was coincided with the oncogenic role of TUG1 reported previously.

A large number of studies have illustrated that miRNAs are vital regulatory factors in cellular processes and involved in the development of HCC.^{35–37} However, the exact function of miRNAs in the progress of HCC is not entirely understood.³⁸ Here, target prediction was performed based on dramatically increased expression of TUG1 in HCC. MiR-29c-3p was predicted as a target of TUG1. The specific target relation between miR-29c-3p and TUG1 was verified by RIP and DLR assays. Recent studies have illustrated that miR-29c-3p may act as a tumor suppressor in a series of

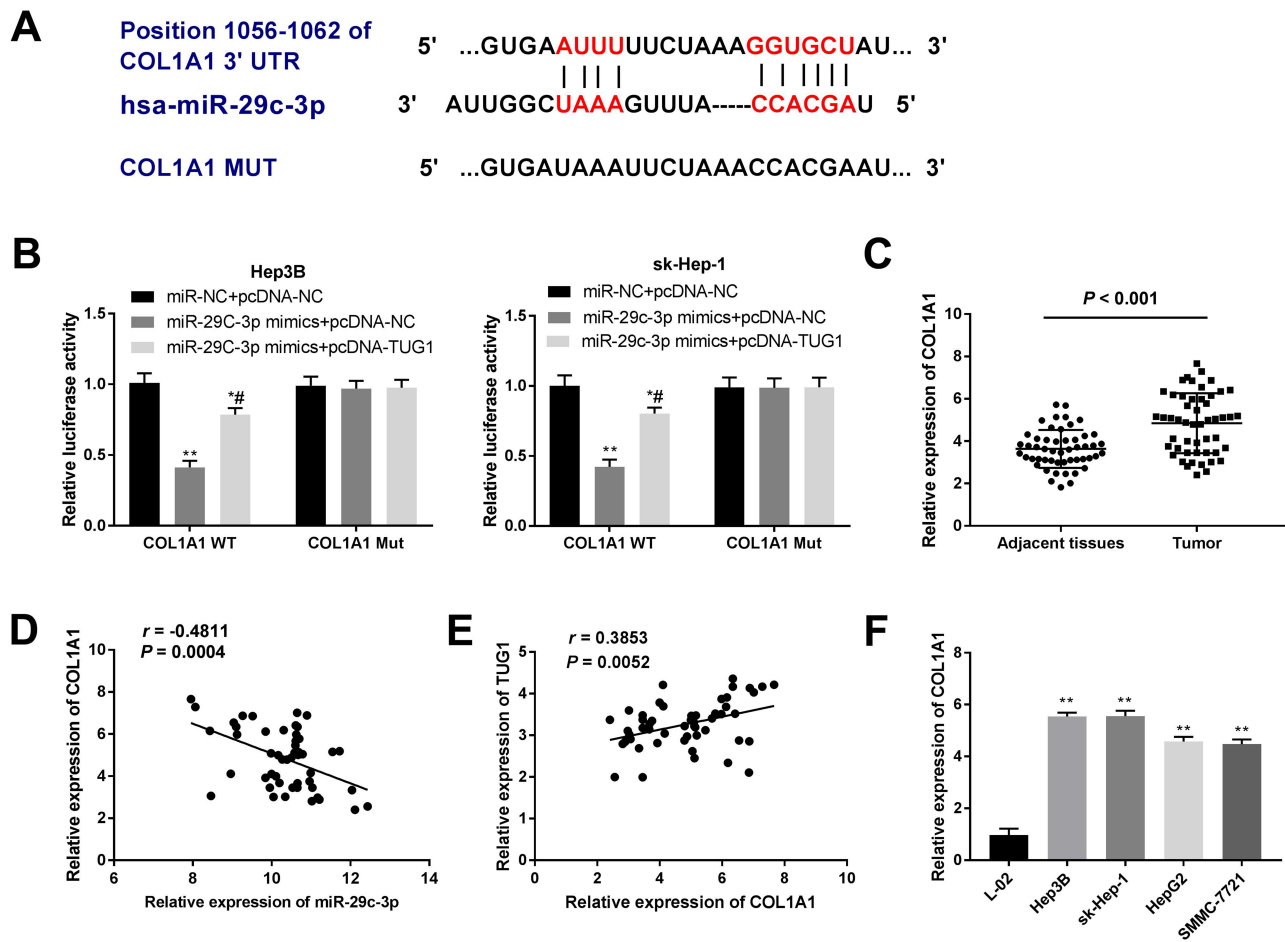


Figure 5 Collagen type 1 alpha 1 (*COL1A1*) was a target gene of microRNA (miR)-29c-3p. **(A)** Bioinformatics analysis showed the predicted binding site between miR-29c-3p and *COL1A1*. **(B)** dual-luciferase reporter (DLR) assay was used to verify the targeting relationship between miR-29c-3p and *COL1A1*. * $P < 0.05$, ** $P < 0.01$ vs the miR-NC + pcDNA-NC group. # $P < 0.05$ vs the miR-29c-3p mimics + pcDNA-NC group. **(C)** The expression of *COL1A1* in 51 paired hepatocellular carcinoma (HCC) tissues and matched adjacent non-tumor tissues was examined by quantitative reverse-transcription PCR (qRT-PCR). $P < 0.001$. **(D)** Correlation analysis between the expression of miR-29c-3p and *COL1A1*. $P = 0.0004$, $r = -0.4811$. **(E)** Correlation analysis between the expression of *COL1A1* and taurine upregulated gene 1 (TUG1). $P = 0.0052$, $r = 0.3853$. **(F)** The expression of *COL1A1* in four HCC cell lines and normal human liver cell line L-02 was assessed by qRT-PCR. ** $P < 0.01$ vs the L-02 cells group. Data were represented as mean \pm standard deviation (SD) of at least two independent experiments.

