European Review for Medical and Pharmacological Sciences 2019; 23: 8956-8963

LncRNA PVT1 aggravates the progression of glioma via downregulating UPF1

Z.-H. LV[1], Z.-Y. WANG[2,3], Z.-Y. LI[4]

[1]Department of Neurosurgery, Harbin Medical University Cancer Hospital, Harbin, China
[2]Reproductive Medicine Center, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China
[3]The Key Laboratory for Reproductive Medicine of Guangdong Province, First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China
[4]Department of Neurosurgery, Nanfang Hospital, Southern Medical University, Guangzhou, China

Zhonghua Lv and *Zengyan Wang* contribute equally to this work

Abstract. – OBJECTIVE: To uncover the influence of plasmacytoma variant translocation 1 (PVT1) on aggravating the progression of glioma via downregulating UPF1.

PATIENTS AND METHODS: The relative levels of PVT1 and UPF1 in glioma tissues were determined. PVT1 level in glioma patients in stage I+II and stage III+IV, and either with metastasis or not was examined as well. The Kaplan-Meier curves were depicted for assessing the survival in glioma patients expressing a high and low level of PVT1. The regulatory effects of PVT1 and UPF1 on the proliferative and migratory abilities of U87 and LN229 cells were evaluated. The subcellular distributions of PVT1 and UPF1 were analyzed, and their interaction was investigated by performing RNA immunoprecipitation (RIP) assay. At last, the mRNA level of UPF1 was determined in U87 and LN229 cells overexpressing PVT1 treated with 50 μM α-amanitin.

RESULTS: PVT1 was upregulated in glioma tissues relative to controls. Its level was higher in glioma patients with advanced stage or accompanied by metastasis. The glioma patients with a high level of PVT1 suffered a worse prognosis. The overexpression of PVT1 accelerated proliferative and migratory abilities of U87 and LN229 cells. UPF1 was conversely downregulated in glioma patients. Its level was negatively correlated to that of PVT1. The overexpression of UPF1 attenuated the proliferative and migratory abilities of U87 and LN229 cells. Both PVT1 and UPF1 were mainly enriched in the cytoplasm. The interaction between PVT1 and UPF1 was identified in the RIP assay. PVT1 prolonged the half-life of UPF1 and inhibited its synthesis.

CONCLUSIONS: PVT1 accelerates the proliferative and migratory abilities of glioma via downregulating UPF1.

Key Words:
Glioma, PVT1, UPF1.

Introduction

Glioma is the most common primary malignancy of the nervous system, accounting for 40-60% of all intracranial tumors[1]. Although therapeutic techniques, such as surgery, radiotherapy, and chemotherapy for glioma have achieved great strides, the median survival of glioma patients is only 9-12 months[2]. Therefore, it is necessary to uncover the pathogenesis of glioma and develop effective diagnostic and therapeutic targets.

Long non-coding RNA (lncRNA) is a nucleotide molecule of more than 200 nt long. Han et al[3] first confirmed the differentially expressed lncRNAs in glioma tissues and normal brain tissues by analyzing the microarray. Later, the potential relationship between lncRNAs and glioma has been well investigated. Zhang et al[4] identified a close relationship between lncRNAs and the occurrence, progression, and differentiation of glioma. Maher et al[5] demonstrated that the abnormally expressed lncRNAs are key regulators influencing cellular behaviors of tumors. Studies have confirmed that lncRNA PVT1 is closely related to lung cancer, breast cancer, liver cancer, and colon cancer. However, the role of PVT1 in glioma is rarely reported.

As an RNA/DNA-dependent ATPase and ATP-dependent RNA helicase, UPF1 is an evolutionarily conserved and ubiquitously expressed phosphoprotein[6]. UPF1 exerts a key role in nonsense-mediated mRNA decay (NMD) and non-NMD RNA degradation[7]. In addition, UPF1 also promotes cell progression into G1/S phase[8]. Chang et al[9] have also found that UPF1 is capable of regulating tumorigenesis. However, there

Corresponding Author: Zhiyong Li, MD; e-mail: braven@aliyun.com

is no report on the correlation between UPF1 and glioma. Therefore, in this study, we investigated the roles of PVT1 and UPF1 in regulating the progression of glioma, which provides new ideas for clinical diagnosis and treatment of glioma.

Patients and Methods

Sample Collection

Cell Culture

U87 and LN229 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) in an incubator with 5% CO_2 at 37°C. The medium was replaced every three days. The glioma cells were treated with 50 μM α-amanitin to block the synthesis of new RNAs.

