European Review for Medical and Pharmacological Sciences 2019; 23: 3733-3741

Long noncoding RNA TPTE2P1 promotes the migration and invasion of hepatocellular carcinoma

J. LIU[1], Z. WANG[1], Y. YIN[1], N. LI[1], N.-Y. YE[1], B. BAO[2], H.-C. GU[3]

[1]Departments of Gastroenterology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, China.
[2]Departments of Gastroenterology, Yizheng People's Hospital, Yizheng, China
[3]Departments of Gastroenterology, Yangzhong Hospital of Traditional Chinese Medicine, Yangzhong, China

Abstract. – OBJECTIVE: Hepatocellular carcinoma (HCC) is a common malignant tumor that poses a serious threat to human health and life. Metastasis is one of the reasons for high rate of relapse. Due to the lack of effective treatment, the prognosis of HCC patients is far from satisfactory. The aim of this study was to investigate the role of long non-coding RNA (lncRNA)-TPTE2P1 in HCC development. Moreover, we aimed to search for new biomarkers which could predict the metastasis and provide novel therapeutic strategies for HCC.

PATIENTS AND METHODS: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to evaluate the expression level of lncRNA-TPTE2P1 in both HCC tissues and cell lines. Wound-healing assay and transwell assay were applied to determine the ability of cell migration. Meanwhile, transwell matrigel assay was applied to detect the invasion of HCC cells. The protein expressions of E-cadherin, Vimentin and N-cadherin in chosen cell lines were detected by Western blotting.

RESULTS: Results found that lncRNA-TPTE2P1 was overexpressed in both HCC tissues and cell lines. Further analysis revealed that overexpression of lncRNA-TPTE2P1 was correlated with tumor size, distant metastasis, differentiation degree, as well as tumor node metastasis (TNM) stage of HCC patients. Subsequent wound-healing assay, transwell assay and Matrigel assay confirmed that down-regulated lncRNA-TPTE2P1 could significantly suppress the invasion and migration of cells. However, up-regulation of lncRNA-TPTE2P1 showed the opposite results. Moreover, lowly expressed lncRNA-TPTE2P1 significantly decreased the protein levels of E-cadherin, Vimentin and N-cadherin. These results indicated that lncRNA-TPTE2P1 might stimulate the migration and invasion of HCC cells by promoting epithelial-mesenchymal transition (EMT).

CONCLUSIONS: In summary, our results suggested that lncRNA-TPTE2P1 functioned as an oncogene in HCC. Meanwhile, lncRNA-TPTE2P1 stimulated HCC cell migration and invasion by promoting EMT. LncRNA-TPTE2P1 might play a vital role in the development and progression of HCC. Our findings demonstrated that lncRNA-TPTE2P1 could serve as an early biomarker of metastasis and therapeutic target for HCC.

Key Words:
LncRNA-TPTE2P1, Hepatocellular carcinoma (HCC), Metastasis, Epithelial-mesenchymal transition (EMT)

Introduction

Hepatocellular carcinoma (HCC) is a common malignant tumor that poses serious threat to human health and life. Siegel *et al*[1] have found that the mortality rate of liver cancer ranks fifth among all cancer-related deaths in the United States. According to the statistics of Chen *et al*[2], around 422.1 thousands of people died of liver cancer in 2015 in our country. Among all types of liver cancer, HCC accounts for about 90%[3]. Due to the lack of effective treatment, the mortality rate of HCC is relatively high. On the other hand, high rate of recurrence and metastasis may also be the major reasons[4]. Although early diagnosis and treatment techniques are constantly improving, the survival time of HCC patients has not been significantly ameliorated. Therefore, it is great importance to search for new early biomarkers of metastasis. Furthermore, the development of therapeutic targets for efficient treatment of HCC is also important.

Corresponding Author: Juan Liu, MD; email: Njliujuan77@163.com

The molecular mechanisms of tumor formation have usually been focused on protein-coding genes. Recent high-throughput sequencing has found that more than 90% of transcriptions are related with non-coding RNAs. As a kind of non-coding RNAs, long non-coding RNAs (lncRNAs) have been identified to participate in a variety of biological processes[5, 6]. Multiple studies have shown that the abnormal expression of lncRNAs is closely related to the development of various malignancies[7-9]. Tumor cells can induce aberrant gene expression, activity or localization of growth promoting and inhibitory factors through abnormal regulation of lncRNAs. Ultimately, this may lead to tumorigenesis and metastasis[10].

