

Original Article

lncRNA TUG1 promotes cell growth and epithelial-mesenchymal transition in human cervical cancer

Yuxia Gao¹, Wenyu Di², Hongyu Li³, Caixia Chen¹, Zhimei Li⁴

¹Department of Gynecological Oncology I, Xinxiang Central Hospital, Xinxiang 453000, Henan, China;

²Department of Pathology, The First Affiliated Hospital of Xinxiang Medical University, Weihui 453100, Henan,

China; ³Department of Gynecological Oncology, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou

450052, Henan, China; ⁴Department of Obstetrics and Gynecology, The People's Hospital of Huixian, Xinxiang 453600, Henan, China

Received July 29, 2016; Accepted August 20, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: Background: Taurine up-regulated gene 1 (TUG1) functions as an oncogene in several human cancers. However, its role in cervical cancer is still unclear. The aim of this study was to explore its effect on cervical cancer progression. Methods: The relative expression level of lncRNA TUG1 was determined by qRT-PCR in a total of 59 patients with cervical cancer. We inhibited TUG1 expression by transfecting TUG1 specific siRNA (si-TUG1). Cell proliferation was determined by using both MTT assay and colony formation assay. Cell cycle and cell apoptosis and were explored by using flow cytometric analysis. Epithelial-mesenchymal transition (EMT) related gene expression was determined by using Western blot. Results: We first reported that TUG1 was significantly overexpressed in cervical cancer tissues; TUG1 up-regulation was associated with advanced clinical features and overall survival time of cervical cancer patients. Function assays reported that inhibition of TUG1 suppressed cervical cancer cell proliferation through promoting cell apoptosis and inhibiting cell cycle progression. In addition, knockdown of TUG1 inhibited EMT phenotype in cervical cancer cells. Conclusion: Our study suggested that TUG1 could promote progression and development of cervical cancer and could act as an oncogene in cervical cancer. TUG1 could be used as a therapeutic target for the treatment of cervical cancer.

Keywords: Cervical cancer, TUG1, growth, progression, ETM

Introduction

Cervical cancer is a common gynecological malignancy that is a leading cause of cancer related mortality among women worldwide [1]. Although, several therapeutic approaches are available for cervical cancer, including surgery, chemotherapy and radiotherapy [2], the prognosis of cervical cancer patients remains unsatisfactory, with a 5-year overall survival of approximate 36% [3]. Therefore, a better understanding of the molecular mechanisms involved in cervical cancer progression will help identify new diagnostic and therapeutic targets.

Approximately 93% of the human genome DNA can be transcribed into RNAs, among which only 2% can be translated into proteins and remaining 98% appears as non-coding RNAs [4]. Long non-coding RNAs (lncRNAs) are transcribed RNA molecules longer than 200 nucle-

otides but have no protein-coding potential [5]. Recent evidence suggested that lncRNAs have a vital role in the regulation of cellular processes such as differentiation, proliferation and metastasis [6, 7]. Aberrant expressions of certain lncRNAs have been confirmed in a variety of cancers, including cervical cancer. For example, Cao et al indicated that down-regulation of lncRNA GAS5 correlated with tumor progression and poor prognosis in cervical cancer [8]. Sun et al showed that down-regulation of MALAT1 inhibited cervical cancer cell invasion and metastasis by inhibition of epithelial mesenchymal transition [9]. Zhang et al found that lncRNA MEG3 was down-regulated in cervical cancer and affected cell proliferation and apoptosis by regulating miR-21 [10]. However, to our knowledge, research of lncRNAs in cervical cancer is still in its infancy.

