Received: 30 April 2022 | Revised: 8 September 2022 | Accepted: 12 September 2022

DOI: 10.1111/cas.15612

ORIGINAL ARTICLE

Cancer Science WILEY

circTOP2A functions as a ceRNA to promote glioma progression by upregulating RPN2

Jikui Sun[1,2,3] | Jinhuan Wang[4] | Meng Li[3] | Shengjie Li[3] | Hanyun Li[5] | Yan Lu[1,6] | Feng Li[3] | Tao Xin[2,3] | Feng Jin[1]

[1]Department of Neurosurgery, Affiliated Hospital of Jining Medical University, & Shandong Provincial Key Laboratory of Stem Cells and Neuro-oncology, Jining, China

[2]Shandong University of Traditional Chinese Medicine, Jinan, China

[3]Shandong Medicine and Health Key Laboratory of Neurosurgery, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, China

[4]Tianjin Cerebral Vascular and Neural Degenerative Disease Key Laboratory, Department of Neurosurgery, Tianjin Neurosurgical Institute, Tianjin Huanhu Hospital, Tianjin, China

[5]Cheeloo College of Medicine, Shandong University, Jinan, China

[6]Medical Research Center, Affiliated Hospital of Jining Medical University, Jining, China

Correspondence
Tao Xin, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, 16766, Jingshi Road, Jinan, 250014, China.
Email: drxintao@yeah.net

Feng Jin, Affiliated Hospital of Jining Medical University, 89, Guhuai Road, Jining, 272029, China.
Email: jinfengsdjn@163.com

Funding information
Key Research and Development Program of Jining Science and Technology, Grant/Award Number: 2020YXNS005; National Natural Science Foundation of China, Grant/Award Number: 81071779, 81972340 and 82173140; Natural Science Foundation of Shandong Province, Grant/Award Number: ZR2016HL34, ZR2021QH256 and ZR2022MH140; Project of Shandong Province Higher Educational Science and Technology Program, Grant/Award Number: J16LL05; Project funded by China Postdoctoral Science Foundation, Grant/Award Number: 2022M711324

Abstract

Competing endogenous RNA (ceRNA)-mediated signaling pathway dysregulation provides great insight into comprehensively understanding the molecular mechanism and combined targeted therapy for glioblastoma. circRNA is characterized by high stability, tissue/developmental stage-specific expression and abundance in brain and plays significant roles in the initiation and progression of cancer. Our previous published data have demonstrated that RPN2 was significantly upregulated in glioma and promoted tumor progression via the activation of the Wnt/β-catenin pathway. Furthermore, we proved that miR-422a regulated the Wnt/β-catenin signaling pathway by directly targeting RPN2. In this study, based on the glioblastoma microarray profiles, we identified the upstream circTOP2A, which completely bound to miR-422a and was co-expressed with the RPN2. circTOP2A was significantly overexpressed in glioma and conferred a poor prognosis. circTOP2A could regulate RPN2 expression by sponging miR-422a, verified by western blot, dual-luciferase reporter gene assay, and RNA pull-down assay. Functional assays including CCK8, transwell and FITC-annexin V were performed to explore the RPN2-mediated role of the circTOP2A effect on the glioma malignant phenotype. Additionally, TOP/FOP and immunofluorescence analysis were used to confirm that sh-circTOP2A could suppress the Wnt/β-catenin pathway partly through RPN2. Finally, a tumor xenograft model was applied to validate the biological function of circTOP2A in vivo. Taken together, our findings reveal the

Abbreviations: circRNA, circular RNA; GBM, glioblastoma multiforme; IGF1, insulin-like growth factor 1; miRNA, microRNA; NHA, normal astrocyte cell; qRT-PCR, quantitative real-time PCR; RBP, RNA-binding protein; WHO, World Health Organization.

critical role of circTOP2A in promoting glioma proliferation and invasion via a ceRNA mechanism and provide an exploitable biomarker and therapeutic target for glioma patients.

KEYWORDS
circTOP2A, glioma, miR-422a, RPN2, Wnt/β-catenin

1 | INTRODUCTION

Glioma is the most common intracranial primary tumor. Among these tumors, GBM, an incurable primary brain tumor with a dismal prognosis in adults, makes up to 50% of the primary malignant brain tumors.[1] High heterogeneity resulting from the complicated molecular network in GBM is responsible for its poor treatment response, including chemoradiotherapy and immunotherapy resistance.[2,3] It is involved in various genetic alterations, such as the mutation of isocitrate dehydrogenase (IDH)1/2 and O^6-methylguanine methyltransferase (MGMT), and the dysregulation of multiple signaling pathways, such as the PI3K/AKT/mTOR, Wnt/β-catenin, and NF/κB pathways,[4,5] However, the aberrant signaling network-mediated molecular mechanism underlying the progression of glioma and the development of more effective treatment options remains still poorly understood.

In the last few decades, noncoding RNAs, including lncRNA-, miRNA-, and circRNA-mediated genetic network dysregulations, have been suggested to serve significant roles in the development and progression of malignancies, including gliomas.[6,7] Recently, circRNA, as a type of regulatory RNA, has attracted great research interest, attributed to the high stability of its ring structure, tissue, or developmental-stage specificity and abundance in exosomes.[8,9] Additionally, high abundance in mammalian brain compared with other tissues, as well as conserved sequences, endow circRNAs with great potential as biomarkers for central nervous system diseases.[10] With the accumulated identification and functional disclosure of circRNAs, it has been described that circRNAs play important roles in cancer progression, recurrence, treatment resistance, and immune escape.[11,12] To date, circRNAs have been found to act as competing endogenous RNA (ceRNA) and have been most widely reported in various types of cancer. For instance, multiple cancer-related ciRS-7 can sponge miR-7 and therefore upregulate the expression of downstream target genes.[8] circRNA HIPK3 sponges multiple miRNAs, including tumor suppressor miR-124, miR-7, miR-4288, and miR-654, and promotes tumor cell proliferation and invasion.[10,13] In addition, circCSNK1G3 enhanced tumor cell proliferation through competitively binding to miR-181b/d in prostate cancer.[14] Even through the molecular mechanisms of circRNAs that participate in glioma occurrence and progression via sponging miRNAs to regulate the target gene expression have been extensively reported, circRNA-mediated signaling pathway network dysregulation remains to be further elucidated.

The Wnt/β-catenin signaling pathway has significant roles in cell proliferation, invasion, angiogenesis, and the differentiation of cancer stem cells in glioma, and is currently considered a potential target for drug design.[15,16] Previously, based on GBM tissue microarray data, we identified an oncogene, RPN2, which was markedly upregulated in glioma and conferred a poor prognosis, and that could activate the Wnt/β-catenin signaling pathway through the suppression of GSK-3β.[17] Furthermore, combining bioinformatics analysis with experiments, we identified that RPN2 was a direct functional target of miR-422a. It is well known that miRNA-mediated dysregulation of mRNAs and their relevant signaling pathways are closely associated with progression, temozolomide (TMZ) resistance, and molecular subtyping of glioma.[18]

In the present study, we based work on our previous expression profiling of circRNA and mRNA in three GBM and in paired adjacent tissues using a microarray containing probes for circRNAs and mRNAs. This was combined with molecular mechanism verification of RPN2 targeted by miR-422a involved in glioma progression via the Wnt/β-catenin pathway, we further identified that hsa_circ_0106819, designated as circTOP2A sponging miR-422a, regulated the expression of RPN2. circTOP2A was correlated with WHO grade and clinical prognosis and promoted cell proliferation by functioning as a ceRNA to upregulate RPN2. Our results indicated that circTOP2A exerted oncogenic potential and that it may be a candidate in the diagnosis and treatment of glioma.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

In total, 52 glioma samples undergoing craniotomy for tumor were obtained from Tianjin Huan Hu Hospital (Tianjin, China). Among the 15 low-grade gliomas, two were WHO grade I (oligodendrogliomas) and 13 were WHO grade II (7 oligodendrogliomas and six diffuse astrocytomas). Of 37 high-grade gliomas, eight were WHO grade III (five mesenchymal oligodendroglioma and three anaplastic astrocytomas) and 29 WHO IV (GBM). None of the patients had received any radiotherapy, chemotherapy, or any other anticancer treatments prior to surgery. Five normal adult brain tissue samples were collected while patients underwent post-trauma surgery for severe traumatic brain injury. All the collected tissues were frozen immediately in liquid nitrogen and stored at −80°C until use.

2.2 | Cell culture and transfection

The U251, U87, A172, LN229, and T98J glioblastoma cells were obtained from the China Academia Sinica Cell Repository. The NHA and low-grade glioma H4 cells were obtained from the Peking Union Medical College Cell Library. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum, (Gibco) and incubated at 37°C with 5% CO_2.

Plasmid-mediated RPN2 and circTOP2A overexpression vector, siRNA targeting circTOP2A, and RPN2, lentivirus vector sh-circTOP2A, miR-422a inhibitor were purchased from GeneChem. The siRNA sequences are shown below, si-circTOP2A: AAATTG TTTTCCCAGGCTG, siRPN2: GGATCGCCCTTTCACAAT and si-NC: TTCTCCGAACGTGTCACGT. Cell transfections were conducted using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer's protocols. The cells were harvested for assays at 48 h after transfection.

