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LABORATORY INVESTIGATION

TRIM44 is indispensable for glioma cell proliferation and cell cycle progression through AKT/p21/p27 signaling pathway

Xia Zhou[1,6] · Yadong Yang[2,3] · Pengcheng Ma[4] · Na Wang[5,7] · Dong Yang[6] · Qiu Tu[1,6] · Bin Sun[6] · Tingxiu Xiang[7] · Xudong Zhao[6,8,9] · Zongliu Hou[1] · Xiangdong Fang[2,3]

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Abstract

Purpose Glioma is one of the lethal cancers which needs effective therapeutic target. TRIM44 has been found playing a carcinogenic role in human tumors such as breast cancer and ovarian cancer. However, the pathophysiological significance of TRIM44 in glioma is still unclear.

Methods Quantitative-PCR and western blot were used to assess the expression of TRIM44 in glioma cells. For cell proliferation, Brdu incorporation and colony formation assays were performed. By Caspase 3 staining and FACS analysis, we revealed that TRIM44 knockdown induced glioma cell apoptosis. A BALB/c nude mouse xenograft model and following immunohistochemical (IHC) staining enables us to explore the effect of TRIM44 deletion on glioma growth in vivo. Western blot of p21, p27 and AKT indicated the possible role of TRIM44 in regulation AKT pathway in glioma.

Results TRIM44 was significantly elevated in glioma cells, and high expression of TRIM44 is related to poor prognostic of glioma patients. *TRIM44* knockdown by shRNAs inhibit glioma cell proliferation, migration, induced cell cycle disruption and further cellular apoptosis in vitro. As well, TRIM44 inactivation obviously inhibit tumor growth in xenograft model. Furthermore, the negative cell cycle regulators p21/p27 are significantly upregulated, while AKT which is known as the main regulator of p21/p27 is inactivated in TRIM44-dificient cells. These results suggested that TRIM44 inactivation disrupted cell cycle progression and inhibit cell proliferation through AKT/p21/p27 pathway in glioma.

Conclusion TRIM44 was associated with oncogenic potential of glioma. Targeting TRIM44 might be beneficial for glioma therapy.

Keywords TRIM44 · Cell cycle · Migration · Proliferation · AKT

Introduction

Glioma is the most malignant, aggressive primary brain tumor in central nervous system [1, 2]. As one of the lethal cancers, glioma is featured by high migration, invasion, proliferation ability, high recurrence and low cure rate [3–5]. Despite aggressive therapeutic approaches including surgical resection, irradiation and chemotherapy management have been explored, the medium survival time of patients is only about 12–16 months [6–9]. The main reason of the poor therapeutic effect is the less understanding of the molecular mechanisms involved in glioma development. Therefore, it is necessary to find more effective biomarkers to improve the diagnosis for glioblastoma.

Tripartite motif containing 44 (*TRIM44*) has been cloned first from cDNA library of mouse brain [10]. As a member of TRIM protein family, TRIM44 is involved in many important biological processes and associated with diverse pathological conditions [11–13]. Like most members in TRIM family, it has been reported that TRIM44 promotes antiviral reaction with virus-induced signaling adaptor (VISA) [14]. While in other studies, TRIM44 has been found to be overexpressed in many malignant tumors including

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✉ Zongliu Hou
HZL579@163.com

✉ Xiangdong Fang
fangxd@big.ac.cn

Extended author information available on the last page of the article

head and neck cancer, gastric carcinoma, esophageal and junctional adenocarcinoma, non-small cell lung cancer and hepatocellular carcinoma [15–19]. Recently, TRIM44 was also found as a poor prognostic factor for breast cancer patients [20]. Hence, TRIM44 was identified as oncogenic target in tumor research [21]. Li et al. reported that TRIM44 knockdown by using MiR-101-3p attenuated glioma cell proliferation, migration in vitro [22]. However, how does TRIM44 influence the glioma cell growth and its mechanism is still unclear. In this study, we found that high TRIM44 expression is negatively related to patients' survival. Also, TRIM44 expression is elevated in glioma cells compared to normal cells. TRIM44 knockdown reduced glioma cell proliferation and migration in vitro and tumor growth in vivo. The ability of glioma cells entry into S phase is damaged in TRIM44 deficient group by cell cycle analysis and Brdu incorporation assay. P21/p27 are known as the most important cyclin-dependent kinase inhibitors and overexpression of p21/p27 were found to prevent cell entry into the S phase [23-26]. TRIM44 knowdown in glioma cells elevated the expression of p21/p27. Additionally, the critical p21/p27 regulator AKT is inactivated after TRIM44 is knockdown while activated in TRIM44 overexpressing cells. Our study revealed the functions of TRIM44 in glioma and its regulation on the AKT/p21/p27 pathway.

Materials and methods

Lentiviral plasmid construction

For TRIM44 knockdown, two individual target sequences have been chosen to target TRIM44 within cDNA. shTRIM44-#1: 5′-CCAGTGAAGAAGAGGACACAT-3′; shTRIM44-#2: 5′-GCCGAAGAAGACAACCAAGAA-3′. 58-bp short hairpin RNA (shRNA) fragments of sense and antisense oligonucleotides containing the target sequence were synthesized and hybridized and then cloned into the pLKO.1 and pLKO-tet-on vector. The recombined clones were validated by DNA sequencing.

Lentivirus packaging and infection of glioma cells

HEK 293T cells were used to produce lentivirus by co-transfection with two packaging plasmids (pCMV△8.9 and pMD2.G) using polyethyleneimine (PEI)-mediated transfection as described previously [27]. Briefly, the supernatant was collected after 48 h and 72 h after transfection. Concentration and purification of virus was performed to remove the serum from the supernatant using ultra-centrifuge. Lentivirus was re-suspended in phosphate-buffer-saline (PBS) after 130,000×*g* of centrifugalizing for 2.5 h. The particles of lentivirus were quantified by real-time PCR using U5 primers [28]. The glioma cells were infected with virus at a multiplicity of infection (MOI) of 10 to avoid cell toxicity.