Taurine upregulated gene 1 (TUG1) was initially characterized by a genomic screening study in

TUG1 promote ETM in cervical cancer

mouse retinal cells [11]. Recent studies suggested that lncRNA TUG1 play important roles in tumor progression. For example, Jiang et al indicated that high TUG1 expression was associated with chemotherapy resistance and poor prognosis in esophageal squamous cell carcinoma [12]. Zhang et al showed that down-regulation of TUG1 inhibited the proliferation, migration, invasion and promotes apoptosis of renal cell carcinoma [13]. Sun et al found that upregulated expression of TUG1 indicated a poor prognosis for colorectal cancer and promoted metastasis by affecting epithelial mesenchymal transition [14]. By contrast, Li et al showed that lncRNA TUG1 acted as a tumor suppressor in human glioma by promoting cell apoptosis [15]. However, the expression pattern and function of TUG1 in cervical cancer have rarely been reported

In this study, we explored the clinical significance of lncRNA TUG1 in cervical cancer and further identify the biological functions of TUG1 on cell growth and epithelial mesenchymal transition (EMT) of cervical cancer cells.

Materials and methods

Patients and sample collection

A total of 59 pairs of cervical cancer tissues and adjacent non-tumor tissues were obtained from patients undergoing surgical procedures at Xinxiang Central Hospital and The First Affiliated Hospital of Xinxiang Medical University, between January 2010 and January 2011. All patients recruited in this study were not subjected to preoperative radiotherapy and/or chemotherapy and were diagnosed as infiltrating carcinoma by pathology. The tumor stage was classified by two experienced gynecological oncologists according to the International Federation of Gynecology and Obstetrics (FIGO) staging system for cervical cancer. After resection, all samples were immediately stored in liquid nitrogen until use. This study was approved by the Human Research Ethics Committee of Xinxiang Central Hospital. Written informed consent was obtained from all patients.

Cell lines and culture conditions

Two cervical cancer cell lines (HeLa and SiHa) were purchased from the Institute of Biochemistry and Cell Biology of the Chinese

Academy of Sciences (Shanghai, China). Cells were cultured in DMEM (Gibco) medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco) in humidified air at 37°C with 5% CO₂.

Cell transfection

TUG1-specific siRNAs (si-TUG1) and the negative control siRNA (si-NC) were purchased from GenePharma. Target sequences for TUG1 siRNAs were as follows: si-TUG1-1, 5'-GTACGTG-TCTTGGAAGTCT-3'; si-TUG1-2, 5'-GCCAAATAAC-TGAAGCTAT-3'. Cells were transfected with siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

Cell proliferation and clonogenic assay

MTT assay was used to detect cell viability. In brief, cells were seeded at 1×10^4 cells/well in a 96-well culture plate. After the required treatments, these cells were incubated with MTT (0.5 mg/ml) for 3 h at 37°C. After medium removal, 100 μ l DMSO solution was added to dissolve formazan crystal. The absorbance at 570 nm wavelength was detected using a microplate reader (Molecular Devices). For clonogenic assay, 500 cells were plated in each well of a six-well plate. When there was visible colony by naked eye, cells were fixed with methanol and stained with 0.1% crystal violet (Sigma). Colonies were then counted.

Flow cytometric analysis of cell apoptosis and cell cycle

Transfected human cervical cancer cells were harvested and stained with propidium iodide according to the Cell Cycle Analysis Kit (Biyuntian), and then assessed by flow cytometer. The percentage of the cells in different phases was counted. For apoptosis analysis, FITC Annexin V Apoptosis Detection Kit (BD Biosciences) was used. The transfected cells were resuspended in binding buffer containing Annexin V-FITC and propidium iodide, and assessed by flow cytometer according to the manufacturer's instructions.