In the current research, it was found that lncRNA-TPTE2P1 was overexpressed in both HCC tissues and cell lines. Further analysis revealed that overexpression of lncRNA-TPTE2P1 was correlated with tumor size, distant metastasis, differentiation degree, as well as tumor node metastasis (TNM) stage of HCC patients. Subsequent wound-healing assay, Transwell assay, and matrigel assay confirmed that down-regulated lncRNA-TPTE2P1 significantly suppressed the invasion and migration of cells. However, up-regulation of lncRNA-TPTE2P1 showed the opposite results. Moreover, lowly expressed lncRNA-TPTE2P1 remarkably decreased the protein levels of E-cadherin, Vimentin and N-cadherin. The above results indicated that lncRNA-TPTE2P1 might stimulate the migration and invasion of HCC cells by promoting epithelial-mesenchymal transition (EMT). All the findings suggested that lncRNA-TPTE2P1 could serve as an early biomarker of metastasis and therapeutic target for HCC.

Patients and Methods

Tissue Specimens

HCC tissues (n=72) and normal tissues (n=66) were collected from patients who received treatment in Zhongda Hospital, School of Medicine, Southeast University from 2010 to 2016. This study was approved by the Ethics Committee of Zhongda Hospital, School of Medicine, Southeast University. Before the study, written informed consents were obtained from all participants. All collected HCC tissue specimens were stored at liquid nitrogen for subsequent use.

Cell Lines and Transfection

Six HCC cell lines, including HepG2, Huh7, MHCC97, Bel7402, SMMC7721 and HCCLM3, were obtained from Shanghai Cell Bank (Shanghai, China). Meanwhile, one normal human liver cell line HL7702 was purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). All cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA) containing 100 U/mL penicillin, 100 μg/mL streptomycin and 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA), and maintained in an incubator with 5% CO_2 at 37˚C.

Lentiviral vector was used to over-express lncRNA-TPTE2P1 in HepG2 cells. Stable transfected cell lines were selected following standard instructions. Plasmid was transfected into Hun7 cells in strict accordance with the instructions of in which Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) to down-express lncRNA-TPTE2P1. Lentiviral vector and plasmids were obtained from GenePharma (Shanghai, China). Stable transfected cell lines were selected, and transfection efficiency was evaluated by quantitative real-time polymerase chain reaction (qRT-PCR).

Isolated RNA and qRT-PCR

Total RNA in cells and tissues was extracted according to the instructions of TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Complementary deoxyribose nucleic acids (cDNAs) were synthesized following the synopsis of Reverse Transcription Kit (TaKaRa, Otsu, Shiga, Japan). Glyceraldheyde 3-phosphate dehydrogenase (GAPDH) was taken as an internal reference. The expression level of lncRNA-PICART1 was detected by qRT-PCR according to the recommended system.

Transwell Assay

Chambers (Millipore, Billerica, MA, USA) were first settled onto 24-well plates. Meanwhile, matrigel (Millipore, Billerica, MA, USA) was unfreeze and evenly spread to the upper-chamber or not. Cells were suspended in serum-free DMEM and inoculated to the upper-chamber with appropriate density and number. Meanwhile, DMEM containing 10% FBS was added to the lower chamber, followed by culture at 37˚C for 14-24 h. Subsequently, the chamber was taken out, and the cells were fixed with methanol and stained with crystal violet. Images were captured with a light microscope. Chambers were decolorized with 33% acetic acid, and the amount of penetrating cells was estimated by optical density (OD) value (490 nm).

Table I. Correlation between lncRNA-TPTE2P1 expression andclinicopathologic characteristics of HCC patients.

Characteristics	n	LncRNA-TPTE2P1		p
		Low expression(n=37)	High expression(n=35)	
Gender				0.808
M	52	27	25	
F	20	10	10	
Age				0.189
<50	32	16	16	
≥50	40	16	24	
Tumor size				0.012*
<5 cm	31	22	9	
≥5 cm	41	15	26	
Distant metastasis				0.031*
No	31	24	7	
Yes	41	13	28	
Differentiation degree				
Low	34	24	10	0.043*
Middle/high	38	13	25	
TNM stage				0.013*
I/II	28	22	6	
III/IV	44	15	29	

Wound Healing Test

Cells infected with Lentivirus or transfected with plasmids were first planted onto 6-well plates. When the cells were grown to an appropriated density, they were scratched by pipette tips. Subsequently, the cells were washed with serum-free DMEM medium and cultured in thermostatic incubator. Finally, the cells were observed continuously at 0 h and 48 h. Images were captured under a light microscope.

