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Original Research

PTBP3 promotes tumorigenesis of glioblastoma by stabilizing Twist1

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ABSTRACT

Objective: Glioblastoma (GBM) is the most common malignancy tumor of central nervous system. PTBP3 was closely associated with the development of tumor. However, the function and molecular mechanism of PTBP3 in GBM is little known.
Methods: qPCR and immunoblotting were used to detect PTBP3 expression levels in glioma tissues and cells. CCK8, Edu, flow cytometry, wound healing, and transwell assays were used to examined the function of PTBP3 in GBM. qPCR, Immunoblotting, and ubiquitination assays were performed to identify the mechanism of PTBP3.
Results: We found that PTBP3 was upregulated in GBM, and high expression of PTBP3 correlated with the poor survival of GBM patients. PTBP3 knockdown reduced proliferation, invasion, and migration of GBM. Conversely, overexpressing PTBP3 has an opposite effect. Moreover, PTBP3 had an effect on the EMT of GBM. More importantly, we found that PTBP3 stabilized Twist1 by decreasing its ubiquitination and degradation. Furthermore, orthotopic xenograft models were used to demonstrate the PTBP3 on the development of GBM *in vivo*.
Conclusion: This study proved that PTBP3 promoted tumorigenesis of GBM by stabilizing Twist1, which provided a new therapeutic target for GBM.

Introduction

Glioma is the most common tumors of central nervous system (CNS), which accounts for approximately 80% of primary brain tumors. Glioblastoma (GBM), WHO grade IV glioma, is the most malignant type [1]. Surgical resection combined with chemo-radiotherapy are now the standard treatment approach for GBM [2]. However, the outcome of GBM patients remains poor, which median survival is about 15 months [3]. Now, the exact cause of the tumorigenesis of GBM is still unclear.

More and more studies have reported that RNA-binding proteins (RBPs) play important roles in the development and progression of cancer [4]. Polypyrimidine tract-binding proteins (PTBPs) are RBPs that are located both in nuclear and cytoplasm [5]. PTBP3, a number of PTBPs, has been reported to be closely associated with different types of cancer. Recent studies showed that PTBP3 was an oncogene to promote progression of non-small cell lung cancer [6], colorectal cancer [5], gastric cancer [7], pancreatic cancer [8], hepatocellular carcinoma [9]. However, the function and the molecular mechanism of PTBP3 in GBM remain unclear.

Epithelial–mesenchymal transition (EMT) is a process in which epithelial cells are transformed into mesenchymal phenotype cells, which causes cells to acquire migratory and invasive ability [10]. EMT plays a vital role in glioma cell proliferation, migration, and invasion, leading to glioma progression [11,12]. EMT is regulated by some crucial transcription factors, which includes ZEB1, ZEB2, Slug, Twist1, and Snail [13,14]. Twist1 is upregulated in malignant glioma, and promote proliferation, migration, invasion and EMT of glioma cells [15–17].

Here, we revealed that PTBP3 was upregulated in GBM and plays a vital role in the proliferation, migration, invasion, and EMT of GBM. Moreover, we elucidated that PTBP3 stabilized Twist1 by decreasing its ubiquitination and degradation. These findings indicate that PTBP3/Twist1 might be a potential therapeutic target for GBM treatment.

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Materials and methods

Clinical glioma samples and database

We obtained transcriptional and clinical data from the Chinese Glioma Genome Atlas (CGGA) database (http:// www. cgga. org. cn/). 36 glioma tissues (12 samples for grade II, 12 samples for grade III, and 12 samples for grade Ⅳ) were obtained from Department of Neurosurgery, Huai'an Hospital Affiliated to Xuzhou Medical University, Second People's Hospital of Huai'an City. The experimental protocol was approved by the Ethics Committee of Huai'an Hospital Affiliated to Xuzhou Medical University, Second People's Hospital of Huai'an City. Written informed consent was obtained from all patients.

Cell lines

The human glioblastoma (GBM) cell lines (A172, U87, T98, and LN229) were bought from ATCC (Rockville, USA). The human GBM cell line of U251 were purchased from Shanghai Institutes for Biological Sciences (Shanghai, China). GBM cells were maintained in DMEM (Thermo Fisher Scientific, Waltham, MA) supplemented with 10% FBS. Normal human astrocytes (NHA) were purchased from Lonza (Basel, Switzerland) and maintained in the provided astrocyte growth media supplemented with rhEGF, insulin, ascorbic acid, GA 1000, L glutamine and 5% FBS. All cells were incubated at 37°C with 5% CO_2.

Quantitative real-time PCR (qPCR)

qRT-PCR assays were performed as our previously study [18]. The expression of PTBP3, ZEB1, ZEB2, Twist1, Slug, and Snail were normalized by that of the GAPDH. The primers used were:

PTBP3, forward: 5'-ACAGCTAATGGGAATGACAGCA-3', reverse: 5'-CTGGCTTCGAAGGTGAGGAG-3';

ZEB1, forward: 5'-CGCAGTCTGGGTGTAATCGTAA-3'; reverse: 5'-GACTGCCTGGTGATGCTGAAA -3';

ZEB2, forward: 5'-AGTGGCAGCAGTCCCTTTAT-3'; reverse: 5'-TCCGTCTTGCAGTCCATCTT-3';

Twist1, forward: 5'-GGCTCAGCTACGCCTTCTC-3'; reverse: 5'-TCCTTCTCTGGAAACAATGA -3';

Slug, forward: 5'-TGCGATGCCCAGTCTAGAAA-3'; reverse: 5'-GTGTCCTTGAAGCAACCAGG -3';

Snail, forward: 5'-CGGACGATGATGTGAACACC-3'; reverse: 5'-TTGCTGTTGTGCTTAACCCC -3';

E-cadherin, forward: 5'-GACAACAAGCCCGAATT-3'; reverse: 5'-GGAAACTCTCTCGGTCCA -3';

Vimentin, forward: 5'-GAGAACTTTGCCGTTGAAGC-3'; reverse: 5'-GCTTCCTGTAGGTGGCAATC-3';

Fibronectin, forward: 5'-CAGTGGGAGACCTCGAGAAG-3'; reverse: 5'- TCCCTCGGAACATCAGAAAC-3';

GAPDH, forward: 5'-GAAGGTGAAGGTCGGAGT-3'; reverse: 5'-GATGGCAACAATATCCACTT-3'.