cancers.^{19,39} Lu et al have found that miR-29c-3p is down-regulated in gallbladder carcinoma, and miR-29c-3p over-expression inhibits cell metastasis.¹⁸ MiR-29c-3p suppresses cell invasion and migration in colon cancer,⁴⁰ and inhibits cell proliferation, apoptosis, and tumor growth in HCC.¹⁹ Another study has also reported that miR-29c-3p, as a direct target gene of P53, inhibits the invasion and migration of colon cancer cells by targeting PHLDB2.⁴⁰ Similar inhibitory effect of miR-29c-3p on HCC was obtained in the current study.

As known, miRNAs can downregulate gene expression through base-pairing with the 3'-UTR of the targeted mRNAs.⁴¹ In this study, we noticed that miR-29c-3p could directly bind to the 3'-UTR of *COL1A1*. The involvement of collagen family members has been determined in the carcinogenesis of several tissue types.⁴² The aberrant expression of

COL1A1 and *COL1A2* has been discovered in some cancers.^{28,43,44} Zang et al⁴⁵ have proved that *COL1A1* and *COL1A2* are differentially expressed in gastric cancer, which can predict poor clinical outcomes in patients with gastric cancer. Preclinical evidence has shown that *COL1A1* is a reliable biomarker and putative therapeutic target for hepatocellular carcinogenesis and metastasis.⁴⁶ Here, DLR assay showed that overexpression of TUG1 reversed the suppressed luciferase activity caused by miR-29c-3p mimics. It is widely believed that lncRNAs act as sponges to regulate the target miRNAs, thus hindering the inhibitory effect of miRNAs on their target genes. Gao et al have reported that HOXC13-AS enhances HMGA2 expression by sponging miR-383-3p in nasopharyngeal carcinoma.⁴⁷ PCA3 up-regulates HMGB1 expression through targeting miR-218-5p in prostate cancer.⁴⁸ HCAL modulates LAPT4B expression via binding