Western blot analysis

Proteins retrieved from cell lysates were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to 0.22 mm nitrocellulose membrane, and incubated with primary antibodies (Abcam). Then the blots

TUG1 promote ETM in cervical cancer

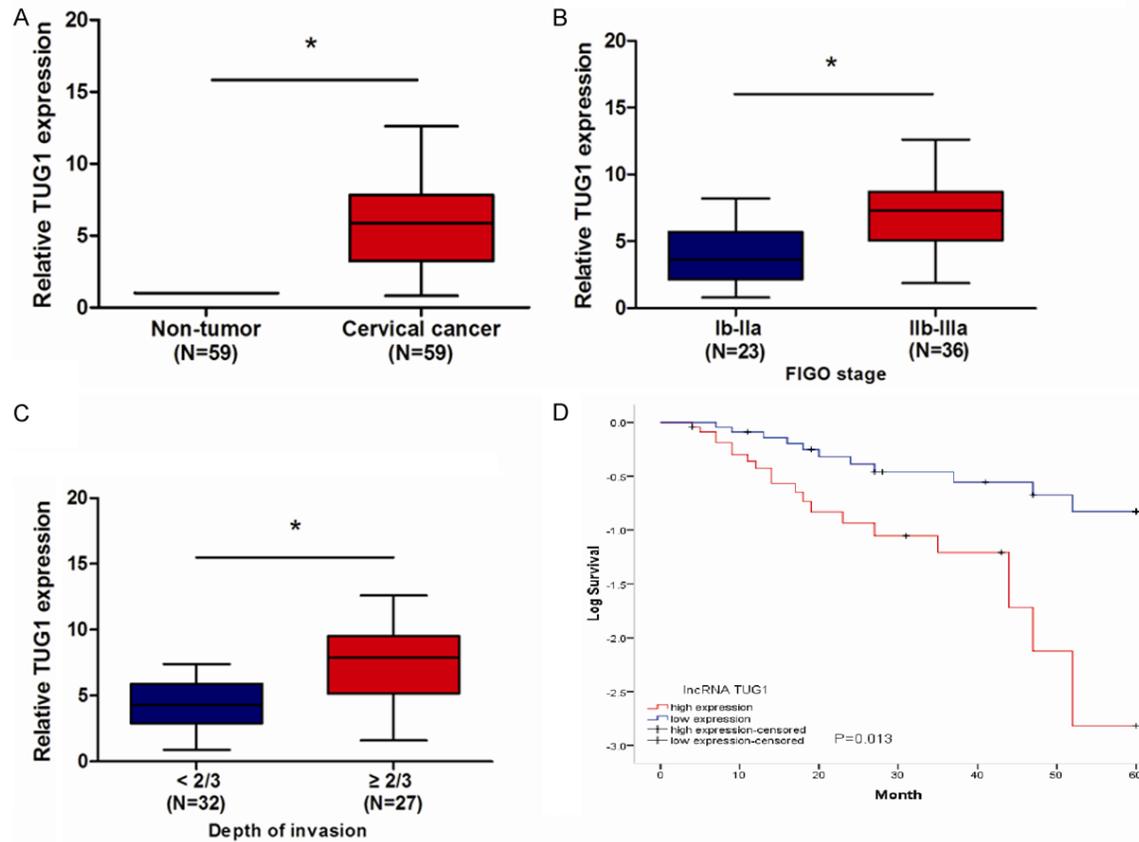


Figure 1. Upregulated expression of lncRNA TUG1 in cervical cancer tissues. A. The expression of lncRNA TUG1 in cervical cancer tissues and adjacent non-tumor tissues. B. The expression of lncRNA TUG1 in different FIGO stage of cervical cancer patients. C. The expression of lncRNA TUG1 in different depth of cervical invasion of cervical cancer patients. D. Kaplan-Meier analyses of correlations between the TUG1 expression and overall survival of 59 cervical cancer patients. *P<0.05.

were incubated with fluorescence labeled secondary antibodies and detected using an Odyssey infrared scanner (Li-Cor Biosciences).

Statistical analysis

Statistical analyses were performed using the SPSS 18.0 software package. The quantitative data were presented as the mean \pm SD. Differences among the groups were assessed by Wilcoxon signed-rank test, Pearson chi-square test, Log-rank test or Student's t test as indicated. Differences were considered to be statistically significant at values of $P < 0.05$.

