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LABORATORY INVESTIGATION - HUMAN/ANIMAL TISSUE

Overexpression of septin 7 suppresses glioma cell growth

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Abstract Our previous study demonstrated that SEPT7 was downregulated at mRNA level in human gliomas. This study is to further examine the expression of SEPT7 in glioma samples and characterizes its role on cell cycle progression and growth of glioma cells. mRNA and protein expression of SEPT7 were detected by RT-PCR, immunohistochemical staining, and western blot analysis in human glioma specimens and normal brain tissues. A pcDNA3-SEPT7 expression plasmid was constructed and transfected into human glioblastoma cell line U251, and cell proliferation and apoptosis were examined. The growth of established U251 and TJ905 subcutaneous xenograft gliomas was measured in nude mice treated with pcDNA3-SEPT7 and U251 xenograft tumors treated with SEPT7 siRNA. SEPT7 expression is negatively correlated with the increase of glioma grade. Overexpression of SEPT7 is able to inhibit cell proliferation and arrest cell cycle progression in the G0/G1 phase both in vitro and in vivo. Knocking down further the already low endogenous expression of SEPT7 in U251 xenograft tumors with siRNA leads to faster tumor growth compared with control tumors. This study demonstrates that SEPT7 is involved in gliomagenesis and suppresses glioma cell growth.

Zhi-fan Jia and Qiang Huang contribute equally to this work.

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Introduction

Septins, which belong to a family of cytoskeleton proteins with GTPase activity, assemble as intracellular filamentous scaffolds. They play putative roles in cytokinesis, cellular morphogenesis, polarity determination, vesicle trafficking and apoptosis [1–4]. SEPT7 is a member of the septin family which has an open reading frame of 1254 nucleotides encoding 418 amino acids, including a GTP-binding motif, located on chromosome 7p14.4-14.1 [5]. The cDNA sequence of mammalian SEPT7 is highly homologous to CDC10 in yeast. CDC10 is involved in cell cycle progression and the cell division cycle of yeast. In fission yeast cells, CDC10 is a component of transcription complex-MBF (multisubunit factor). Together with Res1p and Res2p, CDC10 regulates transcription of genes required from G1/S phase to S phase. When CDC10 is suppressed, MBF complex becomes constitutively activated, leading to the transcriptional activation of specific genes encoding products for S phase and the cyclin-dependent kinases (CDKs) required for the progression through "Start" [6]. In budding yeast cells, the spindle pole body (SPB), which is equivalent to the centrosome in mammalian cells, duplicates in G1/S boundary in fission yeast cells. When CDC10 is inhibited, the SPB undergoes early duplication and subsequent maturation in the G1 phase [7]. Considering that CDC10 inhibits cell cycle progression, we hypothesize that SEPT7 may play a similar role in glioma cell cycle progression.

SEPT7 is abundantly expressed in the central nervous system (CNS) [8], but its functional role has not been

reported. Our previous study on the gene expression profile of 63 gliomas of different grades demonstrated that SEPT7 gene expression is significantly downregulated in a variety of gliomas [9, 10], In our in-vitro studies using human glioma cell lines we showed that overexpression of SEPT7 in vitro in human TJ905 glioma cells inhibits cell proliferation, arrests the cell cycle in theG0/G1 phase, downregulates the expression of cyclin D1, CDk4, cyclin E, and CDk2, upregulates the expression of CDK inhibitors p16 and p21, and induces cell apoptosis [11]. In addition, we showed that SEPT7 overexpression inhibits the invasion and migration ability of human U251 glioma cells, reverses the imbalanced state of MMPs/TIMPs, downregulates the expression of integrin alpha$_V$beta$_3$ and alters the structure of tubulin-alpha [12].

In this study, we would like to further examine the role of SEPT7 in cell proliferation, cell cycle, apoptosis, and cell cycle-related proteins by overexpressing SEPT7 in U251 glioma cells both in vitro and in vivo.

Materials and methods

Sample collection

Forty-seven human freshly resected glioma samples and four normal brain specimens were collected from the Department of Neurosurgery, Tianjin Medical University General Hospital, Tianjin, China. Tissues and clinical information were obtained as part of an Institute Review Board-approved study at the University. The tissue samples included 19 WHO grade II tumors (eight protoplasmic astrocytomas, nine fibrocystic astrocytomas, and two oligoastrocytomas); 13 WHO grade III gliomas (anaplastic astrocytomas); and 15 WHO grade IV tumors (13 glioblastomas and two medulloblastomas). Four normal brain tissues were removed from the lateral temporal lobe during surgery for intractable temporal epilepsy. Each specimen was divided into two portions: one portion was fixed in 10% formalin for routine histopathological examination and immunohistochemical staining; another portion was frozen for RT-PCR and western blot analysis. All the tumors or normal brain tissues were diagnosed by two independent neuropathologists.

Glioma tissue array

The glioma tissue array was prepared by ChaoYin Biotech Company and was composed of 59 glioma specimens and four samples of normal brain tissues. The glioma specimens included one WHO grade I tumor (pilocytic astrocytoma), 12 WHO grade II tumors (nine protoplasmic astrocytomas, two fibrocystic astrocytomas, and one mixed oligoastrocytoma), 28 WHO grade III gliomas (all of these tumors were anaplastic astrocytomas), and 18 WHO grade IV tumors (17 glioblastomas and one medulloblastoma).

Semi-quantitative RT-PCR analysis

U251-MG cells were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Science. TJ905, TJ899, and TJ862 glioblastoma cell lines were established and characterized in our Laboratory [13, 14]. Total RNAs were extracted from glioma samples, normal tissues, and four glioma cell lines (U251, TJ905, TJ899, TJ862) using TRIzol reagents (Invitrogen, Gaithersburg, MD, USA). Isolated RNA was electrophoresed on 1.0% agarose–formaldehyde gel to detect the quality of the RNA. The first strand cDNA was generated using 1 μg total RNA and SuperScript II Reverse Transcriptase (Invitrogen).

PCR amplification was performed using a Perkin–Elmer DNA thermal cycler (PTC-200). The primers for SEPT7 were 5′-tgctccttcaggacatggact-3′ (forward) and 3′-cacagcaagaggtaaacggtc-5′ (reverse); β-actin was amplified for loading control by primers: 5′-tccctggagaagagctacga-3′ (forward) and 3′-gatccacacggagtacttgc-5′ (reverse). The PCR conditions were 94°C for 30 s, 54°C for 30 s, and 72°C for 60 s, for 30 cycles, and final extension of 5 min. PCR products were analyzed by electrophoresis and densitometry data were used for statistical analysis.

Western blot analysis for detection of SEPT7 and cell-cycle regulators

Total proteins of specimens were extracted and the protein concentration was determined by the Lowry method. Antibodies against SEPT7 (sc-20620), CDK2 (sc-748), CDK4 (sc-749), Cyclin D (sc-718), Cyclin E (sc-20684), p21 (sc-756) and p16 (sc-1661), and β-actin (sc-47778) were purchased from Santa Cruz Biotechnology (San Diego, CA, USA). All the primary antibodies were used at a dilution of 1:100. Specific protein was detected using a Super Signal protein detection kit (Pierce, Rockford, IL, USA). After washing with stripping buffer, the PVDF membrane was re-probed with an anti-β-actin antibody (1:500 dilution). Densitometry data were collected for statistical analysis.

