TPPP3 Promote Epithelial-Mesenchymal Transition via Snail1 in Glioblastoma

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Research Article

Keywords: TPPP3, Glioma, EMT, proliferation, migration, aninvasion

Posted Date: March 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1438957/v1

Abstract

Glioma is a common primary malignant brain tumor. Accurate molecular typing is very important for glioma diagnosis, treatment, risk factors identification and prognosis judgment. With the advancement and innovation of molecular biology technology, scientists have discovered that cell movement in organisms is closely related to the regulation of cytoskeletal proteins and microtubule dynamics. We investigated the role and application value of TPPP3, a member of the tubulin polymerization family, in gliomas. The expression of TPPP3 in glioma was higher than that in normal brain tissue (NBT), and increased with the grade of glioma. Inhibition of TPPP3 expression in glioma could reduce the proliferation, migration and invasion of glioma cells. TPPP3 affected the process of EMT by regulating the expression of Snail 1 protein. In clinical data analysis, we found a positive correlation between TPPP3 and Snail 1 protein expression levels in gliomas. Inhibition of TPPP3 expression leads to better survival expectations in glioma patients. The content of this study paves the way for further in-depth exploration of the role of TPPP3 in glioma in the future, and provides new treatment and research directions.

Introduction

Human glioma (Glioma) is a primary central nervous system malignant tumor that originates from neuroepithelial ectoderm in histological classification[1]. It ranks first in the global incidence and is also the most difficult intracranial tumor to overcome[2]. The abnormal proliferation ability, anti-apoptotic characteristics, and aggressive growth characteristics of glioma cells are difficult to cure glioma. Even during high-dose chemotherapy and radiotherapy, tumor cells can continue to infiltrate the surrounding brain tissue[3, 4].The mechanism of invasion of glioma cells is still unclear. Previous studies have shown that glioma cells infiltrate peripheral along the basement membrane of blood vessels or diffusely along channels between extracellular matrix, neurons and astrocytes[5]. Therefore, it is of great importance to study the biological characteristics and pathological mechanism of glioma and find new mechanisms and new perspectives to fight the growth and metastasis of glioma for the treatment of cancer.

Abnormal cytoskeletal structure changes may induce malignant change of normal cells in the body, and affect biological behaviors such as differentiation, proliferation, apoptosis, adhesion, invasion and migration of tumor cells, and even cause drug resistance and chemoradiotherapy resistance of tumor cells[6, 7]. Microtubules are one of the components of cytoskeleton. Tubule-promoting polymerins (TPPPs)are a family of proteins that bind to tubulin monomers, promote tubulin polymerization and stabilize the formed microtubules[8]. TPPP3 has been confirmed to be involved in the regulation of these malignant behaviors in tumor cells in a number of tumor studies. TPPP3, a member of TPPP family, has been confirmed to be involved in the regulation of these malignant behavior transformation in tumor cells in a number of tumor studies[9–12]. Stabilizing the cytoskeleton structure may effectively inhibit the malignant biological effects and behaviors of glioma cells, and inhibit the proliferation, migration and invasion of tumor cells[13–15]. Therefore, exploring the influence of TPPP3 on the malignant phenotype of glioma cells can provide a new entry point for us to deal with the refractory of glioma.

In this study, we first detected the expression of TPPP3 in various grades of glioma tissues. Then, different glioma cell lines were used to carry out related in vitro cell function experiments and detection of malignant behavior-related proteins to verify the effect of TPPP3 on the proliferation, apoptosis, migration and invasion of glioma cells. In addition, we analyzed the role of TPPP3 in epithelial-mesenchymal transformation by detecting EMT-related marker proteins. The specific mechanism of TPPP3 regulating the malignant progression of glioma was elaborated based on in vitro cytofunctional response experiments, and the correlation between TPPP3 and Snail 1 was analyzed. The biological role of TPPP3 in malignant progression of glioma was further clarified by in vivo experiments. Finally, clinical data were used to analyze the effect of TPPP on the survival of glioma patients.

Materials And Methods

Clinical tissues

The glioma samples were taken from patients with glioma in the Department of Neurosurgery, the Affiliated Hospital of Guizhou Medical University. A total of 81 tissues were involved in this study, including 10 normal brain tissues. The experiment involving specimens in this study obtained the consent of the patient and passed the approval of the ethics committee of this hospital.

Cell culture

Human glioma cell lines (U118, A172, LN229, U251) were purchased from the Shanghai Chinese Academy of Sciences Cell Bank. All cells were cultured in DMEM high-sugar medium and FBS (10%), and then placed in a 37°C, 5% CO2 cell incubator.

Real-time fluorescence quantitative PCR

Fully lyse cells or tissues with Trizol reagent, RNA extraction kit (AM1931) was used to extract cells and tissues separately according to the instructions. Follow the steps of the RNA Reverse Transcription Kit (Applied Biosystems™,4368813) to gradually complete the reverse transcription of RNA. The Q-PCR reaction was performed according to the requirements of SYBR Green Master Mix Kit (Applied Biosystems™,4432346), results were calculated according to the Ct value ($2^{\Delta\Delta Ct}$). The primer involved were as follows: β-actin (internal control) primer (forward primer, 5’-CATGTACGTTGCTATCCAGGC-3’; reverse primer, 5’-CTCCTTAATGTCACGCACGAT-3’); TPPP3 primer (forward primer, 5’-GGTCCATTCCTGCGTCGTTC-3’; reverse primer, 5’-GCCCAGTTCTTGCCATTCATC-3. All reagents and kits were purchased from Thermo Scientific.

Western blot analysis

An appropriate amount of RIPA lysate was used to lysate cells or tissues and extract proteins. Protein concentration was measured with BCA kit (Abcam, ab102536). Protein samples and protein markers were loaded in 10% separation gel and then transferred to 0.45-μm PVDF membranes (Millipore, 42029053) activated with methanol. Then membrane was blocked in 5% non-fat milk for 2h and incubated with

primary antibody (Abcam, USA) at 4°C overnight. Then placed into the corresponding secondary antibody (Abcam, USA,1:5000) box and incubated with a shaker at room temperature for 2h. Finally, the ECL working solution (Thermo Scientific™, USA) was prepared according to 1:1, and the membrane was exposed in the chemiluminescence instrument, the results were analyzed by Image J. The involved primary antibodies were as follow and concentration was diluted according to product instructions: TPPP3, N-cadherin, E-cadherin ,Vimentin, Snail 1,Slug,Twist 1,ZEB 1,β-catenin(all from Abcam).

