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Transcription factor TCF3 controls the cell proliferation and migration in glioblastoma multiforme cell lines

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Abstract: TCF3 is a member of the TCF/LEF transcription factor family. Recent studies have demonstrated its potential carcinogenic properties. Here we showed that *TCF3* was upregulated in glioma tissues compared to normal brain tissues. This upregulation of *TCF3* gene probably has functional significance in brain tumor progression. Our studies on glioblastoma multiforme cell lines showed that knock-down of TCF3 induced apoptosis, and inhibited cell migration. Further analysis revealed that down-regulation of TCF3 gene expression inhibits AKT and ERK1/2 activation, suggesting that carcinogenic properties of TCF3 in glioblastoma multiforme (GBM) are partially mediated by phosphatidylinositol 3-kinase/AKT and MAPK/ERK signaling pathways. Considering together, the results of the present study demonstrated that high levels of *TCF3* in gliomas might potentially promote glioma development through AKT and ERK pathways.

Key words: Glioblastoma multiforme, Transcription factor 3, Proliferation, Invasion, and Migration.

Introduction

Gliomas are the most common primary brain tumors in adults. Based on their histological features, the World Health Organization (WHO) has classified gliomas into four grades: grade I (pilocytic astrocytoma), grade II (diffuse astrocytoma), grade III (anaplastic astrocytoma) and grade IV (glioblastoma multiforme) (Louis et al. 2007). Among these brain tumors, Glioblastoma multiforme (GBM) is the most frequent and malignant type in adults. The clinical hallmarks of GBM are its aggressive proliferation and inexorable recurrence despite of multimodal therapies. Current standard-of-care therapy is surgery followed by radiation and chemotherapy (temozolomide). Unfortunately, the median survival rate of GBM patients is only 12–15 months (Dunn et al. 2012). Thus novel therapies are urgently needed.

The poor prognosis of GBM is mainly due to its rapid proliferation and invasion. The mechanism behind these traits is still not clear. Since neural stem cells (NSC) share some features with GBM cells (Hadjipanayis and Van Meir 2009), we hypothesized that NSC-associated transcription factors may affect the proliferation and/or differentiation of GBM cells. Previously we have identified 87 transcription factors that are specifically expressed in neural stem cells (Fu et al. 2009). Among them, TCF3, a member of the Wnt pathway-associated TCF/LEF transcription factor family (Arce et al. 2006), was found to have high expression levels in glioma in this study. Previous works have demonstrated that TCF3 is important for stem cells. During the embryonic development, TCF3 regulates the identity and function of epidermal and embryonic stem cells (Merrill et al. 2004). In mouse embryonic stem (ES) cells, TCF3 competes with Oct4, Sox2 and Nanog for promotor binding

(Cole et al. 2008) and represses ES-cell self-renewal (Tam et al. 2008). TCF3 is an important regulator for stem cell self-renewal or differentiation, depending on cellular context. Thus, we set to explore its function in GBM.

To our knowledge, there is no report about the function of TCF3 on GBM yet. Our studies revealed that malignant gliomas express *TCF3* at elevated levels compared to normal brain tissues. TCF3 may be a tumor promoter in GBM with multiple tumorigenic effects, possibly via AKT and ERK1/2 pathways.

Materials and Methods

Human tissue samples

All human normal brain and glioma tissues from patients were collected in the Department of Neurosurgery, Renmin Hospital of Wuhan University from year 2011 to 2014. All tissues were stored in a liquid nitrogen tank. Eight glioma samples were used in this study, including 3 diffuse astrocytomas (grade II), 3 anaplastic astrocytomas (grade III) and 2 glioblastoma multiforme (grade IV). Three normal brain samples were collected from patients with cerebral trauma. Prior informed written consent was obtained from all patients and the study was approved by the Institutional Research Board of Renmin Hospital.

Cell lines and reagents

Cell lines U251, A172 and HEK-293T cells were kindly provided by Prof. Y. Zhou (Wuhan University, Wuhan, China). These cells were cultured in Dulbecco's modified Eagle's medium (HyClone), supplemented with 10% fetal bovine serum (HyClone) in an incubator containing 5% CO_2 at 37°C.

Lentivirus packaging

RNAi stem-loop DNA oligos containing the sequences targeting TCF3 were designed (Forward oligos: 5'-CCGGGCACCTACCTGCAGATGAAATCTCGAGATTTCA TCTGCAGGTAGGTGCTTT TTG-3', Reverse oligos: 5'-AATTCAAAAAGCACCTACCTGCAGATGAAATCTCGAGATTTCATC TGCAGGTAGGTGC-3') and chemically synthesized, annealed and cloned into the *Age*I/*Eco*RI-digested lentiviral expression plasmid PLKO.1 ZSGreen. This plasmid contains a U6 promoter to express the shRNA and the green fluorescent protein (GFP). A non-silencing sequence (Forward oligo:

5'-CCGGCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGGTTTTTG-3', Reverse oligos: 5'-AATTCAAAAACCTAAGGTTAAGTCGCCCTCGCTCGAGCGAAGGGCGA CTTAACCTTAGG-3') was used as a negative control (NC). Recombinant lentiviruses were produced by transfecting 293T cells with 10μg recombinant PLKO.1 ZSGreen plasmid and 10μg packaging components (5μg PMD2G plasmid and 5μg psPAX2 plasmid) using 20μl of Neofect™ DNA transfection reagent (Neofect tech, Beijing, China) according to the manufacturer's instructions. After 48h, the viral supernatant was harvested and passed through 0.45μm filters, then stored at -80 ℃.

Transduction of glioblastoma cell lines

U251 and A172 cells were seeded at a density of 1 x 10^5 cells/mL on 6-well plates in DMEM medium supplemented with 10% FBS. After 18–20 h incubation, the medium was replaced with a mixture of 0.5 ml viral supernatant and 0.5 ml DMEM culture medium containing 5μg polybrene. After 12h of infection at 37 °C, the medium was replaced by fresh DMEM. At the time point of 48h post-infection, the infection efficiency was evaluated by the expression of GFP.