Immunohistochemical staining

Sections were incubated with antibodies (1:100 dilution) and followed by incubation with biotinylated secondary antibodies and ABC-peroxidase reagent. The immunoreactivity was detected by DAB staining. Ten random visual

fields were chosen for counting immunopositive staining cells per 500-1000 cells under a light microscope with the magnification ×400. Cells were counted by two independent observers and the mean value of the count was used for statistical analysis. The expression levels were scored according to the percentage of positive staining cells as follows: 0: no positive cells, 1: positive cells <25%, 2: positive cells between 26 and 50%, 3: positive cells >50%.

SEPT7 plasmid construction and transfection

The pcDNA3-SEPT7 was constructed by cloning SEPT7 fragment from normal human cDNA into pcDNA3 (Invitrogen) between *Hin*dIII and *Bam*HI sites, and the recombinant plasmid was identified by endonuclease digestion and DNA sequencing.

U251 cells (1×10^5) were cultured in 60-mm dishes, and until they were 50–80% confluent the pcDNA3-SEPT7 or pcDNA3 plasmid was separately transfected into the U251 cells mediated by Lipofectamine 2000 (Invitrogen), as previously described [15]. The stably transfected clones were selected by G418, and identified by RT-PCR and western blotting.

Flow-cytometric analysis

Control cells, and pcDNA3 and pcDNA3-SEPT7 transfected cells in the log phase of growth were harvested. Nuclei of cells were stained with propidium iodide. A total of 10,000 nuclei were analyzed in a FACSCalibur flow cytometer (Becton and Dickinson, Franklin Lakes, NJ, USA) and DNA histograms were analyzed using Modifit software (Becton and Dickinson).

Cell proliferation assay

MTT assay was used for evaluation of cell proliferation rate in pcDNA3-SEPT7-transfected, pcDNA3-transfected,

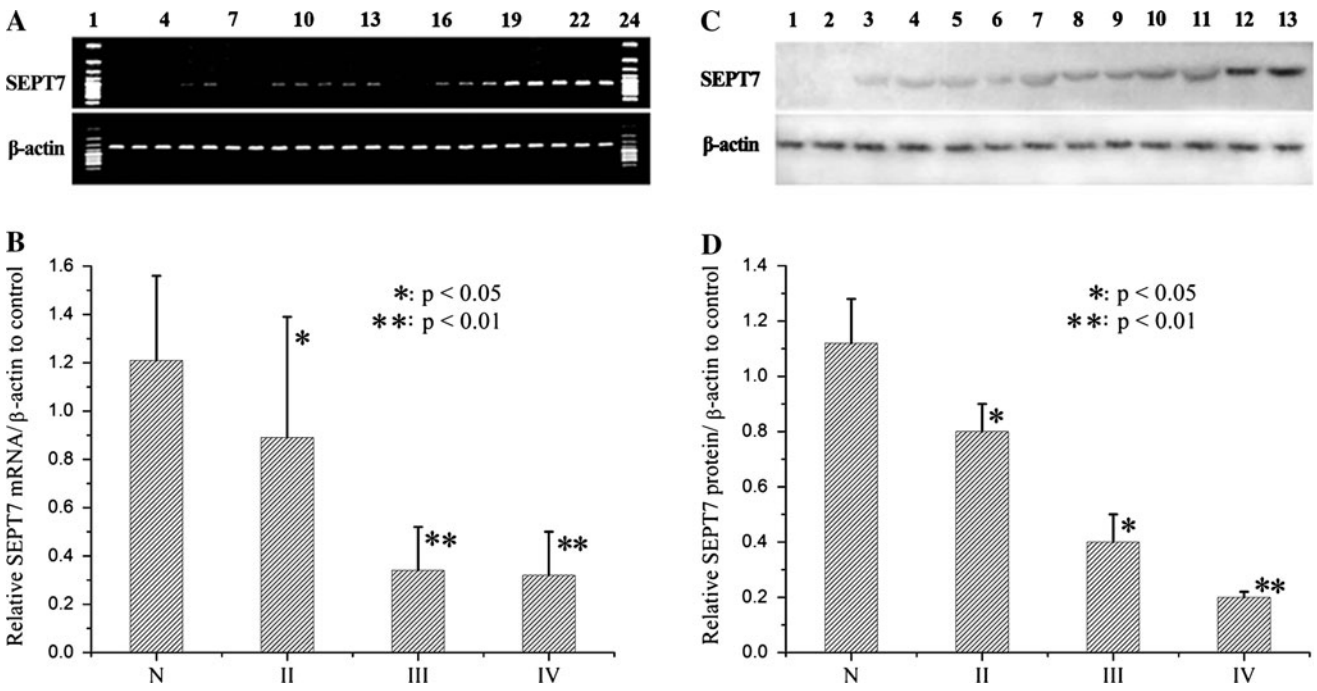


Fig. 1 Analysis of SEPT7 expression in glioma tissues and cell lines. **a**, **b** Expression of SEPT7 mRNA in gliomas and normal brain (*N*) was detected by RT-PCR and data were normalized with *β*-actin. Lanes: 2–6, WHO IV; 7–13, WHO III; 14–21, WHO II; 22 and 23, normal brain tissue; 1 and 24, DNA marker. **c**, **d** Expression of SEPT7 and *β*-actin in gliomas and normal brain tissue was detected by western blotting. Lanes: 1–4, WHO IV; 5–8, WHO III; 9–12, WHO II; 13, normal brain tissue. Data were derived from three independent experiments. *Asterisks* indicate significant difference in SEPT7 expression of gliomas as compared with normal brain tissues. The SEPT7 mRNA expression of WHO grade IV gliomas in lanes 2, 3, 5, and 6 of **a** corresponds to its protein expression in lanes 1–4 of **c**. The SEPT7 mRNA expression of WHO grade III gliomas in lanes 9, 10, 12, and 13 and WHO grade II gliomas in lanes 16–19 of **a** correspond to lanes 5–8 and 9–12 of **c**, respectively. Lane 22 in **a** corresponds to lane 13 of **c** as normal brain tissues. **e**, **f** Immunohistochemical staining of SEPT7 in glioma samples (**e**) and glioma tissue array (**f**) (× 200). **g** Expression of SEPT7 mRNA in glioma cell lines was analyzed by RT-PCR. Lanes: 1, TJ905 cells; 2, TJ899 cells; 3, U251 cells; 4, TJ862 cells; 5, normal brain tissue; 6, DNA marker

