Background. The translationally controlled tumor protein (TCTP) is a multifunctional protein that plays important roles in immune responses, cell proliferation, tumorigenicity and cell apoptosis. Here, we examined the clinical value of TCTP in glioma patient survival and investigated the functional roles and mechanism of TCTP in glioma development.

Methods. TCTP expression was determined through immunohistochemical staining, immunoblotting, and quantitative real-time PCR (qRT-PCR). TCTP or TCF-4 expression was silenced using short hairpin (sh) RNA. In vitro cell proliferation was detected using MTT, BrdU and colony formation assays, and in vivo tumor growth was performed using the xenograft model. TCTP/TCF-4/β-catenin association was detected using a co-immunoprecipitation (co-IP) assay. TCF-4 transcription activity was detected using a TOPflash/FOPflash report gene assay. Wnt/β-catenin-targeted gene expression was detected through Western blotting.

Results. TCTP protein levels were significantly elevated in high-grade gliomas compared with low-grade gliomas and normal brain tissues. Importantly, the expression of TCTP was significantly associated with poorer overall survival and disease-free survival, and TCTP also reduced the survival rate after treatment with radiotherapy and temozolomide (RT-TMZ) for glioma patients. The ectopic expression of TCTP enhanced glioma cell proliferation both in vitro and in vivo, whereas the knockdown of TCTP inhibited this effect. Similarly, the overexpression of TCTP increased β-catenin binding to TCF-4, TOPflash report gene transcription activity, and the expression of Wnt/β-catenin signaling target genes including c-Myc and cyclin D1; notably, the knockdown of TCTP reduced these effects. The knockdown of TCF-4 using shRNA rescued the enhanced cell proliferation induced by the overexpression of TCTP.

Conclusion. TCTP is associated with reduced survival of glioma patients and induces glioma tumor growth through enhanced Wnt/β-catenin signaling.

Keywords: glioma, TCTP, TCF-4, β-catenin.
target genes such as c-Myc and cyclin D1. Various human cancers, including glioma, manifest abnormal β-catenin/TCF/LEF transactivation, reflecting mutations or the ectopic expression of the proteins involved in the Wnt/β-catenin signaling pathway such as β-catenin, GSK-3β, APC, axin, and others. However, the mechanism of β-catenin and TCF-4 involvement in gliomagenesis is poorly understood.

In this study, we observed that TCTP expression was significantly associated with poor overall survival and reduced survival rate after treatment with radiotherapy and temozolomide (RT-TMZ) for glioma patients. The overexpression of TCTP enhanced glioma cell growth, both in vitro and in vivo, through TCF-4 binding and regulation through Wnt/β-catenin signaling. Notably, the knockdown of TCTP inhibited these effects.

Materials and Methods

Ethics Statement

Informed consent was obtained from all patients involved in this study, and the study protocol was approved by the Clinical Research Ethics Committee of Guangdong Medical College. The protocol for all animal studies was approved by the Clinical Research Ethics Committee of Guangdong Medical College.

Immunohistochemical Staining

Human glioma samples were obtained from Affiliated Hospital of Guangdong Medical College. The pathological-grade tumors were defined according to the 2000 WHO criteria. Normal brain samples were obtained from automobile-accident patients without glioma. Tissue sections for the study were cut according to the 2000 WHO criteria. Normal brain samples were obtained from the Department of Pathology, Guangdong Medical College. The protocol for all animal studies was approved by the Clinical Research Ethics Committee of Guangdong Medical College.

Cells

Human glioma cell lines U138, H-4, HS-683, U-87, U-251, U-343, and rat astrocyte D1TNC1 cells were purchased from the American Type Culture Collection (ATCC). The cells were cultured according to the recommendations of ATCC. U-251, U138, H-4, HS-683, and D1TNC1 cells were transfected with the plasmids for full-length, wild-type TCTP or the empty vector, TCTP shRNA or Ctrl shRNA, using Lipofectamine 2000 (Invitrogen). The positive transfectants were selected using 0.8 mg/mL G418 (GIBCO BRL) for 2 weeks to obtain a stable cell line for the overexpression or knockdown of TCTP for use in subsequent assays. For TCF-4 knockdown, Lipofectamine 2000 was used for transfection, and the positive transfectants were selected using 0.8 mg/mL G418 (GIBCO BRL) and 0.4 mg/mL Zeocin (Sigma) for 2 weeks to obtain a stable cell line for the knockdown of TCF-4 to use in relevant experiments.