Cell culture

The human glioma cell line T98G, U87MG and U251MG were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37 °C, and 5% CO_2. The medium was changed every 2–3 days, and cultures were digested using 0.25% trypsin. The human glioma stem cells were maintained adherently in serum free DMEM/F12 Medium supplemented with B27, EGF, bFGF [29, 30]. The stable transfected U251MG-teton-shTRIM44 cell were cultured in tet-negative FBS (Lonsera, U912-001). The glioma cells used in the study were validated using Short Tandem Repeat (STR) profiling.

Western blotting

Cultured cells were harvested and lysed by RIPA buffer containing PMSF and protease inhibitor cocktail for 30 min on ice and mix every 10 min. Protein extracts were quantified by BCA assay and electrophoresis by SDS-PAGE. Blocking is performed by 5% milk for 1 h in the room temperature. The specific proteins were blotted with the following antibodies: anti-TRIM44 (Proteintech, 1/1000), anti-β-Actin (Sigma, 1/10000), anti-total AKT (Cell signaling technology, 1/1000), anti-p-AKT-473 (Cell signaling technology, 1/1000) and anti-p-AKT-308 (Cell signaling technology, 1/1000).

RNA extraction and quantitative PCR

Total RNA was extracted from cultured cells using Trizol Reagent (Sigma, St. Louis, MO, USA) according to the manufacturer's instruction. 2 μg of total RNA were reverse transcribed into cDNA using the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific; Fermentas). Real-time PCR reactions were performed using SYBR Green (Thermo Scientific). Expression of interested gene was quantified by measuring the CT values and normalized to 18S by the $2^{-\Delta\Delta CT}$ method. Primers for quantitative PCR were specific (Supplementary Table 1).

Flow cytometric analysis of cell apoptosis and cell cycle

For apoptosis analysis, an apoptosis detection kit (Annexin V-FITC/PI kit) was used. Cells were harvested and washed in PBS and then stained with Annexin V-FITC and PI according to the manufacture' instruction. The apoptosis rate of cell samples was analyzed by using FACS. For cell cycle

analysis, cells were digested with 0.25% trypsin–EDTA (Life Technologies, USA) and fixed with pre-cooled 70% ethanol for overnight at 4 °C. After washing in PBS for two times, cells were resuspended in staining buffer (PBS with 100 μg/mL RNase A and 50μg/mL propidium iodide) for 0.5 h. Cell cycle analyses were performed by flow cytometry using the BD FACS Vantage™ SE System.

Cell migration assays

The migration ability of glioma cells was determined by using 24-well cell culture inserts (8-μm pore size, Corning, NY, USA). To stop the proliferation, cells were treated with 5 μg/mL mitomycin C for 8 h. After that, 5×10^4 cells suspended in serum-free DMEM were seeded onto the insert. DMEM supplemented with 10% FBS was added to the lower chamber of each Trans-well. After 24 h incubation in 37 °C, the cells on the top of the membrane surface were removed with a cotton swab. Cells on the bottom of the membrane surface were fixed with 4% paraformaldehyde and stained with DAPI.

BrdU incorporation assay

DNA synthesis was determined by using 5-Bromo-2-deoxy-Uridine (BrdU) incorporation assay according to the manufacturer's instructions. Briefly, 10 μM of BrdU was added to the cells and incubated for 1 h. Cells then washed with PBS for two times and fixed with 4% paraformaldehyde (PFA) for 20 min in room temperature. After permeabilization with 0.3% Triton-X-100, the cells were treated with 2 M HCL for 30 min at 25 °C. After blocking with 10% goat serum for 1 h, cells were incubated with anti-BrdU antibody (Abcam, 1:250) for 1 h at room temperature and then with the CY3-labeled secondary antibody.

Soft agar colony formation assays

To mimic the tumor initiation ability, the Soft agar colony formation assays was performed. 0.7%-layer soft agarose (Sigma) were prepared with culture medium and added 1 mL per well for 6-well plate and cover the plates to allow agar mixture to solidify at room temperature for 20 min. Cells transfected with lentivirus were digested and seeded at a concentration of 5000 cells per well. Cells were mixed with 1 mL 0.35%-upper soft agarose and added to the top of the solidified lower layer agarose, then cell/agarose mixture was allowed to solidify at room temperature for 30 min. 1 mL completed culture medium was added to the top of upper layer agarose carefully before placing into a 37 °C humidified cell culture incubator. Fresh medium was added every 3 days for 4 weeks, followed by fixation with 4% formaldehyde and stained the colonies were stained with 0.005% crystal violet. All the recognizable clones in each well was counted and analyzed after enlarge the images. We count almost all the visible clones by enlarge the images to the same size. In detail, for clones distributed equally, we divided the plate into four parts and counted all clones in one part, then we calculate the clone number by multiplying four to represent the plate. For the unequally distributed clones, we count all the clones one by one.

Xenograft mouse models

U251MG/teton-shTRIM44 cells were digested using 0.25% trypsin and 1×10^7 cells/mL resuspended in PBS containing 30% Matrigel (BD Biosciences, USA), then 100 μL suspended 1×10^6 tumor cells were subcutaneously injected into 6-week-old female BALB/c nude mice (Charles River Laboratories, China). Totally, 27 mice were assigned to two groups 13 mice were used in control group and 14 mice were used in dox induced group) equally according to the volume of tumors. 2 mg/mL doxycycline (DOX) was administrated via the drinking water containing 5% sucrose. Tumor volume was calculated based on the formula $0.5 \times \text{length} \times \text{width}^2$ [31]. All animal protocols were approved by the Animal Ethic Committee of the Kunming Institute of Zoology. The data are shown as the means ± SM, and statistical analyses were performed using GraphPad Prism statistical software.