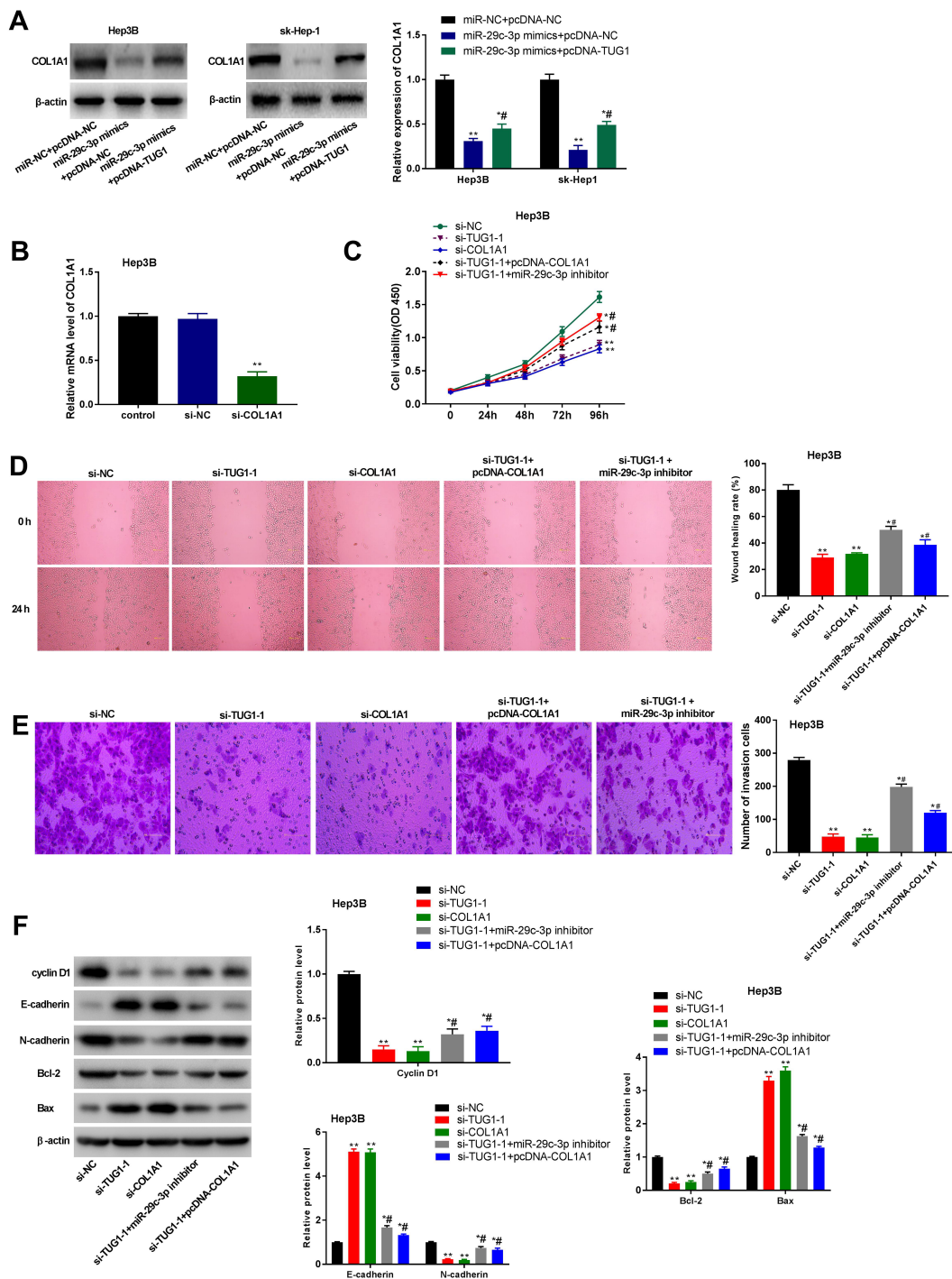


Figure 6 Taurine upregulated gene 1 (TUG1) promotes the proliferation, migration and invasion of hepatocellular carcinoma (HCC) cells via microRNA (miR)-29c-3p/collagen type I alpha 1 (*COL1A1*) axis. **(A)** Western blot assay was used to detect the expression of *COL1A1* in transfected Hep3B and sk-Hep-1 cells. **P* < 0.05, ***P* < 0.01 vs the miR-NC + pcDNA-NC group. #*P* < 0.05 vs the miR-29c-3p mimics + pcDNA-NC group. **(B)** The mRNA level of *COL1A1* was detected by quantitative reverse-transcription PCR (qRT-PCR). **(C)** 3-(4,5-Dimethyl-2-Thiazolyl)-2, 5-Diphenyl-2-H-Tetrazolium Bromide (MTT) assay was applied to measure cell proliferation at 0, 24, 48, 72 and 96 h in transfected Hep3B and sk-Hep-1 cells. **(D)** Wound-healing assay was conducted to evaluate the migration of transfected Hep3B and sk-Hep-1 cells. **(E)** Transwell invasion assay was used to detect the invasion of Hep3B and sk-Hep-1 cells. **(F)** Western blot assay was used to detect the protein levels of cyclin D1, E-cadherin, N-cadherin, Bcl-2 and Bax. **P* < 0.05, ***P* < 0.01 vs the si-NC group. #*P* < 0.05 vs the si-TUG1-I group. Scale bar = 100 μm, ×400 magnification. Data were represented as mean ± standard deviation (SD) of at least two independent experiments.

miR-15a, miR-196a and miR-196b in HCC.⁴⁹ Our results illustrated that overexpression of *COL1A1* and inhibition of miR-29c-3p both reversed the suppressive effects of TUG1