Plasmids, Antibodies, and Chemicals

TCTP cDNA and short hairpin RNA (shRNA) for TCTP and TCF-4 were purchased from Open Biosystems. The TCTP shRNA1 target sequence was 5′-TGATGATCTTCTGTAGGC-3′; the TCTP shRNA2 target sequence was 5′-TCTTGCTTACAAGC-3′; the TCTF4 shRNA1 target sequence was 5′-TCCTGCTACCTTC-3′, the TCF4 shRNA2 target sequence was 5′-TGTCCTACTGACCATCT-3′, and the nonsense shRNA sequence was 5′-TACCATCCGGCACAATCAGC-3′. The rabbit anti-TCTP, anti-TCF-4, and anti-Ki67 antibodies used for the Western blotting, immunoprecipitation, and immunohistochemical assays were purchased from Abcam, and the mouse anti-β-catenin antibody used for the immunoblotting assay was purchased from BD Biosciences. The mouse anti-β-actin and anti-Brdu antibodies used for the immunoblotting assay were purchased from Sigma. The secondary antibodies anti-mouse IgG-HRP and anti-rabbit IgG-HRP were purchased from Sigma. MIT assay reagents were purchased from Sigma (CDG1).

Cell Culture

Human glioma cell lines U138, H-4, HS-683, U-87, U-251, U-343, and rat astrocyte D1TNC1 cells were purchased from the American Type Culture Collection (ATCC). The cells were cultured according to the recommendations of ATCC. U-251, U138, H-4, HS-683, and D1TNC1 cells were transfected with the plasmids for full-length, wild-type TCTP or the empty vector, TCTP shRNA or Ctrl shRNA, using Lipofectamine 2000 (Invitrogen). The positive transfectants were selected using 0.8 mg/mL G418 (GIBCO BRL) for 2 weeks to obtain a stable cell line for the overexpression or knockdown of TCTP for use in subsequent assays. For TCF-4 knockdown, Lipofectamine 2000 was used for transfection, and the positive transfectants were selected using 0.8 mg/mL G418 (GIBCO BRL) and 0.4 mg/mL Zeocin (Sigma) for 2 weeks to obtain a stable cell line for the knockdown of TCF-4 to use in relevant experiments.

qRT-PCR

Total RNA was extracted using the Absolutely RNA Miniprep Kit (Stratagene) and reverse transcribed using ThermoScript RT-PCR System (Invitrogen). The cDNA was used for real-time PCR using the SYBR-Green Master PCR Mix (Applied Biosystems) in triplicate. All RT qPCR primer pairs were purchased from SA Biosciences. PCR, and data collection was performed using the Step-one qPCR System (Stratagene). All quantitations were normalized to an endogenous β-actin control. The relative quantitation value for each target gene was compared with the calibrator (β-actin), which was expressed as 2-(Ct-Cc) (Ct and Cc are the mean threshold cycle differences after normalizing to β-actin). The relative expression levels of the samples are presented using a semi-log plot.

Yeast 2-Hybrid Assay

The full-length cDNA of TCTP was inserted in frame into the GAL4 DNA activation domain vector pACT2 (pAS2-1–TCTP; BD Clontech) and used as the bait for screening a Human Fetal Brain cDNA Library (Cat. No. 630469, BD Clontech). Positive yeast clones were selected through auxotrophy for histidine and β-galactosidase expression. The plasmids were harvested from the positive clones and re-examined for binding to pAS2-1–TCTP in a co-transformation assay according to the manufacturer’s instructions. The cDNA inserts were DNA sequenced.

Immunoblotting and Immunoprecipitation

For immunoblotting, the samples were subjected to SDS-PAGE, transferred to PVDF membranes (Millipore), and detected using appropriate primary antibodies followed by horseradish peroxidase-conjugated goat anti-mouse or rabbit IgG. The blotting signals were detected using SuperSignal West Dura Extended Duration Substrate (Pierce). The quantitative analysis of the immunoblotting signals was obtained via densitometry using LAS-4000 Image Software (Fuji Film).