Western blot

Total protein in cells were extracted by radioimmunoprecipitation assay (RIPA) with Phenylmethanesulfonyl fluoride (PMSF) (Beyotime, Shanghai, China) at a ratio of 100:1. Protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, the membranes were incubated with primary antibodies at 4°C overnight. On the next day, the membranes were incubated with corresponding secondary antibodies at room temperature for 2 h. Primary rabbit antibodies against E-cadherin, Vimentin and N-cadherin were purchased from Proteintech (ProteintechGroup Inc, Rosemont, IL, USA). GAPDH and Rabbit IgG antibodies were purchased from Abcam (Abcam, Cambridge, MA, USA). Expression of relative proteins was determined by Image J software (NIH, Bethesda, MD, USA).

Statistical Analysis

Each experiment was repeated for at least three independent times. All experimental data were exhibited as mean ± SEM. Chi-squared test was used to evaluate the significance of differences in Table I. Independent sample Student's unpaired *t*-test was applied to compare the difference between two groups. $p<0.05$ was considered statistically significant.

Results

LncRNA-TPTE2P1 was Over-Expressed in Both HCC Tissues and Cell Lines

The expression level of lncRNA-TPTE2P1 in HCC tissues and cells was assessed by qRT-PCR. As shown in Figure 1A, the expression level lncRNA-TPTE2P1 in tumor tissues (n=72) was significantly higher than that of normal tissues (n=66). Consistently, lncRNA-TPTE2P1 expression level in different HCC cell lines (HepG2, Huh7, MHCC97, Bel7402, SMMC7721 and HCCLM3) was detected as well. The results illustrated that lncRNA-TPTE2P1 was overexpressed in HCC cell lines when compared with human normal liver cell line HL7702 (Figure 1B). Furthermore, we analyzed the relationship between clinical features and lncRNA-TPTE2P1 expression. It was found that lncRNA-TPTE2P1 was correlated with tumor size, differentiation degree and TNM stage of HCC patients. Moreover, our findings demon-

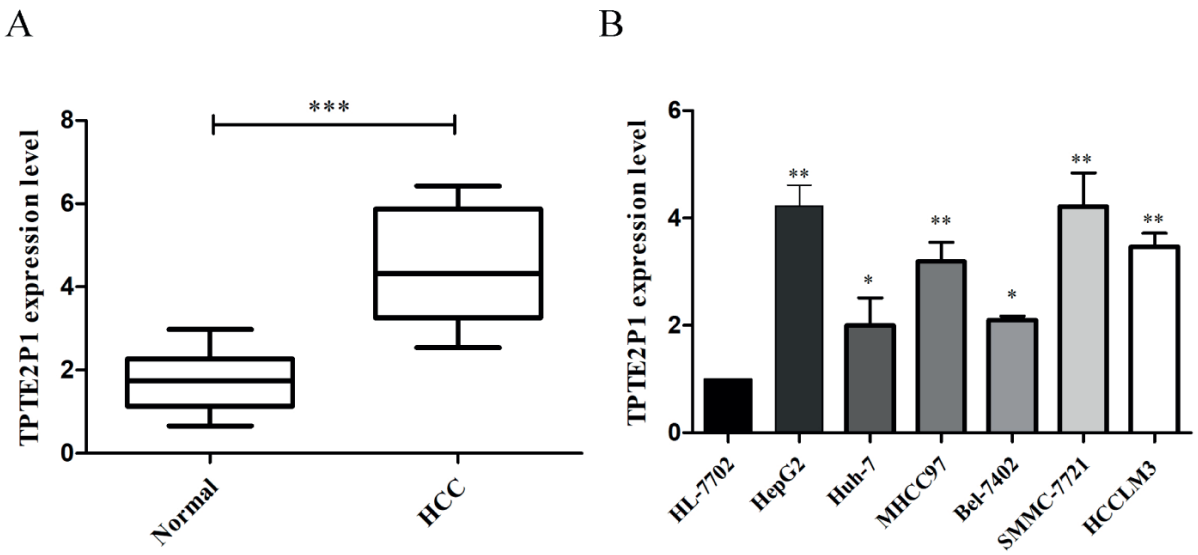

Figure 1. LncRNA-TPTE2P1 was over-expressed in both HCC tissues and cell lines. ***A,*** The relative expression of lncRNA-TPTE2P1 in hepatocellular carcinoma tissues and adjacent normal tissues was detected. ***B,*** QRT-PCR was used to verify the expression of lncRNA-TPTE2P1 in HCC cell lines. *p<0.05, **p<0.01, ***p<0.001. Data were expressed as mean ± SEM.

strated that overexpression of lncRNA-TPTE2P1 indicated significantly higher rate of distant metastasis (Table I). The above results confirmed abnormal overexpression of lncRNA-TPTE2P1 in HCC. Meanwhile, lncRNA-TPTE2P1 expression level was associated with the degree of malignancy and distant metastasis of HCC.