Immunofluorescence

The cells were fixed with 4% paraformaldehyde in the petri dish for 15min. Then add 0.2%Triton X-100(Sigma-Aldrich, Germany) 200uL/ dish permeability 10min. Wash PBS 3 times /2min, add 200uL sealing solution/dish at room temperature for 1h. Dilute the primary antibody (Abcam, USA) with PBS in accordance with the required proportion and incubate at 4℃ overnight. The fluorescence secondary antibody (Abcam, USA) was diluted well, and 200uL/ dish was added, wrapped in tin foil to avoid light, and sealed at room temperature for 2h. 200uL/ dish of DAPI (Abcam,100ng/mL) was added and dyed at room temperature for 10min, with the whole process shielded from light. Finally, photographs are taken under a confocal laser microscope.

Wound-healing assay

The cells were counted and added with DMEM high glucose medium containing 10% FBS to adjust the cell concentration to 200,000 cells/well. The cells were cultured in cell incubator. When the cells grew to 80%~90%, mark "+" at the bottom of the hole with 10uL spear head, remove the old culture medium, then add 900uL DMEM culture medium to each well, take photos with fluorescence microscope. The cells were photographed again at the same location 24 hours later, and their mobility was compared. Cell migration rate = experimental group migration area/control group migration area.

Transewell assay

Add 50μL Matrigel (Corning, USA) to the upper chamber of each Transwell cell (diluted 1:8 in DMEM serum-free medium) and place in an incubator for 30 min. The cell concentration was adjusted to 100,000 cells/well. Transewell added 600uL DMEM containing 1% FBS into the 24-well plate in the lower chamber, and 200uL DMEM medium containing 100,000 cells into the upper chamber (Corning, CLS3470), and cultured in the cell incubator for 24h. The cells were then placed in a 24-well plate containing 600uL 4% paraformaldehyde for 30min and stained with 1% crystal violet (Sigma-Aldrich, V5265) for 30min. Gently wipe the upper chamber with a cotton swab, dry it, and take photos with a fluorescent microscope for counting.

Cell count kit-8(CCK8) assay

When the cells grew to 70%-80% and fused, the adherent cells were digested with trypsin. the adherent cells were collected and counted to prepare cell suspension with a density of 5×104/ mL, and 100μL cell suspension was prepared in a 96-well plate. The culture plate was pre-cultured in the incubator for 24 hours. According to the instruction of CCK8 kit (Dojindo, Japan), 10ul working solution was added to

each well. After incubation for one hour, the absorbance at 450nm was measured with a microplate reader.

Immunohistochemistry

The prepared paraffin sections were stained by Immunohistochemistry. The staining was completed according to the description of the immunohistochemical staining kit (CST, SignalStain® Boost) instructions. The brief steps are as follows: The dewaxed tissue sections were placed in anhydrous ethanol for 15 minutes, 95%, 90%, 80% and 75% ethanol for 10 minutes successively, and finally placed in ddH_2O for 15 minutes for dehydration. The sections were immersed in 1 X citrate repair solution, then heated in the microwave oven until boiling, and kept at temperature (95°-98°) for 10 minutes. Then the sections were cooled for 30 minutes for antigen repair. After inactivation of endogenous peroxidase in tissues, the slices were closed and incubated in diluted primary and secondary antibodies. DAB chromogenic solution was added on the surface of the slices, and the reaction lasted until brownish yellow color appeared. The chromogenic solution was removed, and the slices were cleaned with PBS, then hematoxylin was added for redyeing for 3 minutes. Finally, it is observed and photographed under a microscope.

The stained sections were observed by two pathologists under a 200x microscope and evaluated independently. The immune response score was calculated by the following formula, IS = percentage (0, no target molecule expressed; 1, 1% ~ 10% was positive; 2,11% ~50% is positive; 3, > 50% is positive) x staining intensity (0, no color; 1, weak color rendering; 2, medium color display; 3, strong color). When the IS score is between 3 and 9, it means that the target protein is highly expressed, otherwise the target protein is considered to be low-expressed.

Small hairpin RNA (shRNA) lentiviruses

Cell lines were constructed using lentivirus transduction as described previously[16].Lentivirus (Genechem, China) was packaged in 293T cells and transfected with Lipofectamine 3000 liposome transfection reagent (Thermo Scientific, L3000001). The interference sequence of TPP3 as mentioned in the study of Li et al. sh1:5'-CCGGGCCAATGTGGGCGTCACTAAACTCGAGTTTAGTGACGCCCACATTGGCTTTTTG-3', sh2:5'-CCGGCTGCTCGGGTCATCAACTATGCTCGAGCATAGTTGATGACCCGAGCAGTTTTTG-3', sh3:5'-CCGGAGGAGAGCTTCCGCAAGTTTGCTCGAGCAAACTTGCGGAAGCTCTCCTTTTTTG-3.When the cells in the blank control die and the lentivirus-transfected cells are still alive, the selected cells are expanded and sub-cultured, and cells were collected to detect the expression of the target protein.

Xenograft mouse model

Animal experiments are approved by the Ethics committee and carried out in strict accordance with the requirements. Twenty-four 5-week-old female BALB/c-nude mice were selected from Shanghai experimental animal center of Chinese Academy of Sciences for xenotransplantation experiment. U251 cells (shCrtl, shTPPP3) with a cell density of $5x10^7$ / mL were injected into the right flank of mice. The

size of the tumor was measured with a vernier caliper every 5 days after tumor formation, the tumor volume = (width^2 × length) / 2, the curve was plotted based on time - weight. After 30 days of subcutaneous inoculation, the nude mice were sacrificed by neck-method, and the tumor tissues in vivo were separated for weighing, photographing, and subsequent experiments.

Statistics analysis

In this study, GraphPad Prism software was used for statistical analysis of data. Student's test was used for significant differences among different groups. Kaplan-Meier survival curve and log-rank test were used to analyze survival differences. A *P* value less than 0.05 was considered statistically significant.

Results

The expression of TPPP3 in glioma is higher than that in normal brain tissue (NBT)

We performed surgical separation of glioma tissues from 81 patients, and used real-time fluorescence quantitative PCR to quantitatively analyze the mRNA abundance of TPPP3 in randomly selected surgically separated tissues and normal brain tissues. It was found that the expression of TPPP3 in gliomas was higher than that in normal brain tissue (NBT), and it became more pronounced as the grade of gliomas increased (Fig. 1A). In terms of protein expression level, we used Western blot analysis to detect the expression level of NBT and various grades of glioma tissues, and the conclusion is the same as above (Fig. 1B). We randomly selected 5 pairs of gliomas and normal brain tissues for western blot experiments, and the results suggested that TPPP3 was more highly expressed in tumor tissues (Fig. 1C). As shown in Fig. 1D, TPPP3 expression was found in all glioma cell lines, but was higher in glioma cell lines than in human normal astrocytes (NHA). IHC and IF were performed to further confirm the expression of TPPP3 in different grades of glioma tissue and normal brain tissue. The results presented are consistent with the above results (Fig. 1E, F).

