

## Original Article

# Long non-coding RNA taurine-upregulated gene 1 promotes cells proliferation, migration and invasion while represses apoptosis, and upregulates AURKA expression in hepatocellular carcinoma

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**Abstract:** This study aimed to assess the effect of long non-coding RNA (lncRNA) taurine-upregulated gene 1 (TUG1) on cells proliferation, migration, invasion and apoptosis of hepatocellular carcinoma (HCC) cells. LncRNA TUG1 expression was detected in HCC cell lines (SMMC7721, HepG2 and BEL-7402 cells) and normal liver cells (L-02 cells) by qPCR assay. After the transfection of blank mimic, lncRNA TUG1 mimic, blank inhibitor and lncRNA TUG1 inhibitor plasmids into SMMC7721 cells, CKK8, wound-healing, matrigel assay, AV/PI, qPCR assay and western blot assays were performed to detect cells proliferation, migration, invasion, apoptosis, RNA expression and protein expression respectively. LncRNA TUG1 expression was increased in SMMC 7721 cells, HepG2 cells and BEL-7402 cells compared to L-02 cells. After transfection of lncRNA TUG1 mimic and inhibitor plasmids, cells proliferation, migration and invasion were observed to be increased in lncRNA TUG1 mimic group compared to blank mimic group (NC1), while were decreased in lncRNA TUG1 inhibitor group compared with blank inhibitor group (NC2). As to cells apoptosis, AV/PI assay disclosed that lncRNA TUG1 mimic suppressed cells apoptosis rate than NC1 while lncRNA TUG1 inhibitor promoted cells apoptosis rate than NC2, apoptosis markers (C-Caspase3 and Bcl) protein expression also supported the regulation of lncRNA TUG1 on cells apoptosis. In addition, lncRNA TUG1 positively regulated the protein and mRNA expressions of AURKA, but not SERPINE1 or BRAF. In conclusion, lncRNA TUG1 promotes cells proliferation, migration and invasion while represses apoptosis, and upregulates AURKA expression in HCC cells.

**Keywords:** lncRNA TUG1, AURKA, hepatocellular carcinoma (HCC), cells proliferation, migration, invasion, apoptosis

## Introduction

Liver cancer, one of the most frequently diagnosed cancers, leads to approximately 782500 new cases and 745500 deaths during 2012 all over the world, among which more than 50% of the total number of new cases and deaths occur in China [1, 2]. Most of these primary liver cancer patients (70% to 90%) are diagnosed as hepatocellular carcinoma (HCC), which is considered as the sixth most common cancer and the third leading cause of cancer-related deaths worldwide [3]. Although there are tremendous improvements related to regular surveillance for patients with chronic liver disease, early diagnosis and surgical intervention, the 5-year survival rates of HCC patients is still less than 25%, which might result from its high degree of

malignancy, recurrence and metastasis [4, 5]. According to the biological characteristics of malignant tumor, aberrant cell activities via regulating multiple genes, molecules as well as signal pathways, contribute to the promotion of tumorigenesis and progression of carcinomas, thereby affecting the prognosis of cancer patients [6]. Therefore, further understanding of the molecular mechanism of HCC progression may improve the diagnosis and develop novel therapeutic targets.

Long non-coding RNAs (lncRNAs), a class of RNA with a length longer than 200 nt and exhibited limited or no protein-coding capacity, have been reported to express in different tissues or cells, and drive several cancer phenotypes through the interaction with various cellular

macromolecules, such as DNA, RNA or protein [7, 8]. LncRNA taurine-upregulated gene 1 (TUG1), a novel lncRNA with 6.7-kb nucleotides, has been reported to be frequently upregulated in several carcinomas such as pancreatic cancer, colorectal cancer as well as esophageal squamous cell cancer, and serve as an oncogenic factor contributing to the processes of tumorigenesis and prognosis of these carcinomas, while the biological functions of lncRNA TUG1 in HCC is unclear [9-11]. Hence, the purpose of this study is to assess the effect of lncRNA TUG1 on cells proliferation, migration, invasion and apoptosis of HCC cells.

### Materials and methods

#### *Cells culture*

Human HCC cell lines including SMMC 7721, HepG2 and BEL-7402 as well as human normal liver cell line L-02 were purchased from Shanghai Institutes for Biological Science (Shanghai, China). Cells were inoculated in Roswell Park Memorial Institute (RPMI) 1640 (Sigma-Aldrich, USA) medium containing 10% fetal bovine serum (FBS) (Gibco, USA), 100 U/ml penicillin as well as 100 mg/ml streptomycin, and cultured in a humidified incubator at 37°C with 5% CO<sub>2</sub>.

#### *Detection of lncRNA TUG1 expression in different cell lines*

The lncRNA TUG1 expression by quantitative polymerase chain reaction (qPCR) assay was detected in human HCC cell lines SMMC-7721 cells, HepG2 cells and BEL-7402 cells as well as human normal liver cell line L-02 cells. The cell line, which exhibited the most significant difference in lncRNA TUG1 expression compared to L-02 cells, was selected for further experiments.