Results

TRIM44 is overexpressed in glioma cells and its knockdown inhibits cell proliferation and migration in vitro

To investigate whether TRIM44 expression level is associated with glioma or not. We first analyzed the association between the TRIM44 expression with patients' survival by using the Human protein atlas (https://www.proteinatlas.org/) which incorporates the Cancer Genome Atlas (TCGA) database. We found that patients with high expression of TRIM44 always has poor survival (Fig. 1a). Further western blot analysis indicated that TRIM44 is elevated in glioma cells [U87MG, U251MG and glioblastoma stem cells (GSC-3#)] compared to human normal astrocyte (HAC) (Fig. 1b). To identify the function of TRIM44 in glioma, two shRNAs targeting TRIM44 and a negative control (shCtrl, non-specific scramble shRNA) were synthesized and transfected into glioma cells and glioblastoma stem cells by lentivirus. The growth of both glioma cells and glioblastoma stem cells have been impaired significantly after TRIM44 is knockdown (Fig. 1c, d, Supplementary Fig. 1a–c). Brdu incorporation assay is a measure of cell proliferation. The Brdu assay reflects a strong impairment on proliferation by

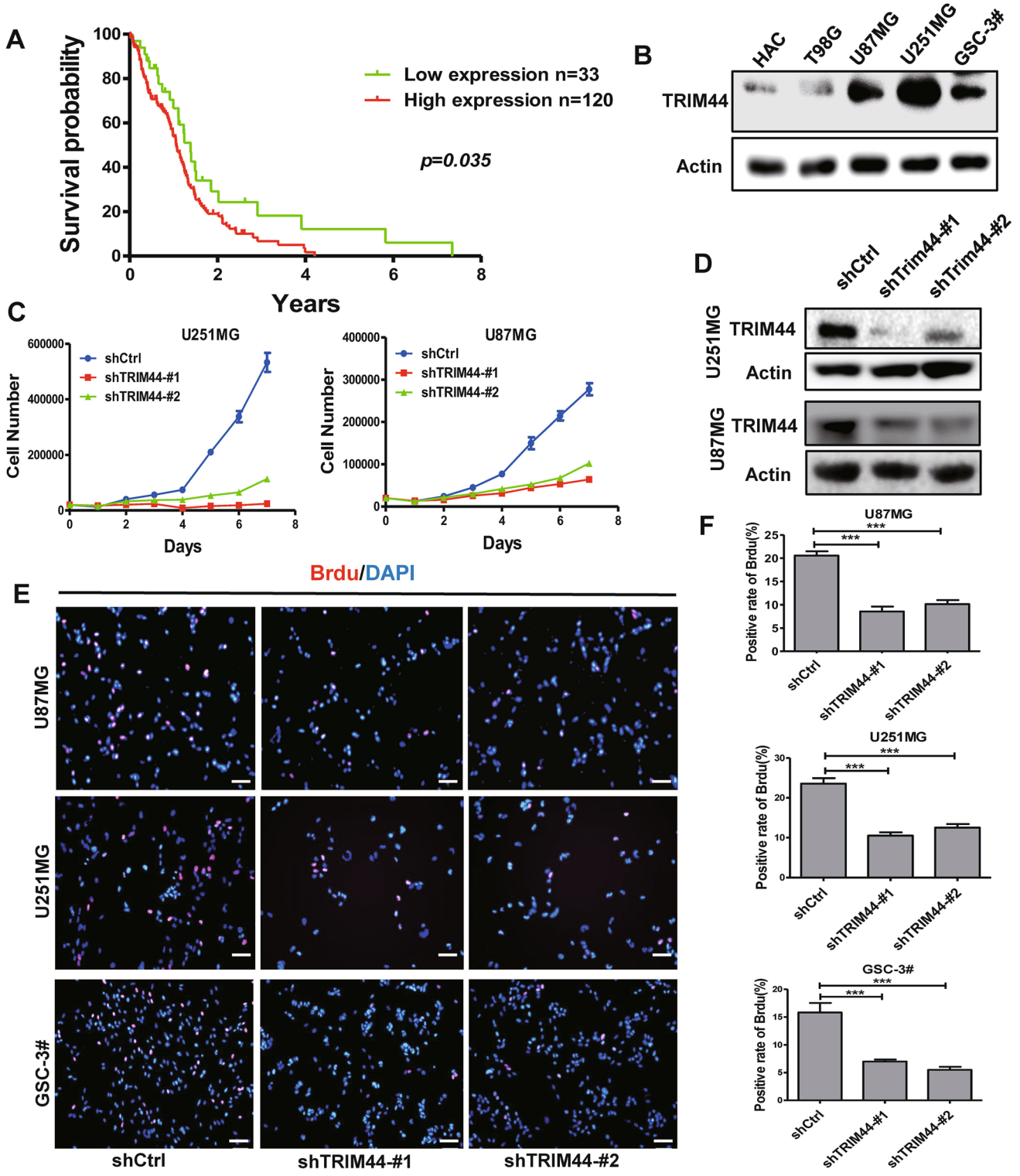


Fig. 1 TRIM44 is overexpressed in human glioma cells and its knockdown impaired glioma cell growth. **a** The survival curve from TCGA database indicated that high level of TRIM44 expression is corelates to worse survival. **b** TRIM44 is overexpressed in glioma cells compared to normal human astrocyte cell line (HAC). **c** Glioma cell growth was impaired after TRIM44 knockdown in glioma cell lines. Cells were counted every day after infected with lentivirus for 24 h. **d** Western blotting analysis of TRIM44 expression after cell infection with shRNAs. Cells from each group were harvested and lysed on the ice three days after infection. **e** TRIM44 knockdown significantly inhibit DNA synthesis in both glioma cell U251MG and U87MG and glioma stem cell (GSC-3#) by using the Click-iT™ EdU Alexa Fluor® 647 Imaging Kit. **f** Statistical quantification of Brdu positive cells in shCtrl group or shTRIM44 groups. The value is presented as the mean ± SEM (*P < 0.05; ***P < 0.001, *t* test). Scale bars: 30 μm