Results

lncRNA TUG1 is up-regulated in cervical cancer

To explore the clinical relevance of lncRNA TUG1 expression in cervical cancer, we per-

formed qRT-PCR assay to detect the TUG1 expression in 59 patients with paired cervical cancer tissues and adjacent non-tumor tissues. Our data showed that the expression level of TUG1 was significantly increased in cervical cancer tissues comparison with the adjacent non-tumor tissues (**Figure 1A**, $P < 0.05$). Next, we analyzed the correlation between the TUG1 expression and clinicopathological features of the 59 cervical cancer patients. The data revealed that TUG1 expression was upregulated in cervical cancer tissues with advanced FIGO stage and depth of cervical invasion (**Figure 1B** and **1C**, $P < 0.05$). Then, the 59 cervical cancer patients were divided into a high TUG1 expression group ($n = 30$) and a low expression group ($n = 29$) based on the median value of relative TUG1 expression. As shown in **Table 1**, lncRNA TUG1 up-regulation was correlated with advanced FIGO stage and depth of cervical invasion ($P < 0.05$). But not correlated

TUG1 promote ETM in cervical cancer

Table 1. Clinicopathological features associated with lncRNA TUG1 expression in 59 cervical cancer patients

Clinicopathological features	Total	TUG1 expression		P value
		Low	High	
Age				
<45	23	10	13	0.486
≥45	36	19	17	
Tumor size (cm)				
<4.0	27	12	15	0.506
≥4.0	32	17	15	
Histologic grade				
G1 + G2	39	22	17	0.119
G3	20	7	13	
FIGO stage				
Ib~IIa	23	16	7	0.012
IIb~IIIa	36	13	23	
Lymph node metastasis				
No	46	25	21	0.133
Yes	13	4	9	
Depth of cervical invasion				
<2/3	32	21	11	0.006
≥2/3	27	8	19	

with other clinicopathological features ($P>0.05$). We further determined the correlation between TUG1 expression and cervical cancer patients' prognosis. Kaplan-Meier survival analysis revealed that cervical cancer patients with high TUG1 expression had a shorter overall survival time than those patients with low TUG1 expression (**Figure 1D**; $P<0.05$). Taken together, these data suggested that TUG1 might play an important role in cervical cancer progression.

Inhibition of lncRNA TUG1 decreases cell proliferation in vitro

To investigate the potential role of lncRNATUG1 in the proliferation of cervical cancer cells, we constructed specific-TUG1 siRNA (si-TUG1) to inhibit TUG1 expression in cervical cancer cells. 48 h after transfection, qRT-PCR showed that si-TUG1 efficiently knocked-down TUG1 expression in HeLa and SiHa cells (**Figure 2A**; $P<0.05$). Then, MTT assay revealed that inhibition of TUG1 significantly suppressed cell proliferation both in HeLa and SiHa cells (**Figure 2B**; $P<0.05$). Similarly, the results of colony formation assay suggested that clonogenic survival

was significantly inhibited following down-regulation of TUG1 in HeLa and SiHa cells by si-TUG1 (**Figure 2C**; $P<0.05$). Those findings indicated that TUG1 could act as an oncogene involved in the promotion of cervical cancer cell proliferation.

Inhibition of lncRNA TUG1 inhibits cell cycle and promotes cell apoptosis progression

Next, flow cytometric analysis was performed to explore whether the effect of lncRNA TUG1 on cell proliferation is mediated by altering cell apoptosis or cell cycle progression. Flow cytometric analysis revealed that depletion expression of TUG1 in HeLa and SiHa cells increased the percentage of cells at G0/G1 phase, and decreased the percentage of cells at S and G2/M phases (**Figure 3A**; $P<0.05$). Furthermore, flow cytometric analysis showed that the proportion of apoptotic HeLa and SiHa cells with TUG1 inhibition was increased compared to control group (**Figure 3B**; $P<0.05$). These results suggested that inhibition of TUG1 suppressed cervical cancer cell proliferation through promoting cell apoptosis and inhibiting cell cycle progression.