#### *Transfection of lncRNA TUG1 mimic and inhibitor plasmids into SMMC 7721 cells*

After washing three times with PBS, SMMC 7721 cells were transfected with blank mimic, lncRNA TUG1 mimic, blank inhibitor and lncRNA TUG1 inhibitor plasmids, and then assigned into NC1 mimic group, lncRNA TUG1 mimic group, NC2 inhibitor group and lncRNA TUG1 inhibitor group. After transfection, the subsequent assays were performed: (1) qPCR assay for lncRNA TUG1 expression at 24 hours (h); (2)

CCK8 assay for cells proliferation at 0, 24 and 48 h; (3) Wound-healing assay for cells migration; (4) Matrigel assay for cells invasion 24 h; (5) Annexin V-FITC (AV)-propidium iodide (PI) assay and C-Caspase 3 expression as well as Bcl-2 expression by western blot assay for cells apoptosis at 48 h; (6) Detection of candidate targeted gene expression by qPCR and western blot assays.

#### *CCK-8 assay for cells proliferation*

Cells were kept in the incubator with 5% CO<sub>2</sub> at 37°C after adding 10 ul CCK8 and 90 ul RPMI 1640 medium. The microplate reader was used for measurement of OD value, and the proliferative-detection was carried out after culturing for 0, 24 and 48 h.

#### *Wound-healing assay for cells migration*

After seeded into 6-well plates, cells were cultured until achieving 90% growth confluence. After that, a sterile pipette tip was used to scrape adherent cells gaps, and microscope (Nikon, Japan) was used for cells observation at 0 h and 24 h. The calculation of migration ratio was as follows: (the wound area at 0 h - wound area at 24 h)/wound area at 0 h (ranging from 0%-100%).

#### *Matrigel assay for cells invasion*

After coated with Matrigel basement membrane matrix (BD, USA) in the Upper site of 8-um pore, 6.5-mm transwell filter chamber (Corning, USA) at 37°C for 2 h, cells (5×10<sup>4</sup>) were seeded in the top chamber of a transwell filter. The 4% paraformaldehyde was used to fix the invasive cells on the lower side of the filter after being culture for 24 h, and 0.5% Crystal violet (Sigma-Aldrich, USA) was used to stain invasion cells. The calculation of invasive cells count in each well was the average of invasive cells count of 5 fields in each well with 100× magnification under microscope.

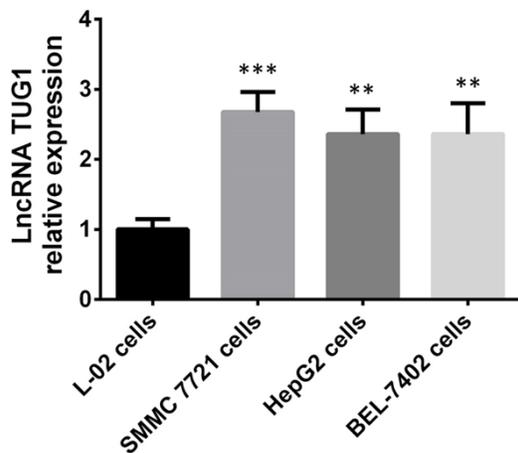
#### *AV/PI assay, C-caspase 3 and Bcl-2 expression for cells apoptosis*

After digested by pancreatin, cells were suspended in 100 ul Binding Buffer supplemented with 5 ul Annexin V-FITC (AV) and kept for 15 minutes at room temperature in the darkness. Before flow cytometry assay, 5 ul Propidium iodide (PI) was added. The analysis of the

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**Table 1.** Antibodies used in this study

Antibody	Company/Country	Dilution ratio
Primary antibody		
Cleaved Caspase-3 Rabbit mAb	CST	1:1000
Caspase-3 Rabbit mAb	CST	1:1000
Anti-Bcl-2 antibody	Abcam	1:1000
Anti-GAPDH antibody	Abcam	1:1000
Anti-PAI1 antibody	Abcam	1:1000
Anti-B-RaB-Raf Rabbit mAb antibody	CST	1:1000
Anti-Aurora A antibody	Abcam	1:1000
Second antibody		
Goat Anti-Rabbit IgGH&L (HRP)	Abcam	1:2000



**Figure 1.** LncRNA TUG1 expression in HCC cells line. Compared to L-02 cells, LncRNA TUG1 expression was increased in SMMC 7721 cells ( $P<0.001$ ), HepG2 cells ( $P<0.01$ ) as well as BEL-7402 cells ( $P<0.01$ ). \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$ .

results was performed by flow cytometry. The cells apoptosis-related protein C-caspase 3 and Bcl-2 expression were detected by western blot assay.

### Detection of candidate targeted gene expression

The detection of mRNA and protein expression of candidate target genes was performed by qPCR and western blot assays, including Aurora kinase A (AURKA), plasminogen activator inhibitor 1 (SERPINE1) and BRAF. Target mRNAs of LncRNA TUG1 were predicted by Rsearch, RNAplex and LncTar database [12-14]. Genes related to HCC pathological processes were subsequently evaluated by DisGenet (www.disgenet.org) [15]. Finally, AURKA, SERPINE1 and

BRAF, potential target genes in HCC were assessed by combining analysis of LncRNA TUG1 predicted target genes and HCC related genes.