Cell transfection

For overexpressing PTBP3, PTBP3 cDNA was cloned and inserted into pCDH1-CMV-MSC-EF1-GFP-Puro vector. For stable knockdown PTBP3, specific short hairpin RNAs (shRNAs) against PTBP3 sequences were constructed. Cells were transfected with the shCtrl, shPTBP3-1, and shPTBP3-2 lentivirus for 48 h. Then, the cells were continuously cultured in the medium containing 2.5 μg/mL puromycin for 10 days. The plasmids were bought from GenePharma (Shanghai, China). Cell transfection was carried out with Lipofectamine 3000 (Invitrogen). The shRNA sequences were described as follows: shCtrl: 5'-CCGGCCTAAGGTTAAGTCGCCCTCTCGA-GAGCGAGGGCGACTTAACCTTAGGTTTTTG-3'; shPTBP3-1: 5'-CCGGACCAGGAAATTCTGTTCTACTCTCGAGAGTAGAACA-GAATTTCCTGGTTTTTTG-3'; shPTBP3-2: 5'-CCGGCAGA-GACTTCACTCGCTTA-GACTCGAGTCTAAGCGAGTGAAGTCTCTGTTTTTG-3';

For knockdown Twist1, cells were transfected with 20 nmol/L Twist1 siRNA (Shanghai, GenePharma). The siRNAs sequences were described as follows: siTwist1: 5'-TCCGCAGTCTTACGAGGAGCT-3'.

Cell proliferation assays

CCK-8 assays were used to test cell viability. Cells were plated in 96-well plates, then cultured for 24, 48, 72, and 96 h. After that, each well was added with 10 μl of CCK-8 solution. After 2 h incubation at 37°C, each well was measured at the 450 nm using a microplate reader.

EdU and colony information assays were used to detect the ability of cell proliferation. EdU was carried out following the manufacturer's protocol by EdU labeling kit (Ribobio). Then, EdU-positive cells were evaluated by fluorescence microscopy (Olympus). The percentage of EdU-positive cells was counted by dividing the number of EdU-positive cells by the total DAPI-stained cells.

Colony information assays were performed according to our previous study [18]. Cells (500 per well) were plated into 6-well culture plates. After 14 d, the cells were fixed in formaldehyde for 15 min and stained with 0.1% crystal violet solution for 20 min. 6-well culture plates were photographed by camera. Colonies with more than 50 cells, was confirmed by manual counting.

Cell cycle assay

Cells were fixed in 75% ethanol for overnight, and then stained with PI (BD, Biosciences) according to the manufacturer's protocol. The percentages of cells in G1, S, and G2 phase were detected by Wincycle-32 bit.

Cell migration and invasion assays

Wound healing assay was performed to detect the migration of cells. Cells were plated in 6-well plates and cultured until fully confluent. Scratches were drawn by 200 μL tip. After PBS washing by PBS, the cells were cultured for 48 h in serum-free medium. Healing of the cell scratches was observed by microscope (Olympus).

Transwell invasion assays were used to evaluate the invasion of cells, which were performed as previously study [19]. Filters were precoated with 10 μg of Matrigel and placed into the 24-well culture plates. Cells in DMEM media without FBS were added to the top chamber. DMEM media with 10% FBS was added to the lower chamber. After 36 h, the invading cells were fixed in 4% formaldehyde for 15min and stained with a 0.3% crystal violet for 20 min. The invading cells was observed by microscope (Olympus) and counted.

Orthotopic xenograft study

Male BALB/c nude mice (5-weeks-old) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. Luciferase-labeled U87 cells transfected with shPTBP3-1 or shCtrl were prepared (7 mice per group), and about 5×10^5 cells were inoculated into the right striatum of nude mice. The tumors were analyzed by IVIS imaging.

Ubiquitination assays

U87 cells transfected with HA-Ub were treated with 20 μM MG132 for 6 h. Lysates were incubated with anti-Twist1 antibody for 3 h and protein A/G agarose beads for a further 8 h at 4°C. Reactions were subjected to IB analysis.

T98 cells co-transfected with HA-Ub and Flag-Twist1 were treated with 20 μM MG132 for 6 h followed. Lysates were incubated with anti-

Myc antibody for 3 h and protein A/G agarose beads for a further 8 h at 4°C. Reactions were subjected to IB analysis.

Statistical analysis

All assays were performed thrice independently. SPSS 18.0 software and graphed by GraphPad Prism 6.0 Software were used to analyze Data. Data were shown as mean ± standard error of mean (SEM). The data were compared by Student's t test between two groups and ordinary one-way analysis of variance (ANOVA) for three or more groups. The statistical significance was defined by $P < 0.05$.

Results

PTBP3 was upregulated in GBM and predicted poor prognosis

To determine role of PTBP3 in GBM, we detected the expression of PTBP3 by CGGA database. We found that PTBP3 were significantly higher in GBM compared with grade II and glioma III (Fig. 1A). In addition, high expression of PTBP3 predicted poor prognosis of patients with GBM according to CGGA data (Fig. 1B). We also investigated PTBP3 expression in 36 clinical samples (12 cases grade II, 12 cases glioma III and 12 cases glioma IV(GBM) tissues). The mRNA and protein levels of PTBP3 were increased observed in GBM tissues compared with grade II and glioma III (Fig. 1C and D). Moreover, we detected mRNA and protein levels of PTBP3 in NHA and GBM cell lines (T98, LN229, U87, A172, and U251). The results demonstrated that mRNA and protein levels of PTBP3 was upregulated in the GBM cells compared with NHA cells (Fig. 1E and F). Therefore, PTBP3 was upregulated in GBM and predicted poor prognosis.