Cell Transfection

The cells were inoculated in a 6-well plate with 4×10^5 cells per well. At 80% confluence, the cells were cultured in 1.5 mL of serum-free medium and 500 μL of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) containing transfection vectors. At 4-6 h, the complete medium was replaced.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA in cells and tissues was extracted using TRIzol method (Invitrogen, Carlsbad, CA, USA) for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). The RNA concentration was detected using a spectrometer. QRT-PCR was then performed based on the instructions of SYBR Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan). The relative level was calculated using the $2^{-\Delta\Delta Ct}$ method. PVT1, forward, 5'-GTCTTGGTGCTCTGTGTTC-3', reverse, 5'-CCCGTTATTCTGTCCTTCT-3'; UPF1, forward, 5'-ACCGACTTTACTCTTCCTAGCC-3', reverse, 5'-AGGTCCTTCGTGTAATAGGTGTC-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward, 5'-CGCTGAGTACGTCGTGGAGTC-3', reverse, 5'-GCTGATGATCTTGAGGCTGTTGTC-3'.

Cell Counting Kit-8 (CCK-8) Assay

The transfected cells were seeded into 96-well plates with 5.0×10^3 cells per well. At the appointed time points, 10 μL of CCK-8 solution (cell counting kit-8, Dojindo Molecular Technologies, Kumamoto, Japan) was added in each well. The absorbance at 450 nm of each sample was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

Transwell

The cell density was adjusted to 1×10^4 cells/ml. 100 μL of suspension was applied in the upper side, while 600 μL of medium containing 10% FBS was applied in the bottom of a transwell chamber (Corning, Corning, NY, USA). After 24 h of incubation, the cells penetrated to the bottom side were fixed in methanol for 15 min, stained with crystal violet for 20 min, and counted using a microscope. The number of migratory cells was counted in 5 randomly selected fields per sample (magnification 200×).

Determination of Subcellular Distribution

Cytoplasmic and nuclear RNAs were extracted using the PARIS kit (Invitrogen, Carlsbad, CA, USA) and subjected to qRT-PCR. U6 was the internal reference of the nucleus, and GAPDH was that of cytoplasm.

MS2-RIP

The cells were subjected to RNA immunoprecipitation (RIP) assay using the Magna RIP RNA Binding Protein Immunoprecipitation Kit (17-700, Millipore, Billerica, MA, USA). The RNA fraction separated by RIP was quantified by NanoDrop ND1000 (Thermo-Fisher Scientific, Waltham, MA, USA). The sequencing libraries were generated using 100 ng RNA from Illumina and TruSeq Stranded Total RNA kit. The samples were sequenced on an Illumina HiSeq 2500 sequencer with a read length of 50 bp. The sequencing process was controlled by the Illumina Data collection software.

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM Corp., Armonk, NY, USA) was used for data analysis. Data were expressed as mean ± standard deviation ($\bar{x}\pm s$). the intergroup data were compared using the *t*-test. The Kaplan-Meier method was introduced for survival analysis. The Spearman correlation test was conducted for evaluating the relationship between the two genes. $p<0.05$ considered the difference was statistically significant.