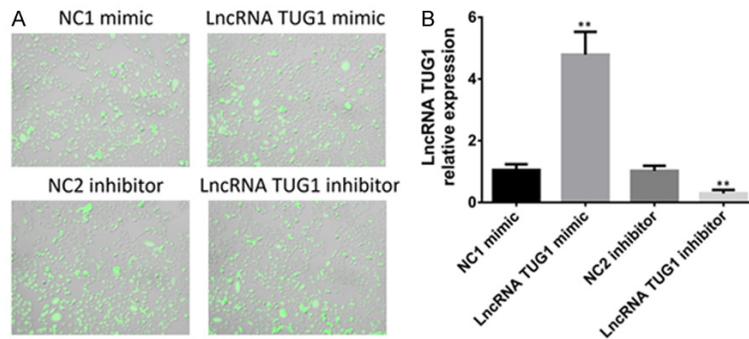
### qPCR assay

LncRNA and mRNA expression were detected by qPCR assay. All procedures were based on the manufacturer's instructions. The TRIzol reagent (Invitrogen, USA) was used to extract total RNAs. The quantitation of RNA was carried out by OD 260 (Takara, Japan). Subsequently, cDNA synthesis was subjected to qPCR with SYBR Green kit (Takara, Japan). The PCR profile was 95°C for 5 min, and 40 cycles of 95°C for 5 s, 61°C for 30 s. GAPDH (mRNAs) or U6 (LncRNAs) were used as the internal reference. Data analysis was carried out using the  $2^{-\Delta\Delta ct}$  method. The primer sequences were as follows: LncRNA TUG1: Forward 5' TAGGAGTGGATGTGTCTGTAGCA 3' Reverse 5' TGGTCGTGGAATATGGTCAATGAG 3'; U6: Forward 5' CTCGCTTCGGCAGCACATA3' Reverse 5'CTCGCTTCGGCAGCACATA3'; GAPDH: Forward 5' GAGTCCACTGGCGTCTTCAC3' Reverse 5' ATCTTGAGGCTGTTGCATACTTCT3'. AURKA: Forward 5' CTGAGGAGGAACTGGCATCAA3' Reverse 5' ATTAGGTAGACTCTGGTAGCATCAT3'; SERPINE1: Forward 5' ACGGCTGGTGTGGTGAAT3' Reverse 5' GCAGTCCASGGATGTCGTAGTAAT3'; BRAF: Forward 5' CGGAGGAGGTGTGGAATA-TCAA3' Reverse 5' GAAGAGGAAGAAGATGTAACGGTAT3'.

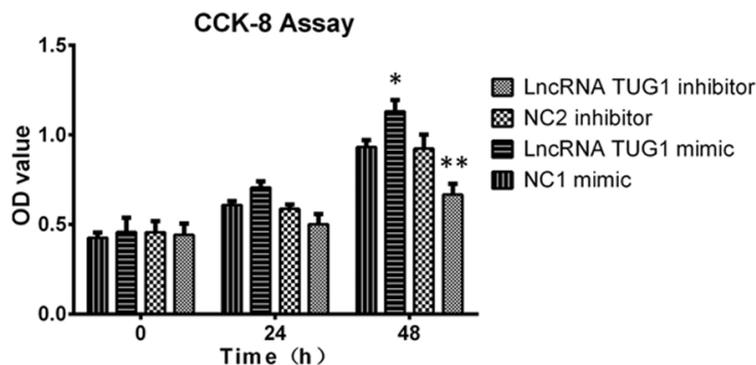
### Western blot assay

Total protein was extracted from lysing cells in 1 ml RIPA buffer (Thermo Fisher Scientific, USA). The protein concentration was performed by bicinchoninic acid (BCA) kit (Pierce Biotechnology, USA) and adjudged according to the standard curve. 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to fractionate 20 µg protein sample, subsequently transferred to polyvinylidene fluoride membranes (Millipore, USA). After blocking by 5% skim milk, the membranes were incubated with the primary antibodies overnight at 4°C overnight (Table 1). After washing, the membranes were incubated with

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**Figure 2.** LncRNA TUG1 expression after LncRNA TUG1 mimic/inhibitor plasmids transfection. A: Transfection rates were all more than 90% in four groups. B: Compared to NC group, the relative expression of LncRNA TUG1 was higher in LncRNA TUG1 mimic group, and lower in LncRNA TUG1 inhibitor group (both  $P<0.01$ ). \* $P<0.05$ , \*\* $P<0.01$ .



**Figure 3.** Cells proliferation after LncRNA TUG1 mimic/inhibitor plasmids transfection. Compared to NC group, cells proliferation was increased in LncRNA TUG1 mimic group ( $P<0.05$ ) and was decreased in LncRNA TUG1 inhibitor group ( $P<0.01$ ). \* $P<0.05$ , \*\* $P<0.01$ .

the secondary antibodies for 1 h at room temperature (Table 1). Protein signaling was detected using an enhanced chemiluminescence (ECL) kit (Millipore, USA).

### Statistics

Statistics was performed using SPSS (SPSS, USA) and GraphPad prim 6.0 (GraphPad, USA). Data were presented in mean  $\pm$  standard deviation. T test was carried out for comparison.  $P<0.05$  (\*) was considered significant,  $P<0.01$  (\*\*) and  $P<0.001$  (\*\*\*) were considered highly statistically significant.

## Results

### LncRNA TUG1 expression in HCC cells line

In order to explore the role of LncRNA TUG1 in HCC cells, qPCR assay was performed. As

shown in Figure 1, LncRNA TUG1 expression was increased in SMMC 7721 cells ( $P<0.001$ ), HepG2 cells ( $P<0.01$ ) as well as BEL-7402 cells ( $P<0.01$ ) compared to L-02 cells.