PTBP3 facilitated proliferation of GBM

To determine the function of PTBP3 in GBM, we selected U87 to establish stable PTBP3 knockdown cells and selected T98 to establish stable PTBP3 overexpression cells (Fig. 2A). The CCK8 assay demonstrated that PTBP3 depletion decreased the proliferation ability of U87 cells (Fig. 2B). Meanwhile, overexpressing PTBP3 increased the proliferation ability of T98 cells (Fig. 2C). Additionally, EdU and colony information assays revealed that PTBP3 inhibition reduced U87 cells proliferation (Fig. 2D, F, H and J). In contrast, overexpressing PTBP3 had the opposite effects (Fig. 2E, G, I and K). Cell cycle progression is important for cancer cell proliferation. PTBP3 knockdown induced cell cycle arrest in G1 phase (Fig. 2L and M).

PTBP3 promoted migration, invasion, and EMT of GBM

Epithelial–mesenchymal transition (EMT) is a process in which epithelial cells are transformed into mesenchymal phenotype cells, which causes cells to acquire migratory and invasive ability [10]. Next, we investigated whether PTBP3 was required for EMT of GBM. EMT process can cause cells to obtain certain features of mesenchymal cells, such as longer cellular projections (pseudopodia)[20]. Then, we detected pseudopodia of GBM cells with PTBP3 knockdown or overexpression. PTBP3 inhibition in U87 cells decreased the length of pseudopodia (Fig. 3A and B). However, overexpressing PTBP3 in T98

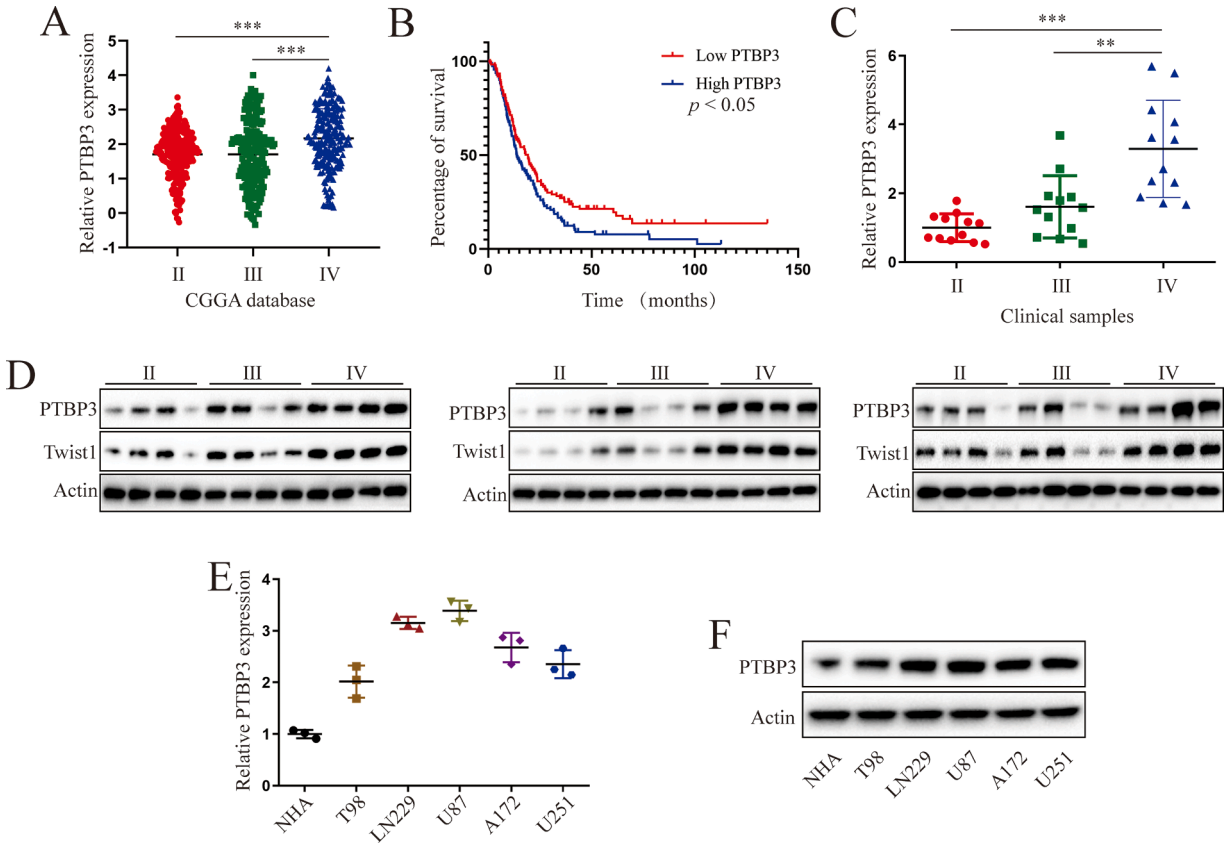

Fig. 1. PTBP3 was upregulated in GBM and predicted poor prognosis.
A. Relative mRNA expression of PTBP3 in Grade II, III and IV glioma samples by CGGA database. **B.** Kaplan-Meier analysis of overall survival of GBM patients with high or low PTBP3 mRNA expression the by CGGA data. **C.** Relative mRNA expression of PTBP3 in Grade II, III and IV glioma samples by clinical data. The expression of PTBP3 was normalized by that of the GAPDH. **D.** The protein levels of PTBP3 and Twist1 in Grade II, III, and IV glioma samples by clinical data. **E.** Relative mRNA expression of PTBP3 in NHA and GBM cells. The expression of PTBP3 was normalized by that of the GAPDH. **F.** The protein levels of PTBP3 in NHA and GBM cells. β-Actin served as the loading control. The data represent the mean of three independent experiments ± SEM. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