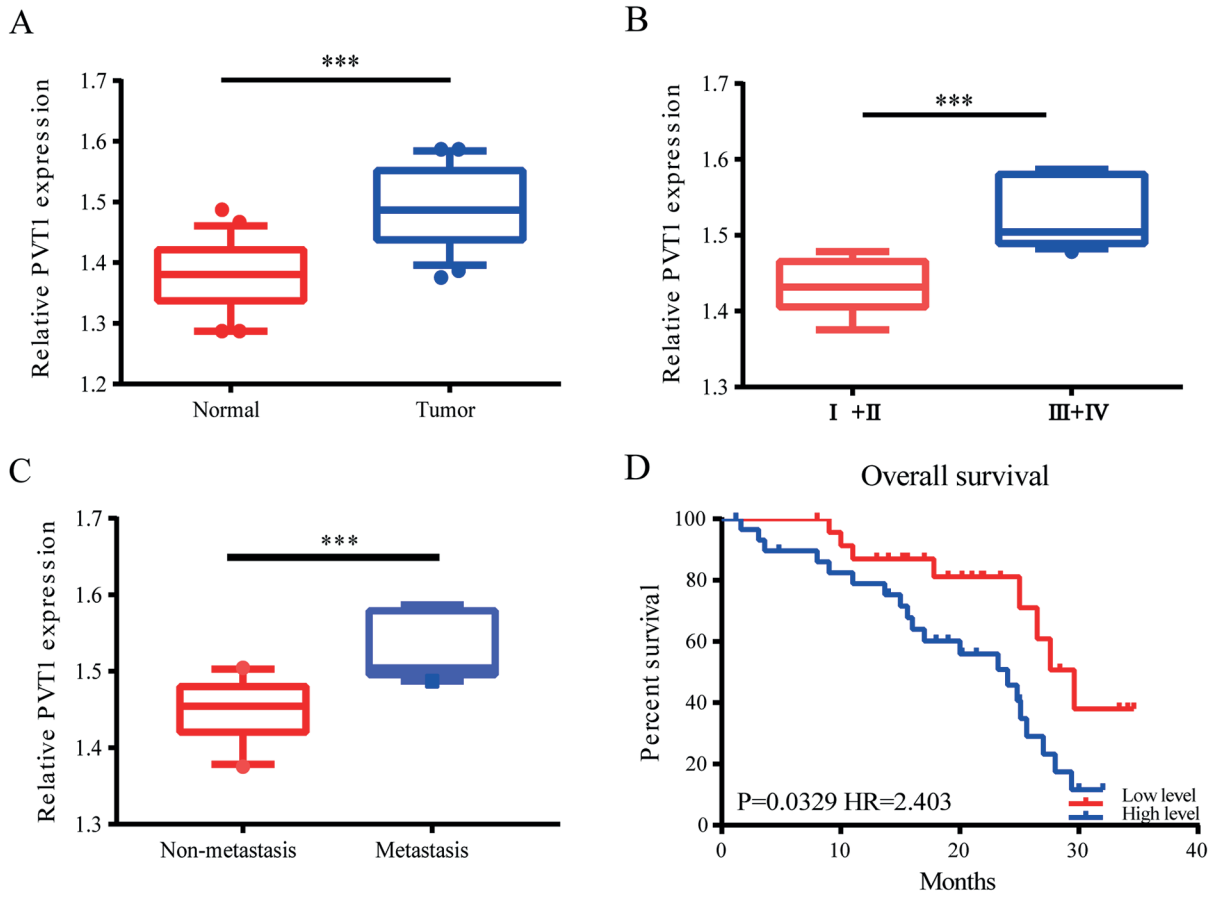

Figure 1. Upregulation of PVT1 in glioma. **A,** The relative level of PVT1 in glioma tissues and adjacent normal ones. **B,** The relative level of PVT1 in glioma patients with stage III+IV and stage I+II. **C,** The relative level of PVT1 in glioma patients either with metastasis or not. **D,** The Kaplan-Meier curves introduced for survival analysis in glioma patients with high level and low level of PVT1.

Results

Upregulation of PVT1 in Glioma

Relative to adjacent normal tissues, PVT1 was found to be upregulated in glioma tissues (Figure 1A). Based on different tumor staging of glioma, a higher level of PVT1 was observed in glioma patients with stage III+IV than those with stage I+II (Figure 1B). Besides, the metastatic glioma patients expressed a higher abundance of PVT1 compared with those non-metastatic ones (Figure 1C). The Kaplan-Meier curves indicated a worse prognosis in glioma patients expressing a high level of PVT1 (Figure 1D). Hence, PVT1 was considered to be involved in the progression of glioma.

PVT1 Stimulated Glioma Cells to Proliferate and Migrate

To uncover the biological role of PVT1 in glioma, we first constructed the overexpression plasmid of PVT1. The transfection of the overexpression plasmid of PVT1 in U87 and LN229 cells markedly upregulated the PVT1 cells, showing an excellent transfection efficacy (Figure 2A). The CCK-8 assay showed the elevated viability in U87 and LN229 cells overexpressing PVT1 than those of the controls (Figures 2B, 2C). Moreover, the migratory potential of the glioma cells was accelerated by the overexpression of PVT1 (Figures 2D, 2E). Hence, PVT1 was proved to accelerate the glioma cells to proliferate and migrate.

Downregulation of UPF1 in Glioma

The overexpression of PVT1 could markedly downregulate UPF1 level (Figure 3A). It is shown that UPF1 was downregulated in glioma tissues relative to controls (Figure 3B). Moreover, a negative correlation was observed between the expression levels of PVT1 and UPF1 in glioma tissues (Figure 3C). Next, we constructed the overexpression