For immunoprecipitation, the cells were lysed using RIPA buffer (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 600 mM NP-40, 1% Triton X-100, 10% glycerol, 1 mm phenylmethylsulfonyl fluoride, 1 mM sodium fluoride, and 1 mM sodium orthovanadate) and incubated with 20 μL of protein-A Sepharose beads (GE Healthcare) and 1 μg of the appropriate primary antibodies.
at 4°C overnight. After washing 3 times with PBS, the samples were analyzed through immunoblotting.

**Cell Proliferation**

For the MTT assay, the cells, including all transfectants, were grown to the exponential phase and detached through trypsin treatment. A total of 2.500 cells/mL were plated onto 96-well tissue culture plates (100 μL complete medium/well) and cultured at 37°C in 5% CO₂. At different time points, 10 μL per well MTT reagent was added and incubated at 37°C for 4 hours. Subsequently, 100 μL DMSO was used to terminate the reaction, and the optical density was determined at OD570/OD630 nm using a multiwell plate reader. The data from 3 independent experiments were analyzed using the Student’s t-test, and P < .05 was considered statistically significant.

For the BrdU assay, 1.5 × 10⁵ cells/mL were seeded into a 3.5 cm-dish. After 1 day, 0.4% FCS was used to arrest cells at the G0 phase. After 2 days, BrdU (30 mg/l) was added to the cells for 40 minutes. After washing 3 times with PBS, the cells were fixed using methanol for 10 minutes and 0.3% H₂O₂ for 30 minutes. A 5% BSA solution was used to block for 1 hour, followed by treatment with formaldehyde at 100°C for 5 minutes. After washing with PBS, anti-BrdU antibody was added to the cells. The color was subsequently developed through incubation with the ImmunoPure Metal Enhanced DAB Substrate kit (Pierce). The number of BrdU-positive cells and total cell number were counted.

**Colony Formation Assay**

The cells were treated with 10 Gy irradiation. A total of 1 × 10⁴ cells were seeded onto a 3.5 cm-dish with 3 duplicate wells and cultured at 37°C in a 5% CO₂ atmosphere. Two weeks later, the cells were fixed using paraformaldehyde for 30 minutes, followed by staining with GIEMSA for 10 minutes. The cells were washed with ddH₂O 3 times to achieve a clean background, and the number of colonies over 50 cells were counted and statistically analyzed.

**Xenograft Model of Tumor Growth**

The transfectants were resuspended at 1 × 10⁷ cells/mL, and an aliquot of 0.1 mL cell suspension was injected subcutaneously into athymic nude mice (SLAC Laboratory Animal, Shanghai, China) (n = 10). The tumor volume was measured at different time points. The tumor volumes were determined through external measurements and calculated according to the equation, \( V = L \times W^2 \times 0.52 \) (V = volume, L = length, and W = width). The data were analyzed using the Student’s t-test, and P < .05 was considered statistically significant.

**Luciferase Reporter Assay**

To evaluate TCF-4/β-catenin transcriptional activity, the luciferase reporter assay was performed using a pair of luciferase reporter constructs, TOPflash and FOPflash (Upstate Biotechnology). TOPflash contains 3 copies of the TCF-4 binding sites, and FOPflash contains mutated TCF-4 binding sites. The cells were transiently transfected in triplicate with 1 of the luciferase reporters and pCMV-β-galactosidase (Promega) using Lipofectamine 2000 (Invitrogen). At 48 hours after transfection, the luciferase activity was determined using a Luciferase Assay System Kit (Promega). The β-galactosidase activity was determined using the Luminescent β-gal Detection Kit (Promega) as an internal control. The luciferase value was normalized to the β-gal value, and the data from 3 independent experiments were analyzed using the Student’s t-test. A value of P < .05 was considered statistically significant.

**Statistical Analysis**

The overall survival is represented in months and is defined as the interval between the date of the surgery and the date of death or last follow-up. Overall survival curves were estimated using the Kaplan–Meier method, and the difference in survival was evaluated using the log-rank test. The P-values of <.05 and .01 were considered statistically significant and very significant, respectively. All computations were performed using R 2.9.0 software (www.r-project.org).