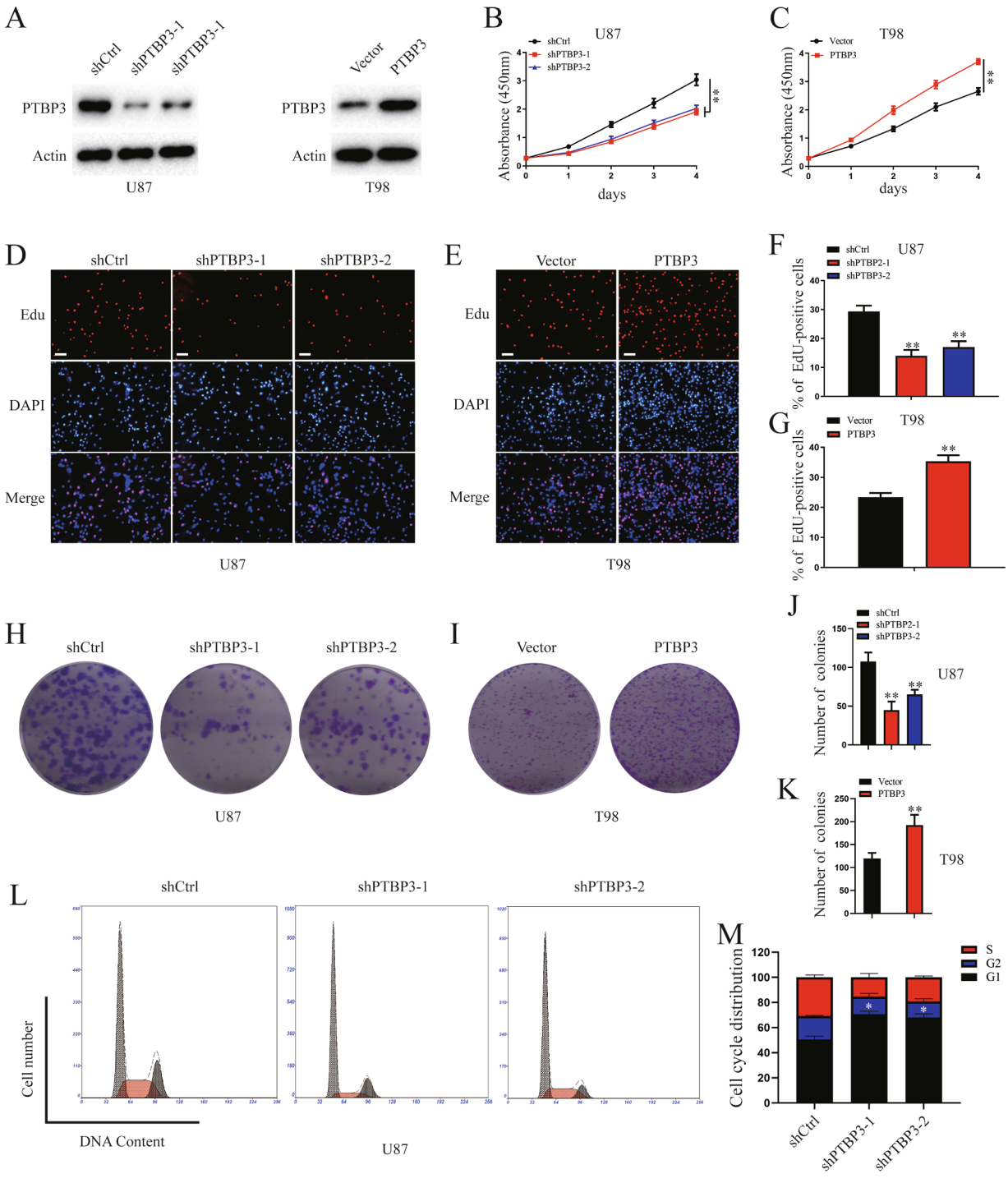

Fig. 2. PTBP3 facilitated proliferation of GBM.
A. The protein levels of PTBP3 in indicated cells. β-Actin served as the loading control. **B and C.** Knockdown or overexpression of PTBP3 on the growth of GBM cells was examined by CCK8 assay. **D-G.** Knockdown or overexpression of PTBP3 on the growth of GBM cells was examined by EdU assay. The percentage of EdU-positive cells was counted by dividing the number of EdU-positive cells by the total DAPI-stained cells. Scale bar: 100 μm. **H-K.** Knockdown or overexpression of PTBP3 on the growth of GBM cells was examined by colony formation assay. **L and M.** Knockdown of PTBP3 on cell cycle distribution of GBM cells. The data represent the mean of three independent experiments ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

cells dramatically increased their length (Fig. 3A and B). Then, wound-healing assay showed that PTBP3 inhibition attenuated migration ability of U87 cells, overexpressing PTBP3 induced migration ability of T98 cells (Fig. 3C–E). Transwell assays showed that PTBP3 inhibition reduced invasion ability of U87 cells, overexpressing PTBP3 induced invasion ability of T98 cells (Fig. 3F and G). Then, we detected levels of EMT-associated makers. PTBP3 inhibition decreased the mesenchymal markers (Fibronectin and Vimentin) and increased epithelial marker (E-cadherin) (Fig. 3H and I). Overexpression of PTBP3 increased the mesenchymal markers (Fibronectin and Vimentin) and decreased epithelial marker (E-cadherin) (Fig. 3H and I). Taken together, PTBP3 accelerates EMT, migration and invasion of GBM.