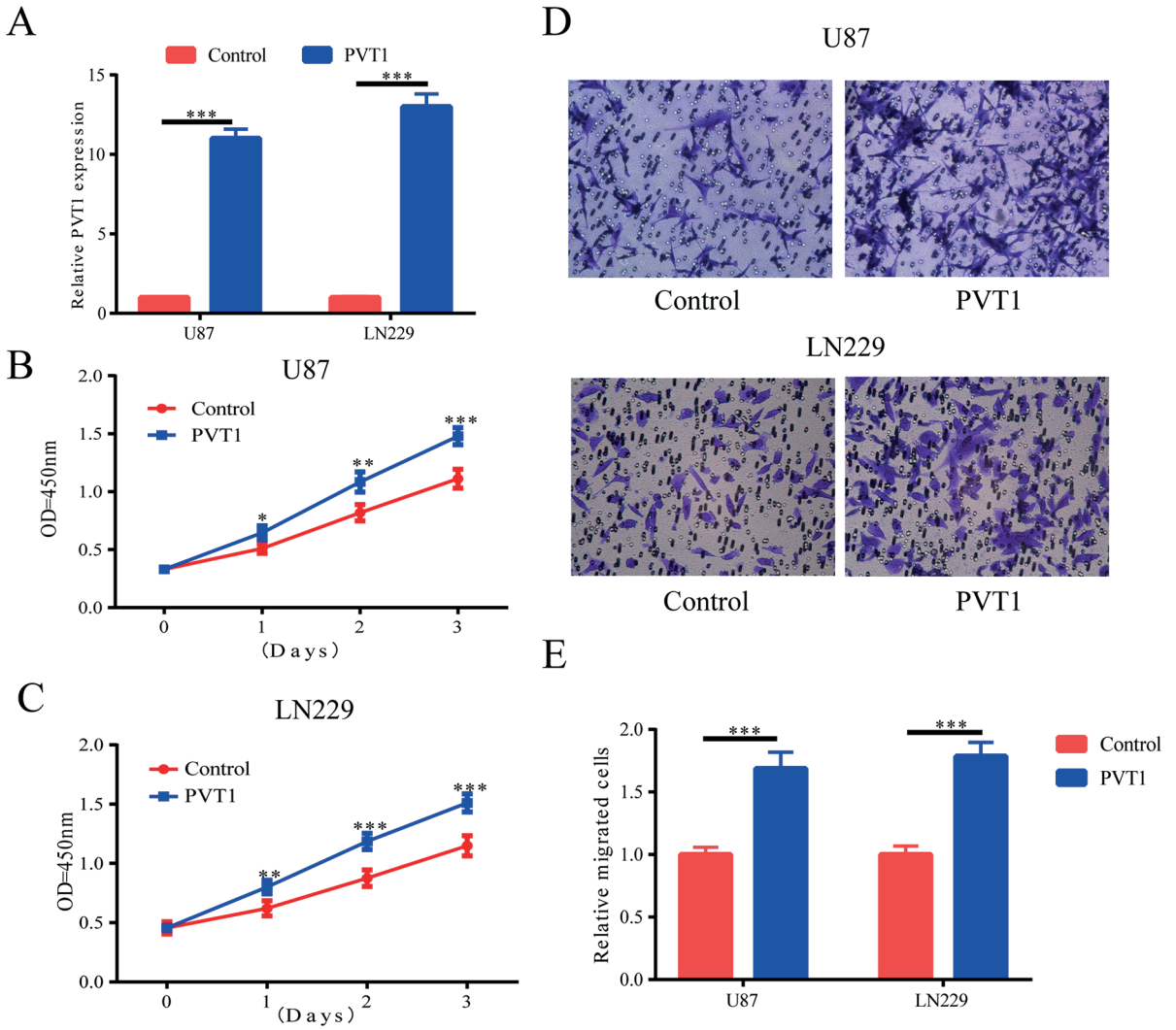

Figure 2. PVT1 stimulated glioma cells to proliferate and migrate. **A,** The transfection efficacy of the overexpression plasmid of PVT1 in U87 and LN229 cells. **B,** The viability in U87 cells transfected with control plasmid or overexpression plasmid of PVT1. **C,** The viability in LN229 cells transfected with control plasmid or overexpression plasmid of PVT1. **D,** The migration in U87 and LN229 cells transfected with control plasmid or overexpression plasmid of PVT1 (magnification: 40×). **E,** The migratory cell number in U87 and LN229 cells transfected with control plasmid or overexpression plasmid of PVT1.

plasmid of UPF1 to further identify its biological role in the progression of glioma. The transfection of the overexpression plasmid of UPF1 sufficiently upregulated UPF1 level in the glioma cells (Figure 3D). After the overexpression of UPF1, the viability in U87 and LN229 cells was markedly reduced (Figures 3E, 3F). The transwell assay revealed the inhibited migratory potential of glioma cells overexpressing UPF1 (Figure 3G). As a result, UPF1 was downregulated in glioma and inhibited glioma cells to proliferate and migrate. UPF1 level was negatively regulated by PVT1.

Interaction Between PVT1 and UPF1

The subcellular distribution of lncRNA determines its regulatory mechanism to a certain extent. Here, we identified that both PVT1 and UPF1 were mainly enriched in the cytoplasm (Figures 4A, 4B). A higher enrichment of PVT1 was found in anti-UPF1 relative to anti-IgG, suggesting the interaction between PVT1 and UPF1 (Figures 4C, 4D). Subsequently, to examine whether PVT1 could regulate the stability of UPF1, U87, and LN229 the cells overexpressing PVT1 were treated with α-amanitin to block the synthesis of new

RNAs. RNA degradation was tested at 0, 6, 12, and 24 h, respectively. The data showed that the surviving transcript in U87 and LN229 cells overexpressing PVT1 was markedly reduced, suggesting that PVT1 prolonged the half-life of UPF1 to inhibit its synthesis (Figures 4E, 4F).

Discussion

Glioma is the most common malignancy of the nervous system. The existence of tumor-related gene heterogeneity and tumor susceptibility have been identified in gliomas[10,11]. Wapinski et al[12] indicated that the pathogenesis of glioma is a complex process involving multiple genes and regulatory networks. Wrensch et al[13] considered that the activation of various oncogenes and the inactivation of the tumor-suppressor genes altogether lead to the occurrence of glioma.