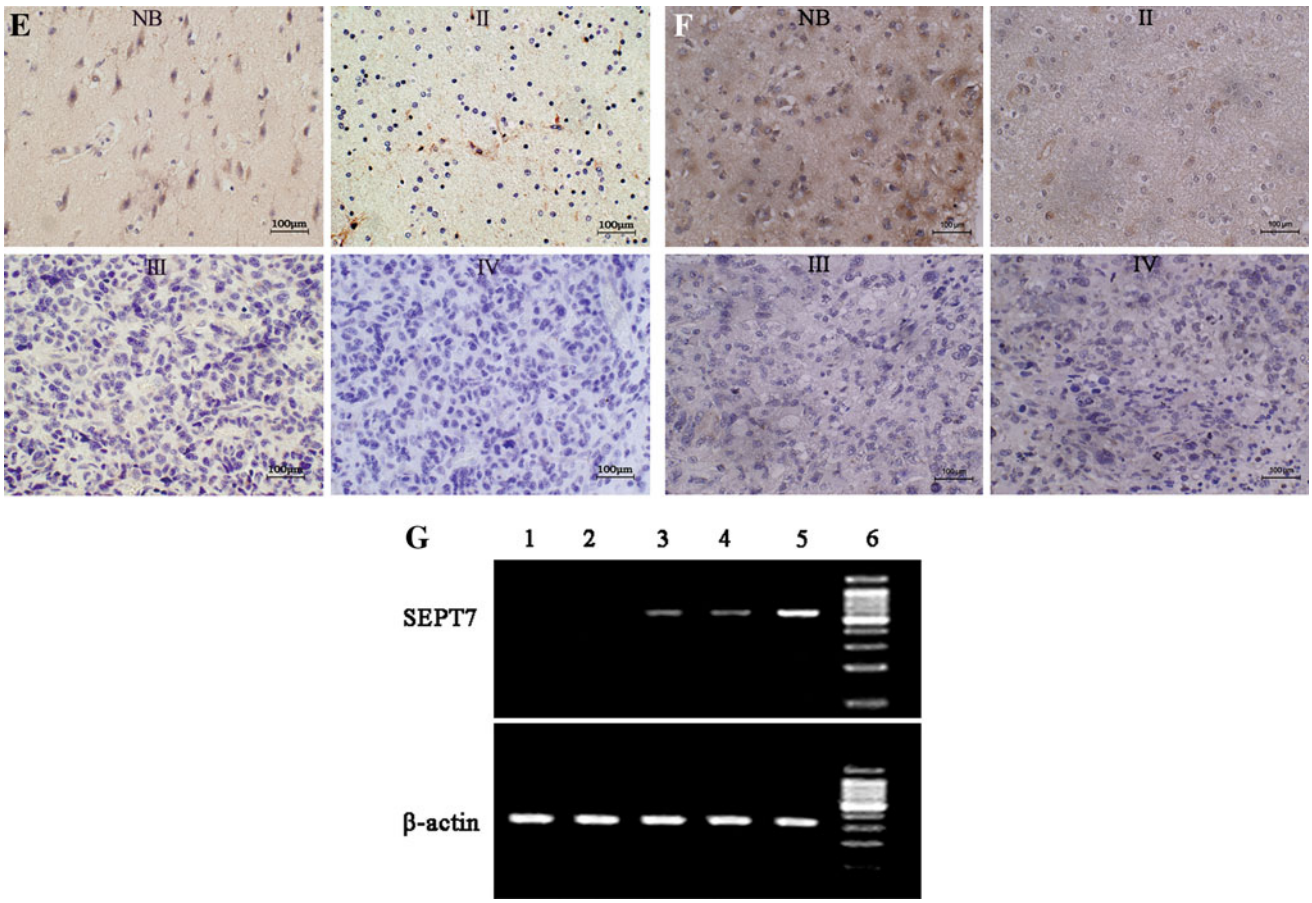


Fig. 1 continued

and parental U251 glioma cells. Briefly, 4×10^3 cells were plated in each well of a 96-well plate. Our designed time points for measurement were: 24, 48, 72, 96, 120, and 144 h. At each time point, 20 µl MTT (5 mg/ml) was added to each well and incubated at 37°C for 4 h, the reaction was stopped by lysing the cells with 200 µl DMSO. Quantification (optical density) was measured at 570 nm. Each group of experiments was repeated three times.

Detection of cell apoptosis

The apoptotic cell death was measured by flow cytometry using an annexinV-PE apoptosis detection kit (Abcam, Cambridge, UK) in parental U251 cells and in cells transfected with pcDNA3 or pcDNA3-SEPT7. Cells were sorted by the FACSCalibur flow cytometer (Becton and Dickinson), and analyzed using CellQuest software (Becton and Dickinson).

Apoptosis of xenograft samples from nude mice was detected by the TUNEL method using an in-situ cell death kit (Roche, Basel, Switzerland) according to supplier's instructions. Apoptotic index (AI) was calculated by two independent investigators who were unaware of the experimental conditions.

Subcutaneous tumor model and gene therapy with SEPT7 and siRNA targeting to SETP7

Six-week-old female immune-deficient nude mice (BALB/c-nu) were purchased from the animal center of the Cancer Institute of the Chinese Academy of Medical Sciences, bred at the facility of laboratory animals, Tianjin Medical University. All the experimental procedures were carried out according to the regulations and internal biosafety and bioethics guidelines of Tianjin Medical University and Tianjin Municipal Science and Technology Commission. The subcutaneous glioma model was established as previously described [16]. When the xenograft tumors grew to approximately 5 mm in diameter, the mice bearing U251 and TJ905 tumors were randomly assigned into three groups: control, pcDNA3, and pcDNA3-SEPT7 groups.

Each group consisted of 10 mice. A mixture of 10 mg plasmid DNA and 10 ml lipofectamine were injected into subcutaneous tumors. Mice in control groups received PBS with lipofectamine. Treatment was given twice more on day 4 and day 7 after the first injection. The tumor volume was measured every three days during the observation period of four weeks, using the formula: Volume = length × width^2/2. All the mice were sacrificed at the end of observation on day 28 and tumors were removed for detecting the expression of SEPT7, CDk4/2, cyclin D1/E, PCNA, MMP2/9, GFAP, BCL-2, and CASPASE-3 by immunohistochemical staining and apoptosis by the TUNEL method.

In addition, the mice bearing U251 tumors with low endogenous expression of SEPT7 were divided into three groups: control group, scramble RNA group, and siRNA targeting SEPT7 group, for observing the effect of SEPT7 silenced further by siRNA duplex. There were 10 mice in each group. In the scramble siRNA group and the SEPT7 siRNA group, lipofectamine and siRNA were injected intratumorally in a multi-site injection manner every four days during an observation period of four weeks. Instead of siRNAs, mice in the control U251 group received PBS injection. The sequences of SEPT7 siRNA, and scramble siRNA used were as follows: SEPT7; sense: CGACUACA UUGAUAGUAAAdTdT; antisense: UUUACUAUCAAU GUAGUCGdTdT; scramble siRNA; sense: UUCUCCGA ACGUGUCACGUTT; antisense: TTAAGAGGCUUGCA CAGUGCA.