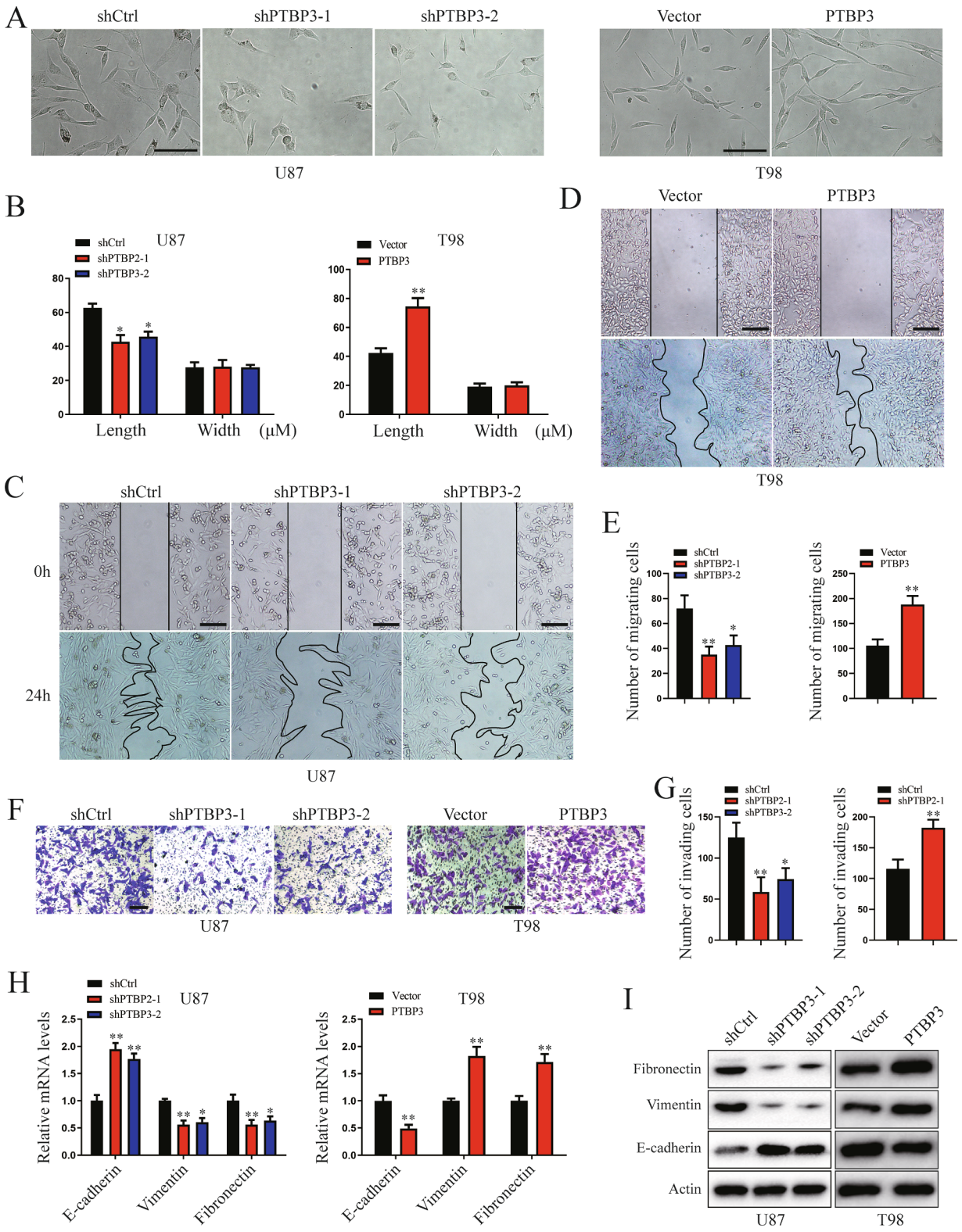

Fig. 3. PTBP3 promoted migration, invasion, and EMT of GBM.
A. Cellular morphologies of indicated GBM cells. Scale bar: 100 μm. **B.** The quantification of GBM pseudopodia length. **C-E.** Knockdown or overexpression of PTBP3 on the migration of GBM cells was examined by wound healing assay. Scale bar: 100 μm. **F and G.** Knockdown or overexpression of PTBP3 on the invasion of GBM cells was examined by Transwell assay. Scale bar: 100 μm. **H.** Relative mRNA expression of E-cadherin, Vimentin, and Fibronectin in indicated GBM cells. The expression of PTBP3 was normalized by that of the GAPDH. **H.** The protein levels of E-cadherin, Vimentin, and Fibronectin in indicated GBM cells. β-Actin served as the loading control. The data represent the mean of three independent experiments ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

PTBP3 stabilized Twist1 in GBM by decreasing ubiquitination and degradation

Next, we tried to reveal the mechanisms of PTBP3 in the development of GBM. We questioned whether PTBP3 impacted the expression of EMT inducers. We showed that the mRNA levels of five well-characterized EMT inducers were not changed, corresponding to PTBP3 overexpression or knockdown (Fig. 4A,B). However, protein level of Twist1were increased or decreased, corresponding to PTBP3 overexpression or depletion, respectively (Fig. 4C). More importantly, the protein levels of Twist1 were positively correlated with the protein levels of PTBP3 in glioma clinical samples (Fig. 1D). We wondered whether PTBP3 regulated Twist1 protein degradation. Previously, studies reported that Twist1 degradation through the ubiquitin–proteasome. MG132, the proteasomal inhibitor, partially reversed expression of Twist1 protein decreased by PTBP3 inhibition (Fig. 4D). Then, we used CHX to inhibit protein synthesis and detected Twist1 protein levels. PTBP3 knockdown in U87 cells led to destabilization of Twist1 protein (Fig. 4E), whereas, overexpressing PTBP3 in T98 cells increase in the stability of Twist1 (Fig. 4F). Next, we found that PTBP3 knockdown dramatically increased Twist1 ubiquitylation in T87 cells (Fig. 4G). Additionally, overexpression of PTBP3 significantly decreased SMAD7 ubiquitination in T98 cells (Fig. 4H).