knockdown on HCC progression in vitro, suggesting that TUG1 could upregulate *COL1A1* expression by sponging miR-29c-3p.

However, this study has some limitations. First, over-expression experiment is needed to reflect the regulatory role of TUG1 in HCC cells more comprehensively. Second, more assays should be added to verify the results on cell proliferation and apoptosis, such as cell cycle assay, and Western blot on apoptosis-related proteins. Third, this study is limited in cellular level and researches on animal models are urgently needed. Fourth, the pathological significance of *COL1A1* in indicating survival and prognosis of patients with HCC is not analyzed. Finally, in-depth mechanisms of TUG1 involving signaling pathways remain need to be studied.

Conclusions

In conclusion, TUG1 was a tumor promoter in HCC. TUG1 positively regulated *COL1A1* via sponging miR-29c-3p. Notably, TUG1 could promote the viability, migration and invasion of HCC cells through regulating miR-29c-3p/*COL1A1* axis. Our study indicated that TUG1/miR-29c-3p/*COL1A1* axis might serve as a therapeutic target for HCC.

Data Sharing Statement

The data in this study can be obtained from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

This study was approved by the Ethics Committee of The Sixth People's Hospital of Qingdao and informed consent was obtained from each patient. All participants agreed to publish the study.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest for this work.