RNA extraction and quantification assay

Total RNA from tissues or cell lines was extracted with TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), and was reverse-transcribed with RevertAid First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). Real-time PCR was performed with All-in-One™ qPCR mix (GeneCopoeia, Guangzhou, China) on an iCycler thermal cycler (Bio-Rad CFX96). Primer sequences for TCF3 were 5'-CAACGAGTCGGAGAACCAGAG-3' (forward) and 5'-GGTCCGTTGGAGAGGTACG-3' (reverse). The PCR program consisted of 40 cycles (10s at 95 °C, 30s at 60 °C and 30s at 72 °C). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was

used as an endogenous control for the data analysis (GAPDH Forward primer: 5′ -GGGAGCCAAAAGGGTCATCA3′ ; Reverse primer：5′ -TGATGGCATGGACTGTGGTC-3′). The $2^{-\Delta Ct}$ or $2^{-\Delta\Delta Ct}$ methods were used to calculate the difference in the expression of *TCF3* between the experimental group and the control group (n=3, means±SEM).

CCK-8 assay

U251 and A172 cells were seeded at a density of $6x10^3$ cells per well on 96-well plates and treated with viral supernatant 24h later. At 24h, 48h and 72h post-infection, cells in 96-well plates were incubated with 10 μl of CCK-8 solution (Zoman, Beijing, China) for 1.5 h at 37°C. The absorbance was detected at a wavelength of 450 nm using a microplate reader (BioTek, EXL800, Vermont, USA).

Immunofluorescence

Round glass slides were placed on the bottom of the wells of a 24-well plate and coated with gelatin. U251 or A172 cells were seeded at a density of $1 x10^4$ cells per well. 48h after TCF3 shRNA or negative control shRNA transfection, slides were initially incubated with primary antibody against ki67 (diluted 1:200, eBioscience Inc., San Diego, CA, USA) in a humidified chamber at 4°C overnight. Goat anti-mouse secondary antibody (labeled with FITC, 1:500 dilutions, Life technologies, Carlsbad, CA, SA) was added and incubated for 1h. Slides were observed under a fluorescence microscope (Nikon) and images were captured with the NIS-Elements BR 4.20.00 software.

Cell cycle assay

48h after infection, U251 and A172 cells were harvested in ice-cold phosphate based saline (PBS) and then fixed with 75% ice-cold ethanol for an additional 24 h at 4˚C. The fixed cells were incubated with propidium Iodide (PI) for 30 min at 4˚C in the dark and then the DNA content was analyzed by a

Beckman Coulter system (EPICS ALTRA II, Fullerton, USA).

Wound healing assay

Infected U251 and A172 cells were seeded at a density of $2x10^5$ cells per well on 6-well plates. At 80% confluence the cells were scratched with a 10µl plastic pipette tip to form a straight wound and cultured for an additional 24 h. The wound closure was measured under a microscope equipped with a camera. Images of 3 random fields were captured at the time of 0, 12 and 24 h after wounding.

Cell migration and invasion assays

For the cell migration and cell invasion assay, Transwell system and 24-well plates were used. Transwell study included both Transwell migration assay and Transwell invasion assay. For the Transwell migration assay, 48h after the infection, U251 or A172 cells were trypsinized and suspended in 100µl of serum-free medium and transferred to the upper chamber ($5x10^4$ cells each insert)(BD Biosciences, San Jose, CA, USA). For the Transwell invasion assay, $5x10^4$ cells were placed on the upper chamber of each insert. The upper chamber was coated with 50µl matrigel (BD Bioscience, San Jose, CA, USA), which was diluted to the concentration of 1 µg/µl with DMEM. Medium supplemented with 20% FBS (600µl) was added to the lower chamber. After a period of incubation (24 h for the migration assay; 48 h for the invasion assay), cells in the upper surface of the membrane were removed with a cotton tip and cells passed through the filter attached to the lower surface were fixed with 4% paraformaldehyde for 30 min. Cells in the lower surface were stained with 0.1% crystal violet (30 min) counted under the microscope (five random fields of view at 10x magnification).

Protein extraction and western blotting

Forty-eight hours after infection, cellular proteins were extracted with RIPA lysis buffer (Beyotime,

ShangHai, China) supplemented with protease inhibitors. The cell lysates were kept on the ice for 10 min, then sonicated for 15s. After centrifugation (13,000×g) for 10 min, the supernatants were collected. For blotting, the supernatant was mixed with 5×loading buffer (250 nM Tris–HCl (PH 6.8), 10% SDS, 0.5% BPB, 50% glycerol, 5% β-mercaptoethanol) and boiled for 10 min. Total protein was separated on 10% SDS–PAGE gels and then transferred to PVDF membranes. The primary antibodies were used as follows: TCF-3 (Cat No. sc-166411, 1:1000; Santa Cruze, CA, USA), Caspase-3 (#9662, 1:1000; Cell Signaling Technology, USA), Cleaved caspase-3 (Cat. #AF7022, 1:1000; Affinity biosciences, USA), Akt (#9272, 1:1000; Cell Signaling Technology, USA), p-Akt (Ser473) Antibody (#9271, 1:1000; Cell Signaling Technology, USA), p44/42 MAPK (Erk1/2) Antibody (#4695, 1:1000; Cell Signaling Technology, USA), pErk1/2 (Thr202/Tyr204) Antibody (#4370, 1:1000; Cell Signaling Technology, USA) and β-actin (Cat no. 10494-1-AP, 1:10,000; Proteintech, Peking, China). Secondary antibodies were horseradish-peroxidase-conjugated secondary anti-mouse IgG (1:10,000; Kerui tech, Wuhan, China), anti-rabbit IgG (1:50,000; Kerui tech, Wuhan, China). The blot was detected using ECL system (BIORAD, USA). Western blot gray values were determined by Image J.