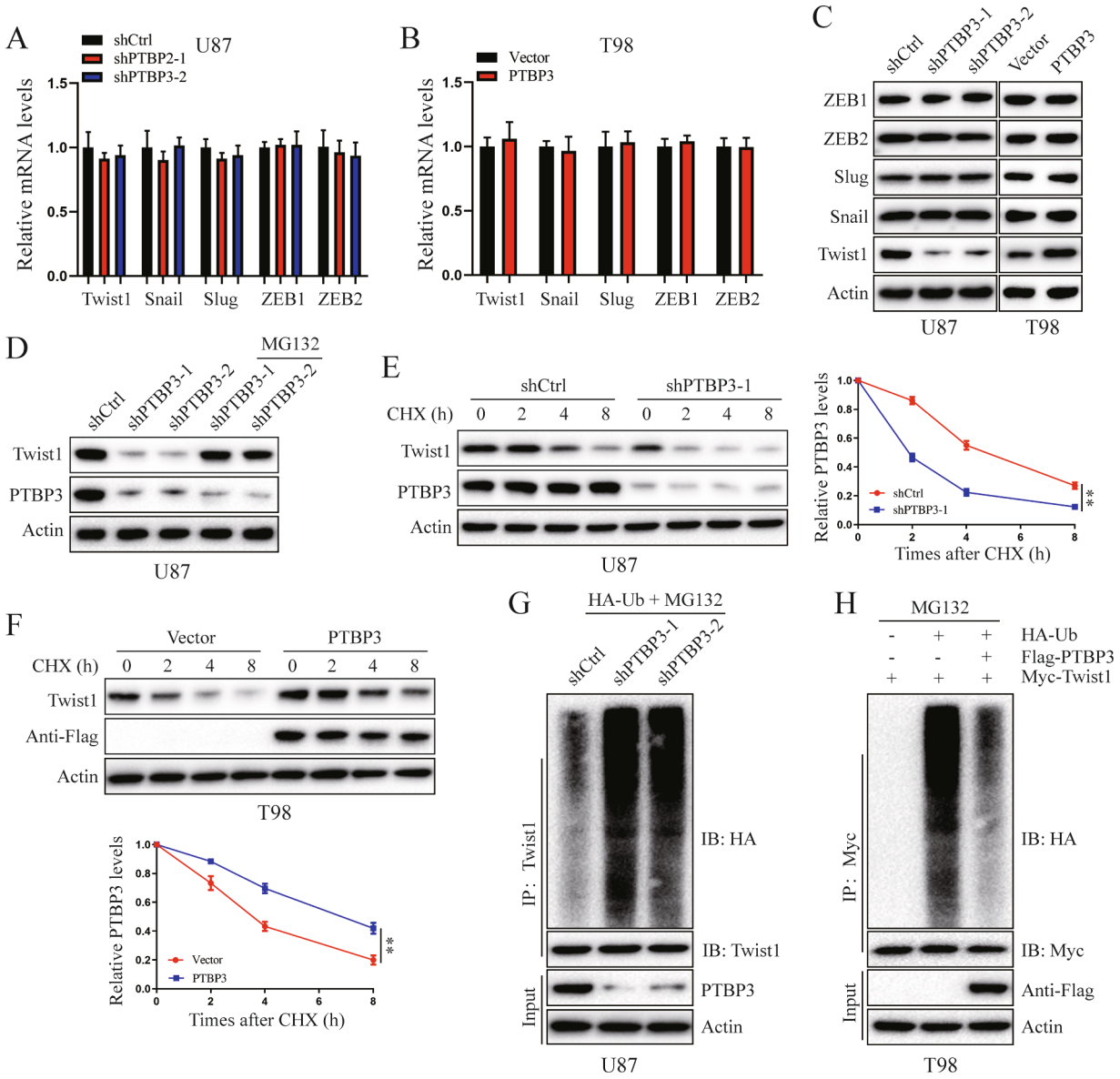

Fig. 4. PTBP3 stabilized Twist1 in GBM by decreasing ubiquitination and degradation.
A and B. Relative mRNA expression of Twist1, Snail, Slug, ZEB1, and ZEB2 in indicated GBM cells. The expression of PTBP3 was normalized by that of the GAPDH. **C.** The protein levels of Twist1, Snail, Slug, ZEB1, and ZEB2 in indicated GBM cells. β-Actin served as the loading control. **D.** GBM cells transfected with PTBP3 shRNA were treated with or without the proteasome inhibitor MG132 (20 μM, 8 h), and then PTBP3 and Twist1 were detected. β-Actin served as the loading control. **E.** U87 cells transfected with the shCtrl or shPTBP3-1 were treated with CHX (10 μg/ml), and collected at the indicated times for western blot. β-Actin served as the loading control. **F.** T98 cells transfected with the Vector or PTBP3 were treated with CHX (10 μg/ml), and collected at the indicated times for western blot. β-Actin served as the loading control. **G.** U87 cells were co-transfected with the shCtrl or sh PTBP3-1 or sh PTBP3-2 and HA-Ub, and cell lysates were subjected to IP with Twist1 antibody, followed by IB with indicated antibodies. Cells treated with 20 μM MG132 for 8 h. **H.** T98 cells were co-transfected with Myc-Twist1, HA-Ub, and Flag-PTBP3, and cell lysates were subjected to IP with Myc antibody, followed by IB with indicated antibodies. Cells treated with 20 μM MG132 for 8 h. The data represent the mean of three independent experiments ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Twist1 was required for PTBP3-mediated proliferation, migration, and invasion of GBM

To determine the requirement for Twist1 in proliferation, migration, and invasion of GBM that is regulated by PTBP3, we performed rescue experiments. Firstly, we explored the expression of EMT-related markers in T98 cells co-transfected with PTBP3 and siRNA of Twist1. Knockdown Twist1 partially reduced the levels of Fibronectin and Vimentin, and partially restored expression of E-cadherin (Fig. 5A). Next, the CCK8 assay was used to examine cell viability. Twist1 knockdown suppressed the effects of PTBP3 overexpression on GBM cell proliferation (Fig. 5B). Then, wound healing and transwell assays showed that the inducing of migration and invasion following overexpressing PTBP3 was rescued by co-transfecting the siRNA of Twist1 (Fig. 5C and D). These results demonstrated that Twist1 was required for the activity of PTBP3 in GBM.

Inhibition of PTBP3 decreased the progression of GBM progression in vivo

The effect of PTBP3 *in vivo* was evaluated by luciferase-labeled U87 orthotopic xenograft model. We found that tumors of the shPTBP3-1 U87 group were much smaller than the control U87 group (Fig. 6A and B). Survival curves showed that the mice of shPTBP3-1 group revealed a better survival compared with the mice of control group (Fig. 6C). Lastly, shPTBP3-1 inhibition decreased protein levels of Twist1, the mesenchymal markers Vimentin and Fibronectin and