2.3 | Generation of circTOP2A knockdown cells

To generate U251 and LN229 cells with stable knockdown of circTOP2A, lentiviruses were produced in HEK293T cells by co-transfection with psh-circTOP2A and packaging plasmids GAG and VSV-G (sh-circTOP2A: AAATTGTTTTCCCAGGCTG). Virus supernatant was harvested at 48 h after transfection, mixed with polybrene (8 μg/ml), and added to U251 and LN229 cells. After the 48 h incubation, 2 μg/ml puromycin was added for 1 week to screen for stable cell clones.

2.4 | Microarray analysis

Three paired samples of tumorous tissues and adjacent nontumorous tissues were used for microarray analysis and circRNA expression profiles were generated by Capitalbio Technology Corporation.

2.5 | RNA and genomic DNA (gDNA) extraction

TRIzol reagent (Invitrogen) was used to extract total RNA from glioma cell lines and freshly frozen clinical samples following the manufacturer's instructions. The nuclear and cytoplasmic fractions were extracted using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific). gDNA was extracted using a genomic DNA isolation kit (Tiangen Biotech) according to the manufacturer's instructions.

2.6 | Actinomycin D and RNase R treatment

The cell culture medium was supplemented with 2 mg/ml actinomycin D or DMSO (Sigma-Aldrich) as a negative control to block the transcription of glioma cells. Total RNA (2 μg) was incubated for 30 min at 37°C with or without 2 U/μg RNase R (Epicenter Technologies).

2.7 | Fluorescence in situ hybridization

Fluorescence in situ hybridization assays were performed to observe the location of circTOP2A in U252 cells. Briefly, after prehybridization at 55°C for 2 h, cell climbing pieces were hybridized with a specific Cy3-labeled circTOP2A probe (Cy3–5′- AATTAAATTG TTTTCCCAGGCTGATAG-3′–Cy3) at 37°C overnight and dyed with 4′,6-diamidino-2-phenylindole (DAPI; Life Technologies). Images were acquired under a confocal fluorescence microscope (Leica, SP8 laser confocal microscopy).

2.8 | RT-PCR and qRT-PCR

For PCR of mRNA and circRNA, RNA was reverse-transcribed using HiScript® II Q RT SuperMix for qPCR (+gDNA wiper; Vazyme). For qPCR of miRNA, cDNA was synthesized using an All-in-One™ miRNA First-Strand cDNA Synthesis Kit (GeneCopoeia). qPCR was performed using ChamQ SYBR qPCR Master Mix (Vazyme) on an ABI Stepone Plus system. The circRNA and mRNA levels were normalized to GAPDH. The miRNA level was normalized to small nuclear U6. The relative expression levels were determined using the $2^{-\Delta\Delta Ct}$ method. Primers are listed in Table S1.

2.9 | Luciferase activity assays

The vectors of luciferase reporters, named circTOP2A-WT and circTOP2A-MUT, RPN2-3'UTR-WT and RPN2-3'UTR-MUT, were synthesized by GenePharma. Briefly, cells were inoculated into a 96-well plate and were co-transfected with miR-422a mimics and si-circTOP2A, respectively. To evaluate the β-catenin/Tcf-4 transcriptional activity, we used the TOP-FLASH and FOP-FLASH luciferase reporter constructs. U251 and LN229 cells with or without circTOP2A stable knockdown were co-transfected with 100 ng of a TOP-FLASH plasmid containing six TCF-binding motifs (Millipore) or 100 ng of a FOP-FLASH control plasmid containing six mutated TCF-binding motifs (Millipore) and RPN2 or not. Dual-luciferase activities were also detected at 48 h after the transfection using the Dual-Luciferase Reporter Assay System (Promega), and the Renilla luciferase activity was used as an internal control.

2.10 | Pull-down assay with biotinylated miRNA

The biotinylated 3′-ends of the miR-422a mimic or control RNA (Geneseed) were transfected into U251 and LN229 cells at a final concentration of 100 nM for 48 h before harvest. Then, the

biotinylated RNA complex was pulled down by incubating the cell lysates with streptavidin-coated magnetic beads at 4°C on a rotator overnight. TRIzol LS reagent (Thermo Fisher Scientific) was used to extract RNA from the input and pull-down beads. The abundance of circTOP2A and RPN2 in bound fraction was determined using qRT-PCR.

2.11 | Western blot analysis

Briefly, total protein of glioma cell lines was extracted using the ExKine Total Protein Extraction Kit (Abbkine) in accordance with its protocol. Equal amounts of protein (30 μg/lane) were separated by 10% SDS-PAGE and subsequently transferred to PVDF membranes (EMD Millipore). After blocking for nonspecific binding, the membranes were incubated with antibodies for RPN2 (1:200 dilution; ab244399; Abcam), TCF4 (1:10,000 dilution; ab76151; Abcam), c-*myc* (1:1000; ab39688; Abcam), cyclin D1 (1:10,000; ab134175; Abcam) or β-actin (1:5000; ab6276; Abcam) overnight at 4°C and followed by incubation with secondary antibodies for 1 h at room temperature. Eventually, an ECL western blotting substrate (Promega) was used to develop the protein bands.

2.12 | Immunofluorescence staining

In brief, cells were fixed with 0.1% paraformaldehyde for 20 min at room temperature and were blocked with 5% BSA (Sigma-Aldrich; Merck KGaA) at room temperature for 2 h. Cells were incubated with anti-β-catenin primary antibody (1:200; cat. no. ab32572; Abcam) overnight at 4°C. After washing with PBS at room temperature, a goat anti-rabbit IgG fluorescent conjugated second antibody (1:10,000; cat. no. A21245; Invitrogen; Thermo Fisher Scientific, Inc.) was added for 1 h at room temperature and samples imaged using a confocal microscope.

2.13 | Immunohistochemical (IHC) staining

Sections underwent dewaxing, re-hydration, antigen retrieval, and blocking, and then were incubated with antibodies against RPN2 (1:200; ab244399; Abcam) and β-catenin (1:500; ab32572; Abcam) overnight at 4°C and then washed three times with PBST. Sections were incubated with HRP-conjugated secondary antibody for 15 min at room temperature and then stained with diaminobenzidene (DAB) and hematoxylin.

2.14 | CCK-8, invasion and apoptosis analysis

Cell viability was analyzed using the Cell Counting Kit-8 (CCK-8, Dojindo) in accordance with the manufacturer's instructions. Corning Transwell insert chambers (8 μm pore size, Corning) and BD Matrigel (BD Biosciences) were used for the cell invasion experiment for different treatments of GBM cells. Cell apoptosis analysis was performed using the FITC-Annexin V Apoptosis Detection Kit (BD Biosciences) in accordance with the manufacturer's instructions, as previously described.[17,19]

2.15 | Xenograft model

Five-week-old female BALB/c nude mice were purchased from the Animal Center of the Cancer Institute, Chinese Academy of Medical Science and housed under SPF conditions (TECNIPLAST S.p.A.). The stably transfected LN229 cells with a knockdown for circTOP2A or a negative control were subcutaneously injected into the right flank of each mouse (10 mice in total). At 2 weeks later, the volumes of tumors were examined every 7 days and after 21 days the mice were sacrificed. Tumors were weighed and processed for further histological analysis and RNA extraction. Tumor volume was calculated as follows: V (volume) = (length × width^2)/2.

2.16 | Statistical analyses

All statistical analyses were performed using GraphPad software version 6.0 (GraphPad Software). Data are presented as the mean ± standard deviation of at least three independent experiments. An unpaired Student's *t*-test and one-way ANOVA were performed to analyze significant differences between two groups or multiple groups, respectively. Fisher's exact test was used to analyze the associations between circTOP2A and clinicopathological characteristics of patients. The survival curves from online databases were plotted using the Kaplan–Meier method and compared using the log-rank test. A p-value < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | The screening and identification of circTOP2A

Based on previous microarray analysis, we identified a significantly high-expressed oncogene RPN2, which was associated with the WHO grade and conferred a poor prognosis for glioma patients. Then, knockdown of RPN2 could inhibit proliferation, invasion and enhanced tumor cell apoptosis and TMZ sensitivity through repression of the Wnt/β-catenin signaling pathway. Furthermore, we demonstrated that RPN2 was a direct target of miR-422a and that miR-422a could regulated the transcriptional activity of β-catenin/TCF4 by directly targeting RPN2, relevant data have been published previously.[17,19] Based on the ceRNA theory related to the malignant progression of glioma, we further explored the upstream circRNAs of the miR-422a/RPN2 axis on the basis of simultaneously analyzed expression profiles of circRNA and mRNA in three paired samples of GBM and matched nontumorous tissues. In order to identify circRNAs

that could competitively bind miR-422a and concurrently regulate the expression of oncogene RPN2, filtered circRNAs were based on the criteria: fold change >2, $p<0.05$, processed signal >200 and cirBase source. The co-expressed circRNAs with RPN2, as well as those that originated from the miRanda database sponging miR-422a, were intersected and subsequently two circRNAs were identified, including has_circ0106819 and has_circ0079864; the detailed screening process is depicted in Figure 1A. Finally, has_circ_0106819 (circTOP2A) were verified using qRT-PCR and Sanger sequencing, and circTOP2A was also one of the most significantly upregulated circRNAs from microarray circRNA profiles, as illustrated in Figure 1B.