**Results**

**Overexpression of TCTP in Human Glioma Tissues and Glioma Cell Lines**

To determine the specific expression of TCTP protein in human glioma, an immunohistochemical analysis was performed. Using an antibody for TCTP staining, we examined tissue samples from 168 participants with a pathological diagnosis of astrocytic glioma. Immunoreactivity for the TCTP antigens was observed in 55% (22/40) of the participants with WHO stage I glioma tissues, 69% (29/42) of the participants with WHO stage II glioma tissues, 78.6% (33/42) of the participants with WHO stage III glioma tissues, 86.4% (38/44) of the participants with WHO stage IV glioma tissues, and 5% (2/40) of the normal brain tissues from automobile accident participants without glioma. Notably, the staining of TCTP was much stronger in high-grade glioma than in low-grade glioma (Fig. 1A and B). To confirm the upregulation of TCTP, real-time qRT-PCR analysis was performed using 40 normal brain tissue samples and 168 glioma tissue samples. Consistent with the results of the immunohistochemical analysis, more TCTP mRNA was detected in high-grade glioma tissues than in low-grade glioma and normal brain tissue samples (Fig. 1C). We next examined the expression of TCTP in glioma cell lines on TCTP antibodies using Western blotting. Compared with normal brain tissue lysate, elevated TCTP expression was observed in all 6 glioma cell lines (Fig. 1D). These results were also confirmed through real-time qRT-PCR analysis (Fig. 1E).

**Expression of TCTP Correlates with Shortened Survival and Decreased Survival Rate after RT-TMZ Therapies**

We further evaluated whether TCTP immunoreactivity correlated with overall survival in 168 participants with glioma. We observed that the upregulation of TCTP predicted shorter overall survival and the disease-free survival of HCC participants (Fig. 2A and B). Multi-variate survival analysis using the Cox proportional hazards model further indicated that the upregulation of TCTP was correlated with a higher hazard ratio (HR) and poor clinical outcomes (overall survival, \( P = .005 \) HR 4.638; disease-free survival, \( P = .016 \) HR 2.986) (Table 1). We therefore investigated the role of TCTP in therapies using radiation and concomitant and adjuvant temozolomide. We observed that the 1-year survival rates were 67.39% (31/46) for cases with negative TCTP antigen and 26.53% (26/98) for cases with positive TCTP antigen (P < .0001, Fig. 2C). Because TCTP-positive patients represent a higher percentage of the high-grade glioma population, we selected 46 participants with positive TCTP antigen to make a valid comparison. This cohort comprised 18 WHO stage I glioma participants, 13 WHO stage II glioma participants, 9 WHO stage III glioma participants, and 6 WHO stage IV glioma participants, which was the same breakdown as

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the participants with negative TCTP antigen. We also observed that TCTP-positive patients have lower survival rate, 30.43% (14/46) compared with 67.39% (31/46) ($P = .0060$, Fig. 2D). These results highlight the clinical importance of TCTP in determining the prognosis for patients with glioma, indicating a new target for glioma therapy.

**TCTP Enhances Glioma Cell Growth In Vitro and In Vivo**

To investigate the biological role of TCTP in glioma cells, we performed gain- or loss-of-function studies. We overexpressed TCTP in U-251 and H-4 cells to express lower endogenous TCTP (Figs 1C, D and 3A). We subsequently examined the role of TCTP in glioma cell proliferation using MTT and BrdU assays. The results showed a significant increase in the growth curve and indicated that cell proliferation was enhanced in vitro after transfection with TCTP in both U-251 and H-4 cells (Fig. 3B–E). To determine whether TCTP enhanced resistance to irradiation in glioma cells, we treated U-251/V, U-251/TCTP, H-4/V, and H-4/TCTP cells with 10 Gy irradiation and then detected their ability to survive using a colony-formation assay. We observed that U-251/TCTP cells and H-4/TCTP cells formed more colonies than vector-control cells with or without irradiation (Figs 4F and 3G). These in vitro and in vivo results demonstrate that TCTP potently promotes glioma cell proliferation and tumor growth.