suppressing TRIM44 in glioma cells (Fig. 1e, f). Colony formation is an important indicator that was commonly used to mimic tumor initiation ability in vitro [32, 33]. By soft agar colony formation assay, we found that the sphere number is impaired significantly after TRIM44 knockdown (Fig. 2a, b) and the diameter of the spheres is much smaller in TRIM44 knockdown group than control (Fig. 2c, d). This result suggested that TRIM44 silence impaired tumor formation ability of glioma cells. As the ability of migration is also an important factor for malignant glioma. We used transwell assay to test the involvement of TIMR44 on cell migration. Suppression of TRIM44 led to significant reduction in cellular migration of U87MG, U251MG and GSC-3# (Fig. 2e, f). In line with the results reporter by Li et al. by using MiR-101-3p mediated TRIM44 knockdown [22], the Epithelial to mesenchymal transition (EMT) markers Vimentin, MMP2, MMP9 and Snail1 are also downregulated in TRIM44 deficient group than control group in both U87MG, U251MG cells (Fig. 2g). All these results above indicate that TRIM44 plays an essential role in maintaining glioma malignancy in vitro.

TRIM44 silence induce glioma cell apoptosis

To evaluate the effect of TRIM44 knockdown on glioma cell apoptosis, the key indicator Caspase 3 was chosen to indicate the activation of apoptosis signal pathway. TRIM44 silence induced cell apoptosis as evidenced by Caspase 3 staining (Fig. 3a, b). Further Annexin V/PI staining followed by fluorescence-activated cell sorting (FACS) analysis showed that TRIM44 deficient cells have a much higher apoptosis rate than control group in both U251MG and U87MG cells (Fig. 3c, d). In U251MG cells, few apoptotic cells in shTRIM44 group were detected in early stage (Q3 in the upper panel of Fig. 3c) and almost all apoptotic cells were found in the late stage (Q2 in the upper panel of Fig. 3c). While in U87MG, about half of the apoptotic cell in shTRIM44 group were detected in early stage of apoptosis (Q3 in the lower panel of Fig. 3c) possibly because of the different apoptosis processes in different cell lines. These data above indicate that TRIM44 knockdown activate Caspase 3 and induce glioma cell apoptosis.

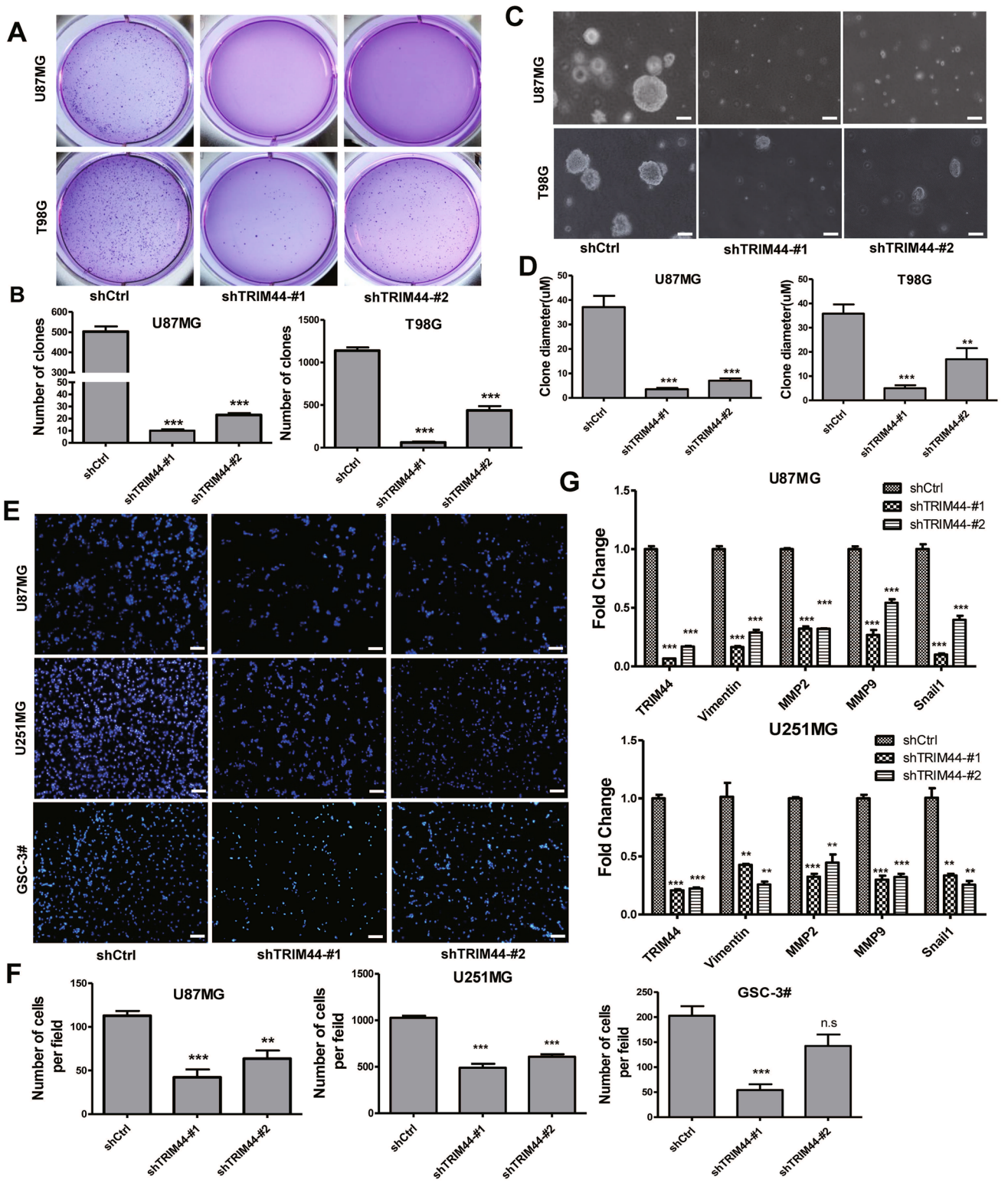
TRIM44 silence inhibits glioma cell proliferation in vivo