References

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin.* 2015;65(1):5–29. doi:10.3322/caac.21254
- Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol.* 2009;27(9):1485–1491. doi:10.1200/JCO.2008.20.7753
- Morise Z, Kawabe N, Tomishige H, et al. Recent advances in the surgical treatment of hepatocellular carcinoma. *World J Gastroenterol.* 2014;20:14381–14392. doi:10.3748/wjg.v20.i39.14381
- Quinn JJ, Chang HY. Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet.* 2016;17(1):47. doi:10.1038/nrg.2015.10
- Bao J, Chen X, Hou Y, et al. LncRNA DBH-AS1 facilitates the tumorigenesis of hepatocellular carcinoma by targeting miR-138 via FAK/Src/ERK pathway. *Biomed Pharmacother.* 2018;107:824–833. doi:10.1016/j.biopha.2018.08.079
- Wang Y, Yang L, Chen T, et al. A novel lncRNA MCM3AP-AS1 promotes the growth of hepatocellular carcinoma by targeting miR-194-5p/*FOXA1* axis. *Mol Cancer.* 2019;18(1):28. doi:10.1186/s12943-019-0957-7
- Wang K, Long B, Zhou L-Y, et al. CARL lncRNA inhibits anoxia-induced mitochondrial fission and apoptosis in cardiomyocytes by impairing miR-539-dependent PHB2 downregulation. *Nat Commun.* 2014;5:1–13.
- Cesana M, Cacchiarelli D, Legnini I, et al. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell.* 2011;147(2):358–369. doi:10.1016/j.cell.2011.09.028
- Afum-Adjei Awuah A, Ueberberg B, Owusu-Dabo E, Frempong M, Jacobsen M. Dynamics of T-cell IFN-gamma and miR-29a expression during active pulmonary tuberculosis. *Int Immunol.* 2014;26:579–582.
- Huang X, She L, Luo X, Huang S, Wu J. MiR-222 promotes the progression of polycystic ovary syndrome by targeting p27 *Kip1*. *Pathol Res Pract.* 2019;215:918–923. doi:10.1016/j.prp.2019.01.038
- Zhang F, Li K, Yao X, et al. A miR-567-PIK3AP1-PI3K/AKT-c-Myc feedback loop regulates tumour growth and chemoresistance in gastric cancer. *EBioMedicine.* 2019;44:311–321. doi:10.1016/j.ebiom.2019.05.003
- Zhu D, Gu L, Li Z, et al. MiR-138-5p suppresses lung adenocarcinoma cell epithelial-mesenchymal transition, proliferation and metastasis by targeting *ZEB2*. *Pathol Res Pract.* 2019;215(5):861–872. doi:10.1016/j.prp.2019.01.029
- Zhu Z, Zhang X, Wang G, Zheng H. Role of microRNAs in hepatocellular carcinoma. *Hepat Mon.* 2014;14(7). doi:10.5812/hepatmon.18672
- Han B, Huang J, Yang Z, et al. miR-449a is related to short-term recurrence of hepatocellular carcinoma and inhibits migration and invasion by targeting *notch1*. *Oncotargets Ther.* 2019;12:10975. doi:10.2147/OTT.S216997
- Xu Q, Liu X, Liu Z, et al. MicroRNA-1296 inhibits metastasis and epithelial-mesenchymal transition of hepatocellular carcinoma by targeting *SRPK1*-mediated PI3K/AKT pathway. *Mol Cancer.* 2017;16(1):103. doi:10.1186/s12943-017-0675-y
- Wang R, Yu Z, Chen F, et al. miR-300 regulates the epithelial-mesenchymal transition and invasion of hepatocellular carcinoma by targeting the FAK/PI3K/AKT signaling pathway. *Biomed Pharmacother.* 2018;103:1632–1642. doi:10.1016/j.biopha.2018.03.005
- Jiang T, Guan L-Y, Ye Y-S, Liu H-Y, Li R. MiR-874 inhibits metastasis and epithelial-mesenchymal transition in hepatocellular carcinoma by targeting *SOX12*. *Am J Cancer Res.* 2017;7:1310.
- Lu K, Feng F, Yang Y, et al. High-throughput screening identified miR-7-2-3p and miR-29c-3p as metastasis suppressors in gallbladder carcinoma. *J Gastroenterol.* 2020;55(1):51–66. doi:10.1007/s00535-019-01627-0
- Wu H, Zhang W, Wu Z, et al. miR-29c-3p regulates *DNMT3B* and *LATS1* methylation to inhibit tumor progression in hepatocellular carcinoma. *Cell Death Dis.* 2019;10(2):48. doi:10.1038/s41419-018-1281-7