LncRNA was once thought to be a transcriptional "noise" without any functions[14,15]. Dinger et al[16] have uncovered the crucial functions of lncRNAs at the transcriptional level, including the regulatory effects on DNA methylation, transcriptional activation, and transcriptional interference. A certain advance has been achieved in glioma analyses. For example, the upregulation

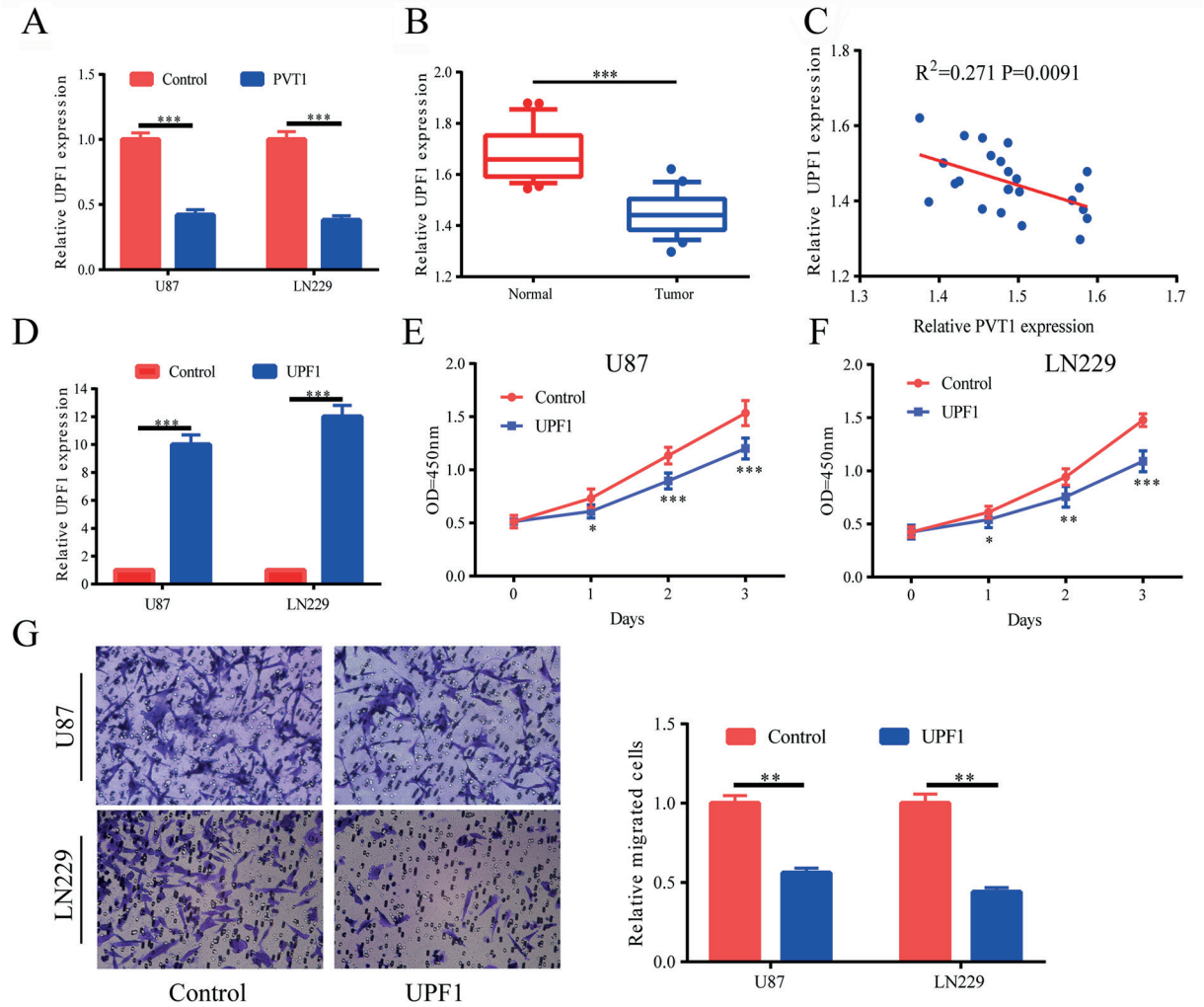

Figure 3. Downregulation of UPF1 in glioma. **A,** The relative level of UPF1 in U87 and LN229 cells transfected with control plasmid or overexpression plasmid of PVT1. **B,** The relative level of UPF1 in glioma tissues and adjacent normal ones. **C,** A negative correlation between the expression levels of UPF1 and PVT1 in glioma tissues. **D,** The transfection efficacy of the overexpression plasmid of UPF1 in U87 and LN229 cells. **E,** The viability in U87 cells transfected with control plasmid or overexpression plasmid of UPF1. **F,** The viability in LN229 cells transfected with control plasmid or overexpression plasmid of UPF1. **G,** Migration in U87 and LN229 cells transfected with control plasmid or overexpression plasmid of UPF1 (magnification: 40×). **H,** Migratory cell number in U87 and LN229 cells transfected with control plasmid or overexpression plasmid of UPF1.