To investigate whether TRIM44 silence reduces tumor growth in vivo, we constructed the most effective shRNA (shTRIM44-#1) into the plko-tet-on plasmid. Knockdown of TRIM44 is achieved by doxycycline treatment in U251MG (Supplementary Fig. 2). $1 \times e^6$ uninduced stable transfected U251MG cells were injected into BALB/c nude mice subcutaneously. After all nude mice formed tumor, mice were divided into two groups equally according to their tumor volume. The average tumor size in control group is $53.23cm^3$ (SD ± 26.61) versus 59.01 cm^3 (SD ± 36.50) in dox induced group. 2 mg/mL doxycycline was added to drinking water to induce TRIM44 knockdown and vehicle only in the control group, the water was changed every two days and tumor volume is measured every week. Remarkably, the tumor growth in TRIM44 silenced group was much slower compared to control group (Fig. 4a). Three weeks later, tumors from both groups were collected for further immunohistochemical (IHC) staining analysis. The volume of tumors in TRIM44 deficient mice are smaller compared to the control group (Fig. 4b, c). By IHC analysis, the protein level of TRIM44 was remarkably decreased in doxycycline induced mouse (Fig. 4d). High expression of KI67 always indicates a high proliferation index in cancer tissues. Here we found that KI67 was dramatically decreased in doxycycline given group, which suggestes a strong suppression to tumor cell proliferation by TRIM44 knockdown (Fig. 4e, f). These results provide strong evidence for the importance of TRIM44 on maintaining tumor growth in vivo.

TRIM44 regulates cell cycle through AKT pathway

Cell cycle progression is essential to keep cell proliferation. To investigate whether the effect of TRIM44 on cell proliferation is due to cell cycle disruption, we performed flow cytometry analysis after propidium iodide (PI) staining. Cell cycle analysis indicated that TRIM44 knockdown caused DNA synthesis inhibition at S phase significantly in both U87MG and U251MG cells (Supplementary Fig. 3). The number of cells at S phase decreased from 15.19% to 3.31% (shTRIM44-#1, $p = 0.0024$) and 5.88% (shTRIM44-#2, $p = 0.0174$) in U87MG and from 14.12% to 5.22% (shTRIM44-#1, $p = 0.0151$) and 5.58% (shTRIM44-#2, $p = 0.0158$) in U251MG (Fig. 5a). The main cell cycle regulators including cyclin-dependent kinase (CDKs) and cyclin-dependent kinase inhibitors (CDKIs) were analyzed. Negative cell cycle regulator p21 and p27 shows significant increase at both transcriptional and protein level. While cell cycle positive regulator cyclinB1 reduced obviously (Fig. 5b, c). There was no significant difference of cyclinD1 in both mRNA expression and protein level (Fig. 5c).

Overexpression of p21/p27 were found to prevent the entry into the S phase [25, 34]. So, we speculated that TRIM44 knockdown inhibits glioma cell growth through p21/p27 related cell cycle arrest. Previous studies reported that AKT signaling pathway regulates cell cycle and cell proliferation by directly targeting p21 and p27 [35–37]. To further investigate the involvement of AKT pathway in p21 and p27 upregulating after TRIM44 silence, total and phosphorylated AKT have been analyzed. Our results indicate that phosphorylated AKT level dramatically



decreased after TRIM44 knockdown in both U87MG and U251MG glioma cells (Fig. 5d). On the contrary, overexpression of the TRIM44 increase phosphorylated AKT levels (Fig. 5e). Collectively, these data suggested that TRIM44 maintain glioma cell growth through activating the AKT/p21/p27 pathway.

◂**Fig. 2** Glioma cells colony formation and migration was impaired after TRIM44 depletion. **a** Soft agar colony formation assay of glioma cell lines T98G and U87MG after TRIM44 knockdown. 5000 cells were plated into 6-well for sphere formation culture. Cells were fixed with paraformaldehyde and stained with crystal violet after 4 weeks. **b** Quantitative analysis of colony number of each group. **c** Clone growth in TRIM44 knockdown groups both in T98G and U87MG cell lines. **d** Quantitative analysis of colony diameter of each group. **e** Cell migration of glioma cells U87MG, U251MG and GSC-3# after TRIM44 knockdown showed by transwell assay. Before adding to the trans-well, cells were cultured in serum free medium for 24 h and treated with 5 μg/mL mitomycin C for eight hours, then digested by 0.25% trypsin. **f** Quantitative analysis of migrated cells of each group. **g** Quantitative PCR analysis of the EMT markers Vimentin, MMP2, MMP9 and Snail1 after TRIM44 knockdown. All the value is presented as the mean ± SEM (**P < 0.01; ***P < 0.001, *t* test). Scale bars: 100 μm

Discussion

Although a growing evidence indicated that TRIM44 plays an important role in several kinds of human diseases, how TRIM44 influence the glioma cell growth and its mechanism is still unclear. In our study, we found the high level of TRIM44 expression is correlated with worse glioblastoma patient's survival. Elevated TRIM44 expression is found in glioma cells. Targeting TRIM44 impaired glioma cell proliferation and migration in vitro, which are the most important hallmarks for tumor malignant development [38, 39]. By soft agar colony formation assay, which is commonly used to indicate tumor-initiating capacity [40–42], We found that TRIM44 knockdown significantly inhibits the clone formation and in vivo tumor growth of glioma cells. These results indicate that TRIM44 may play an oncogenic role in glioma as in other human tumors.