Statistical analysis

Data were presented as mean values ± SEM and analyzed with SPSS version 17.0 (SPSS, Chicago, IL, USA). A Student's t-test was used to determine the significance of the difference between two samples. $p<0.05$ was considered as statistically significant difference. All experiments were carried out in triplicate unless otherwise noted.

Results

TCF3 is upregulated in human glioma tissue samples

We studied the expression levels of *TCF3* in both glioma samples and normal brain tissues with the quantitative real time-polymerase chain reaction (RT-PCR). The results are shown in figure 1. The levels of *TCF3* were significantly higher in human glioma tissue samples compared to normal brain tissues ($p<0.01$). It seems that the levels of *TCF3* expression increase with the uprising of the grades of gliomas (G1-G3: Grade II; G4-G6: Grade III; G7-G8: Grade IV). However, this observation cannot be confirmed by statistics, probably due to the small sample number.

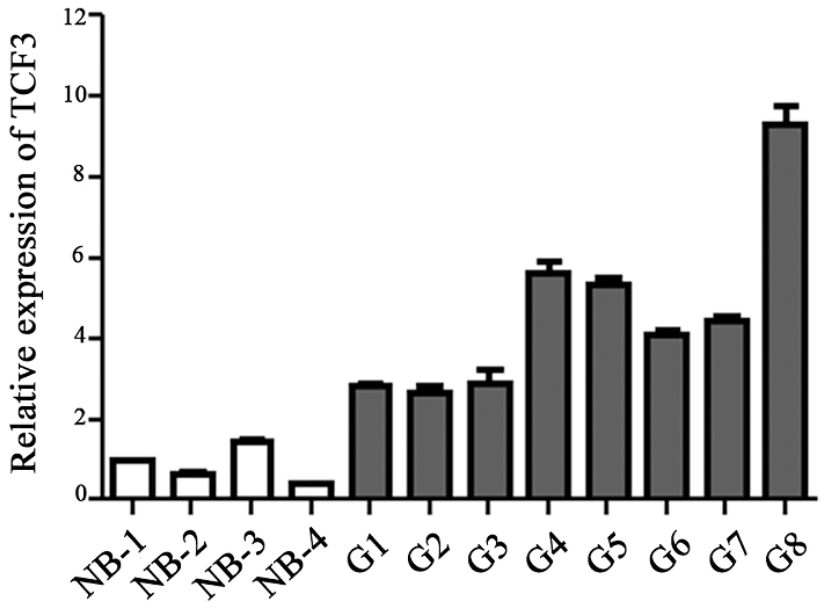

Fig. 1. The expression levels of TCF3 in glioma tissues and normal brain tissues. The data was the relative expression levels of TCF3 mRNA detected by Real-time PCR. 4 normal brain tissues (NB1-

NB4) and 8 glioma tissues (G1-G8. G1-G3: Grade II; G4-G6: Grade III; G7-G8: Grade VI) are included in this analysis. The average of normal brain tissues was set as 1. The $2^{-\Delta\Delta Ct}$ method was used to calculate the TCF3 mRNA expression levels which was normalized against GAPDH mRNA levels. Error bars represent standard deviation (SD) of 3 independent measures.

Suppression of TCF3 resulted in reduction of cell numbers

We applied lentivirus with shRNA to knockdown TCF3 expression in GBM cell lines (U251 and A172 cells). After TCF3 shRNA treatment, we measured the levels of the *TCF3* mRNA 48h later and protein 72h later in U251 and A172 cells. As shown in Figure 2, the suppression efficiencies of TCF3 shRNA are above 80% at mRNA levels (Figure2A) and about 50% at protein levels (Figure 2B and 2C) in both cell lines ($p<0.001$). Next, the effect of TCF3 on cell proliferation was evaluated with a CCK-8 kit at different time points. As shown in figure 3A, TCF3 shRNA treatment resulted in a marked cell number reduction compared to NC shRNA after 24 h ($p<0.05$ in U251 cells and $p<0.01$ in A172 cells; Fig. 3A), 48 h ($p<0.05$ in U251 cells and $p<0.01$ in A172 cells; Fig. 3A) and 72 h ($p<0.05$ in U251cells and $p<0.001$ in A172 cells; Fig. 3A). These results indicated that suppression of TCF3 reduced GBM cells numbers.