TPPP3 affected the epithelia-mesenchymal transition (EMT) process of glioma

In order to reveal the potential effects of TPPP3 on glioma life activities, the GlioVis glioma database platform was used. GSEA gene enrichment analysis suggested that difference genes in the high and low TPPP3 risk groups were mainly in the gene concentration of tumor biological effects such as cell adhesion and migration. In order to verify the predictive information provided by bioinformatics about the biological function of TPPP3 on glioma, we selected existing glioma cell lines in the experiment for analysis. The results suggested that after overexpression of TPPP3, the related proteins of the EMT pathway changed (Fig. 2A). We conducted a functional experiment to test the proliferation ability of glioma cells. Transwell results suggested that the number of cells that crossed the compartment and penetrated the matrix glue and invaded the opposite side of the compartment cellulose membrane increased significantly after TPPP3 was overexpressed (Fig. 2B, C). Wound healing experiments

suggested similar results: When TPPP3 expression was increased, the migration ability of glioma cells was significantly enhanced compared with the control group (Fig. 2D, E). CCK8 results indicated that the proliferation rate of glioma cells after TPPP3 overexpression was significantly higher than control group (Fig. 2F). These data suggest that up-regulation of TPPP3 expression in glioma cells may confer stronger ability of migration and invasion in vitro.

TPPP3 expression silencing inhibited the malignant biological behavior of glioma cells

Among existing glioma cell lines, glioma cell lines LN229 and U251 with relatively high TPPP3 expression levels were selected to establish TPPP3 knockout cell models, which were used for subsequent cell functional experiments in vitro. The effect of TPPP3 knockdown on the migration and invasion of glioma cells was evaluated by wound healing assay and Transwell assay. Transwell assay showed that the migration number of LN229-SHTPPP3 cells was significantly lower than that of maternal cells (Fig. 3A, B). Wound healing experiment results showed that the migration rate of LN229-shTPPP3 was significantly lower than that of maternal cells (Fig. 3C, D). Similarly, Transwell migration experiment and wound healing experiment were performed in U251 and U251-shTPPP3 cells, and similar results were obtained. In addition, 96h CCK-8 assay showed that the proliferation ability of glioma cells with TPPP3 knockdown was significantly reduced compared with control cells (Fig. 3E). These results indicated that TPPP3 knockdown could inhibit the proliferation, migration and invasion of glioma cells.

TPPP3 affected the process of EMT through the key protein snail

The previous data analysis results showed that TPPP3 expression increased with the increase of the degree of malignancy and could target the relatively malignant mesenchymal phenotype, while the transformation of epithelioid cells to mesenchymal phenotype in tumor cells quietly occurred in the progression of tumor malignancy, and this transformation is also the source of tumor invasion and metastasis ability. In LN229 and U251 cell models, the expression levels of common key markers of EMT process in TPPP3 interference group and corresponding control group were detected by western blot analysis. The results indicated that the down-regulation of TPPP3 expression level could inhibit Snail expression, while the expression of other key proteins had no significant effect (Fig. 4A). In order to further verify whether Snail is a key protein for TPPP3 to play a role in the EMT process, we conducted a rescue experiment. The results suggested that the down-regulation of TPPP3 expression level could inhibit the expression of N-cadherin and Vimentin, while the expression of E-cadherin protein was negatively correlated with the expression of TPPP3. On the basis of low expression of TPPP3, overexpression of Snail protein, the experimental results had changed. That is, the effect of down-regulation of TPPP3 on the expression of N-cadherin and Vimentin was negligible. When Snail was overexpressed and TPPP3 was at a low expression level, the negative correlation between E-cadherin protein expression and TPPP3 expression no longer existed (Fig. 4B).

In functional experiments, similar results were also found. The results of Transwell experiments suggested that in glioma cell lines with low expression of TPPP3, the invasive ability of shTPPP3 glioma cell lines had changed from weak to stronger if Snail protein was overexpressed (Fig,4C, D). Wound-healing assay results showed that the migration ability of shTPPP3 glioma cell line was significantly enhanced compared with the control group after overexpression of Snail protein (Fig. 4E, F). In terms of cell proliferation ability, after overexpression of Snail protein, the proliferation rate of shTPPP3 glioma cells was significantly faster than that of control cells (Fig. 4G).

The effect of TPPP3 on the proliferation of glioma cells in vivo

In order to more accurately describe the function of TPPP3 in the occurrence and malignant development of glioma, we verified the function of TPPP3 in vivo. It can be seen from Fig. 5A to C that the glioma cells knocked down TPPP3 had the worst tumorigenesis effect in nude mice, and the tumor volume and weight were significantly smaller than the control group. The results of immunohistochemistry suggested that the expression levels of key marker proteins N-cadherin and Vimentin in the EMT process in mouse glioma tissues with knockdown of TPPP3 expression were low, while the expression of E-cadherin protein was negatively correlated with the expression of TPPP3(Fig. 5D). These in vivo experimental data further illustrate that TPPP3 inhibited the proliferation of glioma cells and affected the EMT process.

Analysis of TPPP3 and Snail protein expression in clinical samples of patients with glioma

We randomly selected some glioma tissue with high and low expression of TPPP3. The results of immunohistochemistry suggest that the expression of Snail changes with the expression of TPPP3(Fig. 6A). There was a positive correlation between the expression of Snail and the expression of TPPP3(Fig. 6B). At the same time, we conducted survival analysis on the existing data and found that among glioma patients, patients with low TPPP3 expression had a longer progression-free survival (Fig. 6C), and the overall survival rate was higher than that of the high TPPP3 group (Fig. 6D). These results were basically consistent with the above experimental results.

Discussion

This study is the first to explore the role of cytoskeletal structure-related factors in the biological behavior of glioma malignancy and its clinical application. We are the first to explore the role of cytoskeletal structure-related factors in the biological behavior of glioma malignancy and their clinical application. Through bioinformation big data analysis and clinical specimens, TPPP3 can be used as a new indicator to evaluate the clinical prognosis of patients with glioma. In addition, this study found relevant mechanisms of TPPP3 regulating tumor cell proliferation, migration and invasion, and EMT transformation in glioma, and discussed the correlation and association between TPPP3 and EMT marker protein Snail 1 in detail. This study will pave the way for further exploration of the role of TPPP3

in glioma, provide direction and ideas, and provide theoretical evidence for TPPP3 to become a potential therapeutic target for glioma.