Loss of control for the cell cycle progress and proliferation is a hallmark of malignant cancer [43, 44]. Here, we found that the DNA synthesis and cell cycle were disrupted in TRIM44-depleted glioma cells. A remarkable up-regulated expression of p21/p27 and down-regulated expression of cyclin B1 both in RNA and protein level were found. As two of the most important cyclin-dependent kinase inhibitors, p21/p27 have been reported to regulate cell cycle negatively [23, 24]. By interacting with cyclins, p21/p27 are found inhibiting their catalytic activity and thus control the cell cycle progression. As a potential S phase promoting cyclin, cyclin B1 but not cyclin D1 was significantly inhibited in TRIM44-deficient glioma cells in our study. This provides powerful evidence to prove the function of TRIM44 in glioma proliferation. Among the cell cycle regulation signaling pathways, AKT pathway has been found participating in cell cycle regulation by directly regulating p21 and p27 [35–37].

AKT signaling pathway is commonly activated in different kinds of human cancers and plays a key role in regulating tumor cell proliferation and apoptosis [45, 46]. Here, we found TRIM44 knockdown decreases AKT phosphorylation, while exogenous overexpression increases the phosphorylation level of AKT thus activates the AKT signaling pathway. Intriguingly, endogenous TRIM44 knockdown only inhibits the Ser473 but not Thr308, while exogenous TRIM44 activate both sites. Either Ser473 or Thr308 phosphorylation was reported enough to activate AKT partially, while full activation requires phosphorylation of both [47]. Gene like *PTEN* was reported regulating AKT pathway by modulating phosphorylation on Ser473 but not Thr308 [48]. TRIM44 activate AKT by phosphorylating Ser473 was previously reported in esophageal cancer [49]. Here, we found that TRIM44 overexpression could activate the AKT not only on Ser473 but also Thr308 in glioma. These results strongly indicate that TRIM44 knockdown likely induces obvious upregulation of CDKIs-p21/p27 through AKT signaling pathway in glioma. The mechanism of how TRIM44 regulate AKT needs further investigation.

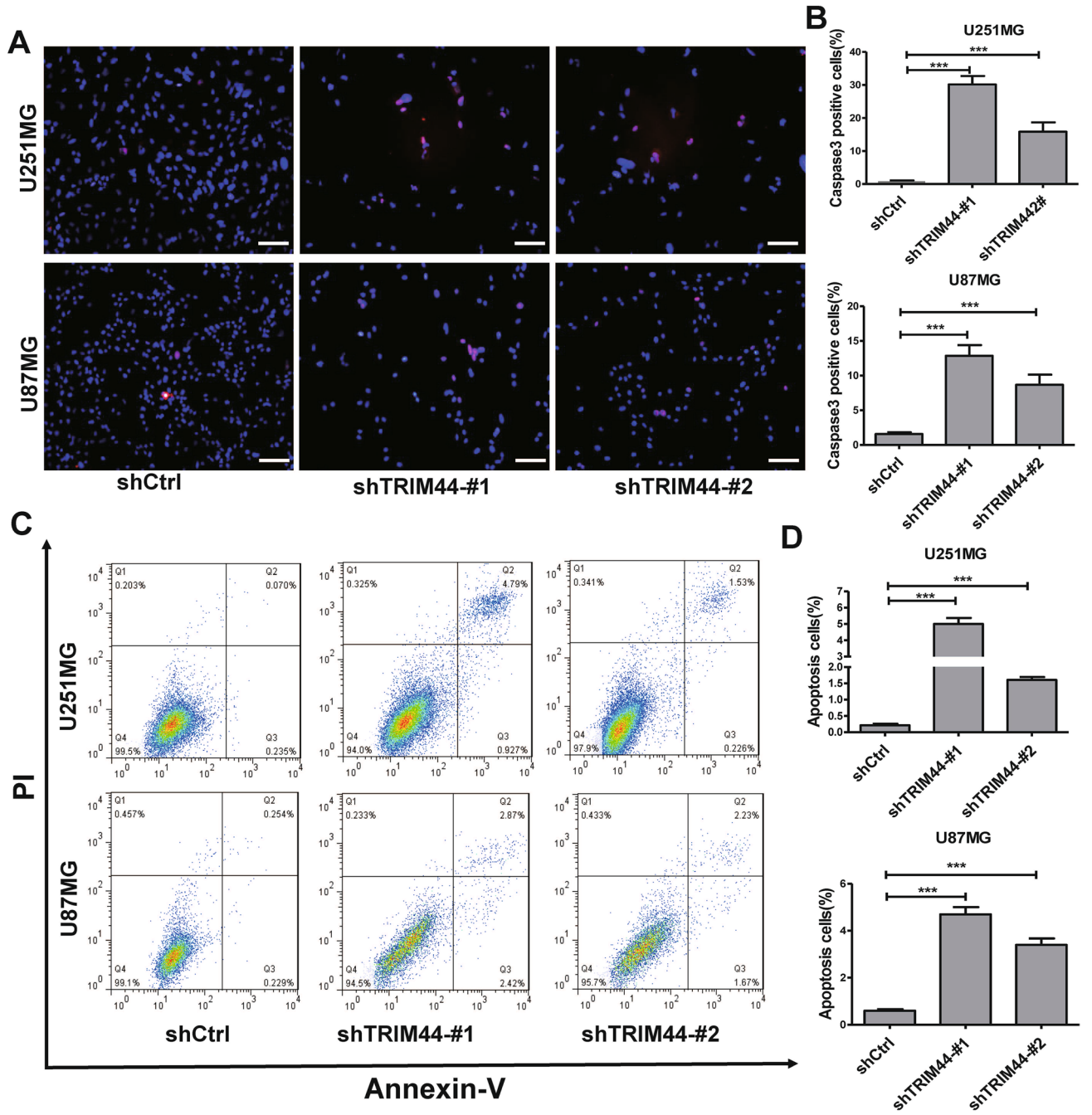


Fig. 3 TRIM44 knockdown induced glioma cell apoptosis. **a** Cleaved (cl)-Caspase 3 staining after TRIM44 knockdown. Three days after virus infection, cells were fixed with PFA and stained cleaved Caspase 3. **b** Statistical quantification of (**a**) to show the apoptosis after TRIM44 knockdown in U251MG and U87MG. **c** Cell apoptosis analysis by Annexin V/PI staining and quantitative analysis. Cell for FACS analysis were collected when the cell appears to morphologic changes. **d** Statistical quantification of apoptotic cells both in Q2 and Q3. The value is the sum of Q2 and Q3. All the value is presented as the mean ± SEM (**P < 0.01; ***P < 0.001, *t* test). Scale bars: 30 um