Statistical analysis

One-way ANOVA was used. Values are means from three independent western blot, apoptosis, or cell-cycle analysis experiments. Results are presented as mean ± SD, $P < 0.05$ is regarded as significant.

Results

SEPT7 is downregulated in gliomas

Both SEPT7 mRNA and protein expression were significantly downregulated in gliomas detected by semi-quantitative RT-PCR and western blotting as compared to those in normal brain tissues. The downregulation of SEPT7 coincides with the lowered expression of SEPT7 in different grades of gliomas. SEPT7 expression was much lower in high-grade than that in low-grade gliomas (Fig. 1a–d). Immunohistochemical staining demonstrated that positive SEPT7 immunoreactivity were located in the cytoplasm of astrocytic tumor cells. Many more SEPT7 immunopositive cells could be found in low-grade tumors than in-high grade counterparts. SEPT7 gene expression

Table 1 Constituent ratio of SEPT7 expression in gliomas and normal brain tissues

Grade	Samples	Positive rate (%)	Positive ratio				χ^2	P
			−	+	++	+++		
N	4	100	0	0	2	2	25.76	<0.05
II	19	68.42	6	6	4	3		
III	13	53.85	6	6	1	0		
IV	15	46.67	8	7	0	0		

Grades II, III, and IV compared with normal brain tissue, $P < 0.01$

Grade II compared with grades III and IV, $P < 0.01$

Table 2 Constituent ratio of SEPT7 expression in glioma tissue array

Grade	Sample	Positive rate (%)	Positive ratio				χ^2 (%)	P
			−	+	++	+++		
N	4	100	0	0	1	3	35.12	<0.05
II	12	66.67	4	5	1	2		
III	28	35.71	18	9	1	0		
IV	18	38.89	11	6	1	0		

Grades II, III, and IV compared with normal brain tissue, $P < 0.01$

Grade II compared with grades III and IV, $P < 0.01$

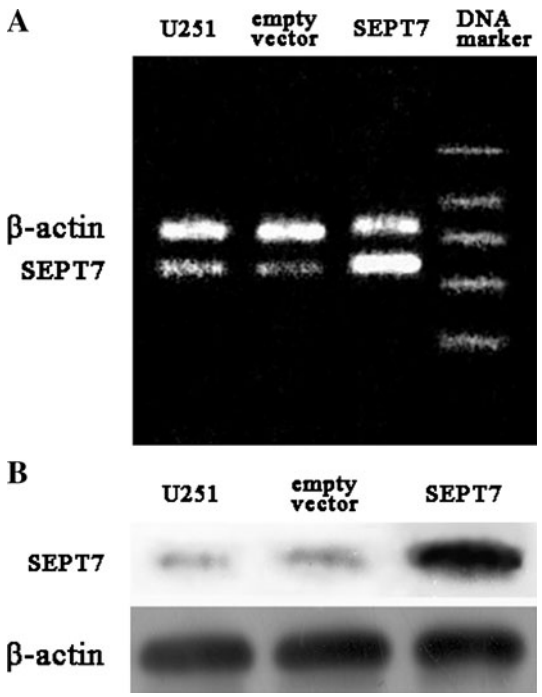
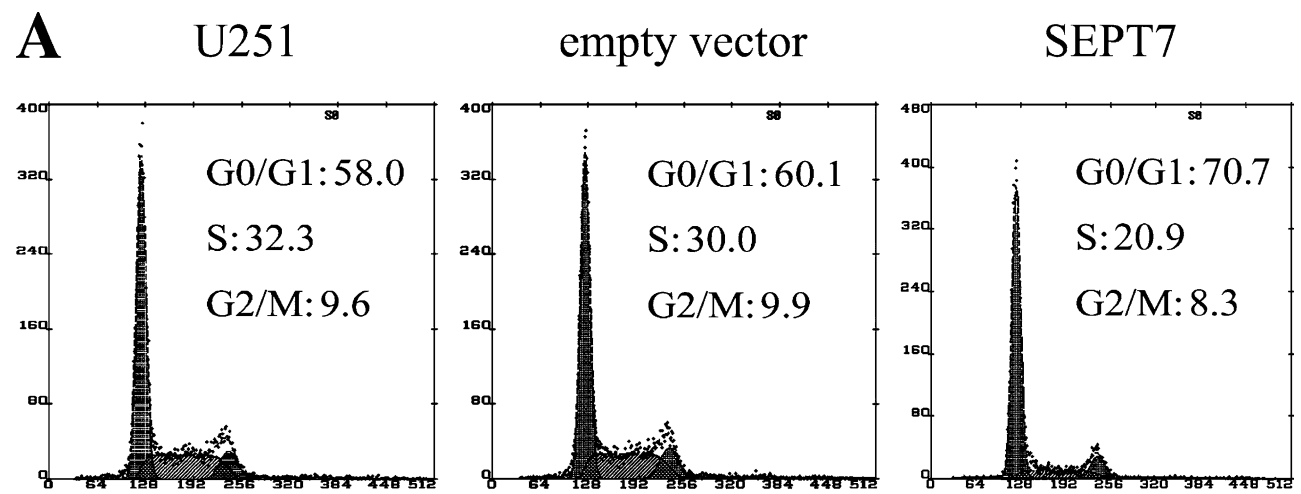
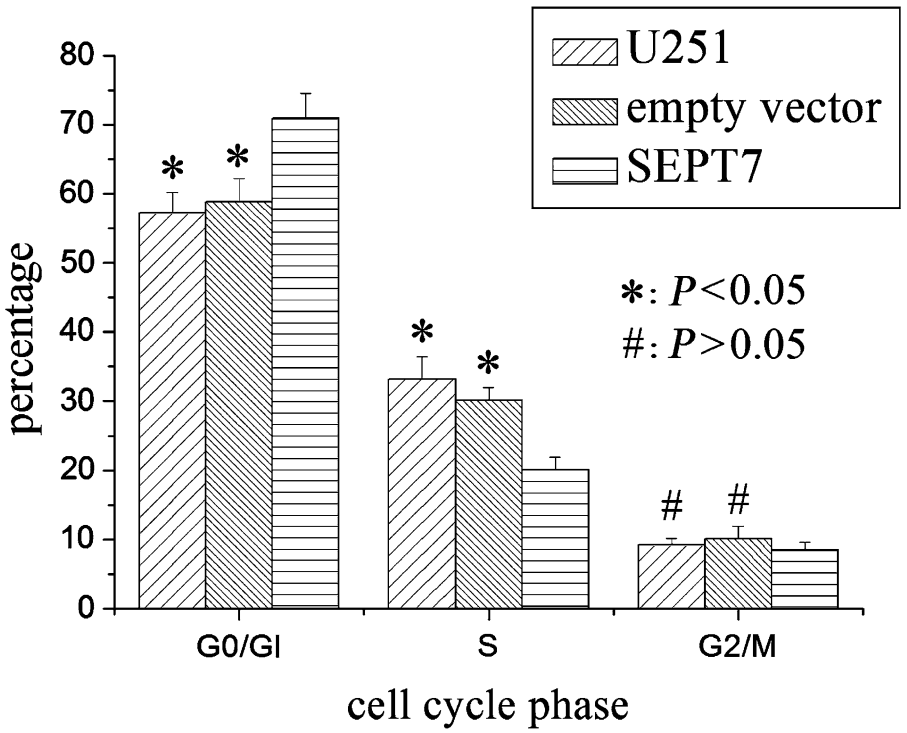