LncRNA-TPTE2P1 Promoted the Migration of HCC cells

To verify metastasis-related functions of lncRNA-TPTE2P1, two cell lines (HepG2 and Huh7) were selected for further study. Lentiviral vector was transfected into HCC cells to obtain stable cells with higher expression of lncRNA-TPTE2P1. Meanwhile, knockdown plasmids were transfected into Sh-TPTE2P1 group to down-express lncRNA-TPTE2P1. Transfection efficiency was verified by qRT-PCR (Figure 2A and 2B). Subsequently, wound healing assay was performed to investigate the migration of HCC cells. It was shown that after lncRNA-TPTE2P1 down-regulation with Sh-TPTE2P1, the migration ability was significantly reduced when compared with negative control group Sh-vector. Meanwhile, overexpression of lncRNA-TPTE2P1 by LV-TPTE2P obviously enhanced the migration ability of HCC cells when compared with negative control group LV-vector (Figure 2C). To further investigate the features, Transwell assay was carried out. As illustrated in Figure 3A, the number of migrated cells in Sh-TPTE2P1 group of HrpG2 was significantly less than that of Sh-vector groups. In contrast, the number of migrated cells was remarkably increased in LV-TPTE2P group when compared with LV-vector group. All the above results demonstrated that lncRNA-TPTE2P1 could promote the migration of HCC cells.

LncRNA-TPTE2P1 Stimulated the Invasion of HCC Cells

Invasion ability is also essential for tumor metastasis. Transwell matrigel assay was conducted to examine the effect of lncRNA-TPTE2P1 on the invasion ability of cells. Our results revealed that down-expressed lncRNA-TPTE2P1 obviously decreased the number of invasive cells when compared with negative control group Sh-vector. On the contrary, up-regulated lncRNA-TPTE2P1 significantly increased the number of invasive cells (Figure 3B). According to the results, we proved that lncRNA-TPTE2P1 stimulated the invasion of HCC cells.

LncRNA-TPTE2P1 Promoted HCC Metastasis via Epithelial-Mesenchymal Transition

Epithelial-mesenchymal transition (EMT) is a unique process that describes changes from polar, immobile epithelial cells to mesenchymal cells. This can lead to an increase in cell mobility[11]. It has been found that EMT plays an important role