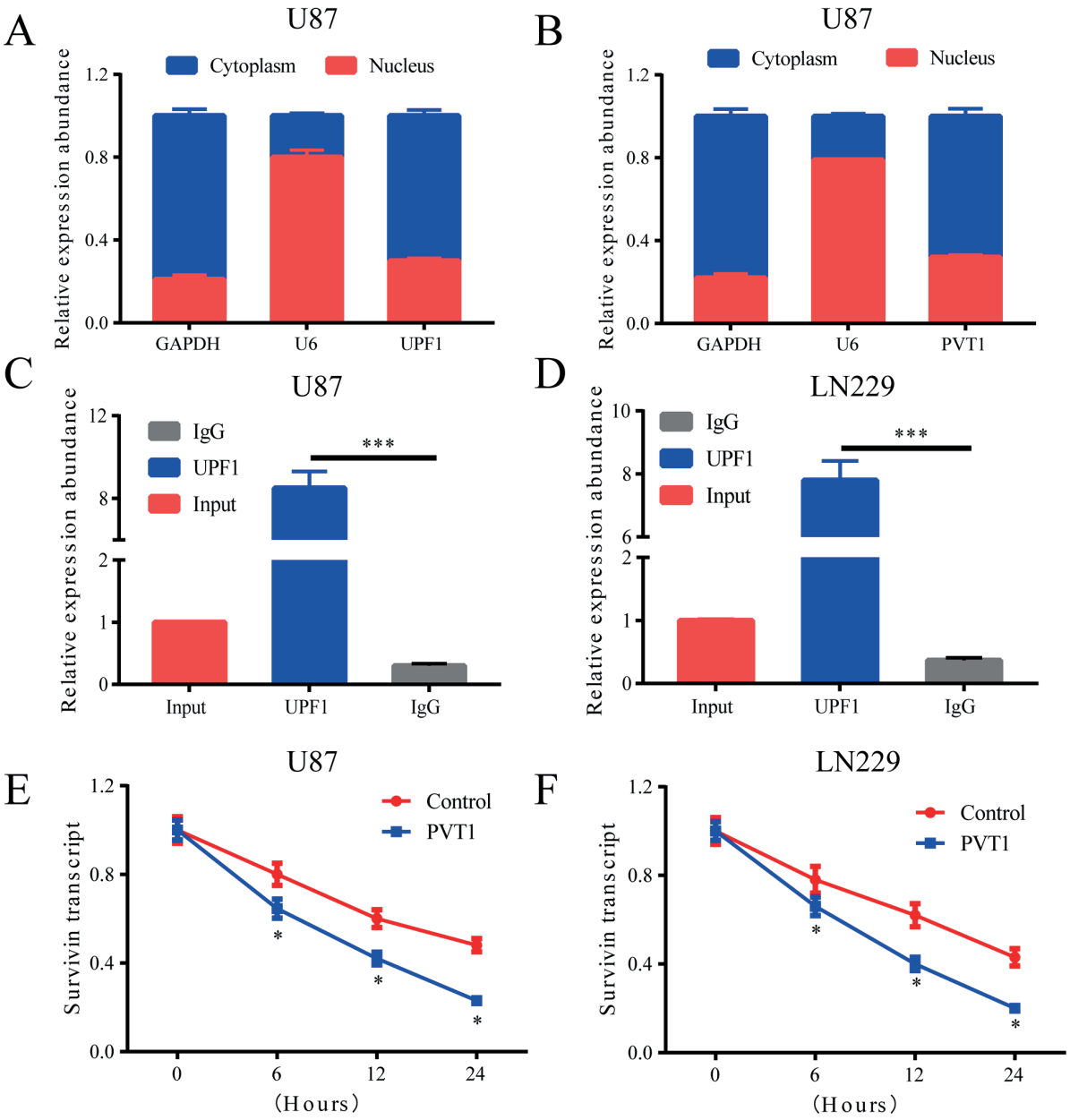

Figure 4. Interaction between PVT1 and UPF1. **A,** Subcellular distribution of UPF1 in U87 cells. GAPDH and U6 were internal references for cytoplasm and nucleus, respectively. **B,** The subcellular distribution of PVT1 in U87 cells. GAPDH and U6 were internal references for cytoplasm and nucleus, respectively. **C,** Immunoprecipitant of PVT1 in input, anti-UPF1 and anti-IgG of U87 cells. **D,** Immunoprecipitant of PVT1 in input, anti-UPF1 and anti-IgG of LN229 cells. **E,** Survivin transcript in U87 cells transfected with control plasmid or overexpression plasmid of PVT1 after treatment of 50 μM α-amanitin. **F,** Survivin transcript in LN229 cells transfected with control plasmid or overexpression plasmid of PVT1 after treatment of 50 μM α-amanitin.

of lncRNA HOTAIR predicts a poor prognosis of glioma, and its level is correlated to the tumor staging[17]. Gupta et al[18] demonstrated that HOTAIR stimulates the malignant growth of glioma by interacting with its target gene nPCR2. Qin et al[19] suggested that lncRNA TSLCA-AS1 is downregulated in glioma, which alleviates the growth of glioma by influencing methylation.