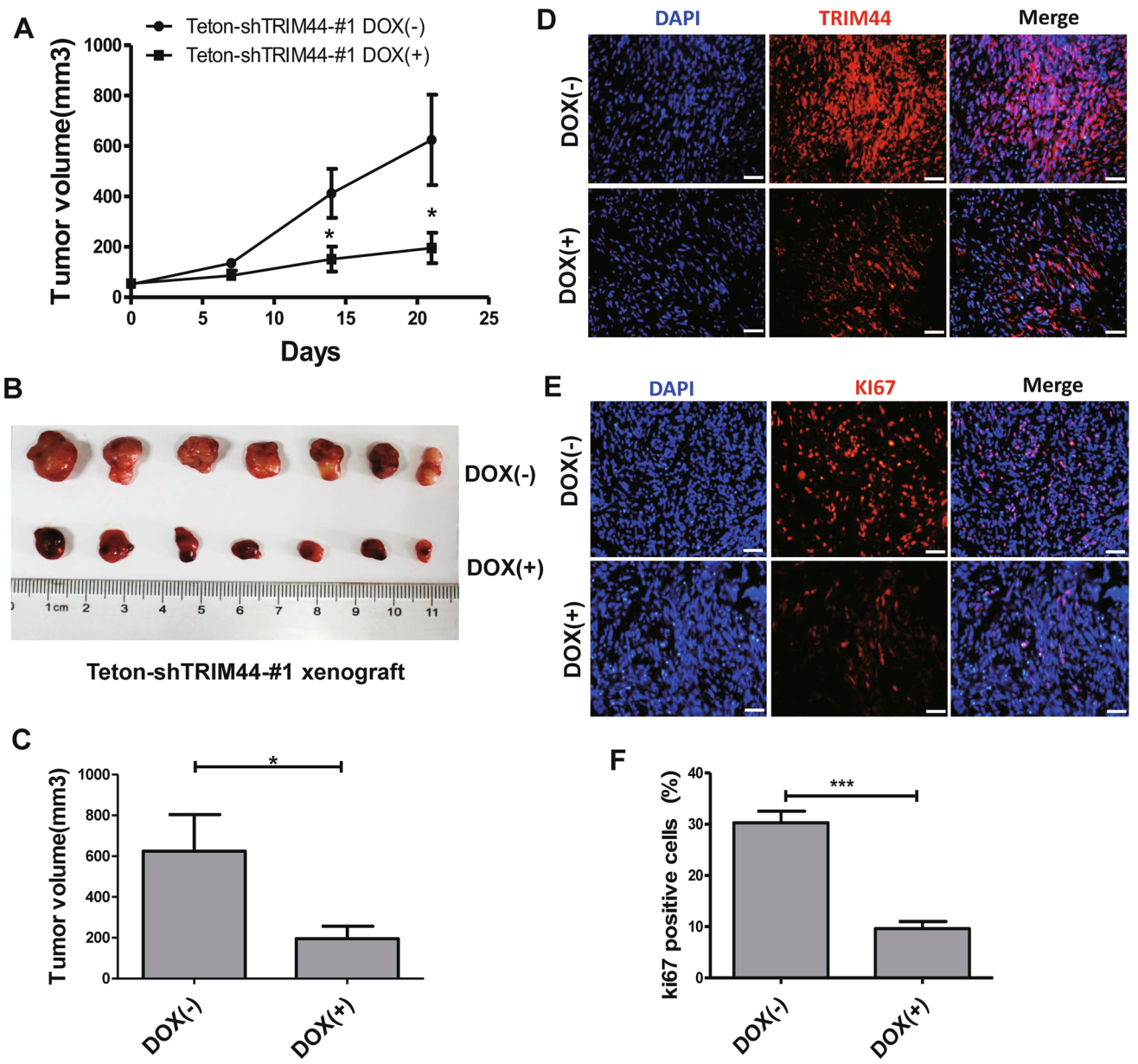


Fig. 4 TRIM44 knockdown inhibits glioma cell proliferation in vivo. **a** Growth curve of the tumors formed by U251MG/teton-shTRIM44 cells in BALB/c nude mice. Doxycycline (DOX) or sucrose was added to the mice drinking water for 30 days after the injection of tumor cells. Tumor sizes were then measured every 3 days. **b** Tumors from two groups were dissected at the end of experiments. **c** Quantification of tumor volumes of both control and TRIM44 knockdown groups. **d** TRIM44 knockdown was confirmed by immunofluorescence staining of paraffin-embedded tumor sections. **e** KI67 staining in paraffin-embedded tumor sections. **f** Quantification of KI67 positive cells in both control and TRIM44 knockdown groups. All the value is presented as the mean ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001, *t* test). Scale bars: 30 μm