20. Young TL, Matsuda T, Cepko CL. The noncoding RNA taurine up-regulated gene 1 is required for differentiation of the murine retina. *Curr Biol*. 2005;15(6):501–512. doi:10.1016/j.cub.2005.02.027
21. Zhang Q, Geng P-L, Yin P, et al. Down-regulation of long non-coding RNA TUG1 inhibits osteosarcoma cell proliferation and promotes apoptosis. *Asian Pac J Cancer Prev*. 2013;14(4):2311–2315. doi:10.7314/APJCP.2013.14.4.2311
22. Sun J, Ding C, Yang Z, et al. The long non-coding RNA TUG1 indicates a poor prognosis for colorectal cancer and promotes metastasis by affecting epithelial-mesenchymal transition. *J Transl Med*. 2016;14(1):42. doi:10.1186/s12967-016-0786-z
23. Xu Y, Wang J, Qiu M, et al. Upregulation of the long noncoding RNA TUG1 promotes proliferation and migration of esophageal squamous cell carcinoma. *Tumor Biol*. 2015;36(3):1643–1651. doi:10.1007/s13277-014-2763-6
24. Koilan S, Hamilton D, Baburyan N, et al. Prevention of liver fibrosis by triple helix-forming oligodeoxyribonucleotides targeted to the promoter region of type I collagen gene. *Oligonucleotides*. 2010;20(5):231–237. doi:10.1089/oli.2010.0244
25. Jiang K, Liu H, Xie D, Xiao Q. Differentially expressed genes *ASP*, *COL1A1*, *FNI*, *VCAN* and *MUC5AC* are potential prognostic biomarkers for gastric cancer. *Oncol Lett*. 2019;17:3191–3202. doi:10.3892/ol.2019.9952
26. Li J, Ding Y, Li A. Identification of *COL1A1* and *COL1A2* as candidate prognostic factors in gastric cancer. *World J Surg Oncol*. 2016;14:297. doi:10.1186/s12957-016-1056-5
27. Zhang Z, Wang Y, Zhang J, Zhong J, Yang R. *COL1A1* promotes metastasis in colorectal cancer by regulating the WNT/PCP pathway. *Mol Med Rep*. 2018;17:5037–5042. doi:10.3892/mmr.2018.8533
28. Hayashi M, Nomoto S, Hishida M, et al. Identification of the collagen type 1 alpha 1 gene (*COL1A1*) as a candidate survival-related factor associated with hepatocellular carcinoma. *BMC Cancer*. 2014;14:108. doi:10.1186/1471-2407-14-108
29. Dong H, Hu J, Zou K, et al. Activation of lncRNA TINCR by H3K27 acetylation promotes trastuzumab resistance and epithelial-mesenchymal transition by targeting MicroRNA-125b in breast cancer. *Mol Cancer*. 2019;18:1–18. doi:10.1186/s12943-018-0931-9
30. Wu D-M, Wang S, Wen X, et al. lncRNA SNHG15 acts as a ceRNA to regulate YAP1-hippo signaling pathway by sponging miR-200a-3p in papillary thyroid carcinoma. *Cell Death Dis*. 2018;9(10):1–15. doi:10.1038/s41419-018-0975-1
31. Gong P, Qiao F, Wu H, et al. lncRNA UCA1 promotes tumor metastasis by inducing miR-203/*ZEB2* axis in gastric cancer. *Cell Death Dis*. 2018;20(12):1–14. doi:10.1038/s41419-018-1170-0
32. Yuan JH, Yang F, Wang F, et al. A long noncoding RNA activated by TGF-beta promotes the invasion-metastasis cascade in hepatocellular carcinoma. *Cancer Cell*. 2014;25:666–681. doi:10.1016/j.ccr.2014.03.010
33. Li Z, Li X, Wu S, Xue M, Chen W. Long non-coding RNA UCA1 promotes glycolysis by upregulating hexokinase 2 through the mTOR-STAT3/microRNA143 pathway. *Cancer Sci*. 2014;105:951–955. doi:10.1111/cas.12461
34. Huang MD, Chen WM, Qi FZ, et al. Long non-coding RNA TUG1 is up-regulated in hepatocellular carcinoma and promotes cell growth and apoptosis by epigenetically silencing of *KLF2*. *Mol Cancer*. 2015;14:165. doi:10.1186/s12943-015-0431-0
35. Mohamed AA, Ali-Eldin ZA, Elbedewy TA, et al. MicroRNAs and clinical implications in hepatocellular carcinoma. *World J Hepatol*. 2017;9(23):1001–1007. doi:10.4254/wjh.v9.i23.1001