The PVT1 gene locates on the sense strand of chromosome 8q24[20]. In tumor cells, the region of chromosome 8q24 is the highest target for DNA replication copy amplification, and its abnormal

amplification often indicates an increased risk of tumors. For example, in breast cancer and metastatic prostate cancer, the copy number variation in the chromosome 8q24 region is closely related to susceptibility to tumor due to the short sequence sites of multiple different nucleotides contained[21,22]. In addition, the chromosome 8q24 region also contains a variety of risk sites associated with many diseases, such as non-syndromic cleft lip and palate and end-stage type 2 diabetic nephropathy[23,24]. Due to the particularity of its structure and location, PVT1 is often closely related to tumorigenesis. This study found that PVT1 was highly expressed in glioma tissues, and its level was higher in glioma patients in an advanced stage or accompanied with metastasis.

NMD is an mRNA quality monitoring mechanism widely found in eukaryotic cells[25]. In tumor research, NMD can exert regulatory mechanisms through targeted gene proteins[26,27]. However, NMD is downregulated under the circumstances of tumor cell nutrient deficiencies and endoplasmic reticulum stress, suggesting that NMD may be inhibited during tumorigenesis[26]. The occurrence of NMD is mainly caused by various polypeptides, such as UPF1, UPF2, UPF3, Y14, SMG1, SMG5, SMG6, and SMG7[28]. Among them, UPF1 is a key regulator in the NMD pathway[29]. Moreover, the expression change of UPF1 may determine the activation or inhibition of NMD[30]. UPF1 is found to be downregulated in pancreatic adenosquamous carcinoma[31]. Consistently, our study showed that UPF1 was lowly expressed in glioma. Furthermore, it is demonstrated that UPF1 was negatively regulated by PVT1, and its synthesis could be inhibited by PVT1. PVT1 accelerated the progression of glioma via downregulating UPF1. Our conclusion provides a novel direction that PVT1/UPF1 could be utilized as targets for the prevention and treatment of glioma.

Conclusions

LncRNA PVT1 is upregulated in glioma, and closely related to poor prognosis of glioma patients. It accelerates the proliferative and migratory abilities of glioma via downregulating UPF1.

Conflicts of interest

The authors declare no conflicts of interest.

References

1) Zheng DH, Wang X, Lu LN, Chen DL, Chen JM, Lin FM, Xu XB. MiR-638 serves as a tumor suppressor by targeting HOXA9 in glioma. Eur Rev Med Pharmacol Sci 2018; 22: 7798-7806.
2) Stupp R, Tonn JC, Brada M, Pentheroudakis G. High-grade malignant glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010; 21 (Suppl 5): v190-v193.
3) Han L, Zhang K, Shi Z, Zhang J, Zhu J, Zhu S, Zhang A, Jia Z, Wang G, Yu S, Pu P, Dong L, Kang C. LncRNA profile of glioblastoma reveals the potential role of lncRNAs in contributing to glioblastoma pathogenesis. Int J Oncol 2012; 40: 2004-2012.
4) Zhang X, Sun S, Pu JK, Tsang AC, Lee D, Man VO, Lui WM, Wong ST, Leung GK. Long non-coding RNA expression profiles predict clinical phenotypes in glioma. Neurobiol Dis 2012; 48: 1-8.
5) Maher EA, Furnari FB, Bachoo RM, Rowitch DH, Louis DN, Cavenee WK, DePinho RA. Malignant glioma: genetics and biology of a grave matter. Genes Dev 2001; 15: 1311-1333.
6) Applequist SE, Selg M, Raman C, Jack HM. Cloning and characterization of HUPF1, a human homolog of the Saccharomyces cerevisiae nonsense mRNA-reducing UPF1 protein. Nucleic Acids Res 1997; 25: 814-821.
7) Imamachi N, Tani H, Akimitsu N. Up-frameshift protein 1 (UPF1): multitalented entertainer in RNA decay. Drug Discov Ther 2012; 6: 55-61.
8) Lou CH, Shao A, Shum EY, Espinoza JL, Huang L, Karam R, Wilkinson MF. Posttranscriptional control of the stem cell and neurogenic programs by the nonsense-mediated RNA decay pathway. Cell Rep 2014; 6: 748-764.
9) Chang L, Li C, Guo T, Wang H, Ma W, Yuan Y, Liu Q, Ye Q, Liu Z. The human RNA surveillance factor UPF1 regulates tumorigenesis by targeting Smad7 in hepatocellular carcinoma. J Exp Clin Cancer Res 2016; 35: 8.
10) Zhu W, Lu L, Li Y, Yao J, Xu B. The effects of p53 Arg72Pro polymorphism on glioma susceptibility: a meta-analysis. Tumour Biol 2014; 35: 3725-3730.
11) Zhou JY, Shi R, Jiang L, Zeng Y, Ma WL. Letter regarding Zhu et al. entitled "Assessment of the association between XRCC1 Arg399Gln polymorphism and glioma susceptibility". Tumour Biol 2014; 35: 6181-6182.
12) Wapinski O, Chang HY. Long noncoding RNAs and human disease. Trends Cell Biol 2011; 21: 354-361.
13) Wrensch M, Fisher JL, Schwartzbaum JA, Bondy M, Berger M, Aldape KD. The molecular epidemiology of gliomas in adults. Neurosurg Focus 2005; 19: E5.
14) Ponjavic J, Ponting CP, Lunter G. Functionality or transcriptional noise? Evidence for selection within long noncoding RNAs. Genome Res 2007; 17: 556-565.
15) Struhl K. Transcriptional noise and the fidelity of initiation by RNA polymerase II. Nat Struct Mol Biol 2007; 14: 103-105.