Fig. 2 Analysis of SEPT7 expression in U251 cells after transfection with SEPT7 expression plasmid. RT-PCR (**a**) and western blotting (**b**) of SEPT7 expression in parental U251 cells and cells transfected with pcDNA3 or pcDNA3-SEPT7 expression plasmid





$P<0.05$: is considered as significant

Fig. 3 Effect of SEPT7 overexpression on cell cycle progression and expression of cell-cycle regulators in U251 cells. **a** Effect of SEPT7 overexpression on cell cycle progression of U251 cells as analyzed by flow-cytometric analysis. **b** Effect of SEPT7 overexpression on the expression of cell-cycle regulators including cyclin D1, cyclin E, CDK2, CDK4, p21, and p16 as detected by western blotting. **c** Effect of SEPT7 overexpression on the expression of CDK2, CDK4, cyclin D1, cyclin E, p21, and p16 as detected by immunocytochemical staining (×200)

was correlated negatively with the ascending order of glioma grades. (Fig. 1e and f; Tables 1, 2). RT-PCR analysis showed that SEPT7 expression was absent in two of the four GBM cell lines (TJ905 and TJ899) and had a lower expression level in U251 and TJ862 cells than in the normal control (Fig. 1g).

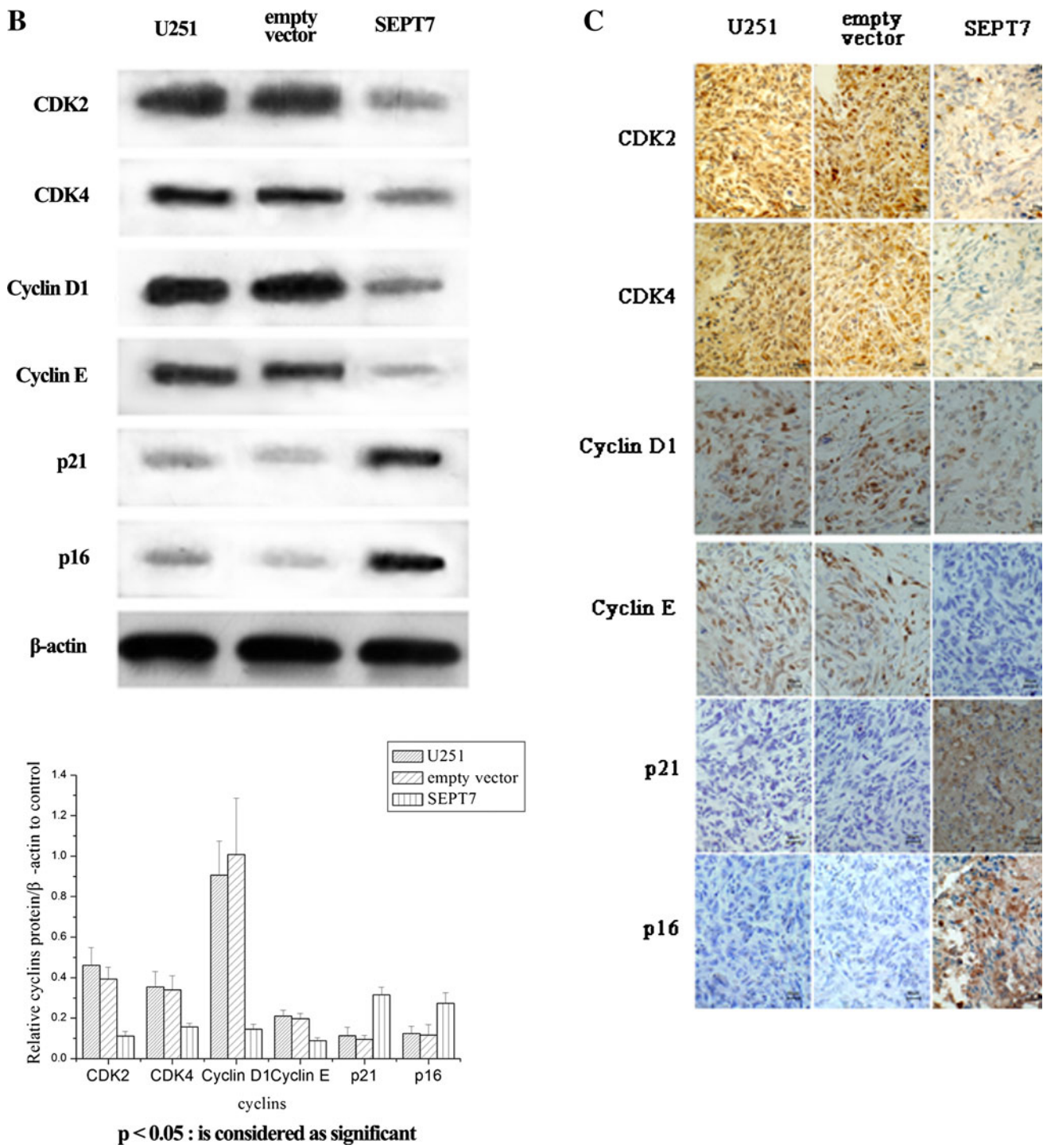


Fig. 3 continued

Construction of pcDNA3-SEPT7 expression plasmid and transfection into U251 cells

The pcDNA3-SEPT7 expression plasmid was constructed and identified by endonuclease digestion and DNA sequencing. RT-PCR and western blotting showed that the expression of SEPT7 mRNA and protein were significantly upregulated in U251 clones stably transfected with pcDNA3-SEPT7 (Fig. 2a, b).