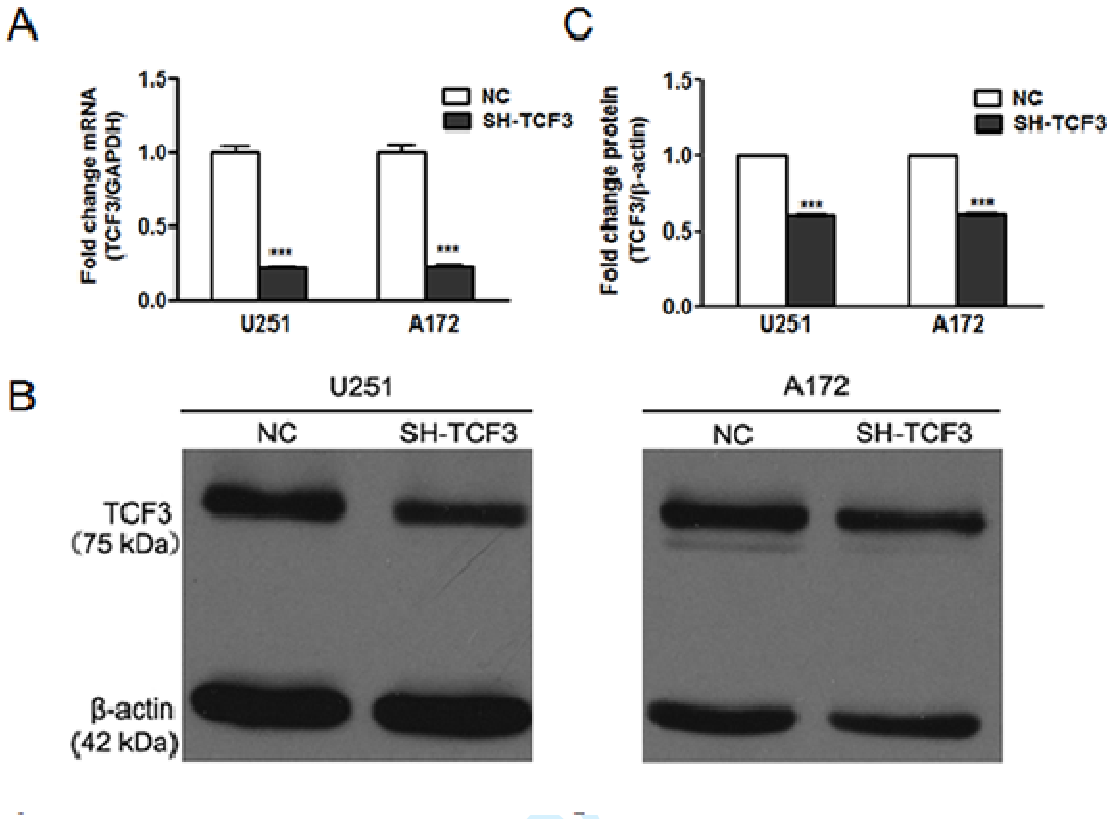

Fig. 2. TCF3 mRNA and protein levels were decreased after SH-TCF3 treatment in U251 and A172 cells. (A) The expression levels of TCF3 mRNA measured by real-time PCR. (B) The protein levels of TCF3 detected by western blot. (C) Histograms showing quantization of western blot and the data was normalized against β-actin. ***$p<0.001$. SH-TCF3, shRNA against TCF3. NC, negative control shRNA

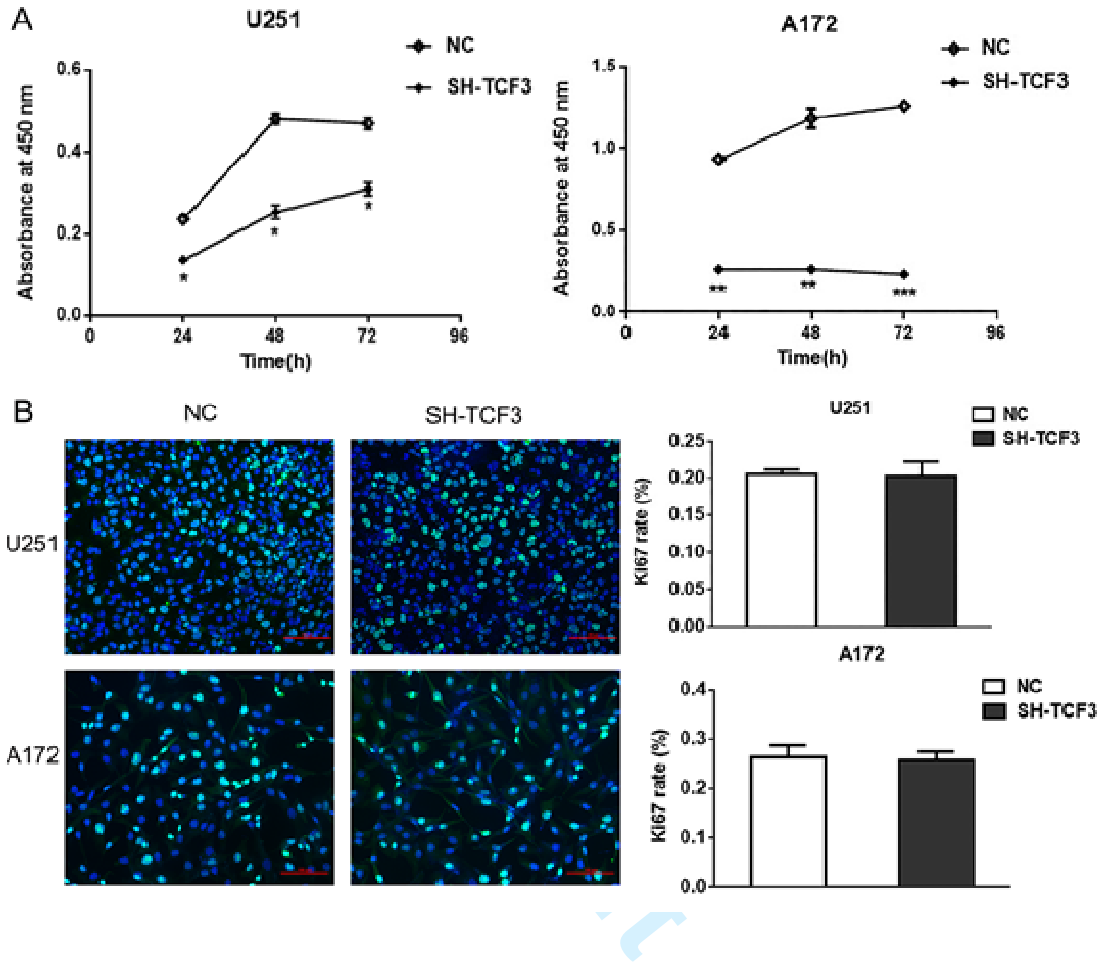

Fig. 3. Knock down of Tcf3 resulted in reduction of cell number but had no effect on cell proliferation. (A) Using CCK-8 kits to measure the cell number in U251 and A172 cells treated with TCF3 shRNA or negative control RNA. The absorbance at 450 nm positively corresponds to the cell number. (B) Images of U251 and A172 cells stained with DAPI (blue) and the antibodies against Ki67 (green). The scale bar shows the length of 100μm. Histograms showing the ratios of Ki67+ cells against all cells. *p<0.05, **p<0.01, ***p<0.001.