### LncRNA TUG1 expression after LncRNA TUG1 mimic/inhibitor plasmids transfection

Transfection rates of SMMC 7721 cells were all more than 90% in NC1 mimic group, LncRNA TUG1 mimic group, NC2 inhibitor group and LncRNA TUG1 inhibitor group (Figure 2A). The relative expression of LncRNA TUG1 was higher in LncRNA TUG1 mimic group than that in NC1 mimic group ( $P<0.01$ ), and lower expression of LncRNA TUG1 was observed in LncRNA TUG1 inhibitor group compared to NC2 inhibitor group ( $P<0.01$ ) (Figure 2B).

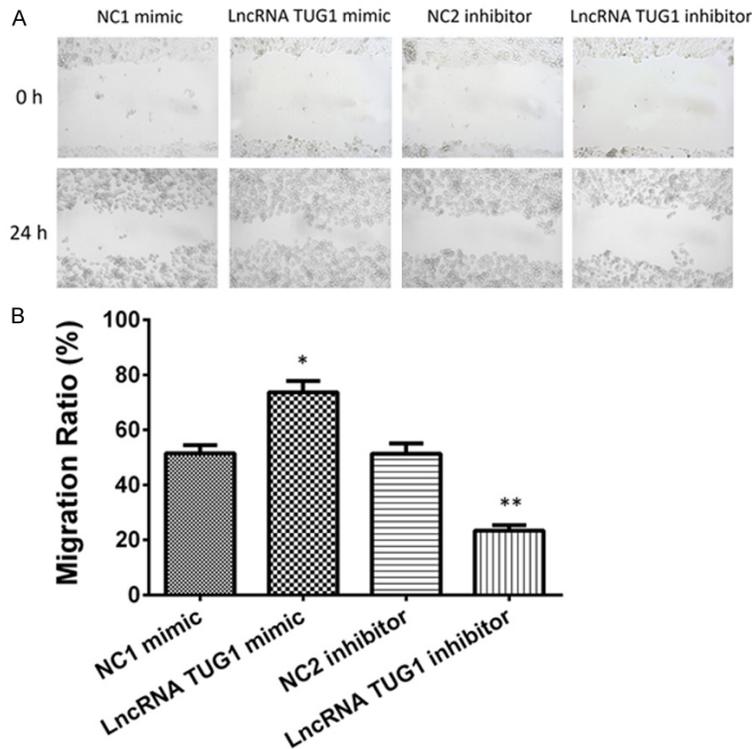
### Cells proliferation after LncRNA TUG1 mimic/inhibitor plasmids transfection

After LncRNA TUG1 mimic/inhibitor plasmids transfection, cells proliferation was evaluated by the CCK-8 assay. Cells proliferation was increased in LncRNA TUG1 mimic group compared to NC1 mimic group ( $P<0.05$ ) and was decreased in LncRNA TUG1 inhibitor group compared to NC2 inhibitor group ( $P<0.01$ ) at 48 h (Figure 3). These suggested that LncRNA TUG1 induced cells proliferation in HCC cells.

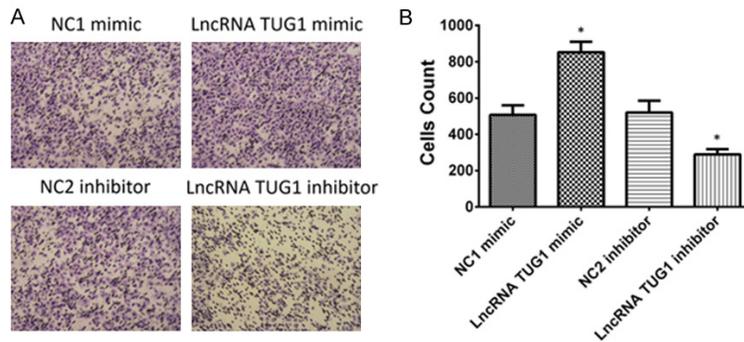
### Cells migration after LncRNA TUG1 mimic/inhibitor plasmids transfection

The wound-healing assay was performed for measurement of cells migration. Compared to NC1 mimic group, the migration ratio of HCC cells was increased in LncRNA TUG1 mimic group ( $P<0.05$ ), while cells migration rate was decreased in LncRNA TUG1 inhibitor group compared to NC2 inhibitor group ( $P<0.01$ ) at 24 h, which indicated that LncRNA TUG1 promoted cells migration in HCC cells (Figure 4A, 4B).

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**Figure 4.** Cells migration after lncRNA TUG1 mimic/inhibitor plasmids transfection. Compared to NC group, the migration ratio of HCC cells was increased in lncRNA TUG1 mimic group ( $P<0.05$ ) and decreased in lncRNA TUG1 inhibitor group ( $P<0.01$ ). \* $P<0.05$ , \*\* $P<0.01$ .



**Figure 5.** Cells invasion after lncRNA TUG1 mimic/inhibitor plasmids transfection. Compared to NC groups, cells invasion was promoted in lncRNA TUG1 mimic group, and was repressed in lncRNA TUG1 inhibitor group (both  $P<0.05$ ). \* $P<0.05$ , \*\* $P<0.01$ .