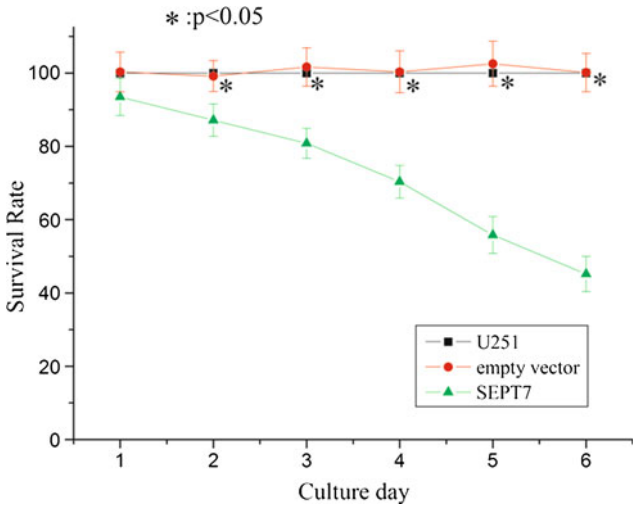


Fig. 4 Effect of SEPT7 overexpression on proliferation of U251 cells as detected by MTT assay. *Black line*: parental cells. *Green line*: cells transfected with SEPT7. *Red line*: cells transfected with pcDNA3

Overexpression of SEPT7 arrests cell cycle in the G0/G1 phase

Although it has been reported that cdc10 (homolog of SEPT7) plays a crucial role in switching of the G1/S phase in yeast [17], the role of SEPT7 on cell cycle progression in glioma cells is still not clear. In the current study, the cell cycle kinetics were examined by flow cytometry in U251 cells transfected with pcDNA3-SEPT7 expression plasmid. Overexpression of SEPT7 resulted in less S phase fraction (SPF) in the total cell population and more cells were arrested in the G0/G1 phase (Fig. 3a). This observation indicated that SEPT7 could delay cell cycle progression in glioma cells, similar to CDC10, that can arrest cell cycle in the G0/G1 phase in yeast [17]. Western blotting was used to detect the crucial cell-cycle regulators involved in the G0/G1 phase, including cyclins, CDKs, and CDK inhibitors. It was demonstrated that the expression of positive regulators driving the G0/G1 phase, for example cyclin D1, cyclin E, CDK4, and CDK2, were downregulated in cells transfected with SEPT7, whereas expression of negative regulators, p21 and p16, were upregulated (Fig. 3b). SEPT7 also arrested the cell cycle at the G0/G1 phase, downregulated CDK2/4 and cyclin D1/E, and upregulated p21 and p16 in TJ905 cells [11].

A similar result was obtained from the tumor specimens in vivo using immunohistochemical staining, showing that SEPT7-overexpressing U251 cells grown in vivo had more intensive staining of p21 and p16 and faint staining of CDK2/4, cyclin D1, and cyclin E than SEPT7 immunonegative cells (Fig. 3c). These results demonstrate that SEPT7 reduces the expression of positive regulators and increases the expression of negative regulators of cell cycle progression.

SEPT7 inhibits glioma cell proliferation

MTT assay showed that U251 cells transfected with pcDNA3-SEPT7 proliferated at a significantly lower rate than control cells. The maximum inhibition (approximately 50%) occurred at the end of observation on day 6 (Fig. 4). A similar result was obtained from TJ905 cells transfected with pcDNA3-SEPT7 [11].

The effect of SEPT7 on tumor cell growth was further studied in an animal model. Tumor volumes of the TJ905 xenograft model in the control, pcDNA3, and pcDNA3-SEPT7 treated groups were 4182 ± 388.8, 3792.75 ± 1035.46, and 667.72 ± 404.95 mm^3, respectively. Meanwhile, tumor volumes of the U251 xenograft model in the corresponding groups were 9850.1 ± 3143.67, 9083.2 ± 3109, and 2911.4 ± 938.11 mm^3, respectively (Fig. 5a, b). These results indicate the significant inhibitory effect of SEPT7 expression on glioma tumor growth.

For further study of the inhibitory effect of SEPT7 on glioma growth in vivo, we extended our observation by knocking down the expression of SEPT7 further in U251 cells with chemically synthesized siRNA duplex which knocks down the expression of SEPT7 in U251 cells effectively (Fig. 5c). It was found that the U251 xenograft gliomas treated with SEPT7 siRNA grew faster than those treated with PBS or scramble siRNA, although there was no statistical significance between them (Fig. 5d, e). It is possible that further blockade of the lowered expression of SEPT7 existing in U251 cells was not enough to efficiently alter the phenotype of cells with significantly reduced SEPT7 expression.

In both TJ905 and U251 xenograft tumors, PCNA was downregulated while GFAP was upregulated (Fig. 7). These results also suggest that exogenous SEPT7 can inhibit the proliferation activity of gliomas and reverse the phenotype of malignant glioma cells toward differentiation.

SEPT7 induces cell apoptosis

Resistance to apoptosis is a key event for the survival of tumor cells. The apoptosis index of U251 cells transfected with pcDNA3-SEPT7 was much higher than that of U251 cells transfected with pcDNA3 and that of control cells (Fig. 6). SEPT7 also induced apoptosis in TJ905 cells [11]. The number of apoptotic cells in xenograft tumors treated with pcDNA3-SEPT7 was also markedly increased as shown by the TUNEL method (Fig. 7). Meanwhile, the anti-apoptotic Bcl-2 protein expression was downregulated and the effector of apoptosis, caspase-3 was upregulated