Knockdown of TCF3 has no effect on cell proliferation

Next we decided to find out whether inhibition of cell proliferation was responsible for reduced

cell numbers. Ki67, a cellular proliferation marker, was applied to study the cell proliferation. There was no difference in Ki67+ cell ratios between cells treated with TCF3 shRNA and cells with NC shRNA (Figure 3B). This result is consistent with the result of cell cycle distribution assay. GBM cells were infected with TCF3 shRNA and NC shRNA for 48h and then went through flow cytometry analysis by Beckman Coulter system (EPICS ALTRA II, Fullerton, USA). The results are shown in Fig. 4. The G0/G1 phase fractions of cells with NC control were 58.0% (n=3) in U251 cells and 78.4% (n=3) in A172 cells, which are similar to those with TCF3 shRNA treatment (61.6% in U251 cells and 80.9% in A172 cells). The average S phase fraction in cells with TCF3 shRNA appeared lower compared to NC-treated cells (17.7% vs. 23.6% in U251 cells and 14.8% vs. 15.8% in A172 cells). But the difference is very small. The results of our experiments suggested that knockdown of TCF3 might have little effect in cell proliferation.

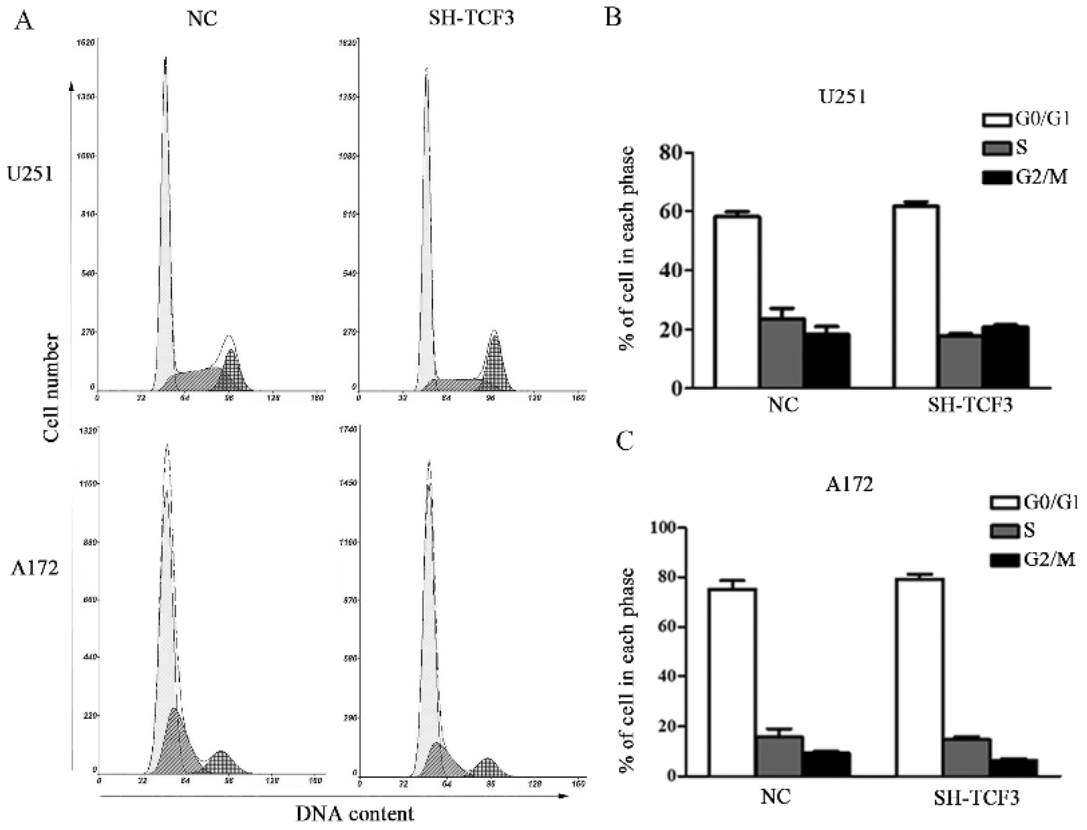

Fig. 4. Effect of TCF3 shRNA on the cell cycle of U251 and A172 cells. (A) Cell cycle distribution detected by FACS. U251 and A172 cells were infected with TCF3 shRNA or NC for 48 h. (B, C) Histograms showing cell cycle phase distribution of both cell lines.

Knockdown of TCF3 promotes apoptosis of GBM cell lines

Since TCF3 plays functional roles in stem cells, we want to find out whether TCF3 is important for cell survival. Apoptosis assays were performed on both U251 and A172 cell lines after the knock-down of TCF3. Seventy-two hours after lentiviruses infection, cells were harvested and the total proteins were subjected to Western blot analyses. We observed significantly increased levels of cleaved caspase-3, which is a hallmark for cell apoptosis, in TCF3 shRNA group as opposed to NC

group in both cell lines (Fig. 5).