3.2 | Characteristics of circTOP2A in glioma

We next assessed the exon structure of circTOP2A, which was derived from exons 1–12 of the *TOP2A* gene located on the chromosome 17. The back-spliced junction of circTOP2A was amplified using divergent primers and confirmed by Sanger sequencing (Figure 2A). The sequence was consistent with circBase database annotation (http://www.circbase.org/). PCR analysis for cDNA and gDNA demonstrated that divergent primers could amplify products from cDNA but not from gDNA (Figure 2B). To check its resistance to RNase R digestion, total RNA was treated with RNase R and the linear isoform levels were used to illustrate the efficacy of the RNase R treatment. The results showed that circTOP2A was resistant to RNase R, proving the circular structure of circTOP2A in GBM tissues (Figure 2C). Additionally, analysis for stability of circTOP2A and TOP2A in U251 cells treated with actinomycin D revealed that circTOP2A had a significantly longer half-life than TOP2A (Figure 2D). To observe cellular localization of circTOP2A, we conducted qRT-PCR analysis of nuclear and cytoplasmic circTOP2A. Results showed that the circTOP2A transcript was preferentially located in the cytoplasm (Figure 2E). Fluorescence in situ hybridization (FISH) further confirmed that circTOP2A was mainly localized in the cytoplasm (Figure 2F). These results confirmed the characteristics of circTOP2A as a circRNA and laid the foundation for the ceRNA function mechanism.

3.3 | circTOP2A is frequently upregulated in glioma and associated with poor prognosis

We first tested endogenous circTOP2A expression in six glioma cell lines and one human normal astrocyte cell (NHA). We found that circTOP2A was upregulated in GBM cell lines compared with NHA and a low glioma cell line (H4 cell; Figure 3A). Based on this result, U251 and LN229 cells were selected for the following circTOP2A loss-of-function assay, whereas U87 cells were selected for a gain-of-function assay. To determine the expression of circTOP2A in clinical specimens, 52 surgically resected specimens of different grades were examined by qRT-PCR to further explore circTOP2A expression. circTOP2A was significantly overexpressed

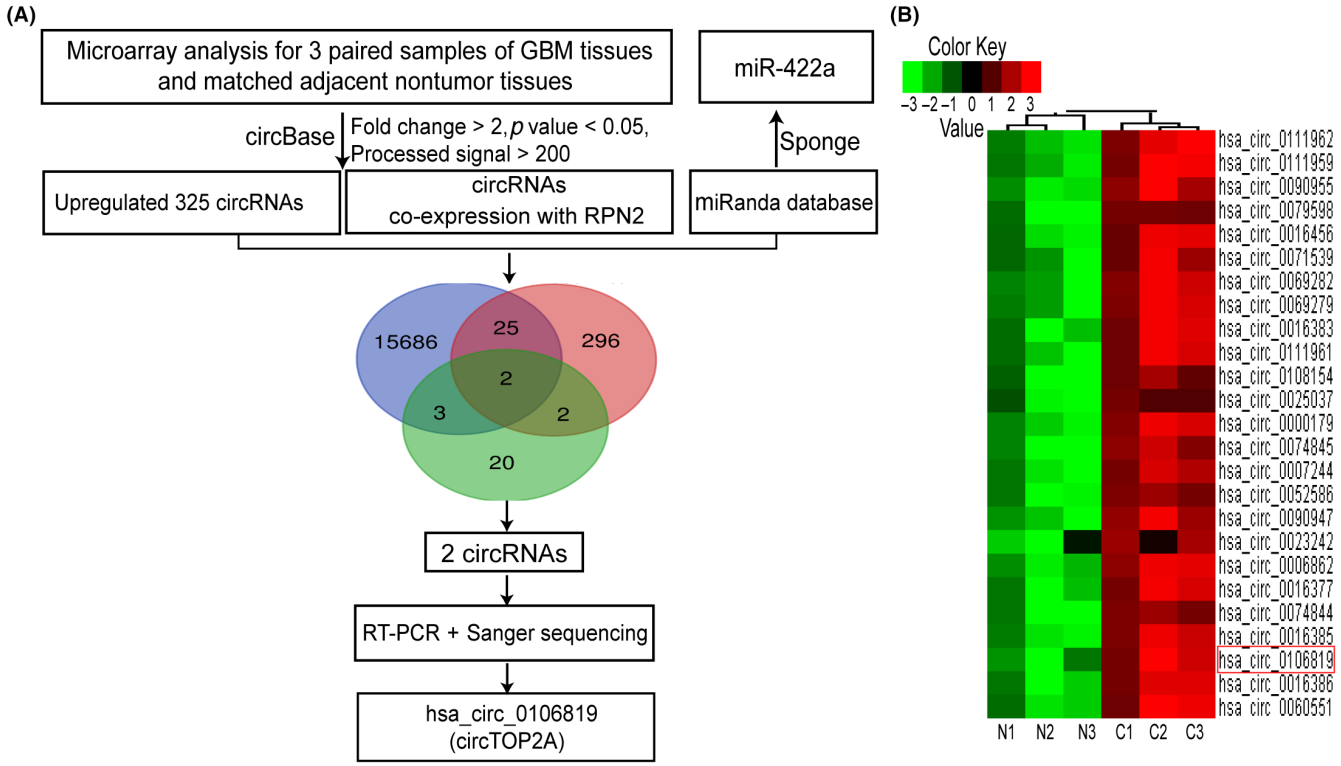

FIGURE 1 A circTOP2A screening process based on glioblastoma multiforme (GBM) microarray. (A) Flow chart delineates the steps for identifying and validating circRNAs in GBM. (B) Heatmap shows the top 25 most upregulated circRNAs in three paired samples of tumorous tissues (C) and corresponding adjacent nontumorous tissues (N) from patients with GBM from array analysis

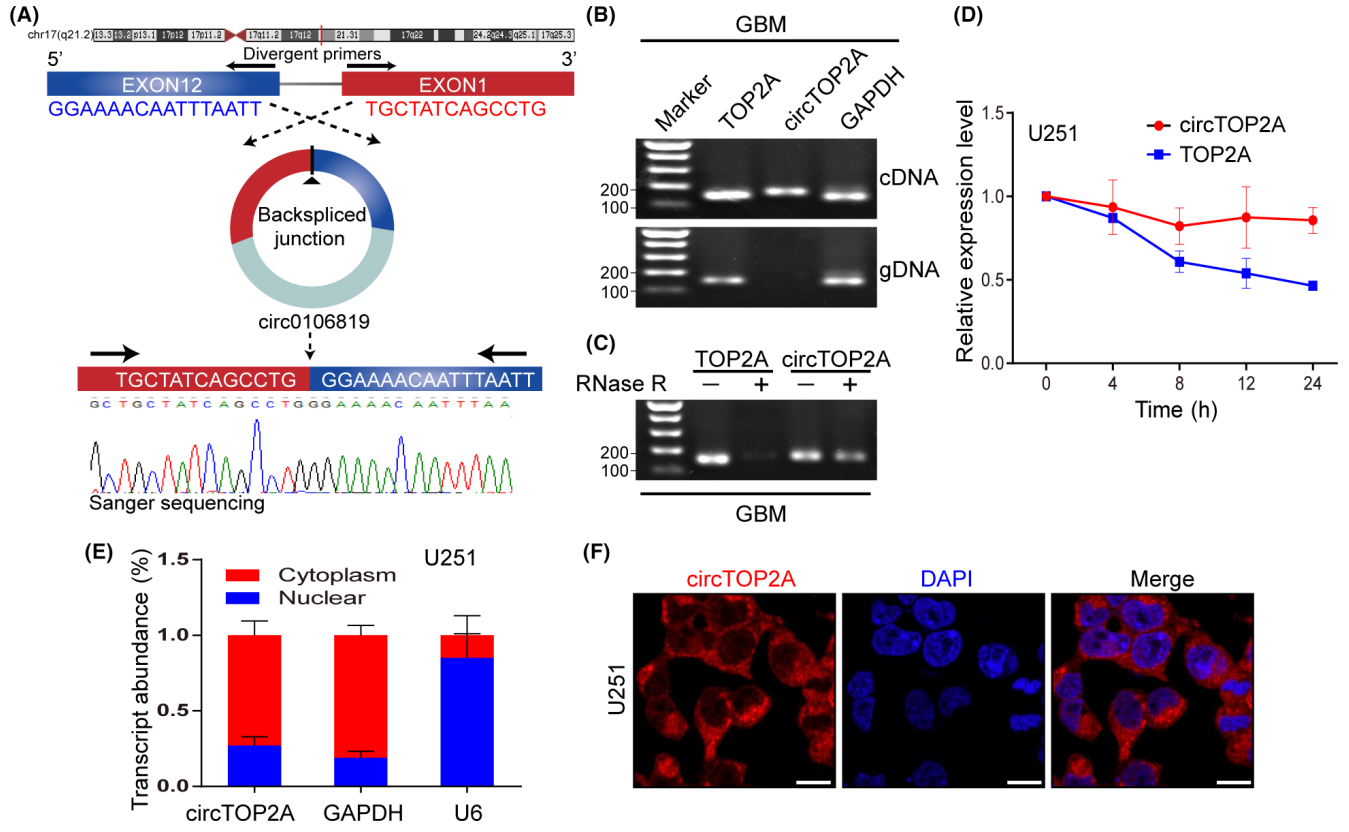

FIGURE 2 Characterization of circTPOP2A in glioma. (A) circTop2A is produced at the *TOP2A* gene locus containing exons 1–12. The back-splice junction of circTOP2A was identified by Sanger sequencing. (B) PCR analysis of circTOP2A and its linear isoform TOP2A in cDNA and gDNA for GBM tissues. (C) PCR analysis of TOP2A and circTOP2A after RNase R treatment for GBM tissues. (D) qRT-PCR to determine the abundance of circTOP2A and TOP2A in U251 cells treated with actinomycin D at the indicated time points. (E) Levels of circTOP2A in the nuclear and cytoplasmic fractions of U251 cells. (F) RNA fluorescence in situ hybridization (FISH) for circTOP2A. The nuclei were stained with DAPI. Scale bars, 20 μm