**TCTP Binds to TCF-4 and Enhances β-catenin/TCF-4 Transcription Activity**

To investigate how TCTP regulates glioma cell proliferation, we performed yeast 2-hybrid screening using full-length TCTP as the bait (pAS2-1-TCTP). Using this genetic screening, we observed that pAS2-1-TCTP bound to GAL4AD-TCF-4 (Fig. 5A). The direct interaction was confirmed through cotransforming GAL4AD-TCF-4 and TCTP shRNA2 to knock down TCTP, and a similar phenotype was observed (Fig. 41–R). These in vitro and in vivo results demonstrate that TCTP potently promotes glioma cell proliferation and tumor growth.
and pAS2-1-TCTP constructs into yeast strain Y190 cultured in SD-/Leu-Trp-His triple-deficient medium for positive β-galactosidase activity. The GAL4AD-TCF-4 yeast clone was DNA sequenced and observed to encode the carboxyl-terminal segment (amino acid residues 25-288) of human TCF-4. This genetic result indicates a direct interaction between TCTP and TCF-4. We next verified the association between TCTP and TCF-4 through a co-immunoprecipitation assay in U-138 cells using anti-TCTP antibody (anti-TCTP) or control rabbit IgG (rIgG) followed by immunoblotting for TCF-4 or TCTP. TCF-4 co-immunoprecipitated with the anti-TCTP antibody but not with rIgG (Fig. 5B).

Because the β-catenin/TCF-4 transcription complex is essential for the Wnt/β-catenin signaling pathway, we examined whether TCTP affected the β-catenin/TCF4 association. Indeed, as shown in Fig. 5C and D, TCF-4 bound more β-catenin when TCTP was overexpressed in U-251 cells and in rat normal astrocyte DI TNC1 cells. Consistently, TCF-4 bound less β-catenin when TCTP was knocked down in U-138 cells (Fig. 5E). We next explored whether TCTP affected β-catenin/TCF-4 transcriptional activity using a luciferase reporter assay. U-251 cells, and rat normal astrocyte DI TNC1 cells were co-transfected with a plasmid, encoding the TCF-4 binding sites’ luciferase reporter (TOPflash) or the control reporter (FOPflash) and the system control plasmid.

Fig. 2. TCTP expression is correlated with low survival rate. (A and B) Kaplan–Meier analyses were performed according to TCTP protein expression scores in glioma participants. Scores of 0-2 and ≥3 were considered negative and positive staining, respectively. The overall survival of participants with positive TCTP expression was evidently shorter (A). The disease-free survival of participants with positive TCTP expression was evidently shorter (B). (C) and (D) The 1-year survival rate after RT-TMZ treatment in glioma participants based on the immunoreactivity scores in glioma participants. Scores of 0-2 and ≥3 were considered negative and positive staining, respectively. Survival rate of total TCTP-positive participants and TCTP-negative participants were compared in (C). (D) To compare fairly, 46 TCTP-positive participants, including 18 WHO stage I glioma participants, 13 WHO stage II glioma participants, 9 WHO stage III glioma participants and 6 WHO stage IV glioma participants, as with the TCTP-negative participants, were selected randomly. Kaplan–Meier analyses were used here.

Table 1. Multivariate cox regression analysis of TCTP expression in glioma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall Survival</th>
<th>Disease-free Survival</th>
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<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P value</td>
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<tr>
<td>TCTP expression</td>
<td>4.638 (1.633-8.465)</td>
<td>.005</td>
</tr>
<tr>
<td>Sex</td>
<td>0.745 (0.165-3.928)</td>
<td>.668</td>
</tr>
<tr>
<td>Clinical stage*</td>
<td>3.622 (0.652-16.369)</td>
<td>.098</td>
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Abbreviations: CI, confidence interval; HR, hazard ratio. Boldface highlights the statistical significance.

*Early (T1/T2) versus advanced stage (T3/T4).
encoding the β-galactosidase reporter. The results showed that the ectopic expression of TCTP increased TOPflash activity but not FOPflash activity (Fig. 5F and G). To further examine whether TCTP was necessary for the activation of TCF-4/β-catenin transcription activity, we knocked down the expression of TCTP in U-138 cells using shRNA. As shown in Fig. 5H, TCTP-shRNA1 decreased the TOPflash activity, but not the FOPflash activity, compared with control shRNA. To further confirm that TCTP regulated β-catenin/TCF-4 transcription activity, we examined the expression of c-Myc and cyclin D1, which are well-characterized targeting genes of the Wnt/β-catenin/TCF-4 signaling pathway. Indeed, the ectopic expression of TCTP triggered the expression of c-Myc and cyclin D1 in U-251 cells and rat normal astrocyte D1 TNC1 cells, whereas TCTP knockdown attenuated the expression of these genes in U-138 cells (Fig. S1). These results clearly indicate that TCTP binds to TCF-4 to recruit β-catenin in association with TCF-4 and enhances β-catenin/TCF-4 transcription activation, leading to the expression of the Wnt targeting genes c-Myc and cyclin D1.