16) Dinger ME, Amaral PP, Mercer TR, Pang KC, Bruce SJ, Gardiner BB, Askarian-Amiri ME, Ru K, Solda G, Simons C, Sunkin SM, Crowe ML, Grimmond SM, Perkins AC, Mattick JS. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Genome Res 2008; 18: 1433-1445.

17) Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, Miyano S, Mori M. Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res 2011; 71: 6320-6326.

18) Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vuver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464: 1071-1076.

19) Qin X, Yao J, Geng P, Fu X, Xue J, Zhang Z. LncRNA TSLC1-AS1 is a novel tumor suppressor in glioma. Int J Clin Exp Pathol 2014; 7: 3065-3072.

20) Gerstein MB, Bruce C, Rozowsky JS, Zheng D, Du J, Korbel JO, Emanuelsson O, Zhang ZD, Weissman S, Snyder M. What is a gene, post-ENCODE? History and updated definition. Genome Res 2007; 17: 669-681.

21) Haiman CA, Le Marchand L, Yamamato J, Stram DO, Sheng X, Kolonel LN, Wu AH, Reich D, Henderson BE. A common genetic risk factor for colorectal and prostate cancer. Nat Genet 2007; 39: 954-956.

22) Wang L, Cai Y, Zhao X, Jia X, Zhang J, Liu J, Zhen H, Wang T, Tang X, Liu Y, Wang J. Down-regulated long non-coding RNA H19 inhibits carcinogenesis of renal cell carcinoma. Neoplasma 2015; 62: 412-418.

23) Birnbaum S, Ludwig KU, Reutter H, Herms S, Steffens M, Rubini M, Baluardo C, Ferrian M, Almeida DAN, Albas MA, Barth S, Freudenberg J, Lauster C, Schmidt G, Scheer M, Braumann B, Berge SJ, Reich RH, Schiefke F, Hemprich A, Potzsch S, Steegers-Theunissen RP, Potzsch B, Moebus S, Horsthemke B, Kramer FJ, Wienker TF, Mossey PA, Propping P, Cichon S, Hoffmann P, Knapp M, Nothen MM, Mangold E. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. Nat Genet 2009; 41: 473-477.

24) Hanson RL, Craig DW, Millis MP, Yeatts KA, Kobes S, Pearson JV, Lee AM, Knowler WC, Nelson RG, Wolford JK. Identification of PVT1 as a candidate gene for end-stage renal disease in type 2 diabetes using a pooling-based genome-wide single nucleotide polymorphism association study. Diabetes 2007; 56: 975-983.

25) Chang YF, Imam JS, Wilkinson MF. The nonsense-mediated decay RNA surveillance pathway. Annu Rev Biochem 2007; 76: 51-74.

26) Lindeboom RG, Supek F, Lehner B. The rules and impact of nonsense-mediated mRNA decay in human cancers. Nat Genet 2016; 48: 1112-1118.

27) Gardner LB. Nonsense-mediated RNA decay regulation by cellular stress: implications for tumorigenesis. Mol Cancer Res 2010; 8: 295-308.

28) Lykke-Andersen J, Shu MD, Steitz JA. Human Upf proteins target an mRNA for nonsense-mediated decay when bound downstream of a termination codon. Cell 2000; 103: 1121-1131.

29) Azzalin CM, Lingner J. The human RNA surveillance factor UPF1 is required for S phase progression and genome stability. Curr Biol 2006; 16: 433-439.

30) Jeong HJ, Kim YJ, Kim SH, Kim YH, Lee IJ, Kim YK, Shin JS. Nonsense-mediated mRNA decay factors, UPF1 and UPF3, contribute to plant defense. Plant Cell Physiol 2011; 52: 2147-2156.

31) Liu C, Karam R, Zhou Y, Su F, Ji Y, Li G, Xu G, Lu L, Wang C, Song M, Zhu J, Wang Y, Zhao Y, Foo WC, Zuo M, Valasek MA, Javle M, Wilkinson MF, Lu Y. The UPF1 RNA surveillance gene is commonly mutated in pancreatic adenosquamous carcinoma. Nat Med 2014; 20: 596-598.