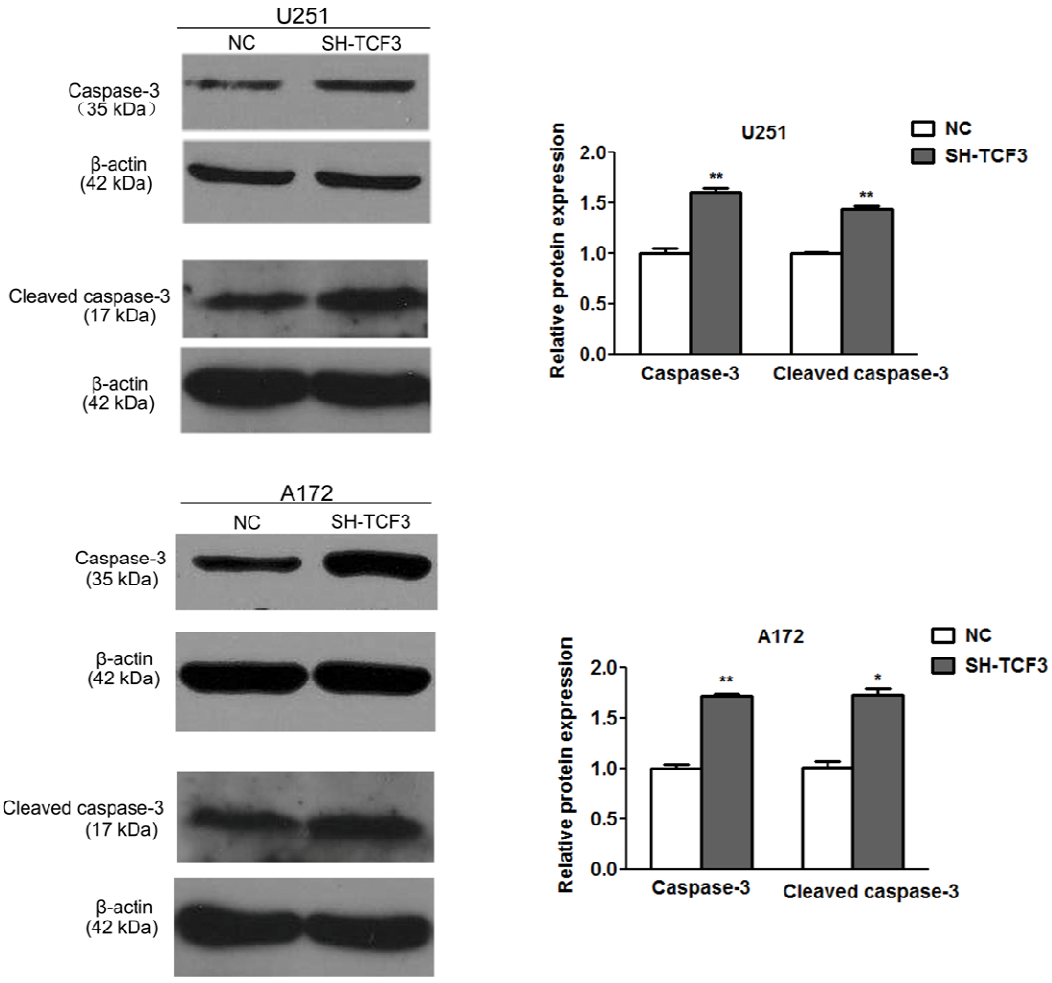

Fig. 5. Expression levels of the caspase-3 and cleaved caspase-3 protein in U251 and A172 cells. (A, C) The results of western blots showing the levels of caspase-3 and cleaved caspase-3 protein increased after TCF3 shRNA treatments in both cells. (B, D) Histograms showing quantization of western blot and the data was normalized against β-actin. Results of at least three independent experiments. *$p<0.05$, **$p<0.01$.

TCF3 suppression inhibits cell migration and cell invasion

One of the prominent features of GBM is its invasiveness. We wondered whether TCF3 would play roles in the cell migration and invasion. Transwell and wound healing assays were used for the cell migration and invasion analysis. The Transwell study showed that after infected with TCF3 shRNA lentiviruses, U251 and A172 cells displayed a substantially suppressed migratory and invasive capacity compared to their respective control groups (Fig. 6A). This inhibitory effect of TCF3 loss on cell invasion was further confirmed by wound healing assays (Fig. 6B). As the levels of TCF3 decreased, the migration of U251 and A172 cells were significantly diminished in comparison with the control.