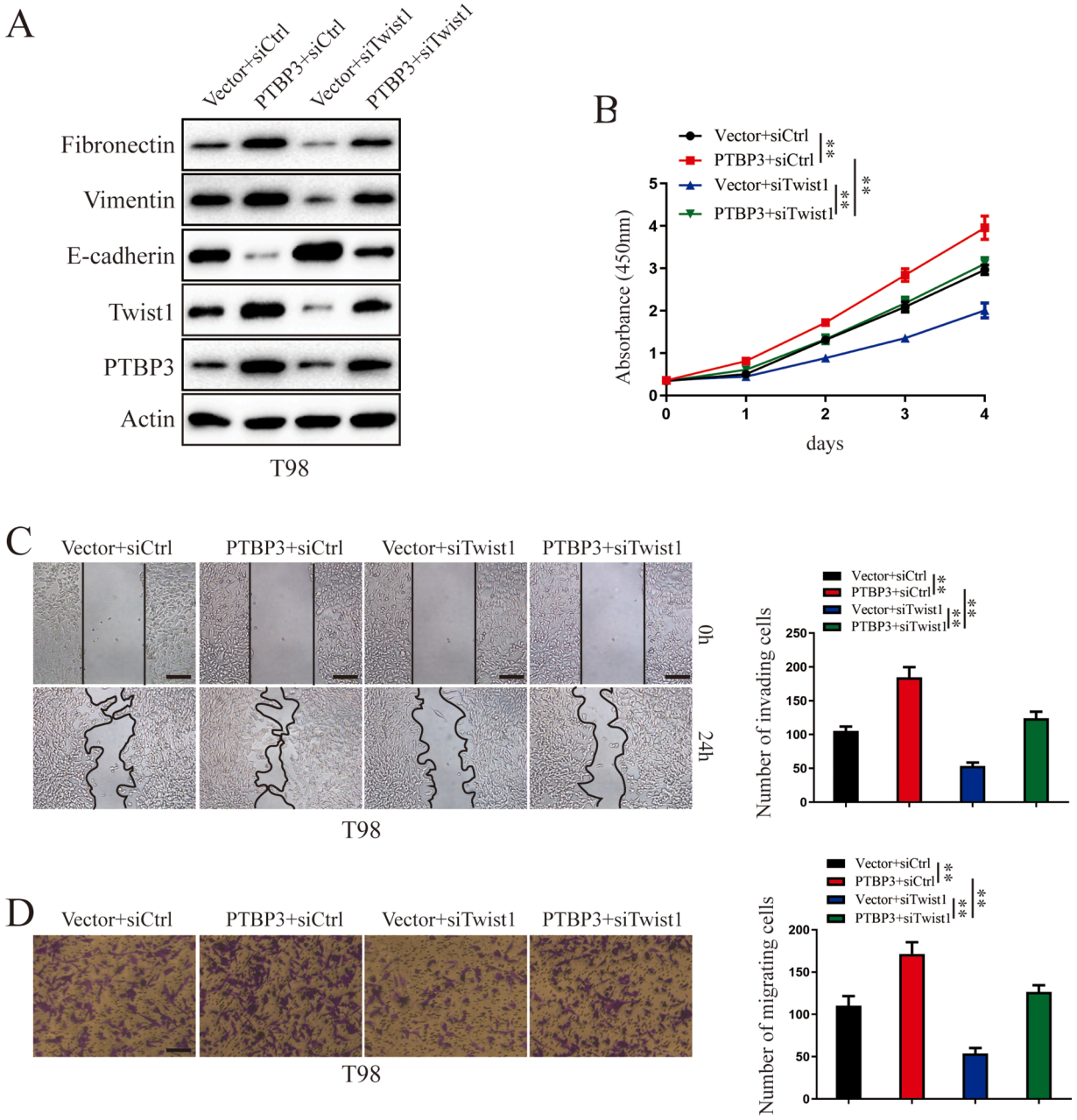

Fig. 5. Twist1 was required for PTBP3-mediated proliferation, migration, and invasion of GBM.
A. The protein levels of PTBP3, Twist1, E-cadherin, Vimentin, and Fibronectin in indicated GBM cells. β-Actin served as the loading control. **B.** The growth of indicated GBM cells was examined by CCK8 assay. **C.** The migration of indicated GBM cells was examined by wound healing assay. Scale bar: 100 μm. **D.** The invasion of indicated GBM cells was examined by Transwell assay. Scale bar: 100 μm. The data represent the mean of three independent experiments ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