While our results revealed that TPPP3 played a key role in glioma EMT, the deeper mechanism remains unclear. Glioma is the tumor type with the highest rate of intracranial disability and mortality. According to the histopathological characteristics of glioma, the WORLD Health Organization classified glioma into grade I- , which represents the degree of malignancy. Grade I- is low-grade glioma (LGG), and grade III- is high-grade glioma (HGG)[17]. The actin cytoskeleton is a molecular framework that provides physical support for cell structure and proliferation[14]. Studies reported the relationship between the cytoskeleton and glioma, that was, AVIL, a regulator of the cytoskeleton, could drive the tumorigenesis of glioblastoma[18]. In normal cells, the cytoskeleton, actin (microfilaments), microtubules (MTs) and intermediate filaments are highly integrated, and their functions are coordinated. More and more research indicated that the mutation and abnormal expression of cytoskeleton and cytoskeleton-related proteins played an important role in the ability of cancer cells to resist chemotherapy and metastasis. The role of microtubules in tumor cell metastasis was revealed. People are becoming more and more interested in the interaction between the key proteins of microtubule interaction and the cytoskeleton of actin[19]. Microtubules, the main component of the cytoskeleton, are heterodimers formed by the polymerization of tubulin and are found in all eukaryotic organisms[20, 21]. It is involved in many physiological functions of cells, including extension and guidance of neurons within the nerve growth cone, maintenance of cell morphology, transport and migration of organelles, and spinning-chain formation necessary for chromatin separation[22]. There are a series of proteins in the cell that interact with tubulin to regulate the function of microtubules[21]. These proteins are called microtubules interacting proteins and their main functions include promoting the aggregation or assembly of microtubules and enhancing the stability of microtubules[23].As a newly discovered protein family in eukaryotes, tubulin polymerization promoting protein family belongs to MP protein family[24]. TPPP3 protein can promote microtubule polymerization[25].

Studies have found that interfering TPPP3 expression can inhibit Hela cell colonization, block G1/S phase, and promote cell apoptosis[26]. In addition, in nude mouse models, studies have found that Lewis Lung Carcinoma cells that inhibit TPPP3 expression formed subcutaneous tumors in mice smaller and slower than the control[27]. Vinay Shukla et al. found that TPPP3 plays an important role in the demulsification process, and inhibition of TPPP3 can induce mitochondria-dependent apoptosis[28]. These results suggest that inhibition of TPPP3 expression can interfere with the process of mitosis and cell cycle and thus inhibit the proliferation of tumor cells. The role of TPPP3 in some malignancies is also gradually being discovered. Yang et al. believed that TPPP3 involves a variety of immune-related pathways and wad related to the level of immune infiltration. And TPPP3 could be considered as a biomarker for predicting the prognosis and immune invasion of head and neck squamous cell carcinoma[10]. TPPP3 plays different roles in different tumors. Inhibit the proliferation, invasion and migration of cervical endometrial carcinoma[11], and nasopharyngeal carcinoma[12]. by different ways. But in breast cancer [29]and non-small cell lung cancer[9], there was a carcinogenic effect. Our research

found that TPPP3 was an oncogene in glioma cells, which promoted the malignant biological behavior of glioma cells.

Epithelial-mesenchymal transition (EMT) is the source that enables tumor cells to acquire strong invasion ability and thus promote tumor progression and metastasis[30]. The characteristics of invasive osmotic growth of glioblastoma are closely related to the remodeling of tumor extracellular matrix EMT[31, 32]. Metastasis and diffusion of cancer cells is considered to be a key step in tumor progression, similar to the transition from epithelial to mesenchymal transformation observed during embryonic development. EMT involves a change in cell morphology in which epithelial cells loosen their attachment to neighboring cells, lose apical basal polarity, become elongated and show increased movement, forming a cascade of invasion and metastasis[30, 33, 34]. The role of EMT in the development and progression of glioma is a hot research topic[35].It was reported that the new functional glioma stem cell marker LGR5 played a role in glioma by promoting EMT[36]. Xie et al. found that Eukaryotic translation Factor 1δ promotes proliferation, migration and invasion of glioma cells through EMT[37]. It had also been reported that IDH1 mutation promoted glioma cell proliferation and migration by inducing EMT[38]. Some scientists have elaborated on the role of EMT in the prognostic value of glioma patients and whether it plays a role in the immune microenvironment of glioma[39]. To assess whether the effect of TPPP3 on glioma motility is related to EMT, we examined the expression of EMT-related markers (Vimentin, N-cadherin, e-cadherin). The results showed that Vimentin and N-cadherin expression were down-regulated after TPPP3 expression was decreased, while e-cadherin expression was up-regulated. This finding suggests that the regulation of glioma motility by TPPP3 may be related to epithelial mesenchymal transformation. Our results verified that TPPP3 may induce EMT by regulating Snail 1 protein expression. This further clarified the biological role of TPPP3 in the malignant progression of glioma. It has also been reported that RND3 promotes Snail 1 degradation and inhibits migration and invasion of glioblastoma cells, which also confirms Snail 1's role in glioma cells[40]. Another report confirmed the role of Snail1 in the process of MicroRNA-22 regulating the proliferation, drug sensitivity and metastasis of human glioma cells[41]. These findings suggested the role of Snail 1 in EMT and glioma, which made our findings more credible.

After the analysis of clinical data, we learned that there was a positive correlation between TPPP3 and Snail 1, and the role of TPPP3 in the survival of glioma patients was also presented. Our results suggest that TPPP3 may have a role in gliomas. However, we still need more evidence to support the application value of TPPP3 in glioma, and the detailed mechanism of its participation in the malignant biological behavior of glioma needs to be explored.

Declarations

Acknowledgments: Not applicable.

Founding: This study was funded by the Guizhou Provincial Natural Science Foundation [No.: QianKeHe (2016) support 2905], the Science and Technology Foundation approved by Guizhou Provincial Health

Commission (No. gzwkj [2022-090]), the Guizhou Science and Technology Foundation (No. [2019]1437), and the Department of Education of Guizhou Province (No. KY [2021]313).

Declaration of interests: Authors declare no conflicts of interest.