### Cells invasion after lncRNA TUG1 mimic/inhibitor plasmids transfection

After plasmids transfection, cells invasion was detected by Matrigel assay. Cells invasion was promoted in lncRNA TUG1 mimic group compared to NC1 mimic group ( $P<0.05$ ), and was

repressed in lncRNA TUG1 inhibitor group compared to NC2 inhibitor ( $P<0.05$ ) (Figure 5A, 5B). These results suggested that lncRNA TUG1 accumulated cells invasion in HCC cells.

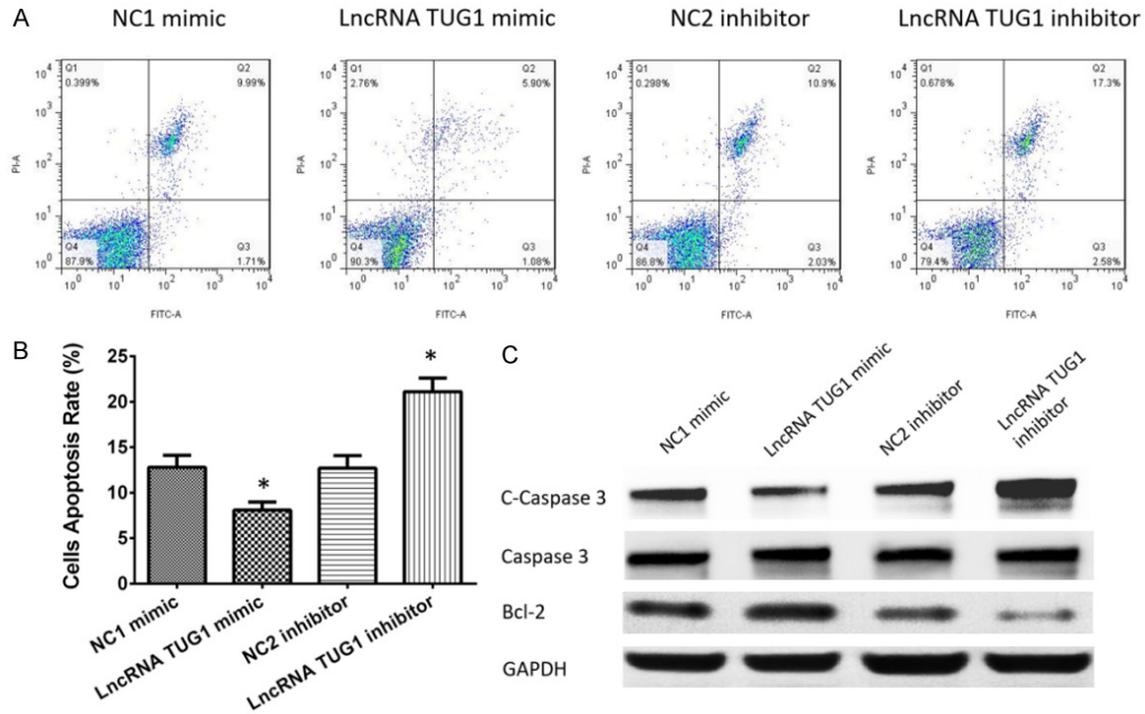
### Cells apoptosis after lncRNA TUG1 mimic/inhibitor plasmids transfection

In order to explore cells apoptosis after plasmids transfection, AV/PI assay was carried out. The knockdown of cells apoptosis rate was observed in lncRNA TUG1 mimic group compared to NC1 mimic group ( $P<0.05$ ), also, the upregulation of cells apoptosis was found in lncRNA TUG1 inhibitor group compared to NC2 inhibitor group ( $P<0.05$ ) (Figure 6A, 6B). Cells apoptosis-related protein was further detected by Western Blot assay. The expressions of C-Caspase were inhibited by lncRNA TUG1 mimic, and were promoted by lncRNA TUG1 inhibitor. And the expression of Bcl-2 was induced by lncRNA TUG1 mimic and was repressed by lncRNA TUG1 inhibitor (Figure 6C). These results indicated that lncRNA TUG1 inhibited cells apoptosis in HCC cells.

### Expression of candidate target genes after lncRNA TUG1 mimic/inhibitor plasmids transfection

After plasmids transfection, lncRNA TUG1 mimic increased both mRNA and protein expressions of AURKA ( $P<0.05$ ), and lncRNA TUG1 inhibitor decreased their expressions ( $P<0.05$ ) compared to NC group, which suggested the positive regulation of lncRNA TUG1 on AURKA in HCC cells (Figure 7A, 7D). No difference of mRNA and protein expressions of SERPINE1 and BRAF between groups was observed (Figure 7B-D).

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**Figure 6.** Cells apoptosis after lncRNA TUG1 mimic/inhibitor plasmids transfection. A, B: Cells apoptosis rate was decreased in lncRNA TUG1 mimic group compared to NC1 mimic group, and increased in lncRNA TUG1 inhibitor group compared to NC2 inhibitor group. C: lncRNA TUG1 decreased C-Caspase 3 expression and increased Bcl-2 expression in HCC cells. \* $P < 0.05$ , \*\* $P < 0.01$ .