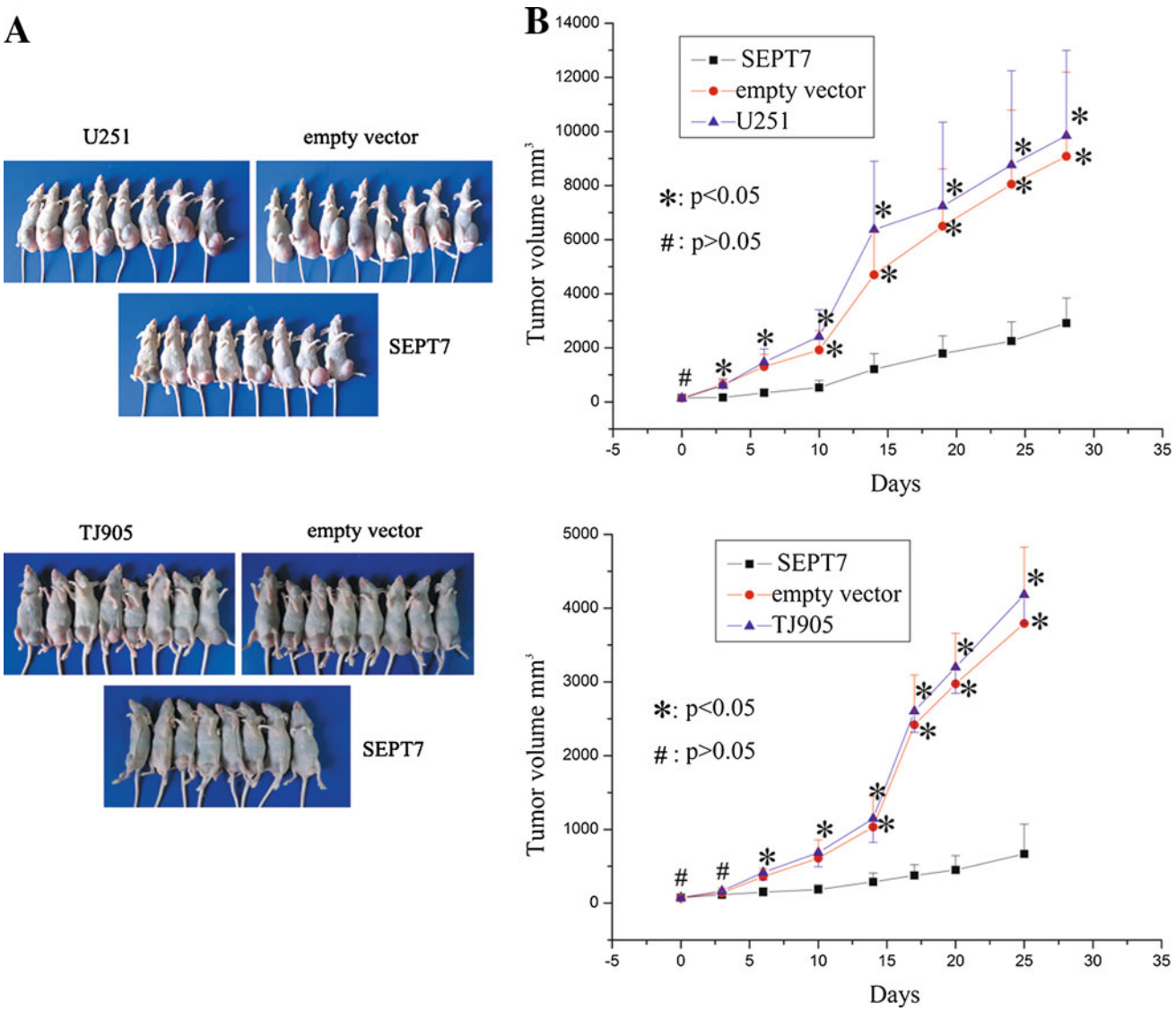


Fig. 5 Effect of SEPT7 on xenograft tumors from U251 cells and TJ905 cells. The tumor volumes are represented in the graphs. *Blue line*, control group; *Red line*, pcDNA3 group; *Black line*, SEPT7 group. (**a**, **b**) Growth of xenograft tumors from TJ905 or U251 cells after treatment with SEPT7. (**c**) Expression of SEPT7 in U251 cells was knocked down after treatment with SEPT7 siRNA detected by Western blotting, (**d**, **e**) Growth of xenograft tumors derived from U251 gliomas cells after treatment with SEPT7 siRNA

(Fig. 7). These data suggest that overexpressing SEPT7 in glioma cells promotes cells apoptosis.

Discussion

Septins have been identified in all eukaryocytes. Although yeast septins are better understood, the function of mammalian septins remains largely undefined. In the past several years, septins have been considered to be involved in oncogenesis [8]. It has been demonstrated that SEPT9 is amplified and overexpressed in diverse types of tumors and promotes cell proliferation, soft agar clonal survival, and vascularization in prostate cancer [18, 19]. The SEPT4 isoform (also named ARTS), being absent in most cases of childhood ALL (acute lymphoblastic leukemia), can promote apoptosis in leukemic (HL-60 and K562) and lung cancer cells (A549) by inhibition of XIAP [20, 21]. SEPT2 (previously known as Nedd5) has been reported to be expressed in various human brain tumor cell lines and astrocytomas, and the maximum expression level is observed in the G2/M phase [22]. Overall, septins exert their effects on cell division, proliferation, and apoptosis, all of which may be involved in tumorigenesis.

The relationship between SEPT7 and gliomagenesis has not been extensively investigated. Limited studies on

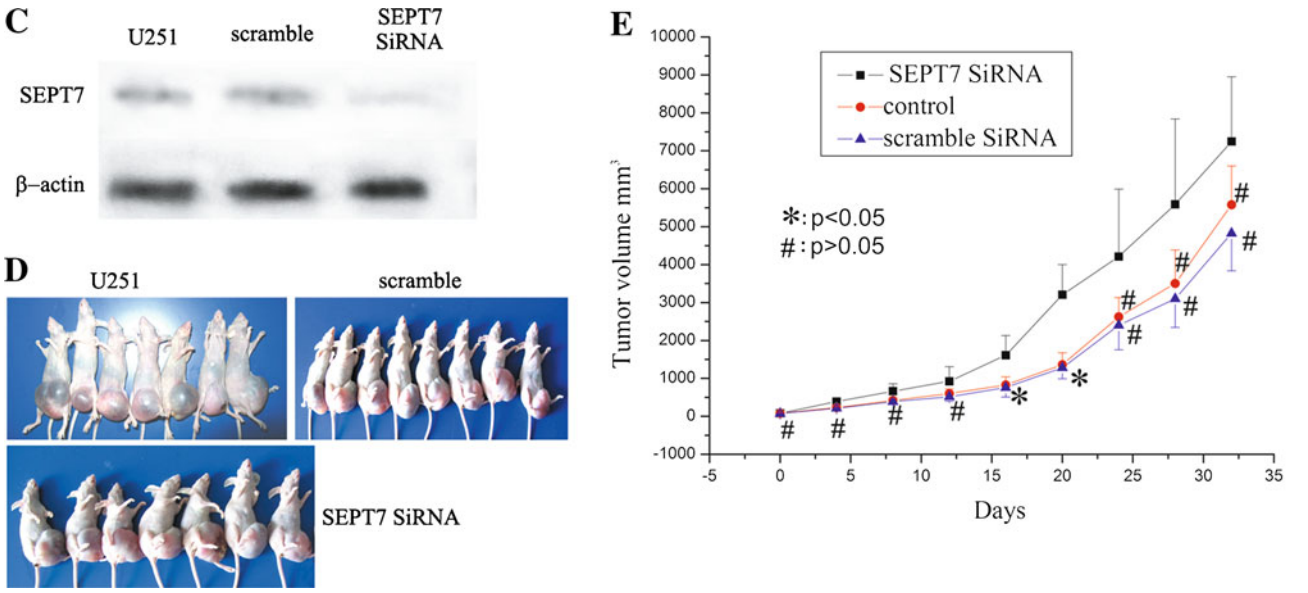


Fig. 5 continued

SEPT7 expression in brain tumors are exclusively carried out by cDNA microarray, and show that SEPT7 is much less expressed in brain tumors than in normal brain tissues [8, 12]. Another study demonstrates that neuroblastoma patients with higher SEPT7 mRNA expression might have better prognosis [23]. There has been no reported study on SEPT7 protein expression in brain tumors or its functional role in gliomagenesis. In the current study, we investigated SEPT7 expression at both the RNA and protein levels using a larger number of human glioma tissue samples. Data from RT-PCR and Western blot analysis suggest that SEPT7 expression is generally downregulated in gliomas or even absent in some high-grade tumors.