Effects of lncRNA TUG1 on EMT in cervical cancer

To explore the effect of lncRNA TUG1 on EMT of cervical cancer cells, Western blot were performed to explore the expression of EMT-related markers in HeLa and SiHa cells after transfection with si-TUG1. As shown in **Figure 4**, decreased expression of TUG1 in HeLa and SiHa cells remarkably decreased the expression of Fibronectin and Vimentin and meanwhile greatly increased the expression of E-cadherin in comparison with control groups ($P<0.05$). Those data suggested that decreased expression of TUG1 could significantly inhibit the EMT process of cervical cancer.

Discussion

The prognosis of cervical cancer remains quite poor, because it is an aggressive malignance with rapid growth rate and high chance of regional and distant metastasis [16]. Thus, early diagnosis is helpful to improve the prognosis of cervical cancer. Recently, lots of lncRNAs have been characterized, and numerous pieces of evidence showed that they play

TUG1 promote ETM in cervical cancer

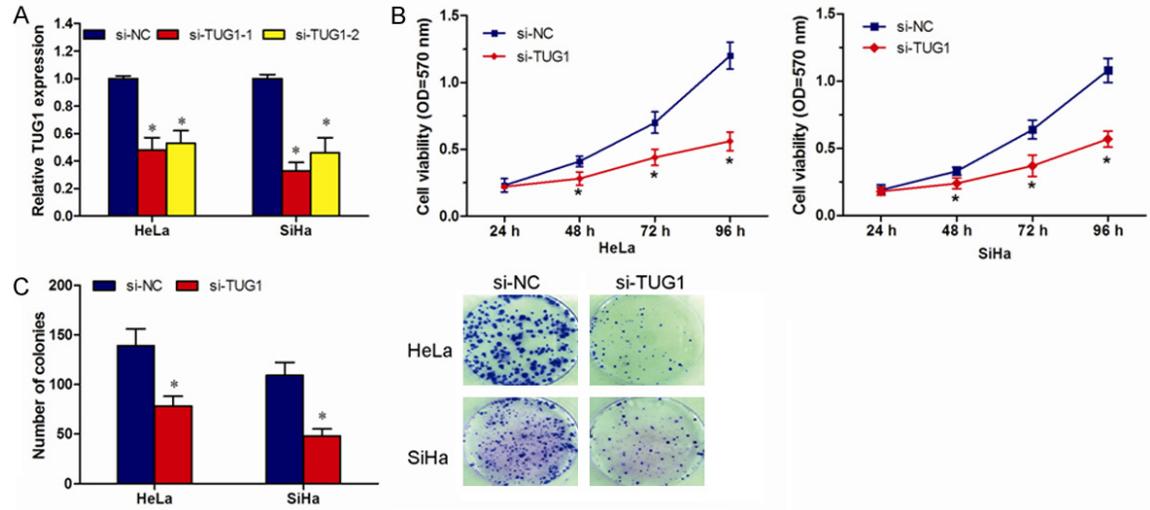


Figure 2. Inhibition of lncRNA TUG1 inhibits cervical cancer cell proliferation. A. qRT-PCR showing the expression of TUG1 in si-TUG1 transfected HeLa and SiHa cells was decreased compared with control cells (si-NC). B. MTT assay showing inhibition of TUG1 suppressed proliferation of HeLa and SiHa cells. C. Colony-formation assay showing silencing of TUG1 significantly decreased the colony-forming ability of HeLa and SiHa cells. *P<0.05.