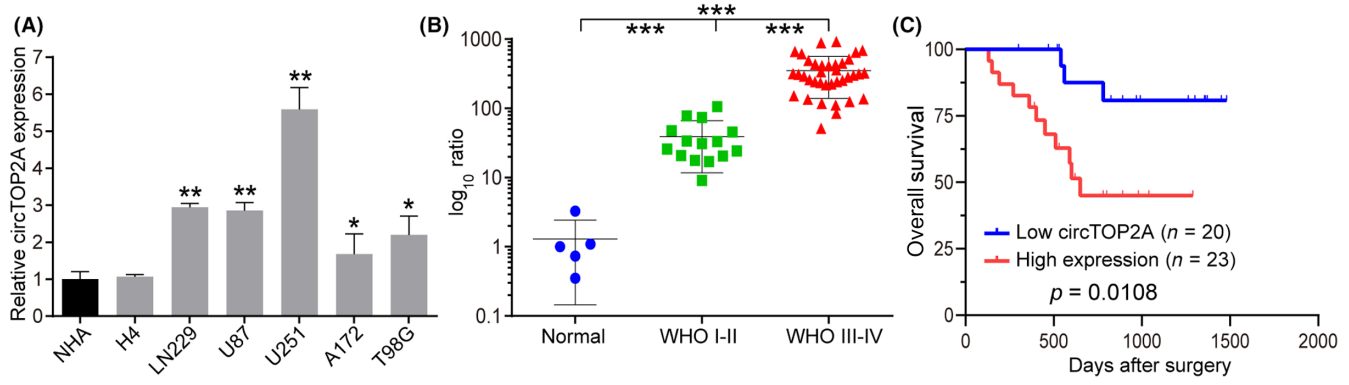

FIGURE 3 circTOP2A is markedly upregulated in GBM and contributes to the poor prognosis. (A) Relative expression levels of circTOP2A in seven glioma cell lines were analyzed by qRT-PCR and compared with normal brain astrocyte cells. (B) Relative expression levels of circTOP2A in 52 glioma specimens of different WHO grades and five normal brain tissues were analyzed by qRT-PCR. (C) Based on the expression of circTOP2A, the overall survival curve was generated using Kaplan–Meier methods and analyzed using the log-rank test. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$

in glioma specimens compared with five normal brain tissues and the expression levels of circTOP2A of WHO III and WHO IV glioma specimens were also markedly higher than that of WHO I and WHO II, implying that circTOP2A expression was positively correlated with WHO grade (Figure 3B). The clinicopathological characteristics of 52 glioma specimens are summarized in Table 1; circTOP2A expression was closely associated with age and histology except for gender. Moreover, patients with high circTOP2A expression (n = 23) had shorter overall survival (OS) than those with low circTOP2A expression (n = 20; Figure 3C). Together, these

TABLE 1 The relationship between circTOP2A and clinicopathological characteristic expression of glioma patients

Variable	No. of cases	circTOP2A		*p*-value
		Low	High	
Gender				
Female	28	15	13	0.2776
Male	24	9	15	
Age (years)				
<45	17	13	4	0.0032
≥45	35	11	24	
Histology				
A	6	6	0	<0.001
O	9	9	0	
AO	5	5	0	
AA	3	1	2	
GBM	29	3	26	

Note: A: astrocytoma; AA: anaplastic astrocytoma; AO: Anaplastic oligodendrocytoma; GBM: primary glioblastoma; O: oligodendroglioma.

results suggested that circTOP2A may act as a critical oncogene in glioma progression.

3.4 | circTOP2A relieves repression of miR-422a on RPN2

Our previous published data proved that RPN2 was a direct functional target of miR-422a.[19] It has been confirmed that circRNAs can serve as miRNA sponges to regulate downstream targets.[20,21] In addition, based on previous research, combined with microarray data, miRanda, circBase, and the circBank database, we screened and identified circTOP2A acting as a sponge for miR-422a. We next determined whether circTOP2A regulated the expression of RPN2 by competitively binding it to miR-422a. We first constructed the circTOP2A luciferase reporter system. miR-422a mimics were co-transfected with the circTOP2A luciferase reporter into U251 and LN229 cells. We observed that miR-422a was able to reduce luciferase activity (Figure 4B). Given that miR-422a could bind to the 3′UTR region, wild-type and mutated putative binding sites of RPN2 transcripts were transfected into U251 and LN229 cells when circTOP2A expression was knocked down, mediated by siRNA. The knockdown verification of circTOP2A in cells is displayed in Figure 4C. Luciferase reporter assays showed that the luciferase activities of the RPN2 wild-type reporter were significantly reduced when transfected with si-circTOP2A, compared with the mutated luciferase reporter (Figure 4D), highlighting the regulatory effect of circTOP2A on miR-422a. Then we used biotin-coupled miR-422a mimics for pull-down assays to detect competitive binding activities of circTOP2A and RPN2 for miR-422a in U251 and LN229 cells. We noted that circTOP2A and RPN2 were markedly enriched in the miR-422a captured fraction compared with the negative control (Figure 4E). Finally, western blot assay results demonstrated that knockdown of circTOP2A could significantly reduce the protein levels of RPN2, but this was partly rescued by the miR-422a inhibitor in U251 and LN229 cells (Figure 4F), whereas circTOP2A overexpression could upregulate RPN2 levels, while also being partly rescued by miR-422a mimics in U87 cells (Figure 4G). These results indicated that circTOP2A regulated the expression RPN2 by acting as sponge for miR-422a.

3.5 | circTOP2A promotes glioma proliferation and invasion through RPN2

As circTOP2A regulated RPN2 expression and participated in malignant progression, and circTOP2A was co-expressed with oncogene RPN2 in GBM microarray analysis data, we next majorly explore whether circTOP2A could affect the glioma malignant phenotype through RPN2. First, we construct lentivirus-mediated knockdown of circTOP2A (sh-circTOP2A) to establish U251 and LN229 cell lines that had stable knockdown of circTOP2A, according to efficient sequencing by siRNA. Similarly, sh-circTOP2A could significantly reduce the protein expression levels of RPN2 (Figure 5A). Using a plasmid vector, we succeeded in overexpressing RPN2 levels in U251 and LN229 cells that had stable knockdown of circTOP2A expression to investigate the RPN2-mediated role in malignant phenotypes regulated by sh-circTOP2A (Figure 5A). CCK8 and annexin V-FITC assays indicated that the repression of circTOP2A by sh-circTOP2A could markedly inhibit tumor cell proliferation and enhance cell apoptosis compared with a negative control (Figure 5B,C). Transwell assay demonstrated that the inhibition of circTOP2A could repress the tumor cell invasive ability (Figure 5D). However, the restoration of RPN2 expression could partly reverse these inhibitory effects by sh-circTOP2A (Figure 5B-D). In addition, a circTOP2A overexpression plasmid and RPN2 siRNA were constructed and transfected or co-transfected into the U87 cells; the identification of circTOP2A overexpression is illustrated in Figure S1A. The RPN2 expression in U87 cells after single transfection of circTOP2A or co-transfection of circTOP2A and siRPN2 is shown in Figure S1B. CCK8 and transwell assays indicated that circTOP2A significantly promoted cell growth and invasion and that knockdown of RPN2 could abrogate the effects of circTOP2A on promoting cell proliferation and invasion (Figure S1C,D). Furthermore, a CCK8 assay was performed to determine the miR-422a-mediated role in circTOP2A on glioma proliferation. The results indicated that the repression of miR-422a could also reverse the inhibitory role of sh-circTOP2A, whereas miR-422a upregulation reversed the enhanced role of circTOP2A on cell proliferation (Figure S2).

3.6 | The inhibition of circTOP2A suppresses the Wnt/β-catenin pathway partly via RPN2

Our previous published data have indicated that RPN2 could activate the Wnt/β-catenin pathway, we further determined whether