TCF-4 Knockdown Inhibits the TCTP-induced Proliferation of Glioma Cells

To determine whether TCF-4 is necessary for TCTP-regulated glioma cell proliferation, we downregulated the expression of TCF-4 in TCTP-overexpressing U-251 and H-4 cells using shRNA1. Compared with control shRNA, TCF-4 shRNA1 decreased the protein expression of TCF-4 (Fig. 6A) and inhibited the proliferation of TCTP-overexpressed U-251 (Fig. 6B) and H-4 cells (Fig. 6C) according to an MTT assay. However, TCF-4 shRNA1 had less effect on U-251/Ctrl shRNA cells and H-4/Ctrl shRNA cells (Fig. 6D and E). Further, the in vivo tumor growth and Ki67-positive cells were dramatically inhibited using TCF-4 shRNA1 compared with control shRNA (Ctrl shRNA) in both TCTP-overexpressing U-251 and H-4 cells (Fig. 6F and G). To confirm these results, another shRNA (TCF-4-shRNA2) targeting TCF-4 was used, and similar effects were observed (Fig. 6H–N). These results demonstrate that TCF-4 is necessary for the proliferation of glioma cells induced by TCTP.
Fig. 4. TCTP knockdown inhibits glioma cell proliferation in vitro and in vivo. (A) U-138 and HS-683 cells transfected with control shRNA (Ctrl shRNA) or TCTP shRNA1. The TCTP levels were detected through immunostaining with the TCTP antibody, and actin was used as a loading control. (B) In vitro growth of U-138/Ctrl shRNA and U-138/TCTP shRNA1 cells measured using MTT assays. (C) In vitro growth of HS-683/Ctrl shRNA and HS-683/TCTP shRNA1 cells measured using MTT assays. (D) In vitro growth of U-138/Ctrl shRNA and U-138/TCTP shRNA1 cells measured using BrdU assays. (E) In vitro growth of HS-683/Ctrl shRNA and HS-683/TCTP shRNA1 cells measured using BrdU assays. (F) The colony formation assay was performed using U-138/Ctrl shRNA and U-138/TCTP shRNA1 cells treated with or without irradiation. (G) The colony formation assay was performed using HS-683/Ctrl shRNA and HS-683/TCTP shRNA1 cells treated with or without irradiation. (H) Average tumor volume (left) and tumor TCTP/Ki67 staining (5 weeks after implantation, right) in athymic nude mice subcutaneously inoculated with U-138/Ctrl shRNA or U-138/TCTP shRNA1 cells. (I) Average tumor volume (left) and tumor TCTP/Ki67 staining (5 weeks after implantation, right) in athymic nude mice subcutaneously inoculated with HS-683/Ctrl shRNA or HS-683/TCTP shRNA1 cells. (J) U-138 and HS-683 cells transfected with control shRNA (Ctrl shRNA) or TCTP shRNA2. TCTP levels were detected through immunostaining with the TCTP antibody, and actin was used as a loading control. (K) In vitro growth of U-138/Ctrl shRNA and U-138/TCTP shRNA2 cells measured using MTT assays. (L) In vitro growth of HS-683/Ctrl shRNA and HS-683/TCTP shRNA2 cells measured using MTT assays. (M) In vitro growth of U-138/Ctrl shRNA and U-138/TCTP shRNA2 cells measured using BrdU assays. (N) In vitro growth of HS-683/Ctrl shRNA and HS-683/TCTP shRNA2 cells measured using BrdU assays. (O) The colony formation assay was performed using U-138/Ctrl shRNA and U-138/TCTP shRNA2 cells measured with or without irradiation. (P) The colony formation assay was performed using HS-683/Ctrl shRNA and HS-683/TCTP shRNA2 cells treated with or without irradiation. (Q) Average tumor volume (left) and tumor TCTP/Ki67 staining (5 weeks after implantation, right) in athymic nude mice subcutaneously inoculated with U-138/Ctrl shRNA or U-138/TCTP shRNA2 cells. (R) Average tumor volume (left) and tumor TCTP/Ki67 staining (5 weeks after implantation, right) in athymic nude mice subcutaneously inoculated with HS-683/Ctrl shRNA or HS-683/TCTP shRNA2 cells. For (A-G and K-P); the results represent at least 3 separate experiments. For H, I, Q, and R, n = 10 mice/group. Error bar: ± S.D. *P < .05.
Discussion