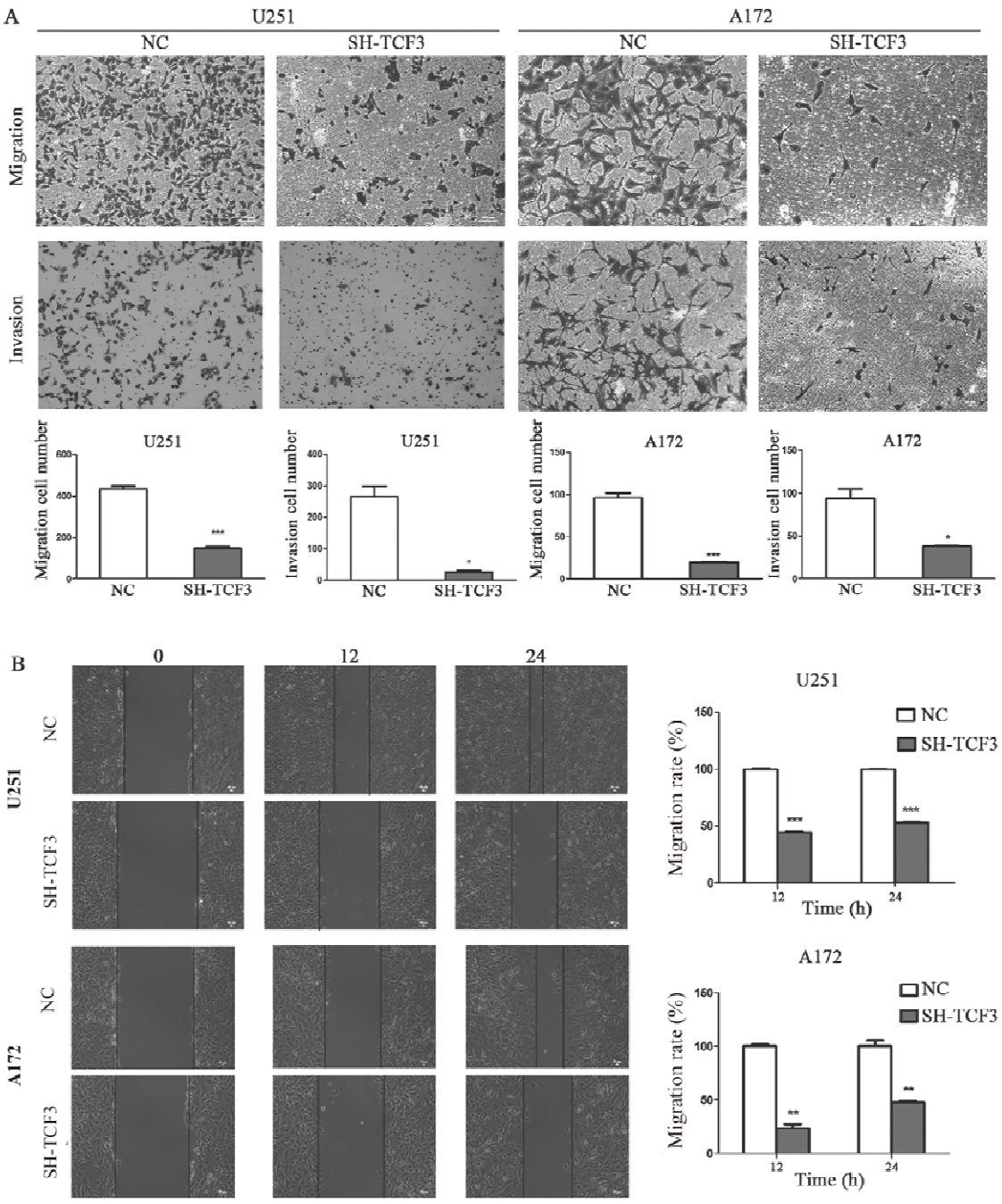

Fig. 6. Knock-down of TCF3 inhibited the migration and invasion of U251 and A172 cells. (A) Representative images of the migration and invasion assay. The migration or invasion cell numbers were the total cell number counted on the lower surface. *p<0.05; **p<0.01. (B) the wound healing assay showed delayed closure of scratching lines in TCF3 shRNA treated cells compared with normal

control at the 12 and 24 h time points in both cells. The mean percentages of wound closure at 12 and 24 h after wounding were significantly decreased in U251 and A172 cells infected with TCF3 shRNA lentiviruses. Histograms showing relative migration rate of TCF3 shRNA treated cells when normal control was set as 100% in U251 and A172 cells. **p<0.01; ***p<0.001.

TCF3 regulates the phosphorylation of AKT and ERK1/2 in GBM cell lines

In order to investigate molecular components in TCF3 tumor-promoting function, we sought to identify target genes of TCF3 in the context of glioblastoma cell lines. Previous studies suggested that the migration and invasion of glioma cells are associated with PI3K/AKT signaling pathway (Jin et al. 2013; Golding et al. 2009). Moreover, the ERK signaling pathway has a close relationship with the migration (Ding et al. 2016) of tumor cells. We found that TCF3 shRNA significantly inhibited the phosphorylation of AKT and ERK1/2 in both cell lines (Fig. 7), indicating that TCF3 might regulate the migration and invasion of GBM via AKT and ERK signaling pathways.

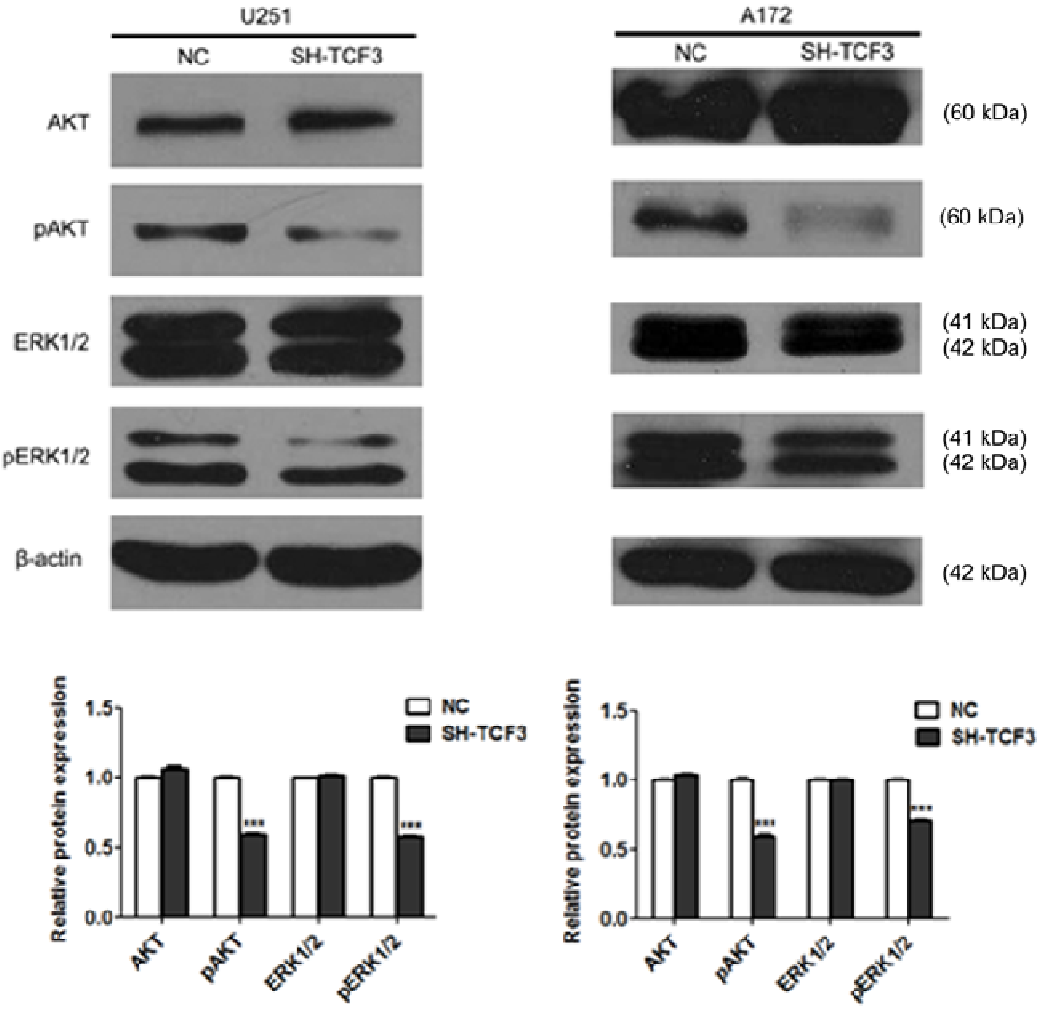

Fig. 7. Knock-down of TCF3 down-regulated pAkt (Ser473) and pErk1/2 (Thr202/Tyr204) levels in U251 and A172 cells. Western blot showed that down-regulation of TCF3 suppressed pAkt (Ser473) and pErk1/2 (Thr202/Tyr204) phospho-protein levels. Histograms showing relative mean values of 3 independent experiments. ***p<0.001.