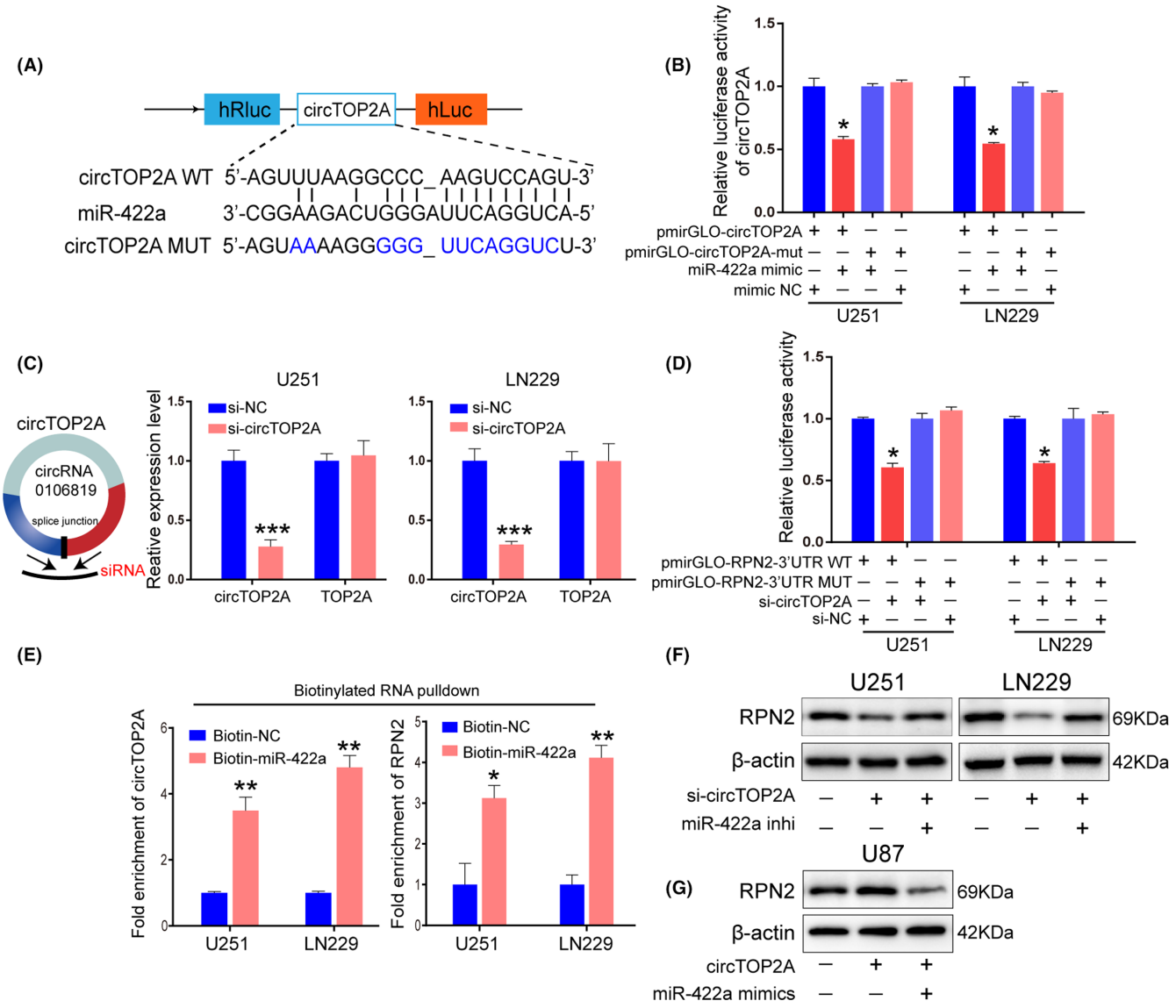

FIGURE 4 circTOP2A relieves repression of miR-422a on *RPN2*. (A) The schematic model shows the putative binding sites of miR-422a for circTOP2A. (B) Luciferase activity of circTOP2A in U251 and LN229 cells transfected with miR-422a mimics that show putative binding to the circTOP2A sequence. (C) circTOP2A siRNA was transfected into U251 and LN229 cells, and the expression levels of cicTOP2A and TOP2A were detected by qRT-PCR. (D) Luciferase reporter activity of RPN2-3′UTR in U251 and LN229 cells with circTOP2A knockdown. (E) circTOP2A and RPN2 were pulled down and enriched with 3′-end biotinylated miR-422a. (F) Western blotting was used to detect the RPN2 expression levels in U251 and LN229 cells transfected with si-circTOP2A alone or co-transfected with miR-422a inhibitor (miR-422a inhi). (G) Western blotting was used to detect the RPN2 expression level in U87 cells transfected with circTOP2A alone or co-transfected with miR-422a mimics. $^{*}p < 0.0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$

circTOP2A could regulate the Wnt/β-catenin pathway through RPN2. TOP/FOP luciferase reporter analysis indicated that the knockdown of circTOP2A markedly inhibited TOP activity with no apparent change in FOP activity (Figure 6A). The downstream key factors of the Wnt/β-catenin pathway, including TCF4, cyclin D1, and c-*myc*, were analyzed by western blot and qRT-PCR in U251 and LN229 cells after stable knockdown of circTOP2A and co-transfection with RPN2. The results showed that sh-circTOP2A could dramatically suppress TCF4, c-*myc* and cyclin D1 expression (Figure 6B,D). Additionally, β-catenin levels in the nucleus were markedly reduced following circTOP2A knockdown as shown by immunofluorescence assays (Figure 6C). However, it was evident that the restoration of RPN2 following circTOP2A downregulation could also reverse the sh-circTOP2A-mediated inhibitory effect on Wnt/β-catenin signaling (Figure 6A–C). Taken together, these data suggested that the downregulation of circTOP2A could attenuate β-catenin/TCF4 transcriptional activity, partially via RPN2.

3.7 | circTOP2A enhances the growth of xenograft tumors of GBM cells in vivo

To investigate the effect of circTOP2A on tumor growth in vivo, we established a nude mice xenograft model by implanting LN229 cells

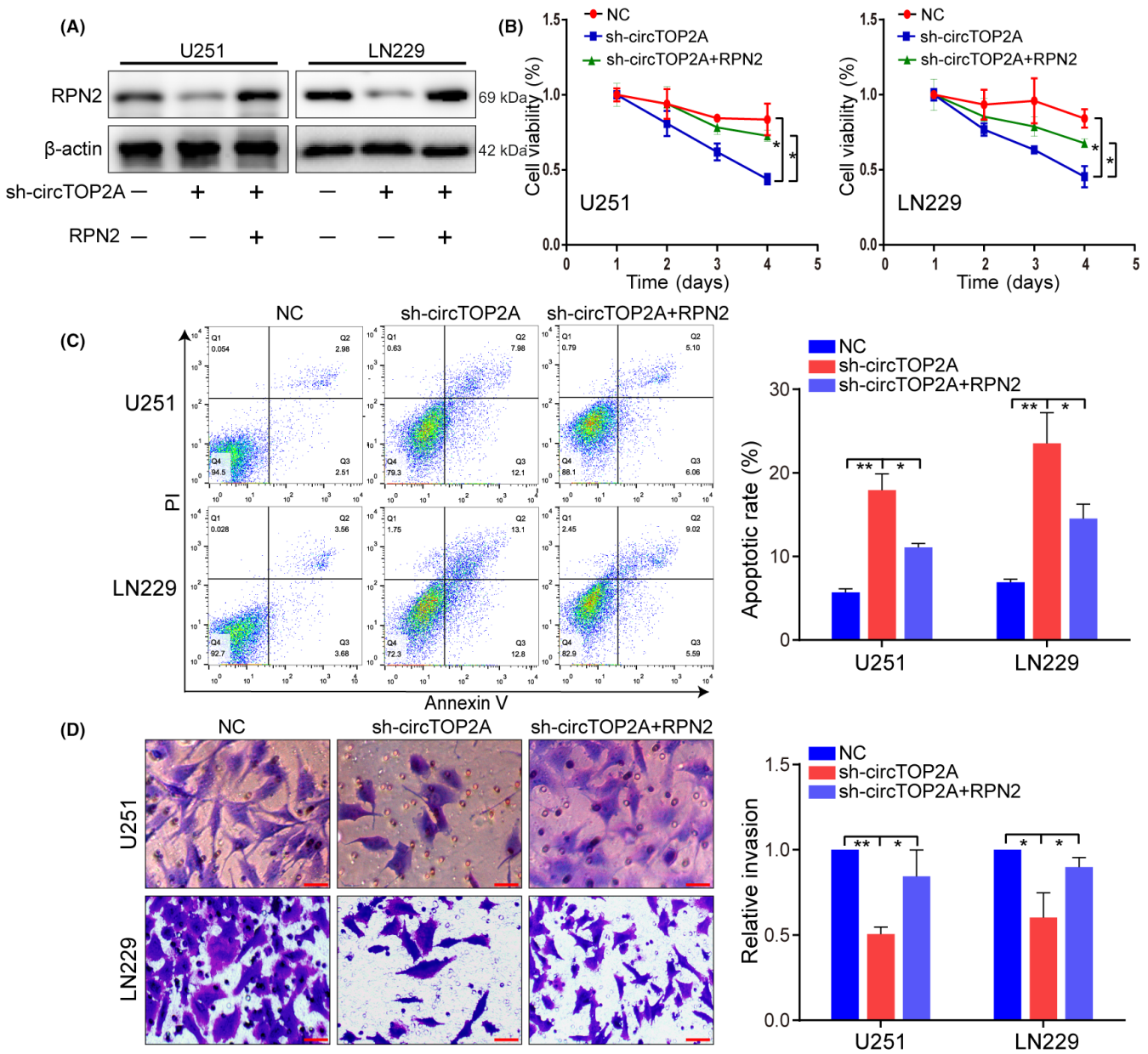

FIGURE 5 circTOP2A promotes glioma proliferation and invasion through RPN2. (A) RPN2 protein expression level assay using western blotting in U251 and LN229 cells after transduction with sh-circTOP2A lentivirus or co-transfected with RPN2 overexpression plasmid. (B–D) CCK8 (B), annexin V-FITC assays (C) and transwell assay (D) analysis in U251 and LN229 cells after transduction with sh-circTOP2A lentivirus or co-transfected with RPN2 overexpression plasmid. Scale bars, 100 μm.*$p < 0.0.05$, **$p < 0.01$

with stable knockdown of circTOP2A. The results indicated that the xenograft tumors formed by circTOP2A-deficient LN229 cells had a significantly smaller size than those of the control group (sh-NC; Figure 7A). The tumor volumes were monitored from 14 days after LN229 cell injection. We found that sh-circTOP2A drastically decreased the tumor growth of LN229 cells (Figure 7B). The tumor weight was also significantly lower than in the sh-NC group (Figure 7C). After harvesting the subcutaneous tumor tissues, total RNA from the xenograft tumors was extracted, and qRT-PCR was used to detect the expression of circTOP2A, miR-422a, and RPN2. The results confirmed the decreased circTOP2A and RPN2 expression, whereas miR-422a was increased in tumors from the sh-circTOP2A group (Figure 7D). In addition, immunohistochemistry was performed. The results demonstrated that expression levels of RPN2 and Wnt pathway key factor β-catenin were decreased in sh-circTOP2A xenograft tumors (Figure 7E). These findings revealed that circTOP2A promoted the malignant progression of glioma through the RPN2-mediated Wnt/β-catenin pathway in vivo.