In this study, we showed that TCTP expression is frequently upregulated in glioma (72.62%, 122/168), particularly in high-grade glioma (stages III and IV, 82.56% and 71/86, respectively), and there was a positive correlation between TCTP expression and advanced clinicopathological features. In addition, TCTP immunoreactivity is inversely correlated with overall survival and disease-free survival in patients diagnosed with glioma and reduces the survival rate after RT-TMZ therapy, further underscoring the clinical significance of TCTP in the pathogenesis, prognosis, and treatment of glioma. Functional analysis through gain- or loss-of-function studies showed that TCTP overexpression enhanced glioma cell proliferation and in vivo tumor growth, whereas TCTP knockdown using shRNA inhibited glioma cell proliferation and in vivo tumor growth. We observed that TCTP binds to TCF-4 and enhances β-catenin association with TCF-4, which increases TCF-4/β-catenin transcription activity.

The canonical Wnt/β-catenin signaling pathway plays important roles in embryonic development, adult tissue homeostasis, and tumor genesis. Recently, several reports have shown that the aberrant activation of Wnt/β-catenin/TCF signaling is an important
contributing factor in glioma development. β-catenin and TCF-4 were upregulated in glioma tissues compared with normal brain tissues, and the knockdown of β-catenin by small interfering RNA (siRNA) in human glioma cells inhibited cell proliferation and invasion, inducing apoptotic cell death and delaying tumor growth.27 –30

It has been reported that TCF-4, the oligodendrocyte-related transcription factor, is required for the maturation of oligodendrocyte progenitors.31 However, the mechanism of β-catenin and TCF-4 involvement in gliomagenesis is poorly understood. The results of the present study show that TCTP binds to TCF-4, increasing the
association of β-catenin with TCF-4 and thereby enhancing Wnt/β-catenin signaling transcription activation. More importantly, TCTP shRNA markedly inhibited glioma cells proliferation. Thus, our results suggested TCTP as a new target for glioma therapy through inhibiting Wnt/β-catenin signaling activation. Notably, even in normal rat astrocyte cells, TCTP still enhanced Wnt/β-catenin signaling activation. Whether TCTP is a regular Wnt/β-catenin signaling regulator, besides in glioma, deserves to be studied.

Recently, TCTP has received much attention as a key player in tumor reversion. In tumor reversion, some cancer cells lose their malignant phenotype. TCTP inhibits MDM2 autoubiquitination and promotes the MDM2-mediated ubiquitination of p53, which ultimately leads to p53 degradation and thus inhibits cancer cell apoptosis and promotes the cancer cell cycle. In our study, participants with positive TCTP showed a decreased 1-year survival rate after RT-TMZ therapy and TCTP over-expression enhanced glioma cell proliferation after 10 Gy irradiation treatment in vitro, which indicated that TCTP may support glioma cancer cell survival by inhibiting p53 regulated apoptosis pathway and activating glioma cell proliferation through Wnt/β-catenin signaling pathway. More evidence is needed to confirm this hypothesis. More interestingly, since TCTP contributed important roles both in Wnt/β-catenin signaling and p53 apoptosis pathways, the crosstalk between these 2 pathways deserves further investigation. Understanding the molecular mechanisms underlying TCTP in these different pathways will provide further target sites for cancer therapies.

**Funding**

Support for this work includes funding from the National Nature Science Foundation of China (grant numbers 31171219, 81271213, 81070878, 81271214 and 81261120404), the Natural Science Foundation of Guangdong Province (No. S2012100008222) and the Science and Technology Innovation Fund of Guangdong Medical College (No. STIF 201101).

**Acknowledgements**

We thank Isaac N. for critical reading and editing of the manuscript.

**Conflict of interest statement** None declared.

**References**