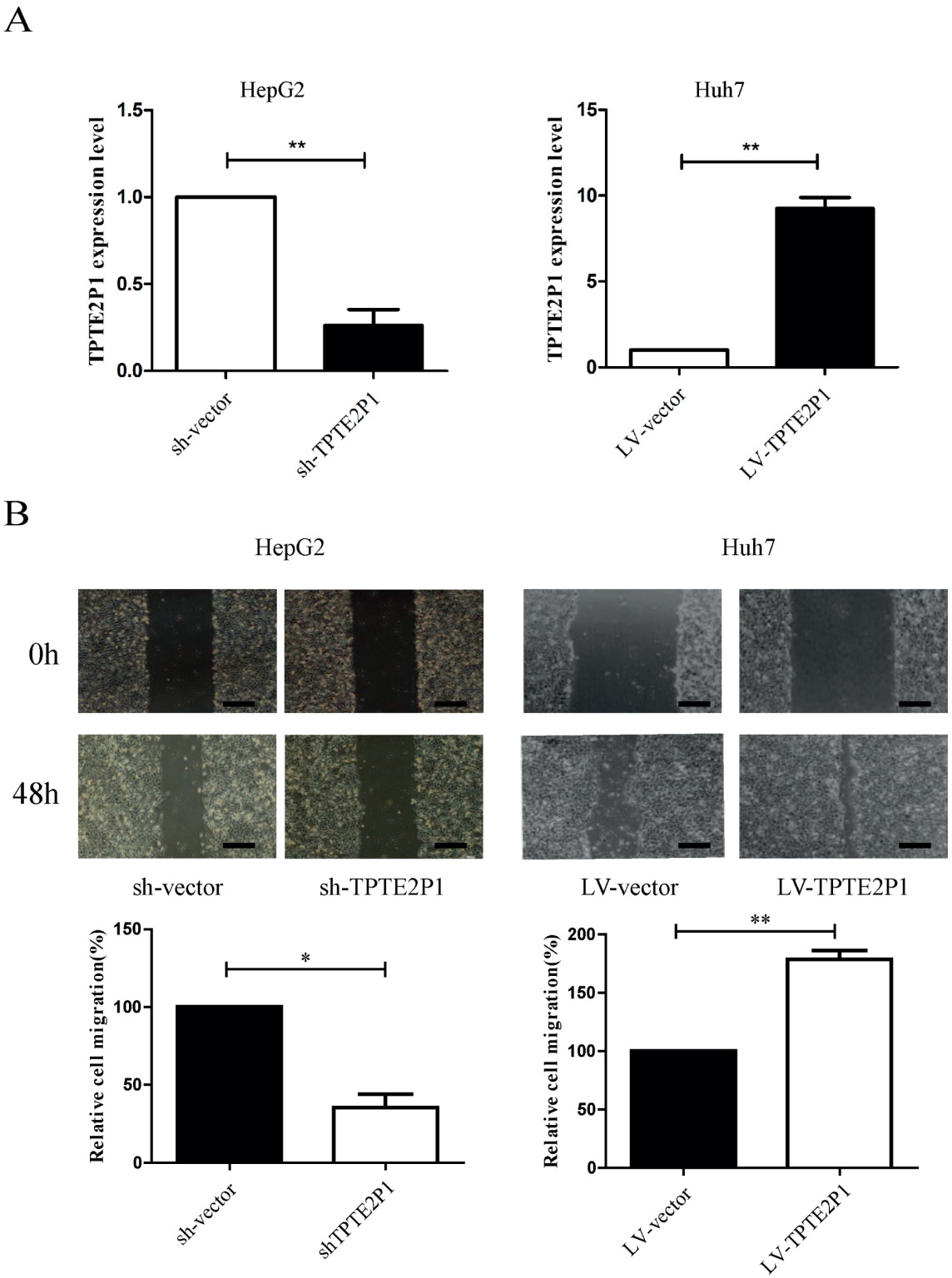

Figure 2. LncRNA-TPTE2P1 promoted the migration of HCC cells. ***A,*** Transfection efficiency was evaluated by qRT-PCR. ***B,*** Wound healing assay was performed in transfected cells. *$p<0.05$, **$p<0.01$, ***$p<0.001$. Data were presented as mean ± SEM of three independent experiments.