4 | DISCUSSION

Our previous study identified the oncogene *RPN2* as one of the most significant upregulated genes; we found that RPN2 was closely

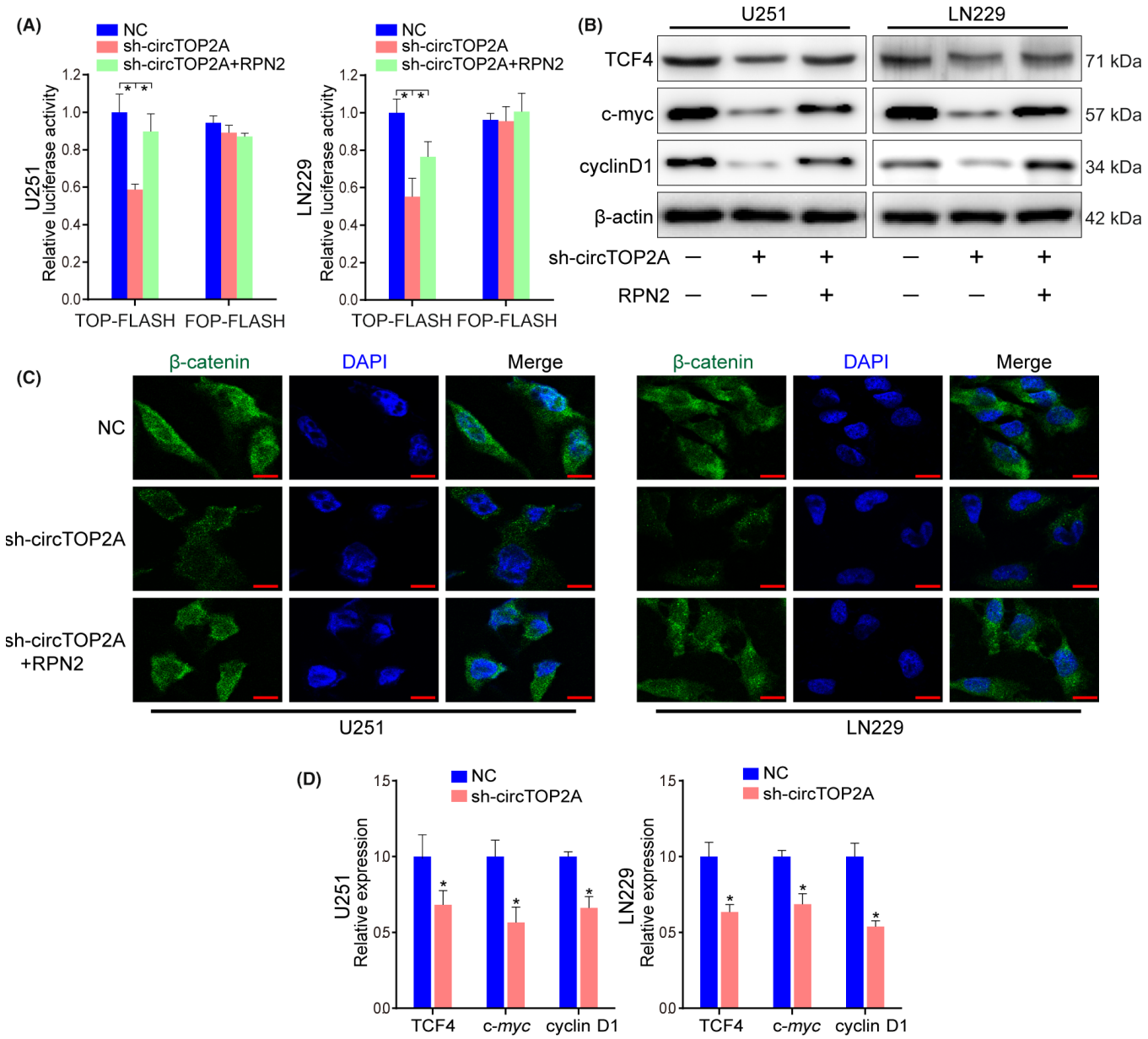

FIGURE 6 Inhibition of circTOP2A suppresses the Wnt/β-catenin pathway via RPN2. (A) TOP/FOP luciferase reporter assays were performed in U251 and LN229 cells after stable knockdown of circTOP2A or not, transfected with TOP/FOP plasmid, or co-transfected with TOP/FOP and RPN2 plasmid. (B) Western blot detection of Wnt/β-catenin pathway downstream factors TCF4, cyclin D1 and c-*myc* expression levels in U251 and LN229 cells after stable knockdown of circTOP2A or not or co-transfected with RPN2. (C) Immunofluorescence assays were performed to examine β-catenin expression in the nucleus and cytoplasm in different treated cells. (D) qRT-PCR was performed to detect TCF4, cyclin D1, and c-*myc* expression levels following circTOP2A knockdown. Scale bars, 100 μm. $^{*}p < 0.05$

related to WHO grade and clinical prognosis. Additionally, knockdown of RPN2 could suppress glioma proliferation and invasion and enhanced TMZ sensitivity in vitro and in vivo. Mechanistically, *RPN2* knockdown suppressed the Wnt/β-catenin signaling pathway, at least partially through GSK-3β activation. Then, we also verified the new miR-422a-mediated RPN2 inactivation mechanism and if RPN2 was a direct target of miR-422a; the relevant data have been previously published.[17,19] Several circRNA-mediated ceRNA axes have been implicated in indispensable oncogenic or tumor suppressive roles during tumor development and progression.[22] Accordingly, in this study, combined microarray circRNA expression and co-expression profiling of circRNA with RPN2, with miRanda database binding to miR-422a, we focused on the identification of the circRNAs that regulated the expression of RPN2 by sponging miR-422a. The results identified has_circ_0106819, designated as circTOP2A. There was a report that has_circ_0043548, also designated as circTOP2A, promoted GBM proliferation and aggressiveness via miR-346/SUSD2 signaling. However, the circTOP2A that we identified and verified