### Discussion

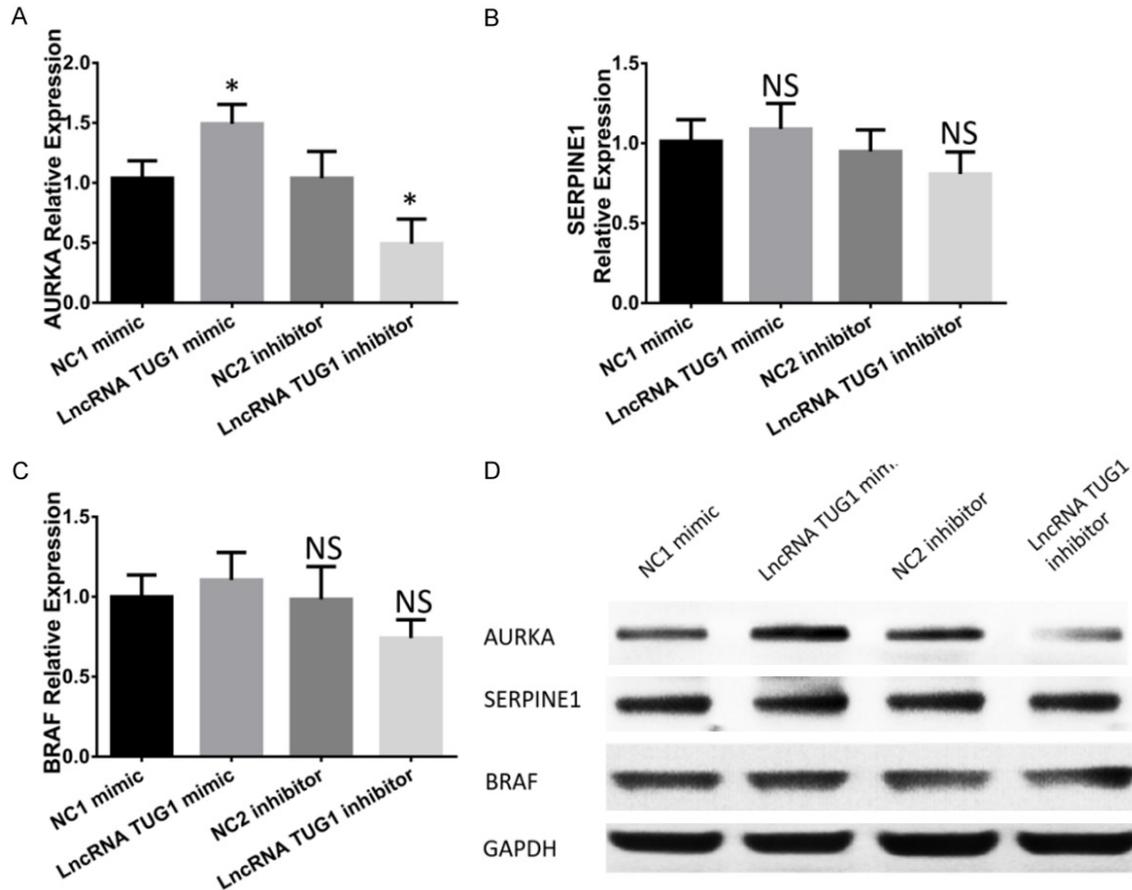
In the current study, we observed that (1) lncRNA TUG1 expression was increased in SMMC 7721 cells, HepG2 cells and BEL-7402 cells compared to L-02 cells; (2) lncRNA TUG1 induced cells proliferation, cells migration as well as cells invasion, and inhibited cells apoptosis in HCC cells; (3) lncRNA TUG1 positively regulated AURKA expression in HCC cells.

lncRNAs, as important functional transcripts, play important roles in the tumorigenesis, metastasis, chemoresistance, prediction of therapy outcomes and viral hepatitis-related progression through regulating multiple genes and pathways in HCC [16-19]. These data suggest that lncRNAs serve as important regulators in HCC pathogenesis.

lncRNA TUG1, a 7598-bp lncRNA located on chromosome 22q12.2, has been reported dys-regulated in several carcinomas [20]. For instance, a previous study discloses that lncRNA TUG1 is upregulated in HCC, which promotes cells proliferation and represses cells

apoptosis by targeting Hedgehog signalling pathways interacted with miR-132 [21]. Another interesting study reveals that overexpression of lncRNA TUG1 is expressed in HCC tissues compared to matched normal tissues, and it promotes cells proliferation, colony formation, tumorigenicity but suppresses cells apoptosis in HCC cells through inhibiting transcription of Kruppel-like factor 2 (KLF2) [22]. Also, lncRNA TUG1 has been reported to correlate with the promotion of cells proliferation, migration and/or invasion in some kinds of carcinomas, including breast cancer, pancreatic cancer and renal cell cancer [9, 23, 24]. These researches indicate that lncRNA TUG1 acts as a regulator involving in the pathology of some cancer through mediating cells activities. Although these two previous studies demonstrate that lncRNA TUG1 promotes cells proliferation and inhibits cells apoptosis in HCC cells, the function of lncRNA TUG1 in other cells activities of HCC cells is still unclear. Therefore, in this study, we explored the functions of lncRNA TUG1 in HCC cells proliferation, migration, invasion as well as apoptosis, and found that lncRNA TUG1 promoted HCC cells proliferation,

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**Figure 7.** Expression of candidate target genes after lncRNA TUG1 mimic/inhibitor plasmids transfection. lncRNA TUG1 mimic increased both mRNA (A) and protein (D) expressions of AURKA ( $P < 0.05$ ), and lncRNA TUG1 inhibitor decreased their expressions ( $P < 0.05$ ) compared to NC group, No difference of mRNA and protein expressions of SERPINE1 (B, D) and BRAF (C, D) between groups was observed. \* $P < 0.05$ , \*\* $P < 0.01$ .

cells migration and cells invasion, while it repressed cells apoptosis. These suggested that lncRNA TUG1 affects HCC pathology via mediating cells proliferation, cells migration, cells invasion as well as cells apoptosis.