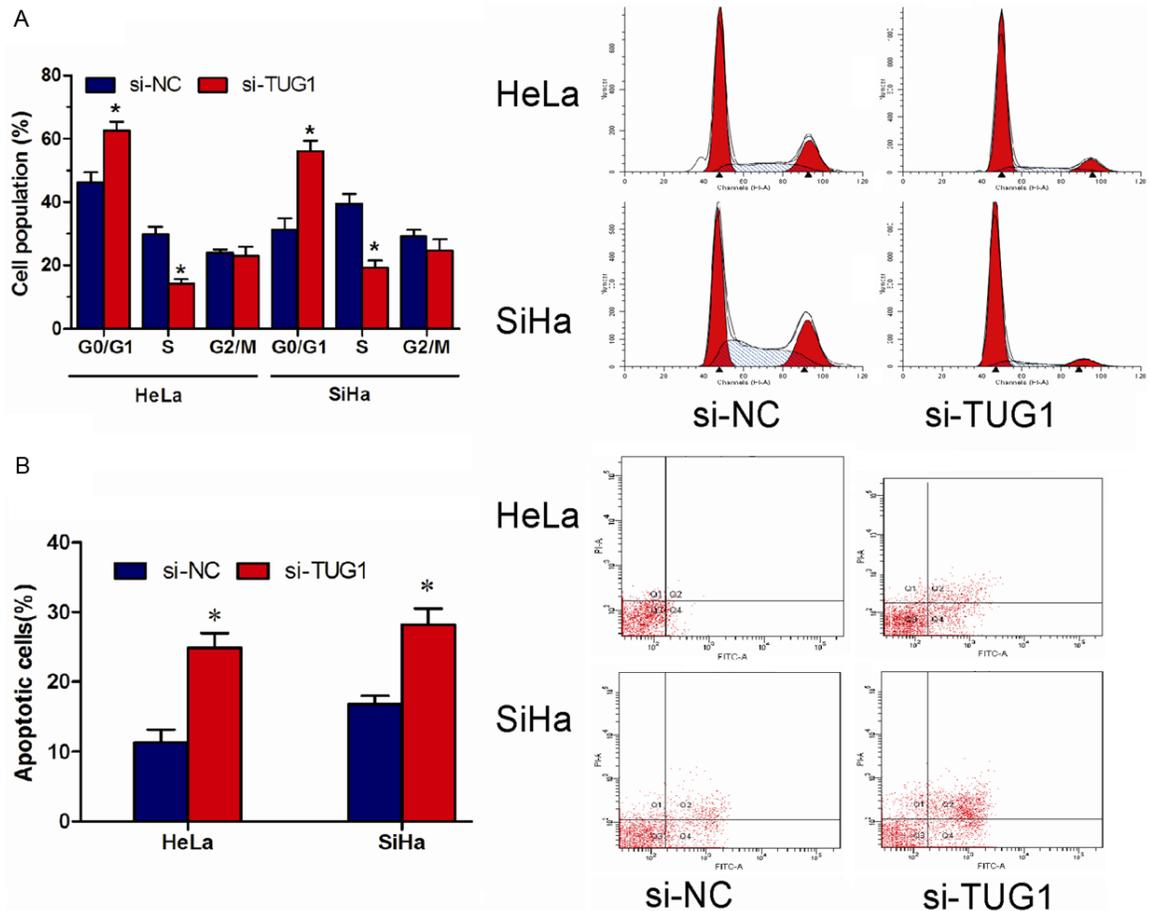


Figure 3. IncRNA TUG1 regulated cervical cancer cell apoptosis and cell cycle in vitro. A. Down-regulated expression of TUG1 arrested HeLa and SiHa cells in G1/G0 phase compared to the si-NC group. B. Inhibition of TUG1 promoted apoptosis in HeLa and SiHa cells compared to the si-NC group. *P<0.05.