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Figures

Figure1

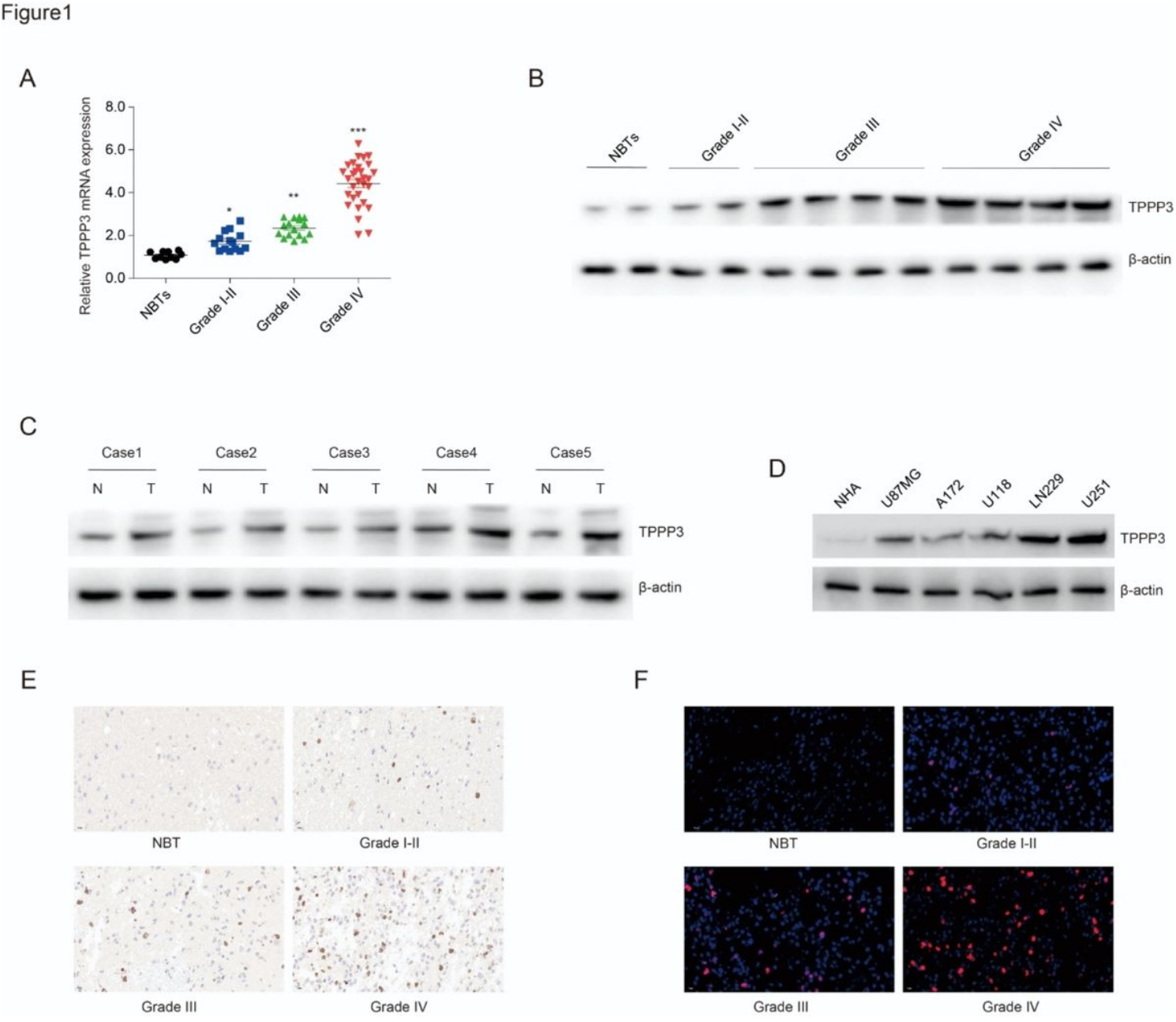

Figure 1

TPPP3 was highly expressed in glioma tissues and glioma cells, and increased with the increase of glioma grade. (A)The expression of TPPP3 in different grades of glioma and brain tissue at the mRNA level was detected by qRT-PCR assay. (B)Western blot detection the expression of TPPP3 in clinical glioma specimens of various grades. (C)The expression of TPPP3 in 5 glioma tissues and normal tissues was analyzed by Western Bolt. (D) The level of protein expression of TPPP3 in each line of glioma cells. (E, F) The expression of TPPP3 in different grades of glioma tissues was independently confirmed by immunohistochemistry and immunofluorescence. Data from at least three independent experiments were quantified. *P<0.05, **P<0.01, ***P<0.001