AURKA, a serine-threonine kinase, is located on chromosome 20q 13.2, which is interacted with the centrosome to influence chromosomal separation and mitotic spindle stability during mitosis [25]. To date, AURKA serves as an oncogenic factor to drive tumorigenesis and tumor progression in various carcinomas, including HCC, gastric cancer and breast cancer [26-28].

For example, AURKA has been reported to promote HCC cells invasion via facilitating epithelial-mesenchymal transition (EMT) and cancer stem cell (CSC) properties by targeting PI3K/

AKT pathway [28]. As to gastric cancer, AURKA accumulates cells proliferation via the promotion of EMT by targeting the Wnt/ $\beta$ -catenin and PI3K/Akt signaling pathways [29]. Also, AURKA induces cells migration and invasion via the active regulation of AURKA/Akt/focal adhesion kinase (FAK) pathway in head and neck squamous cell cancer [30]. These data illustrate that AURKA affects cells activities through targeting multiple genes or pathways, thereby contributing to the development and progress of various cancer, such as HCC. In the present study, we found the positive regulation of lncRNA TUG1 on AURKA expression in HCC cells, suggested that lncRNA TUG1 might affect cell biological behaviors through targeting AURKA in HCC. Therefore, further study exploring the targeting genes of lncRNA TUG1 in HCC cells is greatly needed.

In conclusion, lncRNA TUG1 promotes cells proliferation, migration and invasion while represses apoptosis, and upregulates AURKA expression in HCC cells.

### Disclosure of conflict of interest

None.

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### References

- [1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; 65: 87-108.
- [2] Forner A, Llovet JM and Bruix J. Hepatocellular carcinoma. *Lancet* 2012; 379: 1245-1255.
- [3] Maluccio M and Covey A. Recent progress in understanding, diagnosing, and treating hepatocellular carcinoma. *CA Cancer J Clin* 2012; 62: 394-399.
- [4] Berzigotti A, Reig M, Abraldes JG, Bosch J and Bruix J. Portal hypertension and the outcome of surgery for hepatocellular carcinoma in compensated cirrhosis: a systematic review and meta-analysis. *Hepatology* 2015; 61: 526-536.
- [5] Ding G, Li W, Liu J, Zeng Y, Mao C, Kang Y and Shang J. LncRNA GHET1 activated by H3K27 acetylation promotes cell tumorigenesis through regulating ATF1 in hepatocellular carcinoma. *Biomed Pharmacother* 2017; 94: 326-331.
- [6] Becht E, Giraldo NA, Germain C, de Reynies A, Laurent-Puig P, Zucman-Rossi J, Dieu-Nosjean MC, Sautes-Fridman C and Fridman WH. Immune contexture, immunoscore, and malignant cell molecular subgroups for prognostic and theranostic classifications of cancers. *Adv Immunol* 2016; 130: 95-190.
- [7] Schmitt AM and Chang HY. Long noncoding RNAs in cancer pathways. *Cancer Cell* 2016; 29: 452-463.
- [8] Ni W, Zhang Y, Zhan Z, Ye F, Liang Y, Huang J, Chen K, Chen L and Ding Y. A novel lncRNA uc.134 represses hepatocellular carcinoma progression by inhibiting CUL4A-mediated ubiquitination of LATS1. *J Hematol Oncol* 2017; 10: 91.
- [9] Zhao L, Sun H, Kong H, Chen Z, Chen B and Zhou M. The lncrna-TUG1/EZH2 axis promotes pancreatic cancer cell proliferation, migration and EMT phenotype formation through sponging Mir-382. *Cell Physiol Biochem* 2017; 42: 2145-2158.
- [10] Wang L, Zhao Z, Feng W, Ye Z, Dai W, Zhang C, Peng J and Wu K. Long non-coding RNA TUG1 promotes colorectal cancer metastasis via EMT pathway. *Oncotarget* 2016; 7: 51713-51719.
- [11] Xu Y, Wang J, Qiu M, Xu L, Li M, Jiang F, Yin R and Xu L. Upregulation of the long noncoding RNA TUG1 promotes proliferation and migration of esophageal squamous cell carcinoma. *Tumour Biol* 2015; 36: 1643-1651.
- [12] Wenzel A, Akbasli E and Gorodkin J. Rsearch: fast RNA-RNA interaction search using a simplified nearest-neighbor energy model. *Bioinformatics* 2012; 28: 2738-2746.
- [13] Tafer H and Hofacker IL. RNAplex: a fast tool for RNA-RNA interaction search. *Bioinformatics* 2008; 24: 2657-2663.
- [14] Li J, Ma W, Zeng P, Wang J, Geng B, Yang J and Cui Q. LncTar: a tool for predicting the RNA targets of long noncoding RNAs. *Brief Bioinform* 2015; 16: 806-812.
- [15] Pinero J, Bravo A, Queralt-Rosinach N, Gutierrez-Sacristan A, Deu-Pons J, Centeno E, Garcia-Garcia J, Sanz F and Furlong LI. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res* 2017; 45: D833-D839.
- [16] Xiao J, Lv Y, Jin F, Liu Y, Ma Y, Xiong Y, Liu L, Zhang S, Sun Y, Tipoe GL, Hong A, Xing F and Wang X. LncRNA HANR promotes tumorigenesis and increase of chemoresistance in hepatocellular carcinoma. *Cell Physiol Biochem* 2017; 43: 1926-1938.
- [17] Li SP, Xu HX, Yu Y, He JD, Wang Z, Xu YJ, Wang CY, Zhang HM, Zhang RX, Zhang JJ, Yao Z and Shen ZY. LncRNA HULC enhances epithelial-mesenchymal transition to promote tumorigenesis and metastasis of hepatocellular carcinoma via the miR-200a-3p/ZEB1 signaling pathway. *Oncotarget* 2016; 7: 42431-42446.
- [18] Tang S, Tan G, Jiang X, Han P, Zhai B, Dong X, Qiao H, Jiang H and Sun X. An artificial lncRNA targeting multiple miRNAs overcomes sorafenib resistance in hepatocellular carcinoma cells. *Oncotarget* 2016; 7: 73257-73269.
- [19] Huang JF, Guo YJ, Zhao CX, Yuan SX, Wang Y, Tang GN, Zhou WP and Sun SH. Hepatitis B virus X protein (HBx)-related long noncoding RNA (lncRNA) down-regulated expression by HBx (Dreh) inhibits hepatocellular carcinoma metastasis by targeting the intermediate filament protein vimentin. *Hepatology* 2013; 57: 1882-1892.
- [20] Zhu J, Shi H, Liu H, Wang X and Li F. Long non-coding RNA TUG1 promotes cervical cancer