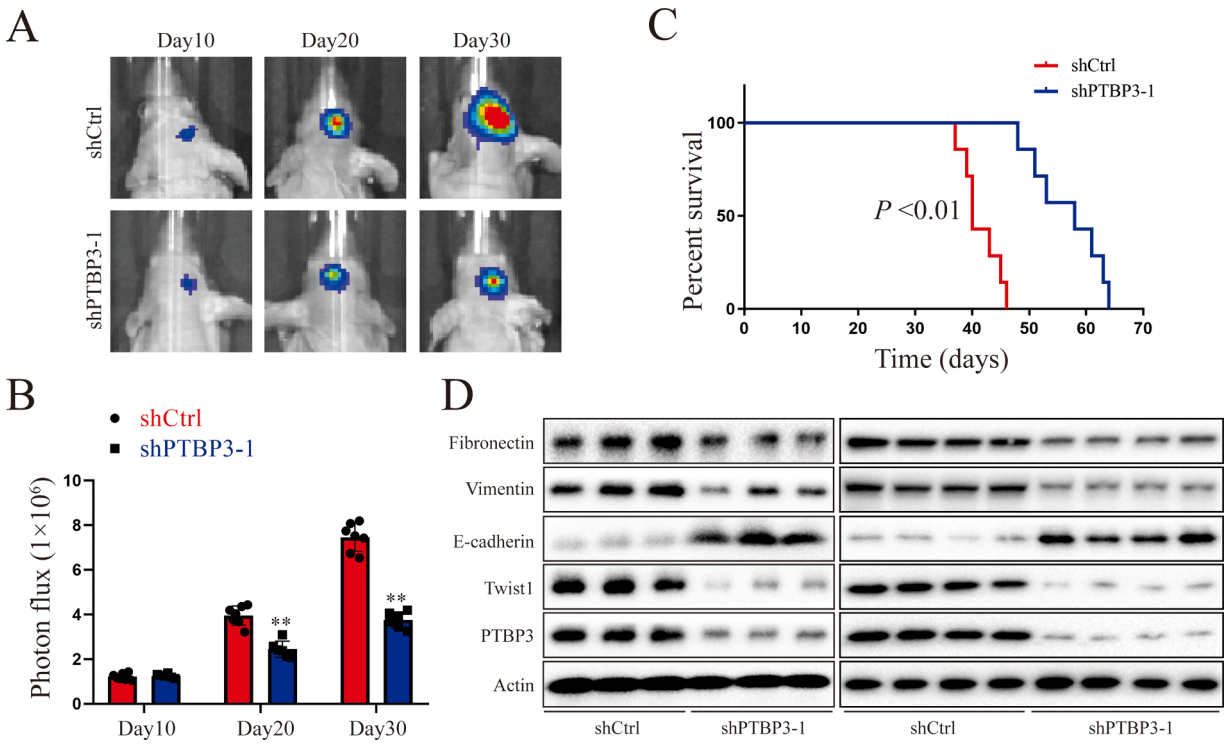

Fig. 6. Inhibition of PTBP3 decreased the progression of GBM progression in vivo.
A and B. Bioluminescence images of orthotopic xenograft tumors transfected with shCtrl or shPTBP3-1 U87 cells and then injected into nude mice. **C.** Survival curves of shCtrl or shPTBP3-1 group nude mice. **D.** The protein levels of PTBP3, Twist1, E-cadherin, Vimentin, and Fibronectin in intracranial tumors. β-Actin served as the loading control. The data represent the mean of three independent experiments ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

increased protein level of epithelial marker E-cadherin *in vivo* (Fig. 6D). These results confirmed that PTBP3 depletion inhibited GBM tumorigenesis *in vivo*.

Discussion

Glioblastoma (GBM) is the most malignant type glioma, which median survival is about 15 months [21]. Therefore, researching the potential target for GBM therapy is urgently needed. PTBP3, a member of PTB family, is reported to acts as an oncogene in several cancer types. PTBP3 contributes to colorectal cancer growth and metastasis [5]. PTBP3 promotes epithelial-mesenchymal transition in breast cancer [4]. PTBP3 contributes to the metastasis of gastric cancer [7]. PTBP3 promotes malignancy and hypoxia-induced chemoresistance in pancreatic cancer [8]. In addition, PTBP3 promotes migration of non-small cell lung cancer [6]. A study reported that MicroRNA-210 regulated cell proliferation and apoptosis by targeting regulator of PTBP3 [22], which just detected the mRNA levels PTBP3 in 15 GBM and five normal brain tissue samples, and demonstrated the function of PTBP3 in proliferation in one GBM cell lines. However, the function and the molecular mechanism of the PTBP3 in GBM are not fully elucidate. In this study, we proved that PTBP3 was also an oncogene in GBM. Firstly, we found that PTBP3 was upregulated in GBM tissues by the data from CGGA database and the clinical samples. Then, we demonstrated that the mRNA and protein expression of PTBP3 were also upregulated in GBM cell lines. More importantly, high expression of PTBP3 positively correlated with poor prognosis of patients with GBM by CGGA data.

The poor outcome for GBM patients mainly depends on high proliferative and infiltrative nature of GBM cells [23]. Recent studies proved that PTBP3 promoted the progression in some cancers. Then, we tried to reveal the function of PTBP3 in GBM cell proliferation, migration, and invasion. The results proved that PTBP3 knockdown markedly reduced the proliferation of GBM cells, and induced GBM cell cycle arrest in G1. In contrast, overexpression of PTBP3 promoted the proliferation of GBM cells. In addition, knockdown of PTBP3 suppressed the invasion and migration of GBM cells. Meanwhile, overexpression of PTBP3 had an opposite effect. *In vivo* experiments showed that PTBP3 inhibition inhibited the growth of intracranial GBM and prolonged the nude mice survival.

Given that our data prove that PTBP3 promoted the proliferation, invasion, and migration of GBM, we next sought to reveal the molecular mechanisms of PTBP3 in GBM. Epithelial–mesenchymal transition (EMT) plays the vital roles in the development and progression of different types of cancer, including glioma [24]. In breast cancer, PTBP3-mediated regulation of ZEB1 mRNA stability promotes epithelial-mesenchymal transition. PTBP3 promotes migration of non-small cell lung cancer through regulating E-cadherin in EMT. In this study, we demonstrated that PTBP3 knockdown decreased the length of pseudopodia of GBM cells, decreased the mesenchymal markers (Fibronectin and Vimentin), and increased epithelial marker (E-cadherin). Meanwhile, overexpressing PTBP3 increased the length of pseudopodia of GBM cells, increased the mesenchymal markers (Fibronectin and Vimentin), and decreased epithelial marker (E-cadherin). To further explore the mechanism, we focused on the EMT inducer. Twist-related protein 1 (Twist1) is reported as an oncogenic transcription factor and an inducer of EMT [25]. Twist1 is upregulated in GBM and promotes the proliferation, migration, invasion, and EMT of GBM [26,27]. In the present study, we found that PTBP3 overexpression or knockdown just increased or decreased the protein levels of Twist1 in five well-characterized EMT inducers. However, mRNA level of Twist1 were not changed in PTBP3 overexpression or knockdown GBM cells. As we all known, Twist1 is reported to be degraded through proteasome pathway [28]. More importantly, we shown that PTBP3 inhibition led to destabilization of Twist1 protein, and increased Twist1 ubiquitination and proteasome degradation. Overexpressing PTBP3 increased stabilization of Twist1 protein, and reduced Twist1 ubiquitination and proteasome degradation.

Conclusion

In summary, PTBP3 was upregulated in GBM tissues and cells. PTBP3 depletion decreased proliferation, migration, and invasion of GBM cells. Meanwhile, overexpressing PTBP3 increased proliferation, migration, invasion of GBM cells. Moreover, PTBP3 had an effect on the EMT of GBM cells. Furthermore, our findings revealed the mechanism between PTBP3 and Twist1, and the roles of PTBP3/Twist1 axis in GBM progression. Therefore, the PTBP3/Twist1 axis could serve as a potential target treatment for GBM.

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CRediT authorship contribution statement

Peng Xie: Funding acquisition, Investigation, Writing – original draft. **Yueqing Zhang:** Investigation. **Rui Chen:** Formal analysis, Data curation. **Jinyu Zheng:** Writing – original draft. **Gang Cui:** Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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