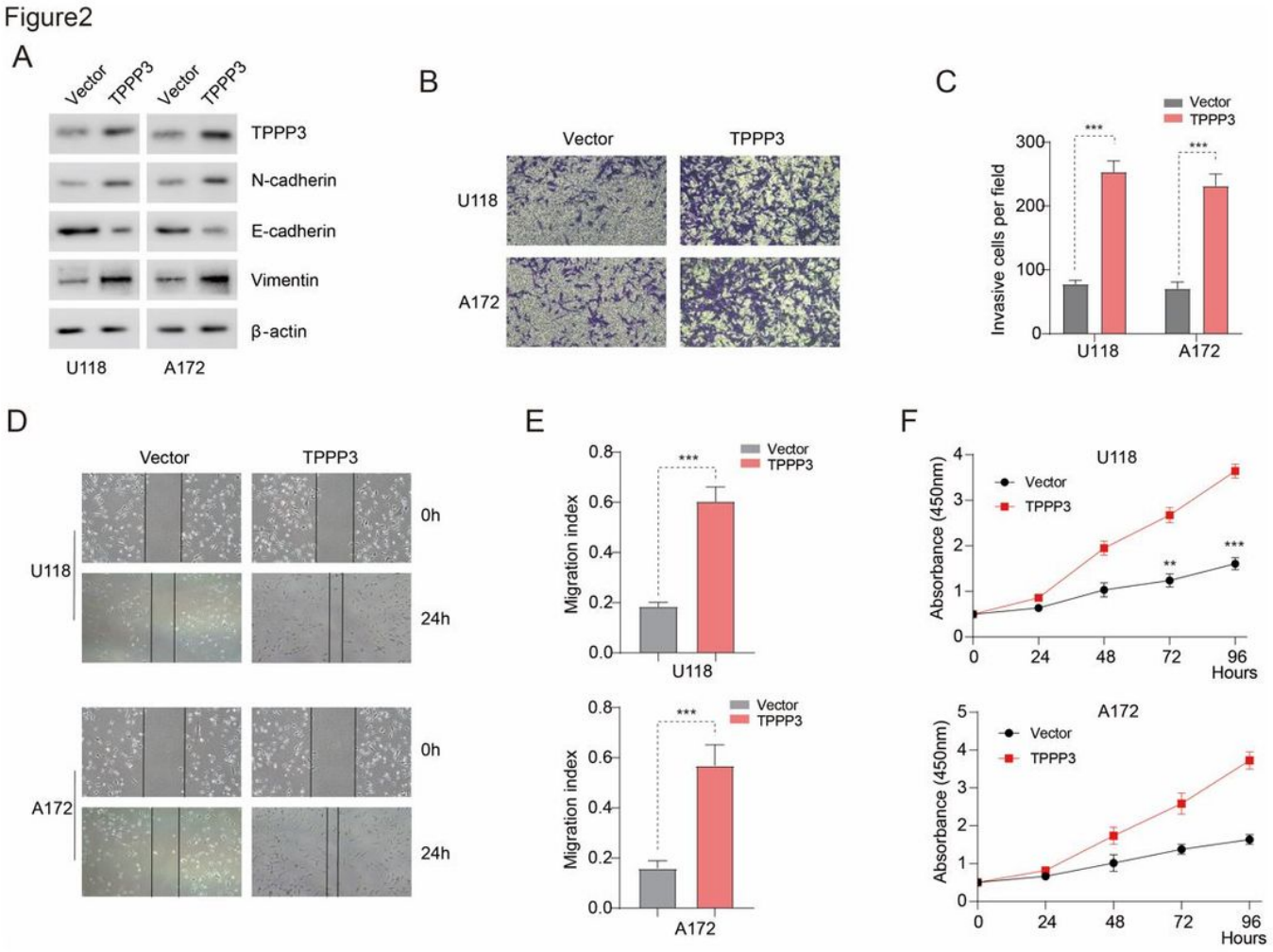

Figure 2

TPPP3 was associated with the EMT process of glioma cells and promoted the proliferation, migration and invasion of glioma cells. (A) Western blot detection the effect of TPPP3 overexpression on the expression of EMT pathway related proteins. (B, C) The effect of TPPP3 overexpression on glioma cells invasion was analyzed by Transwell assay. (D, E) Wound healing assay quantified the effect of TPPP3 overexpression on glioma cell migration. (F) CCK8 assay detected the proliferation of glioma cells after overexpression of TPPP3. Data from at least three independent experiments were quantified. ***P<0.001

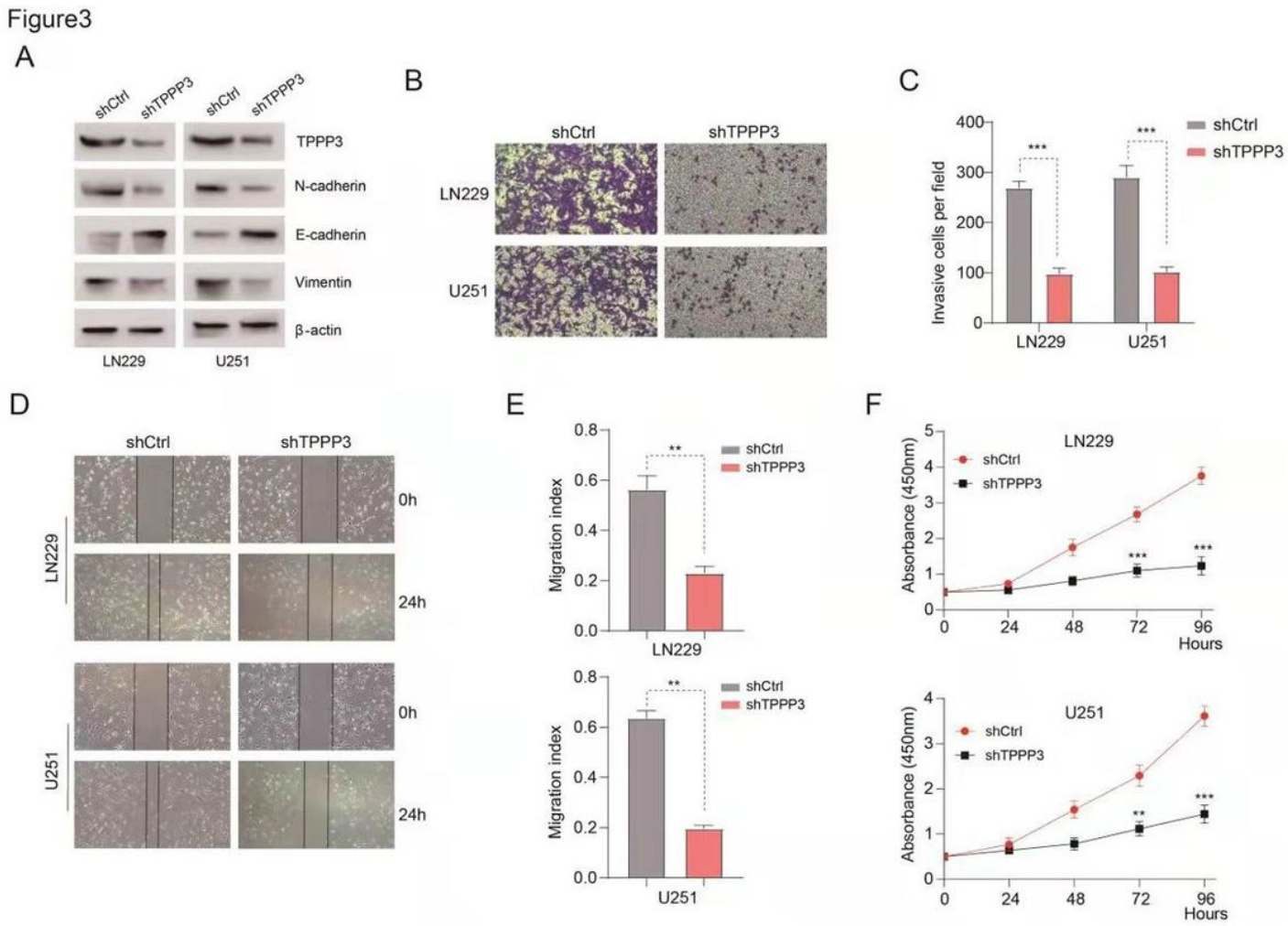

Figure 3

Knockdown of TPPP3 expression inhibited the EMT process and malignant biological behavior of glioma cells. Glioma cell lines with TPPP3 knockdown expression were constructed for subsequent experiments. A. Changes in the expression of key proteins in the EMT process of glioma cells after knocking down the expression of TPPP3. (B, C) The invasion ability of glioma cells was quantitatively analyzed by Transwell experiment. (D, E) The number of migrated glioma cells was quantitatively analyzed by the wound-healing assay. (F) CCK8 assay detection the proliferation of glioma cells. Data from at least three independent experiments were quantified. $^{**}P<0.01$, $^{***}P<0.001$