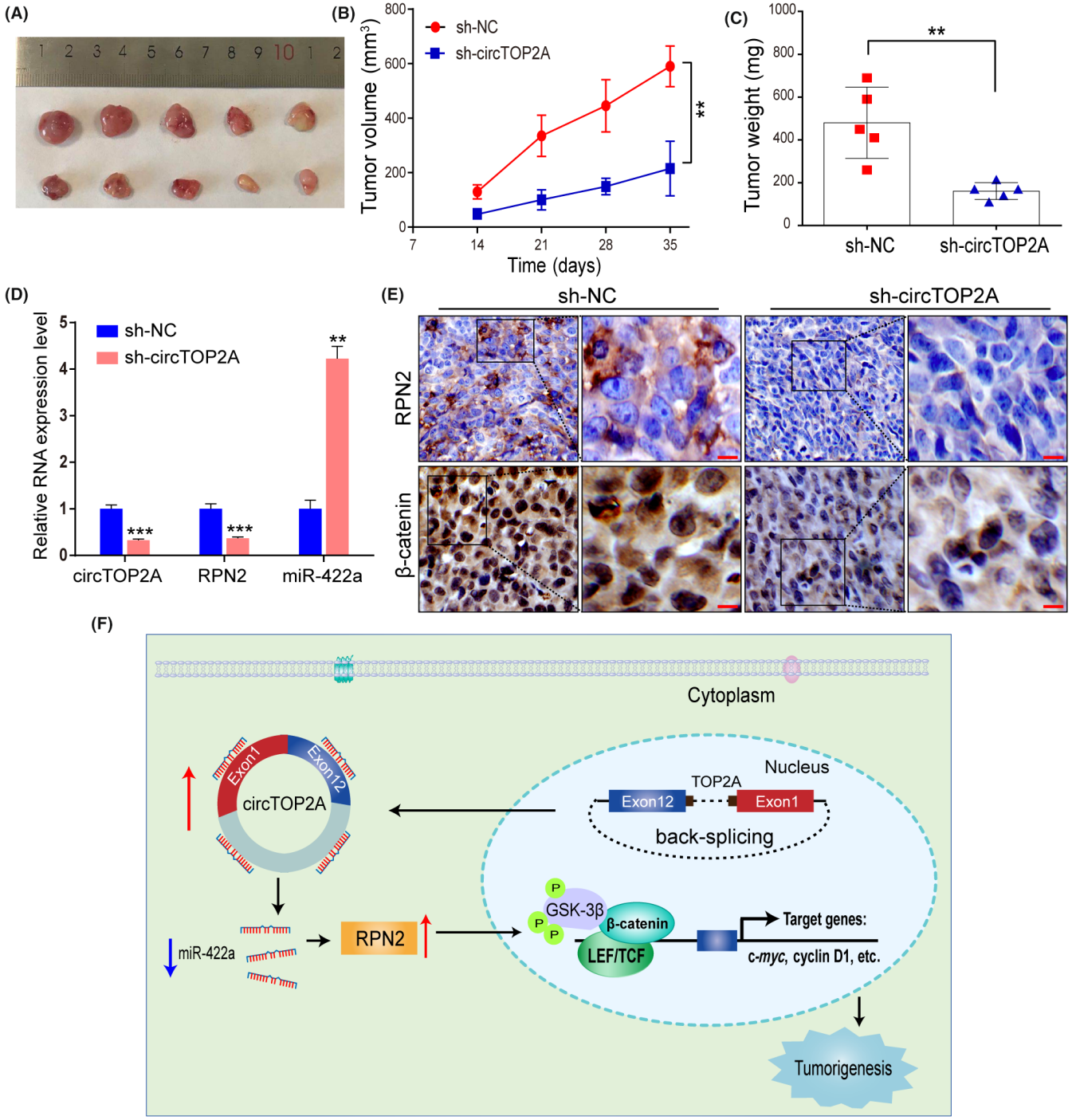

FIGURE 7 circTOP2A enhances the growth of xenograft tumors of GBM cells in vivo. (A) Nude mice were subcutaneously injected with negative control (sh-NC) or shRNA-circTOP2A stably transfected LN229 cells. After 35 days, tumors were dissected and imaged. (B) Tumor volume was measured every 7 days, and a tumor growth curve was plotted. (C) Tumor weight was calculated on the day the mice were killed. Data represent mean ± SD ($n = 5$ each group). (D) Expression levels of circTOP2A, miR-422a, and RPN2 in xenograft tumors were determined using qRT-PCR. (E) Changes in RPN2 and β-catenin expression in xenograft tumors were detected by immunohistochemistry (IHC) staining. (F) Schematic illustration of the regulation of the Wnt/β-catenin pathway by the circTOP2A–miR-422a–RPN2 axis. $^{**}p < 0.001$; $^{***}p < 0.0001$

was derived from exons 1–12 of the *TOP2A* gene and the spliced variant of circ-TOP2A was 1621 nucleotides long, whereas circ-TOP2A (designated as has_circ_0043548) was spliced from exons 7–23 of *TOP2A* and the spliced variant of circ-TOP2A was 2424 nucleotides long.[23] Therefore, the circTOP2A that we focused on in this study was completely different from the variant reported previously. A series of functional experiments demonstrated that circ-TOP2A acts as a ceRNA that competitively binds to miR-422a, and then abolishes the inhibitory effect of miR-422a on the target gene *RPN2*. Moreover, we also mainly explored the RPN2-mediated role

of the circTOP2A-regulated Wnt/β-catenin pathway, revealing that circTOP2A promoted glioma cell growth via a ceRNA mechanism.

Aberrant RPN2 overexpression has been verified to be frequently found in a variety of tumors such as colorectal, breast, gastric, ovarian cancer, and osteosarcoma, and is associated with cancer progression and chemotherapy resistance.[24–28] Bioinformatics analysis by Zhou et al. from the GEO database identified that *RPN2* might be the significant gene among the top 10 hub genes serving as valuable prognostic biomarkers for GBM.[29] Additionally, Heroux et al. also reported that *RPN2* was a significant biomarker of GBM using mass spectrometry-based label-free quantitative proteomics analysis.[30] All the data indicated that RPN2 plays significant roles as a critical oncogene in multiple tumors, including glioma. In the present study, circTOP2A was co-expressed with RPN2 and could regulate the Wnt/β-catenin signaling pathway. It has been documented that RPN2 promoted epithelial–mesenchymal transition in laryngeal squamous cell carcinoma and the viability of nasopharyngeal cancer through the activation of the PI3K/AKT and JAK/STAT3 pathways, respectively.[31,32] However, Wnt/β-catenin signaling forms a crosstalk with the PI3K/AKT and JAK/STAT3 pathways, therefore, we speculated that circTOP2A may regulate *RPN2* expression to subsequently affect multiple cancer-related signaling pathways through a ceRNA mechanism. To date, ceRNA-mediated RPN2 dysregulation has been reported to be involved in the progression of glioma. circ_0037655 relieved sevoflurane-induced glioma growth inhibition in vivo by mediating the miR-130a-5p/RPN2 axis.[33] RPN2, as a target of miR-378e, was positively regulated via circNFIX by competitively sponging miR-378e.[34] All the research indicated that RPN2 may be regulated by various circRNAs via competitively binding to multiple miRNAs.

Accumulating data suggest that miR-422a plays a tumor suppressor role in a variety of malignant tumors including glioma. miR-422a markedly suppressed glioma cell proliferation and invasion by targeting PIK3CA, IGF1, and the IGF1 receptor (IGF1R), which are new prognostic biomarkers for human glioblastoma.[35–37] Our data provides evidence that miR-422a shares common binding sites with circTOP2A and RPN2, and the luciferase reporter analysis confirmed that miR-422a could bind to circTOP2A and RPN2. Subsequent biotinylated miRNA pull-down assays showed the competitive binding activities of circTOP2A and RPN2 for miR-422a and the further rescue experiment showed that circTOP2A significantly attenuated the inhibitory effects of miR-422a on RPN2. However, whether circTOP2A can regulate other genes targeted by miR-422a, such as IGF1R and PIK3CA, remains to be further elucidated. In addition, to date, miR-422a acting as an MRES-mediated ceRNA network has also been reported. circCRIM1 competitively sponges miR-422a to block the inhibitory effect of miR-422a on FOXQ1 and subsequently contributes to nasopharyngeal carcinoma cell metastasis, epithelial-mesenchymal transition (EMT), and docetaxel chemoresistance.[38] A report on GBM demonstrated that circNT5E acts as a sponge for microRNA-422a to abolish the miR-422a inhibitory role on NT5E, SOX4, PI3KCA, p-Akt, and p-Smad2 to promote glioblastoma tumorigenesis.[39] In our study, we identified a new circTOP2A sponging miR-422a and verified the miR-422a-mediated role in circTOP2A for regulating the expression of RPN2. Identification of the circTOP2A–miR-422a–RPN2 axis expands the understanding of the underlying mechanism of glioma progression.

In addition to the ceRNA mechanism, circRNAs can interact with different RNA-binding proteins to form specific circRNA–protein complexes (circRNPs) that subsequently regulate the subcellular localization of proteins, as well as the transcription of related genes and some of them can encode functional proteins.[40–42] Our results demonstrated that circTOP2A was one of the most upregulated genes in GBM and that it plays a significant oncogenic role in glioma progression. Whether circTOP2A interacts with RNA-binding protein or in sponging other miRNAs needs to be further explored via RNA pull-down and proteome analyses. Moreover, the expression circTOP2A was closely associated with WHO grade, molecular subtype, and poor prognosis. However, our clinical sample size was extremely limited and further expanding the sample size with various molecular subtyping to detect the circTOP2A expression is quite necessary. In addition, circRNAs are abundant in exosomes and potential clinical applications.[9,43,44] Han reported that exosomal circ-HIPK3 facilitates tumor progression and temozolomide resistance by regulating the miR-421/ZIC5 axis in glioma.[45] The particular traits, including long half-life as well as abundance in the brain and exosomes, tend to infer that circTOP2A expression detection from blood and cerebrospinal fluid (CSF) from glioma patients provides excellent insights into noninvasive diagnosis and distinguishes different gliomas subtypes, as well as provides an early treatment assessment. Finally, a subcutaneous transplanted model was used to evaluate the tumor growth inhibitory effect of sh-circTOP2A in vivo. However, the model neglected factors such as the blood–brain barrier, a tissue microenvironment similar to that of clinical specimens.[46] Therefore, orthotopic xenografts can exhibit clinical characteristics such as in vivo invasion and metastatic patterns closely mimicking the glioma.[46] This may be another limitation of our study.

In conclusion, our data revealed that circTOP2A competitively binds to miR-422a to eliminate the suppressive effect of miR-422a on *RPN2*, thereby promoting glioma cell proliferation and invasion in vitro and in vivo. These findings provide new insights into understanding the development and progression of glioma, and provide a potential therapeutic approach for glioma patients.

AUTHOR CONTRIBUTIONS

JS, TX, and FJ conceived and designed the experiments. JS, HL, and YL performed the in vitro experiments. JW provided the clinical specimens. JS, SL, and HL performed the in vivo experiment. ML, SL, and FL helped with the bioinformatics analysis and data analysis. JS, FL, and FJ reviewed and revised the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

We thank Tianjin Huanhu Hospital for providing clinical samples and the research platform for our study. This study was supported by the National Science Foundation of China (Nos.

81071779, 81972340, 82173140), Shandong Provincial Natural Science Foundation (No. ZR2016HL34, No. ZR2021QH256, ZR2022MH140), Project of Shandong Province Higher Educational Science and Technology Program (No. J16LL05), the Key Research and Development Program of Jining Science and Technology (No. 2020YXNS005) and Project funded by China Postdoctoral Science Foundation (2022M711324).

DISCLOSURE

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Data are available on request to the authors.

ETHICAL APPROVAL

Approval of the research protocol by an Institutional Reviewer Board and Informed Consent. The present study was reviewed and approved by the Institutional Review Board of Tianjin Huanhu Hospital, and written informed consent was obtained from all participating patients.

ANIMAL STUDIES

All experimental procedures involving the use of animals were approved by the Animal Ethical and Welfare Committee of Tianjin Huanhu Hospital of Nankai University.