36. Zhao X, Yang Z, Li G, et al. The role and clinical implications of microRNAs in hepatocellular carcinoma. *Sci China Life Sci*. 2012;55(10):906–919. doi:10.1007/s11427-012-4384-x
37. Peng S, Geng J, Sun R, Tian X, Wei H. Polyinosinic-polycytidylic acid liposome induces human hepatoma cells apoptosis which correlates to the up-regulation of RIG-I like receptors. *Cancer Sci*. 2009;100:529–536. doi:10.1111/j.1349-7006.2008.01062.x
38. Thurnherr T, Mah W-C, Lei Z, et al. Differentially expressed miRNAs in hepatocellular carcinoma target genes in the genetic information processing and metabolism pathways. *Sci Rep*. 2016;6(1):20065. doi:10.1038/srep20065
39. Zhang S, Jin J, Tian X, Wu L. hsa-miR-29c-3p regulates biological function of colorectal cancer by targeting *SPARC*. *Oncotarget*. 2017;8(61):104508. doi:10.18632/oncotarget.22356
40. Chen G, Zhou T, Li Y, Yu Z, Sun L. p53 target miR-29c-3p suppresses colon cancer cell invasion and migration through inhibition of *PHLDB2*. *Biochem Biophys Res Commun*. 2017;487(1):90–95. doi:10.1016/j.bbrc.2017.04.023
41. Bartel DP. MicroRNAs: target recognition and regulatory functions. *cell*. 2009;136(2):215–233. doi:10.1016/j.cell.2009.01.002
42. Ramaswamy S, Ross KN, Lander ES, Golub TR. A molecular signature of metastasis in primary solid tumors. *Nat Genet*. 2003;33(1):49–54. doi:10.1038/ng1060
43. Ibanez de Caceres I, Dulaimi E, Hoffman AM, et al. Identification of novel target genes by an epigenetic reactivation screen of renal cancer. *Cancer Res*. 2006;66(10):5021–5028. doi:10.1158/0008-5472.CAN-05-3365
44. Bonazzi VF, Nancarrow DJ, Stark MS, et al. Cross-platform array screening identifies *COL1A2*, *THBS1*, *TNFRSF10D* and *UCHL1* as genes frequently silenced by methylation in melanoma. *PLoS One*. 2011;6(10):e26121. doi:10.1371/journal.pone.0026121
45. Zang S, Guo R, Xing R, et al. Identification of differentially-expressed genes in intestinal gastric cancer by microarray analysis. *Genomics Proteomics Bioinformatics*. 2014;487(6):276–283. doi:10.1016/j.gpb.2014.09.004
46. Ma HP, Chang HL, Bamodu OA, et al. Collagen 1A1 (*COL1A1*) is a reliable biomarker and putative therapeutic target for hepatocellular carcinogenesis and metastasis. *Cancers*. 2019;11:786.
47. Gao C, Lu W, Lou W, Wang L, Xu Q. Long noncoding RNA HOXC13-AS positively affects cell proliferation and invasion in nasopharyngeal carcinoma via modulating miR-383-3p/*HMG2* axis. *J Cell Physiol*. 2019;234:12809–12820. doi:10.1002/jcp.27915
48. Zhang G, He X, Ren C, Lin J, Wang Q. Long noncoding RNA PCA3 regulates prostate cancer through sponging miR-218-5p and modulating high mobility group box 1. *J Cell Physiol*. 2019;234(8):13097–13109. doi:10.1002/jcp.27980
49. Xie C-R, Wang F, Zhang S, et al. Long noncoding RNA HCAL facilitates the growth and metastasis of hepatocellular carcinoma by acting as a ceRNA of LAPT4B. *Mol Ther Nucleic Acids*. 2017;9:440–451. doi:10.1016/j.omtn.2017.10.018

Cancer Management and Research

Publish your work in this journal

Cancer Management and Research is an international, peer-reviewed open access journal focusing on cancer research and the optimal use of preventative and integrated treatment interventions to achieve improved outcomes, enhanced survival and quality of life for the cancer patient.

Submit your manuscript here: <https://www.dovepress.com/cancer-management-and-research-journal>

Dovepress

The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.