Discussion

To the best of our knowledge, this is the first work on the relationship between TCF3 and gliomas. Through a genome-wide screening, we identified TCF3 as a specific transcription factor for neural stem cells development. It is also highly expressed in the glioma tissue. In order to investigate the involvement of *TCF3* gene in GBM, we used shRNA to knockdown the expression of TCF3 in human GBM cell lines. The down regulation of TCF3 led to the reduction of U251 and A172 cell numbers. Further analysis showed that TCF3 knockdown has little effect on the cell cycle, but increases the cell apoptosis. So TCF3 can promote cell survival. We also found that TCF3 promotes cell migration and invasion as TCF3 knockdown caused a significant reduction of cell mobility. TCF3 may promote glioblastoma cells survival and migration via AKT and ERK pathway as our experiments showed that the phosphorylation levels of Akt and ERK were significantly reduced after the addition of TCF3 shRNA.

TCF3 belongs to LEF/TCFs family. They are sequence-specific DNA binding transcription factors, functioning in the Wnt signaling pathway by recruiting β-catenin to Wnt target genes. Mammals have four TCF/LEF family members TCF7 (officially TCF1), TCF3 (officially TCF7L1), TCF4 (officialy TCF7L2), and LEF1 (Sokol 2011; Wu et al. 2012). Aberrant expression of LEF/TCFs contributes to many human tumors: TCF1 in endometrial cancer (Rebouissou et al. 2004), TCF3 in breast cancer (Slyper et al. 2012), TCF4 in colon cancer (Shin et al. 2014), LEF1 in glioblastoma (Liu et al. 2012). TCF3 seems to mostly act as a transcriptional repressor (Kim CH 2000) and repress Wnt target genes in various tissues (Wu et al. 2012; Yi et al. 2011), which is different from its other family members

such as TCF7 and LEF1. However, it can also act as an activator in pathways other than Wnt signaling pathway (Pereira et al. 2006; Korinek et al. 1998). So, it is not surprising that many TCF3-regulated genes are apparently unrelated to Wnt signaling (Cole et al. 2008; Nguyen H 2006; Yi et al. 2011). TCF3 can silence or activate proteins involved in cytoskeletal structure, cell–cell communication and cell–matrix interactions (Yang and Weinberg 2008; Brabletz S and Brabletz T 2010), which is consistent with our result that TCF3 regulates cell mobility.

The function roles of TCF3 in cancer is still not very clear. It is possible that TCF3 plays different functions in different tissues. In mouse ES cells, TCF3 can compete with Oct4, Sox2 and Nanog for promoters of many genes and repressing ES-cell self-renewal (Tam et al. 2008). This might explain the results that high levels of TCF3 repress proliferation of colorectal cancer (CRC) cells (Chiaro et al. 2012). In our experiments, TCF3 showed little effect on cell cycles, which suggests that TCF3 may not promote tumor progress via cell growth. Other reports showed that TCF3 overexpression led to the activation of genes associated with Epithelial Mesenchymal Transition (EMT), including both structural genes and regulators such as ZEB1/2, Twist, and miR-200 (Brabletz S 2010; Yang J 2008). This line of experiments is consistent with our data that TCF3 promotes cell migration and invasion. On the other hand, Hoang *et al* (2006) found that TCF3 promotes genes associated with an undifferentiated state in embryonic skin progenitors.

The PI3K signaling pathway has been well studied in many cancers (Yuan and Cantley 2008), including glioblastomas. It drives many glioma-relevant processes, including survival, proliferation, migration and invasion (Engelman et al. 2006). A central node in the PI3K signaling is the serine-theonine kinase AKT. In order to be fully activated, Akt requires phosphorylation of two specific

amino acid residues, theonine 308 and serine 473. Elevated AKT phosphorylation has been observed in up to 85% of glioblastoma cell lines and patient samples (Wang H 2004). Upon activation, Akt phosphorylates downstream target proteins, including mTOR, GSK-3, mdm2, and BAD located at the cytoplasm and nucleus (West et al. 2002), ultimately affecting a number of processes involved in survival, cell growth, metabolism, proliferation, and metastasis.

ERK1 and ERK2 are two members of the MAPK family. The MAPK/ERK signaling pathway regulates cell proliferation, differentiation, and angiogenesis in various tumor types. The MAPK pathway members play important roles in glioma cell proliferation and differentiation (Cuevas et al. 2006; Mandell et al. 1998). Elevated phosphorylation levels of ERK1/2 have been observed in neovasculature around glioma and necrosis.

In many types of tumors, both MAPK/ERK and PI3K signals are activated. In thyroid cancers, the MAPK/ERK and/or the PI3K pathways are activated, which changes the expression levels of many transcription factors involved in cellular proliferation and apoptosis (Nikiforov 2002). In Non small-cell lung cancer (NSCLC), both p-ERK and p-AKT are often overexpressed (Bunn and Thatcher 2008). Baoyu Lv *et al* (2015) also has demonstrated that EMT process can be triggered by the SDF-1/CXCR4 axis in glioblastoma. Then PI3K/AKT and ERK pathways are activated, resulting in the tumor cell invasion and proliferation. However, we have not found any reports indicating that TCF3 activates PI3K/AKT and/or MAPK/ERK signaling pathways.

In summary, our data suggests for the first time that the over-expression of *TCF3* mRNA can be associated with malignant gliomas. Overexpressed TCF3 could promote glioma development by inhibiting cell apoptosis, enhancing the migration and invasion of tumor cells. Those functions of

TCF3 are probably mediated by activation of the PI3K/AKT and MAPK/ERK signaling pathways. TCF3 could be a potential target for GBM.

Acknowledgement

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