Figure4

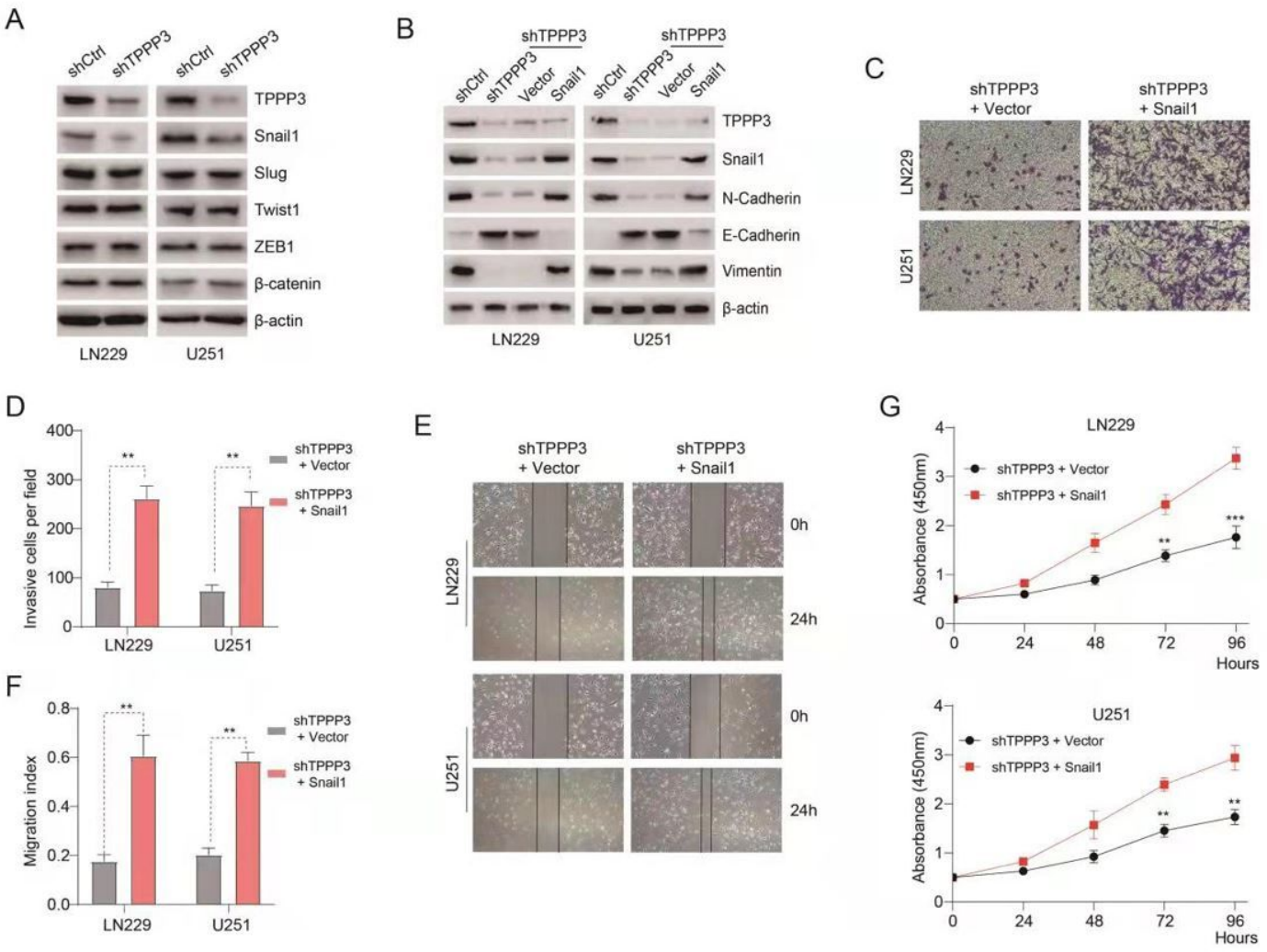

Figure 4

TPPP3 induced epithelial-mesenchymal transition by regulating the expression of Snail 1 protein. (A)Western Blot analysis the effect of knocking down the expression of TPPP3 on the expression of common key proteins in the EMT process. (B) The effect of overexpression of Snail 1 in cells with knockdown of TPPP3 on the expression of E-cadherin, N-cadherin and Vimentin, the key markers of EMT process. (C, D) Snail 1 was overexpressed in TPPP3 knockdown cells, and the invasion ability of glioma cells was quantitatively analyzed by Transwell experiment. (E, F) The wound healing experiment tested the migration ability of glioma cells after overexpression of Snail 1 in TPPP3 knockdown cells. (G) The CCK8 experiment analyzed the effect of overexpression of Snail 1 in TPPP3 knockdown cells on the proliferation of glioma cells. Data from at least three independent experiments were quantified. ** $P<0.01$