TUG1 promote EMT in cervical cancer

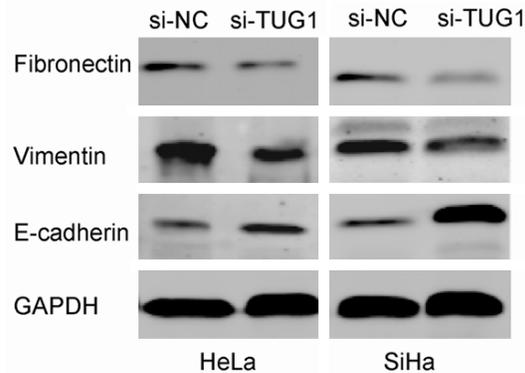


Figure 4. Effect of TUG1 on EMT related-gene expression in cervical cancer cells. Western blot analysis of EMT-related gene expression in si-TUG1 transfected HeLa and SiHa cells. * $P < 0.05$.

an important role in cancer pathogenesis, indicating that they could provide new insights into the biology of this disease [6]. LncRNA dysregulation contributes to a range of biological functions and provides a cellular growth advantage, resulting in progressive and uncontrolled tumor growth [17]. Effective control of both cell growth and motility is critical to the prevention of tumorigenesis and successful cancer therapy [18]. So, identification of cervical cancer associated lncRNAs and investigation of their clinical significance and functions are great clinical value for the diagnosis and treatment of cervical cancer.

Pervasive studies showed that lncRNA TUG1 was aberrantly expressed and participated in tumor development and progression. In the present study, we explored the expression of TUG1 in cervical cancer tissues and adjacent non-tumor tissues. Furthermore, we conducted a series of experiments to investigate the function of TUG1 acted in cervical cancer progression. We found that lncRNA TUG1 was significantly overexpressed in cervical cancer tissues and TUG1 overexpression was associated with advanced clinical features and overall survival time of cervical cancer patients. Function assays reported that inhibition of TUG1 suppressed the cervical cancer cell proliferation through inhibiting cell cycle progression and promoting cell apoptosis. Those findings suggested that lncRNA TUG1 play important roles in cervical cancer progression.

EMT is a process whereby cancer cells lose their epithelial properties to acquire a mesenchymal phenotype and become motile and

invasive, which is closely associated with metastasis [19]. Recent studies suggested that lncRNA play critical roles in EMT progression. For example, Wang et al showed that lncRNA CPS1-IT1 suppressed the metastasis of hepatocellular carcinoma by regulating HIF-1 α activity and inhibiting epithelial-mesenchymal transition [20]. Wang et al found that upregulation of lncRNA H19 indicated a poor prognosis in gallbladder carcinoma and promotes epithelial mesenchymal transition [21]. Xu et al suggested that down-regulation of lncRNA MALAT1 induced epithelial-to-mesenchymal transition via the PI3K-AKT pathway in breast cancer [22]. In our study, we found that knockdown of TUG1 remarkably decreased the expression of fibronectin and vimentin and meanwhile greatly increased the expression of E-cadherin in cervical cancer cells, indicating that the effects of lncRNA TUG1 on cervical cancer cell growth was partly associated with EMT process.

In conclusion, we suggested that lncRNA TUG1 significantly contributed to cervical cancer progression. Suppression of TUG1 inhibited cervical cancer cell growth and EMT.

Therefore, lncRNA TUG1 may be considered as a novel molecular target for the diagnosis and treatment of cervical cancer.

Acknowledgements

The project was supported by the Science and Technology Department of Zhengzhou (No. 153PKJGG165) and the Key Laboratory of Gynecological Oncologists of Zhengzhou City (N2013SC0763).

Disclosure of conflict of interest

None.

Address correspondence to: Caixia Chen, Department of Gynecological Oncology I, Xinxiang Central Hospital, No. 56 Jinsui Road, Xinxiang 453000, Henan, China. E-mail: geningjiang65@gmail.com

References

- [1] Siegel R, Naishadham D and Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; 63: 11-30.
- [2] Arbyn M, Raifu AO, Autier P and Ferlay J. Burden of cervical cancer in Europe: estimates for 2004. *Ann Oncol* 2007; 18: 1708-1715.
- [3] Schiffman M, Castle PE, Jeronimo J, Rodriguez AC and Wacholder S. Human papillomavirus