## LncRNA in hepatocellular carcinoma

- progression by regulating the miR-138-5p-SIRT1 axis. *Oncotarget* 2017; 8: 65253-65264.
- [21] Li J, Zhang Q, Fan X, Mo W, Dai W, Feng J, Wu L, Liu T, Li S, Xu S, Wang W, Lu X, Yu Q, Chen K, Xia Y, Lu J, Zhou Y, Xu L and Guo C. The long noncoding RNA TUG1 acts as a competing endogenous RNA to regulate the Hedgehog pathway by targeting miR-132 in hepatocellular carcinoma. *Oncotarget* 2017; 8: 65932-65945.
- [22] Huang MD, Chen WM, Qi FZ, Sun M, Xu TP, Ma P and Shu YQ. 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.
- [23] Fan S, Yang Z, Ke Z, Huang K, Liu N, Fang X and Wang K. Downregulation of the long non-coding RNA TUG1 is associated with cell proliferation, migration, and invasion in breast cancer. *Biomed Pharmacother* 2017; 95: 1636-1643.
- [24] Zhang M, Lu W, Huang Y, Shi J, Wu X, Zhang X, Jiang R, Cai Z and Wu S. Downregulation of the long noncoding RNA TUG1 inhibits the proliferation, migration, invasion and promotes apoptosis of renal cell carcinoma. *J Mol Histol* 2016; 47: 421-428.
- [25] Bao Z, Lu L, Liu X, Guo B, Zhai Y, Li Y, Wang Y, Xie B, Ren Q, Cao P, Han Y, Jia W, Chen M, Liang X, Wang X, Zeng YX, He F, Zhang H, Cui Y and Zhou G. Association between the functional polymorphism Ile31Phe in the AURKA gene and susceptibility of hepatocellular carcinoma in chronic hepatitis B virus carriers. *Oncotarget* 2017; 8: 54904-54912.
- [26] Mesic A, Markocic E, Rogar M, Juvan R, Hudler P and Komel R. Single nucleotide polymorphisms rs911160 in AURKA and rs2289590 in AURKB mitotic checkpoint genes contribute to gastric cancer susceptibility. *Environ Mol Mutagen* 2017; 58: 701-711.
- [27] Golmohammadi R, Namazi MJ, Going JJ and Derakhshan MH. A single nucleotide polymorphism in codon F31I and V57I of the AURKA gene in invasive ductal breast carcinoma in Middle East. *Medicine (Baltimore)* 2017; 96: e7933.
- [28] Chen C, Song G, Xiang J, Zhang H, Zhao S and Zhan Y. AURKA promotes cancer metastasis by regulating epithelial-mesenchymal transition and cancer stem cell properties in hepatocellular carcinoma. *Biochem Biophys Res Commun* 2017; 486: 514-520.
- [29] Liu X, Li Z, Song Y, Wang R, Han L, Wang Q, Jiang K, Kang C and Zhang Q. AURKA induces EMT by regulating histone modification through Wnt/beta-catenin and PI3K/Akt signaling pathway in gastric cancer. *Oncotarget* 2016; 7: 33152-33164.
- [30] Wu J, Yang L, Shan Y, Cai C, Wang S and Zhang H. AURKA promotes cell migration and invasion of head and neck squamous cell carcinoma through regulation of the AURKA/Akt/FAK signaling pathway. *Oncol Lett* 2016; 11: 1889-1894.