ORCID

Feng Jin https://orcid.org/0000-0001-8233-2575

REFERENCES

1. Liao W, Fan S, Zheng Y, et al. Recent advances on glioblastoma multiforme and Nano-drug carriers: a review. *Curr Med Chem*. 2019;26:5862-5874.
2. Davis ME. Glioblastoma: overview of disease and treatment. *Clin J Oncol Nurs*. 2016;20:S2-S8.
3. Lauko A, Lo A, Ahluwalia MS, Lathia JD. Cancer cell heterogeneity & plasticity in glioblastoma and brain tumors. *Semin Cancer Biol*. 2022;82:162-175.
4. Aldape K, Zadeh G, Mansouri S, Reifenberger G, von Deimling A. Glioblastoma: pathology, molecular mechanisms and markers. *Acta Neuropathol*. 2015;129:829-848.
5. Kanu OO, Hughes B, Di C, et al. Glioblastoma multiforme oncogenomics and signaling pathways. *Clin Med Oncol*. 2009;3:39-52.
6. Shahzad U, Krumholtz S, Rutka JT, Das S. Noncoding RNAs in glioblastoma: emerging biological concepts and potential therapeutic implications. *Cancer*. 2021;13:1555.
7. Slack FJ, Chinnaiyan AM. The role of non-coding RNAs in oncology. *Cell*. 2019;179:1033-1055.
8. Chen LL. The expanding regulatory mechanisms and cellular functions of circular RNAs. *Nat Rev Mol Cell Biol*. 2020;21:475-490.
9. Wang S, Zhang K, Tan S, et al. Circular RNAs in body fluids as cancer biomarkers: the new frontier of liquid biopsies. *Mol Cancer*. 2021;20:13.
10. Sun J, Li B, Shu C, Ma Q, Wang J. Functions and clinical significance of circular RNAs in glioma. *Mol Cancer*. 2020;19:34.
11. Xu T, Wang M, Jiang L, et al. CircRNAs in anticancer drug resistance: recent advances and future potential. *Mol Cancer*. 2020;19:127.
12. Yan L, Chen YG. Circular RNAs in immune response and viral infection. *Trends Biochem Sci*. 2020;45:1022-1034.
13. Zheng Q, Bao C, Guo W, et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat Commun*. 2016;7:11215.
14. Chen S, Huang V, Xu X, et al. Widespread and functional RNA circularization in localized prostate cancer. *Cell*. 2019;176(831–843):e822.
15. He L, Zhou H, Zeng Z, Yao H, Jiang W, Qu H. Wnt/beta-catenin signaling cascade: a promising target for glioma therapy. *J Cell Physiol*. 2019;234:2217-2228.
16. Shahcheraghi SH, Tchokonte-Nana V, Lotfi M, Lotfi M, Ghorbani A, Sadeghnia HR. Wnt/beta-catenin and PI3K/Akt/mTOR signaling pathways in glioblastoma: two Main targets for drug design: a review. *Curr Pharm des*. 2020;26:1729-1741.
17. Sun J, Ma Q, Li B, et al. RPN2 is targeted by miR-181c and mediates glioma progression and temozolomide sensitivity via the wnt/beta-catenin signaling pathway. *Cell Death Dis*. 2020;11:890.
18. Hassan A, Mosley J, Singh S, Zinn PO. A comprehensive review of genomics and noncoding RNA in gliomas. *Top Magn Reson Imaging*. 2017;26:3-14.
19. Sun J, Chen Z, Xiong J, et al. MicroRNA422a functions as a tumor suppressor in glioma by regulating the Wnt/betacatenin signaling pathway via RPN2. *Oncol Rep*. 2020;44:2108-2120.
20. Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. *Nature*. 2013;495:384-388.
21. Hsiao KY, Lin YC, Gupta SK, et al. Noncoding effects of circular RNA CCDC66 promote colon cancer growth and metastasis. *Cancer Res*. 2017;77:2339-2350.
22. Li J, Sun D, Pu W, Wang J, Peng Y. Circular RNAs in cancer: biogenesis, function, and clinical significance. *Trends Cancer*. 2020;6:319-336.
23. Sang J, Li X, Lv L, Zhang C, Zhang X, Li G. CircTOP2A acts as a ceRNA for miR346 and contributes to glioma progression via the modulation of sushi domaincontaining 2. *Mol Med Rep*. 2021;23:255.
24. He Q, Li Z, Yin J, et al. Prognostic significance of autophagy-relevant gene markers in colorectal cancer. *Front Oncol*. 2021;11:566539.
25. Tominaga N, Hagiwara K, Kosaka N, Honma K, Nakagama H, Ochiya T. RPN2-mediated glycosylation of tetraspanin CD63 regulates breast cancer cell malignancy. *Mol Cancer*. 2014;13:134.
26. De Souza R, Zahedi P, Badame RM, Allen C, Piquette-Miller M. Chemotherapy dosing schedule influences drug resistance development in ovarian cancer. *Mol Cancer Ther*. 2011;10:1289-1299.
27. Fujiwara T, Takahashi RU, Kosaka N, et al. RPN2 gene confers osteosarcoma cell malignant phenotypes and determines clinical prognosis. *Mol Ther Nucleic Acids*. 2014;3:e189.
28. Zhang H, Jiang H, Zhang H, Liu J, Hu X, Chen L. Ribophorin II potentiates P-glycoprotein- and ABCG2-mediated multidrug resistance via activating ERK pathway in gastric cancer. *Int J Biol Macromol*. 2019;128:574-582.
29. Zhou L, Tang H, Wang F, et al. Bioinformatics analyses of significant genes, related pathways and candidate prognostic biomarkers in glioblastoma. *Mol Med Rep*. 2018;18:4185-4196.
30. Heroux MS, Chesnik MA, Halligan BD, et al. Comprehensive characterization of glioblastoma tumor tissues for biomarker identification using mass spectrometry-based label-free quantitative proteomics. *Physiol Genomics*. 2014;46:467-481.
31. Wang B, Liu W, Jiang X, et al. Overexpression of ribophorin II is required for viability of nasopharyngeal cancer cells by regulating JAK1/STAT3 activation. *Immunopharmacol Immunotoxicol*. 2021;43:471-477.
32. Zhou J, Zhang J, Zhang W, et al. Ribophorin II promotes the epithelial-mesenchymal transition and aerobic glycolysis of laryngeal squamous cell carcinoma via regulating reactive oxygen species-mediated Phosphatidylinositol-3-kinase/protein kinase B activation. *Bioengineered*. 2022;13:5141-5151.

33. Zhu J, Xie B, Huang G, Li Y, Liu Z. Sevoflurane represses the progression of glioma by the regulation of circ_0037655/miR-130a-5p/RPN2 axis. *Metab Brain Dis*. 2022;37:787-799.
34. Ding C, Wu Z, You H, et al. CircNFIX promotes progression of glioma through regulating miR-378 e/RPN2 axis. *J Exp Clin Cancer Res*. 2019;38:506.
35. Liang H, Wang R, Jin Y, Li J, Zhang S. MiR-422a acts as a tumor suppressor in glioblastoma by targeting PIK3CA. *Am J Cancer Res*. 2016;6:1695-1707.
36. Maris C, D'Haene N, Trepant AL, et al. IGF-IR: a new prognostic biomarker for human glioblastoma. *Br J Cancer*. 2015;113:729-737.
37. Wang H, Tang C, Na M, et al. miR-422a inhibits glioma proliferation and invasion by targeting IGF1 and IGF1R. *Oncol Res*. 2017;25:187-194.
38. Hong X, Liu N, Liang Y, et al. Circular RNA CRIM1 functions as a ceRNA to promote nasopharyngeal carcinoma metastasis and docetaxel chemoresistance through upregulating FOXQ1. *Mol Cancer*. 2020;19:33.
39. Wang R, Zhang S, Chen X, et al. CircNT5E acts as a sponge of miR-422a to promote glioblastoma tumorigenesis. *Cancer Res*. 2018;78:4812-4825.
40. Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. *Nucleic Acids Res*. 2016;44:2846-2858.
41. Liu Y, Li Z, Zhang M, et al. Rolling-translated EGFR variants sustain EGFR signaling and promote glioblastoma tumorigenicity. *Neuro Oncol*. 2021;23:743-756.
42. Tsitsipatis D, Grammatikakis I, Driscoll RK, et al. AUF1 ligand circPCNX reduces cell proliferation by competing with p21 mRNA to increase p21 production. *Nucleic Acids Res*. 2021;49:1631-1646.
43. Li Y, Zheng Q, Bao C, et al. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. *Cell Res*. 2015;25:981-984.
44. Cheng J, Meng J, Zhu L, Peng Y. Exosomal noncoding RNAs in glioma: biological functions and potential clinical applications. *Mol Cancer*. 2020;19:66.
45. Han C, Wang S, Wang H, Zhang J. Exosomal circ-HIPK3 facilitates tumor progression and temozolomide resistance by regulating miR-421/ZIC5 Axis in glioma. *Cancer Biother Radiopharm*. 2021;36:537-548.
46. Yogi K, Thorat R, Shirsat N. In vivo tumorigenicity assays using subcutaneous and orthotopic xenografts in immunodeficient mice. *Methods Mol Biol*. 2022;2423:153-164.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sun J, Wang J, Li M, et al. circTOP2A functions as a ceRNA to promote glioma progression by upregulating RPN2. *Cancer Sci*. 2023;114:490-503. doi: 10.1111/cas.15612