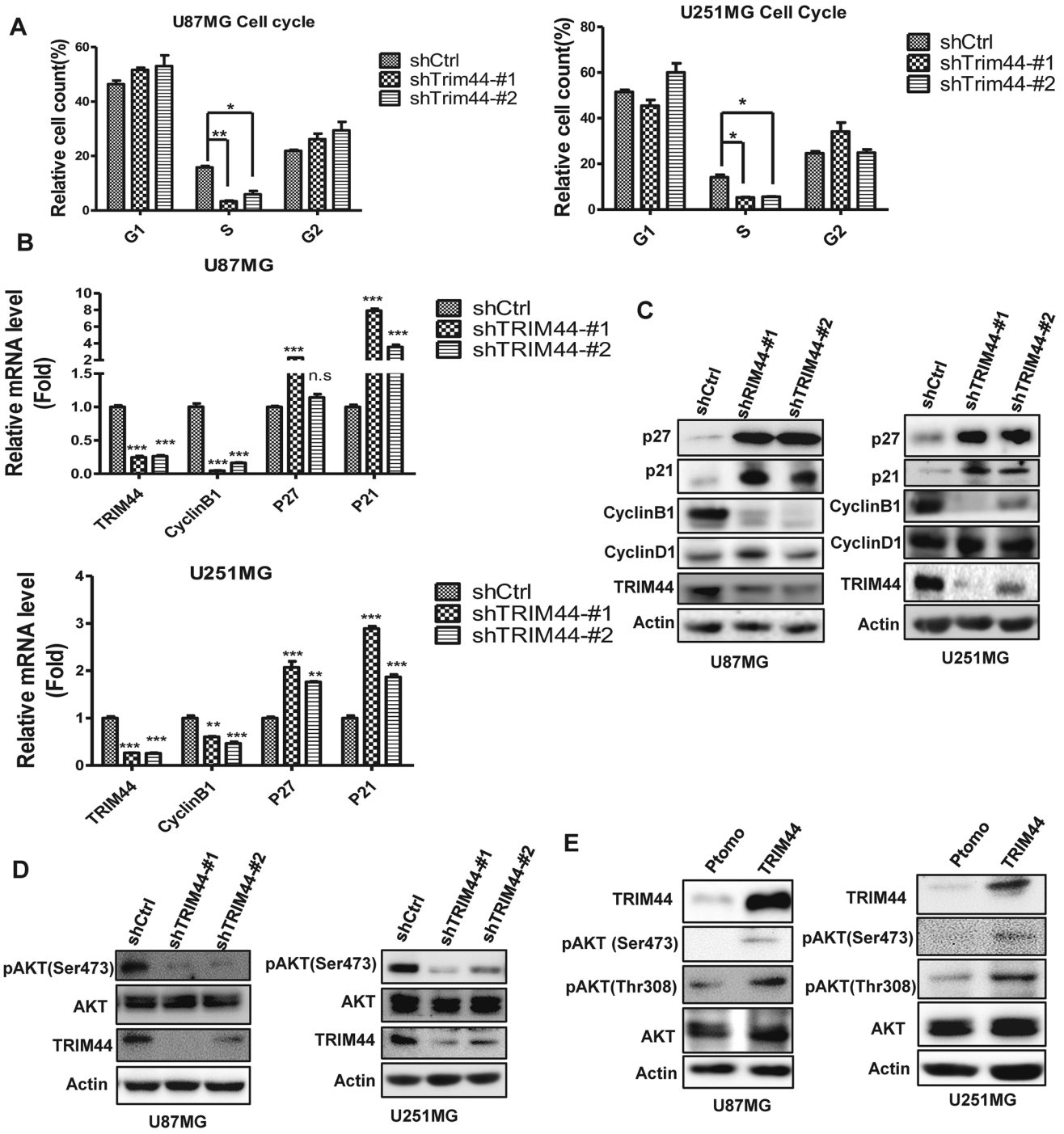


Fig. 5 TRIM44 regulates glioma cell cycle through AKT/p21/p27 pathway. **a** Flow cytometric analysis of cell cycle after TRIM44 knockdown in both U87MG and U251MG cells. Cells were fixed by 70% alcohol overnight at 4℃, followed by 50 μg/mL propidium iodide (PI) staining for 30 min. Cell cycle analysis were performed by flow cytometry using the BD FACSVantage™ SE System. **b** Quantitative PCR analysis of the p21 and p27 after TRIM44 knockdown. **c** Western blot was used to determine the expression of cell-cycle related proteins in the lysates of TRIM44-wild type and TRIM44 silent cells; β-actin was used as a loading control. **d** Total AKT and phosphorylated AKT(Ser473) were monitored after TRIM44 silenced. **e** Total AKT and phosphorylated AKT (Ser473 and Thr308) were monitored after TRIM44 overexpressed in glioma cells

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Author contributions XZ and YY performed most experiments of the work. PM helped in the experiment. NW helped with the lentivirus preparation and in vitro experiments. DY, QT, and BS helped with the paraffin section and immunochemical staining. XZ, TX, ZH and XF designed the experiments and wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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Affiliations

Xia Zhou[1,6] · Yadong Yang[2,3] · Pengcheng Ma[4] · Na Wang[5,7] · Dong Yang[6] · Qiu Tu[1,6] · Bin Sun[6] · Tingxiu Xiang[7] · Xudong Zhao[6,8,9] · Zongliu Hou[1] · Xiangdong Fang[2,3]

1 Key Laboratory of Tumor Immunological Prevention and Treatment of Yunnan Province, Central Laboratory of Yan'an Hospital, Affiliated to Kunming Medical University, 245 East Ren Ming Road, Kunming 650051, Yunnan, China

2 CAS Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, No. 1 Beichen West Road, Chaoyang District, Beijing 100101, China

3 University of Chinese Academy of Sciences, Beijing 100049, China

4 State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China

5 Oncology and Hematology Department, Mianyang Hospital of T.C.M., Mianyang 621000, Sichuan, China

6 Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Sciences and Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, Yunnan, China

7 Chongqing Key Laboratory of Molecular Oncology and Epigenetics, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

8 Kunming Primate Research Center, Chinese Academy of Sciences, Kunming 650223, Yunnan, China

9 KIZ-SU Joint Laboratory of Animal Model and Drug Development, College of Pharmaceutical Sciences, Soochow University, Suzhou 215000, Jiangsu, China