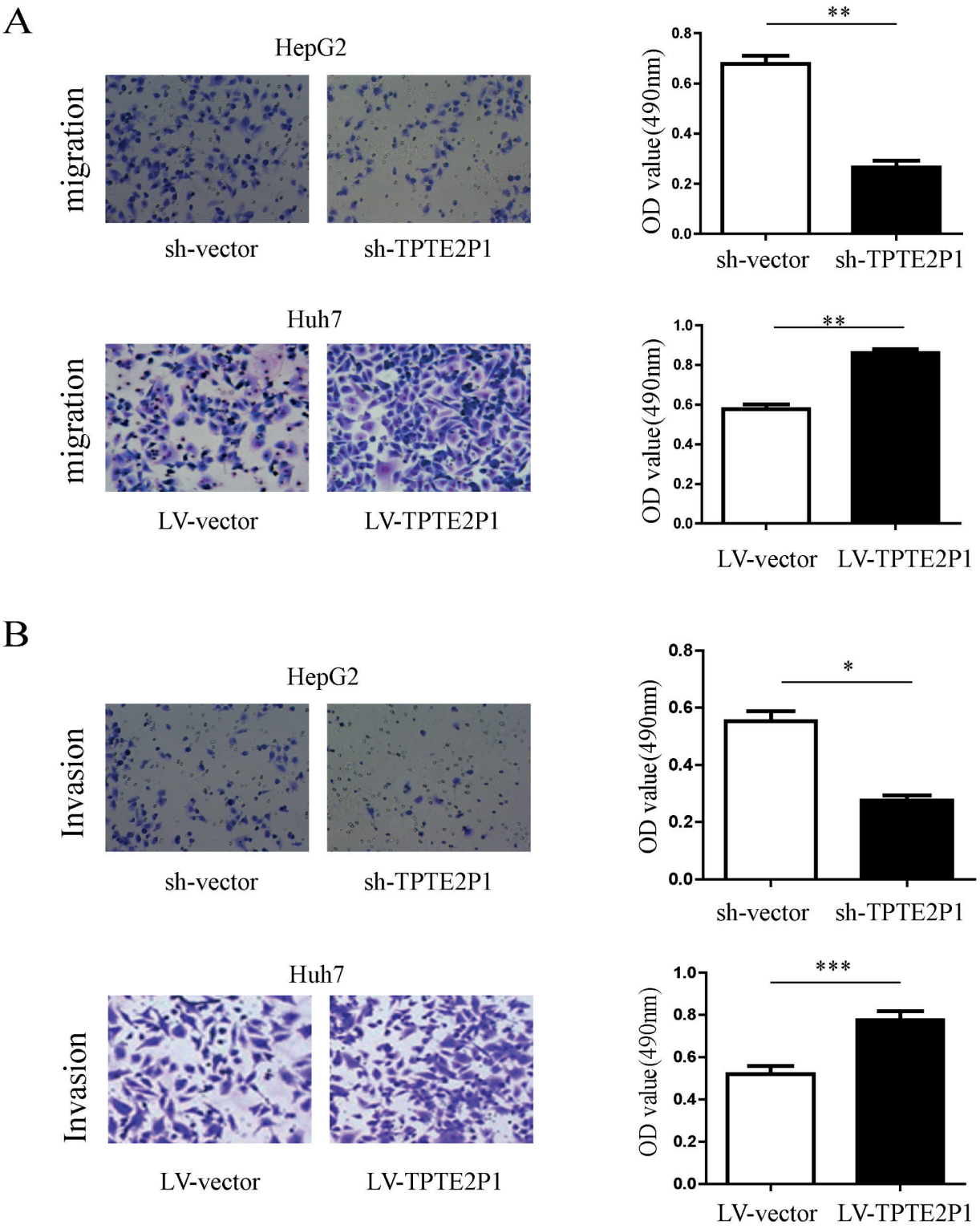

Figure 3. LncRNA-TPTE2P1 stimulated the invasion of HCC cells. ***A,*** Transwell assay was performed in transfected cells to evaluate migration ability. ***B,*** Invasive ability of cells was detected by transwell matrigel assay. *$p<0.05$, **$p<0.01$, ***$p<0.001$. Data were expressed as mean ± SEM.

in the process of tumor metastasis[12]. To illustrate the underlying mechanism of lncRNA-TPTE2P1 in HCC development, we confirmed lncRNA-TPTE2P1 exerted its function by regulating EMT. As shown in Figure 4A, knockdown of lncRNA-TPTE2P1 significantly increased the expression of E-cadherin, whereas decreased the levels of N-cadherin and Vimentin in HepG2 cells. All changes in protein levels suggested that EMT was suppressed. However, up-regulated lncRNA-TPTE2P1 exhibited the opposite effects (Figure 4B). Hence, these results indicated that highly expres-