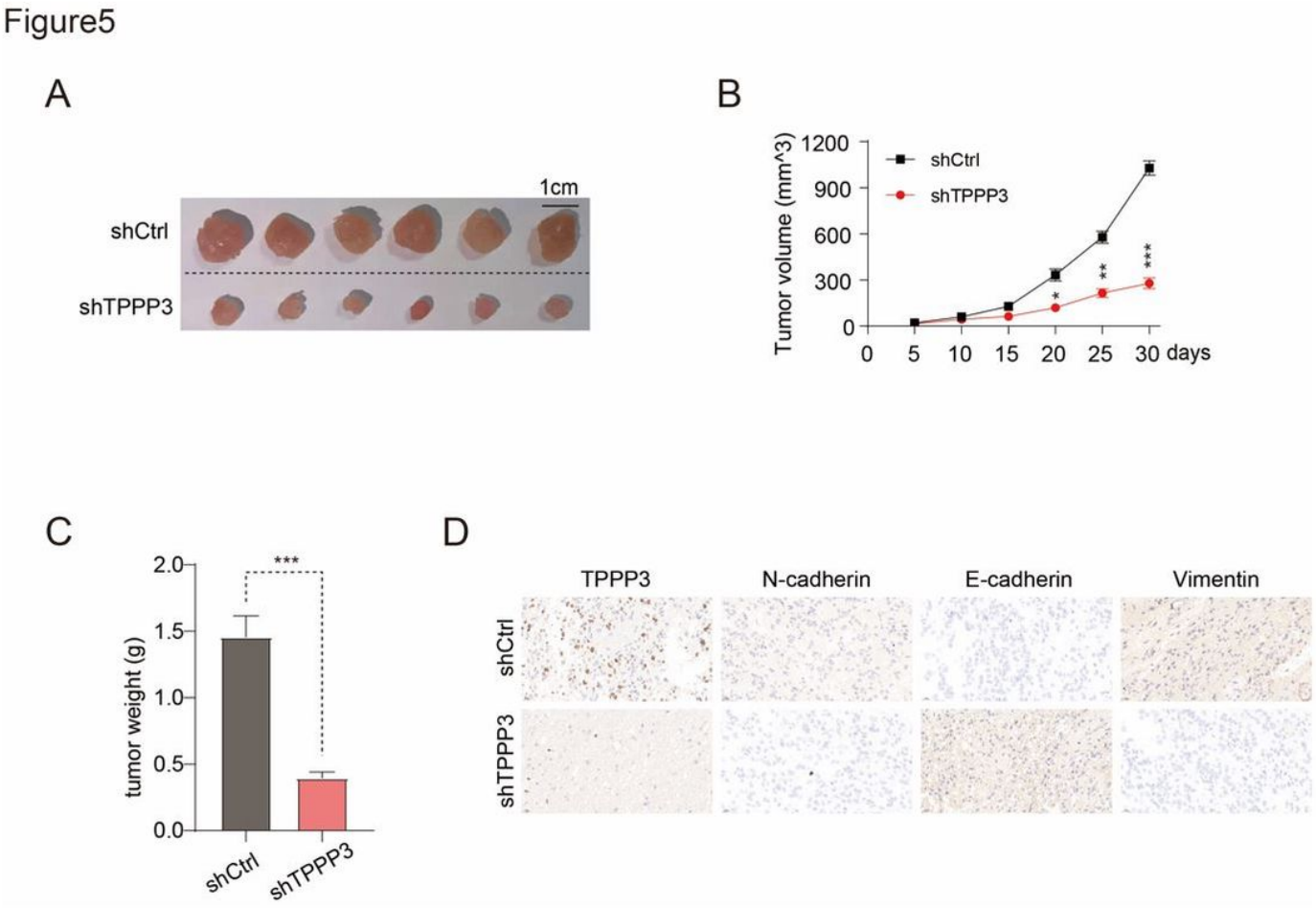

Figure 5

Knockdown of TPPP3 expression significantly inhibited the proliferation of glioma cell lines in vivo. An animal model with TPPP3 knockdown expression was constructed for subsequent experiments. (A)Photos of subcutaneous tumors of mice in the experimental group and the control group. (B)Tumor volume of experimental and control mice at different time. (C)Tumor weight of experimental and control mice. (D) The expression of key proteins in THE TPPP3 and EMT process in tumor tissues of the two groups was detected by immunohistochemistry.

Figure6

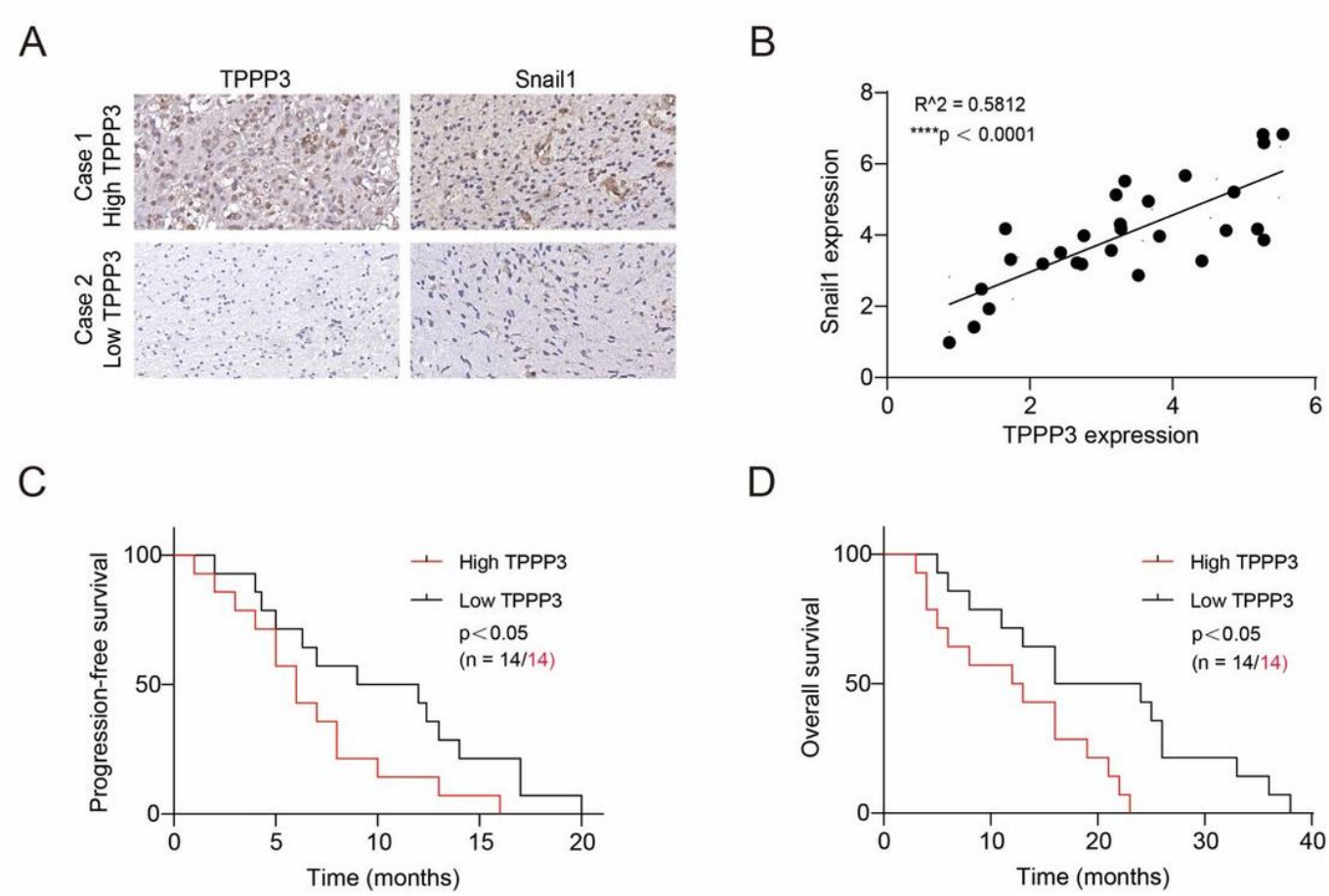

Figure 6

Data analysis of clinical significance of TPPP3 in glioma. (A) Immunohistochemical images of representative glioma tissues with high and low TPPP3 expression. (B) Correlation analysis between TPPP3 and Snail 1 in glioma tissues. (C, D) The expression level of TPPP3 in glioma affected the survival of patients.