Int J Clin Exp Pathol 2016;9(10):10327-10333

TUG1 promote ETM in cervical cancer

- and cervical cancer. *Lancet* 2007; 370: 890-907
- [4] Mattick JS and Makunin IV. Non-coding RNA. *Hum Mol Genet* 2006; 15 Spec No 1: R17-29.
- [5] Mercer TR, Dinger ME and Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 2009; 10: 155-159.
- [6] Gibb EA, Brown CJ and Lam WL. The functional role of long non-coding RNA in human carcinomas. *Mol Cancer* 2011; 10: 38.
- [7] Fatica A and Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet* 2014; 15: 7-21.
- [8] Cao S, Liu W, Li F, Zhao W and Qin C. Decreased expression of lncRNA GAS5 predicts a poor prognosis in cervical cancer. *Int J Clin Exp Pathol* 2014; 7: 6776-6783.
- [9] Sun R, Qin C, Jiang B, Fang S, Pan X, Peng L, Liu Z, Li W, Li Y and Li G. Down-regulation of MALAT1 inhibits cervical cancer cell invasion and metastasis by inhibition of epithelial-mesenchymal transition. *Mol Biosyst* 2016; 12: 952-962.
- [10] Zhang J, Yao T, Wang Y, Yu J, Liu Y and Lin Z. Long noncoding RNA MEG3 is downregulated in cervical cancer and affects cell proliferation and apoptosis by regulating miR-21. *Cancer Biol Ther* 2016; 17: 104-113.
- [11] Young T, Matsuda T and Cepko C. The noncoding RNA taurine upregulated gene 1 is required for differentiation of the murine retina. *Curr Biol* 2005; 15: 501-512.
- [12] Jiang L, Wang W, Li G, Sun C, Ren Z, Sheng H, Gao H, Wang C and Yu H. High TUG1 expression is associated with chemotherapy resistance and poor prognosis in esophageal squamous cell carcinoma. *Cancer Chemother Pharmacol* 2016; 78: 333-9.
- [13] 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.
- [14] Sun J, Ding C, Yang Z, Liu T, Zhang X, Zhao C and Wang J. 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: 42.
- [15] Li J, Zhang M, An G and Ma Q. LncRNA TUG1 acts as a tumor suppressor in human glioma by promoting cell apoptosis. *Exp Biol Med (Maywood)* 2016; 241: 644-9.
- [16] Berman ML, Keys H, Creasman W, DiSaia P, Bundy B and Blessing J. Survival and patterns of recurrence in cervical cancer metastatic to periaortic lymph nodes (a Gynecologic Oncology Group study). *Gynecol Oncol* 1984; 19: 8-16.
- [17] Prensner JR and Chinnaiyan AM. The emergence of lncRNAs in cancer biology. *Cancer Discov* 2011; 1: 391-407.
- [18] Bergers G and Benjamin LE. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 2003; 3: 401-410.
- [19] Zavadil J, Haley J, Kalluri R, Muthuswamy SK and Thompson E. Epithelial-mesenchymal transition. *Cancer Res* 2008; 68: 9574-9577.
- [20] Wang TH, Yu CC, Lin YS, Chen TC, Yeh CT, Liang KH, Shieh TM, Chen CY and Hsueh C. Long noncoding RNA CPS1-IT1 suppresses the metastasis of hepatocellular carcinoma by regulating HIF-1 α activity and inhibiting epithelial-mesenchymal transition. *Oncotarget* 2016; [Epub ahead of print].
- [21] Wang SH, Wu XC, Zhang MD, Weng MZ, Zhou D and Quan ZW. Upregulation of H19 indicates a poor prognosis in gallbladder carcinoma and promotes epithelial-mesenchymal transition. *Am J Cancer Res* 2016; 6: 15.
- [22] Xu S, Sui S, Zhang J, Bai N, Shi Q, Zhang G, Gao S, You Z, Zhan C and Liu F. Downregulation of long noncoding RNA MALAT1 induces epithelial-to-mesenchymal transition via the PI3K-AKT pathway in breast cancer. *Int J Clin Exp Pathol* 2015; 8: 4881.