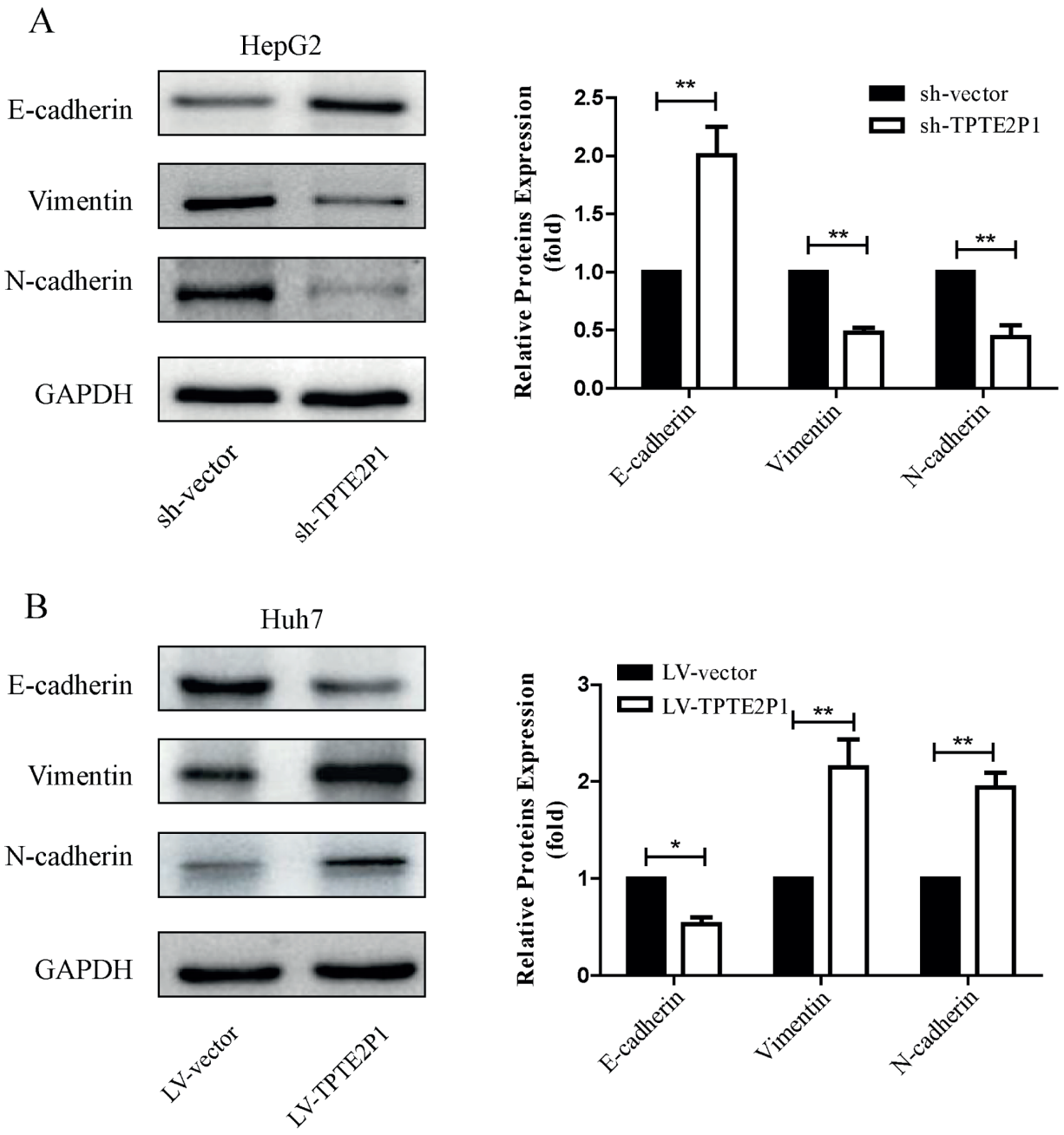

Figure 4. LncRNA-TPTE2P1 promoted metastasis of cells via epithelial-mesenchymal transition. ***A-B,*** Western blotting was used to detect the protein levels of E-cadherin, N-cadherin and Vimentin in transfected HepG2 and Huh7 cells, respectively. *$p<0.05$, **$p<0.01$. Data were presented as mean ± SEM of three independent experiments.

sed lncRNA-TPTE2P1 promoted the migration and invasion of HCC cells by activating EMT signaling pathways.

Discussion

HCC is a common malignant tumor worldwide, especially in China[3]. Metastasis and recurrence have always been important factors affecting the therapeutic effect of HCC. This is a complex process involving multiple steps and various regulatory factors. Therefore, it is important for the advancement of novel early metastasis biomarkers and therapeutic targets for efficient treatment of HCC.

LncRNA is an RNA molecule that does not encode proteins. However, it is widely involved in various biological processes of cells. In the Human Genome Project, only 3% of the genes encode proteins. Meanwhile, lncRNAs account for 62% of the genome transcripts[13]. It can be speculated that the non-coding region may regulate the expression of genes and the synthesis of proteins by expressing lncRNAs, thereby actively participating in regulation of biological functions[14]. In normal liver tissues, many lncRNAs are usually undetectable or exert very low expression levels. However, they are highly expressed in HCC tissues. Further studies have demonstrated that these lncRNAs are mainly involved in regulating gene expression and further regulating the proliferation, differentiation and apoptosis, invasion and metastasis of HCC cells[15-19]. Several studies have reported that lncRNA-TPTE2P1 is highly expressed in gallbladder cancer and colorectal cancer[20, 21]. Nevertheless, it is still needed to figure out the functional role of lncRNA-TPTE2P1 in HCC, as well as its underlying mechanism. Herein, in the current study, a series of physiological assays were involved to evaluate the exact role of lncRNA-TPTE2P1 in HCC. Our results indicated that lncRNA-TPTE2P1 functioned as an oncogene in HCC.

Our results found that lncRNA-TPTE2P1 was overexpressed in both HCC tissues and cell lines. Further analysis revealed that overexpression of LncRNA-TPTE2P1 was correlated with tumor size, distant metastasis, differentiation degree, as well as TNM stage of HCC patients. Subsequent wound-healing assay, transwell assay, and matrigel assay revealed that down-regulated lncRNA-TPTE2P1 could significantly suppress the invasion and migration of HCC cells. However, up-regulation of lncRNA-TPTE2P1 showed the opposite results.

EMT, as a complex and multi-path development process, plays an important role in tumor metastasis. The migration of mesenchymal epithelium is greater than that of epithelial cells. Previous studies have shown that EMT promotes tumor metastasis. In this study, we found that lowly expressed lncRNA-TPTE2P1 significantly suppressed the protein levels of E-cadherin, Vimentin and N-cadherin. This indicated that lncRNA-TPTE2P1 might stimulate the migration and invasion of HCC cells by promoting EMT. Taken together, all data suggested that lncRNA-TPTE2P1 functioned as an oncogene in HCC, which could accelerate the migration and invasion of cells *via* EMT.