Because CDC10, a homolog of SEPT7 in yeast, plays a key role in the switching of the G1/S phase [24], we hypothesize SEPT7 may have a similar role in cell cycle progression, although there is no direct evidence to validate such a hypothesis. However, this study provides convincing evidence that SEPT7 suppresses glioma cell growth both in vitro and in vivo and induces cell cycle arrest at G0/G1 phase. By contrast, U251 xenograft gliomas treated with siRNA targeting SEPT7 grow more quickly. As shown by our results, cyclinD1/CDK4 and cyclin E/CDK2 driving the G1/S transition in early and late G1 stage [25], respectively, are downregulated by overexpression of SEPT7, whereas p16 and p21, the CDK inhibitors suppressing the activity of cyclinD1/CDK4 and cyclin E/CDK2 complex [26], are upregulated. Thus, the molecular mechanism of SEPT7 suppression of cell proliferation in gliomas is likely to be its effect on modulation of the expression and activity of positive and negative cell-cycle regulators. Recently, a novel link between mammalian septins and SOCS7/NCK, which plays a role in the DNA damage checkpoint response, has been reported. SOCS7, as a nucleo-cytoplasmic shuttling protein binds, to septin 2/6/7 complex and is required for septins in the regulation of NCK, and nuclear accumulation of NCK is necessary for DNA damage-induced cell cycle arrest through interactions with the DNA damage pathway integrated by ATM/ATR. This finding may also imply that SEPT7 is involved in the cell cycle modulation, and should be investigated further [27].

Forced overexpression of SEPT7 may induce apoptosis through downregulation of Bcl-2 and upregulation of caspase-3, and increased cell apoptosis also contributes to the inhibitory effect of SEPT7 on glioma cell growth. But the mechanism of induction of apoptosis is complex and involves many signaling pathways. Although the expression of SEPT7 and caspase 3 is elevated, the apoptosis rate ($\approx$15%) is not increased high enough to show numerous apoptotic cells in tumor specimens. Therefore, we believe that the inhibitory effect on cell proliferation may be a more important factor in the suppression of glioma cell growth by SEPT7. Moreover, the upregulation of GFAP in TJ905 and U251 xenograft tumors treated with SEPT7 implicates that SEPT7 is capable of reversing the phenotypes of malignant gliomas toward differentiation [16].

In summary, this study demonstrates the SEPT7 is involved in malignant glioma cell growth. However, more studies should be carried out for better understanding of the signaling cascade of SEPT7, the interplay between SEPT7 signaling and other transduction pathways, and the molecular mechanism for the modulation of SEPT7 expression.

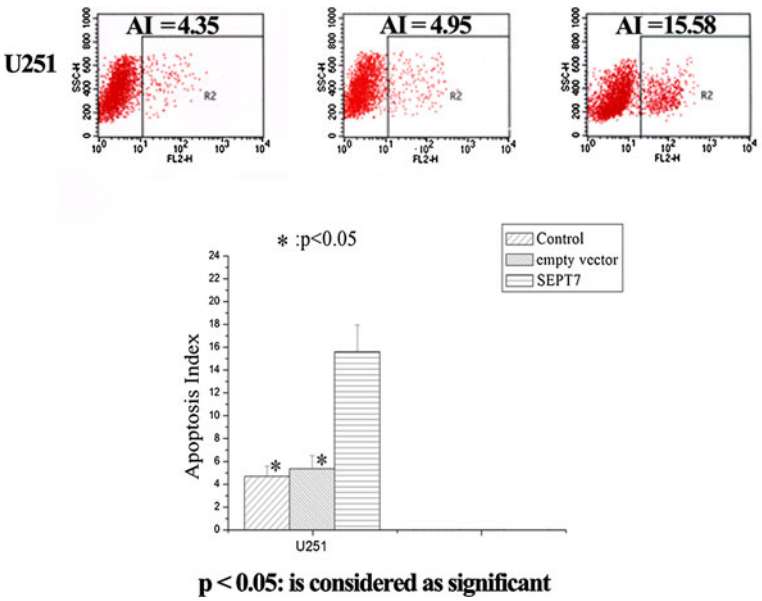


Fig. 6 Effect of SEPT7 overexpression on apoptosis of U251 cells. Data are mean values from three independent experiments

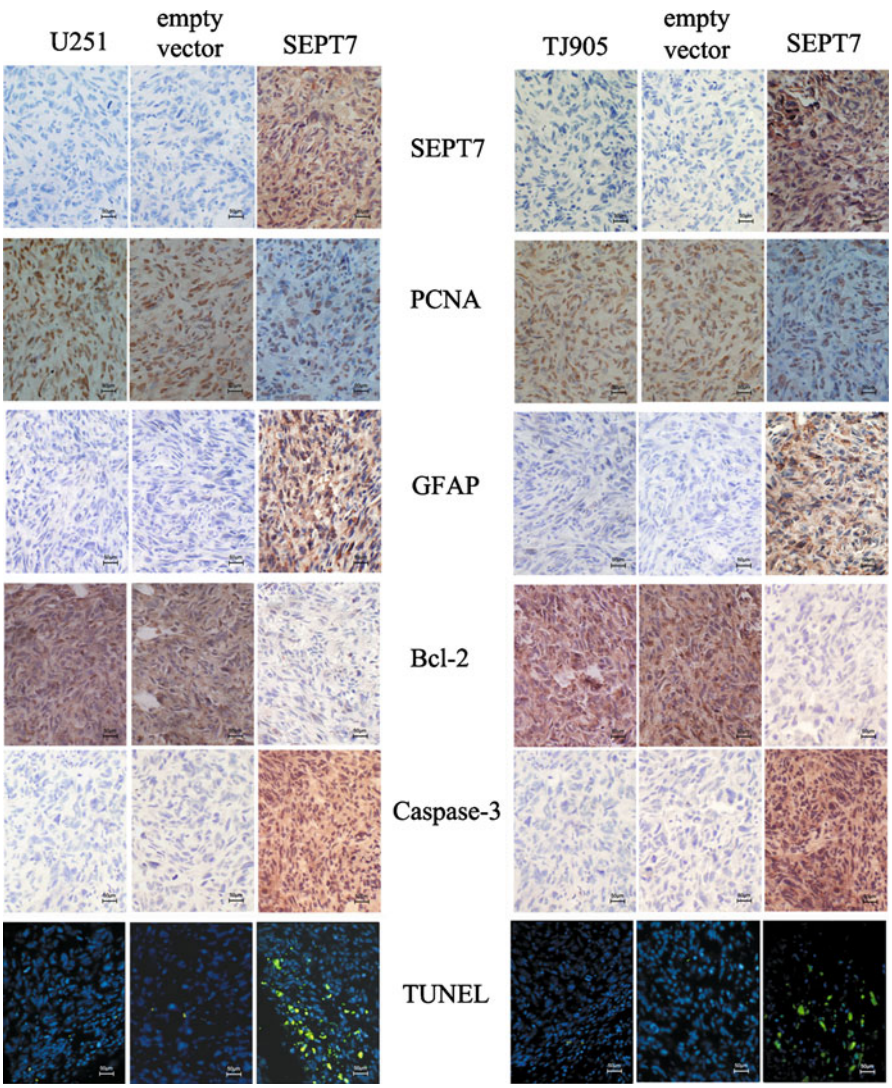


Fig. 7 Immunohistochemical staining of SEPT7, PCNA, GFAP, Bcl-2, and caspase-3, and TUNEL staining in xenograft tumors from U251 cells and TJ905 cells treated with SEPT7 (×200)

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