Conclusions

With the development of molecular biology technology, the abnormal expression of specific genes in human body has been found to be closely related to the occurrence and development of malignancies. Gene therapy of tumors has become a research hotspot in recent years. Our results illustrated that lncRNA-TPTE2P1 functioned as an oncogene in HCC. Meanwhile, the regulation of EMT might be the underlying mechanism of lncRNA-TPTE2P1. Our findings might bring a novel insight of biomarkers and therapeutic strategies in HCC. Moreover, further molecular mechanism and clinical diagnosis and treatment significance of lncRNA-TPTE2P1 in HCC needs to be studied in the future.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
2) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
3) Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. Nat Rev Gastroenterol Hepatol 2010; 7: 448-458.
4) Harlan LC, Parsons HM, Wiggins CL, Stevens JL, Patt YZ. Treatment of hepatocellular carcinoma in the community: disparities in standard therapy. Liver Cancer 2015; 4: 70-83.

5) Harries LW. Long non-coding RNAs and human disease. Biochem Soc Trans 2012; 40: 902-906.
6) Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.
7) Bach DH, Lee SK. Long noncoding RNAs in cancer cells. Cancer Lett 2018; 419: 152-166.
8) Yang H, Wang S, Kang YJ, Wang C, Xu Y, Zhang Y, Jiang Z. Long non-coding RNA SNHG1 predicts a poor prognosis and promotes colon cancer tumorigenesis. Oncol Rep 2018; 40: 261-271.
9) Jiao ZY, Tian Q, Li N, Wang HB, Li KZ. Plasma long non-coding RNAs (lncRNAs) serve as potential biomarkers for predicting breast cancer. Eur Rev Med Pharmacol Sci 2018; 22: 1994-1999.
10) Li C, Cui Y, Liu LF, Ren WB, Li QQ, Zhou X, Li YL, Li Y, Bai XY, Zu XB. High expression of long non-coding RNA MALAT1 indicates a poor prognosis and promotes clinical progression and metastasis in bladder cancer. Clin Genitourin Cancer 2017; 15: 570-576.
11) Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 2009; 28: 15-33.
12) Brabletz T, Kalluri R, Nieto MA, Weinberg RA. EMT in cancer. Nat Rev Cancer 2018; 18: 128-134.
13) Sahu A, Singhal U, Chinnaiyan AM. Long noncoding RNAs in cancer: from function to translation. Trends Cancer 2015; 1: 93-109.
14) Gentilini D, Scala S, Gaudenzi G, Garagnani P, Capri M, Cescon M, Grazi GL, Bacalini MG, Pisoni S, Dicitore A, Circelli L, Santagata S, Izzo F, Di Blasio AM, Persani L, Franceschi C, Vitale G. Epigenome-wide association study in hepatocellular carcinoma: Identification of stochastic epigenetic mutations through an innovative statistical approach. Oncotarget 2017; 8: 41890-41902.
15) Cui M, Xiao Z, Wang Y, Zheng M, Song T, Cai X, Sun B, Ye L, Zhang X. Long noncoding RNA HULC modulates abnormal lipid metabolism in hepatoma cells through an miR-9-mediated RXRA signaling pathway. Cancer Res 2015; 75: 846-857.
16) Quagliata L, Matter MS, Piscuoglio S, Arabi L, Ruiz C, Procino A, Kovac M, Moretti F, Makowska Z, Boldanova T, Andersen JB, Hammerle M, Tornillo L, Heim MH, Diederichs S, Cillo C, Terracciano LM. Long noncoding RNA HOTTIP/HOXA13 expression is associated with disease progression and predicts outcome in hepatocellular carcinoma patients. Hepatology 2014; 59: 911-923.
17) Tang L, Zhang W, Su B, Yu B. Long noncoding RNA HOTAIR is associated with motility, invasion, and metastatic potential of metastatic melanoma. Biomed Res Int 2013; 2013: 251098.
18) Yu G, Lin J, Liu C, Hou K, Liang M, Shi B. Long non-coding RNA SPRY4-IT1 promotes development of hepatic cellular carcinoma by interacting with ERRalpha and predicts poor prognosis. Sci Rep 2017; 7: 17176.
19) Jariwala N, Sarkar D. Emerging role of lncRNA in cancer: a potential avenue in molecular medicine. Ann Transl Med 2016; 4: 286.
20) Lv W, Wang L, Lu J, Mu J, Liu Y, Dong P. Downregulation of TPTE2P1 Inhibits Migration and Invasion of Gallbladder Cancer Cells. Chem Biol Drug Des 2015; 86: 656-662.
21) Dai X, Xie Y, Dong M, Zhao J, Yu H, Zhou B, Xu Y, Yu Y, Cao Y, Zhang Y. The long noncoding RNA TPTE2P1 promotes the viability of colorectal cancer cells. J Cell Biochem 2018; 1-9.