Mol Cell Biochem (2013) 377:87–96
DOI 10.1007/s11010-013-1573-2

TNF receptor-associated factor 6 regulates proliferation, apoptosis, and invasion of glioma cells

**Zhang Peng · Yue Shuangzhu · Jiang Yongjie ·
Zhang Xinjun · Liu Ying**

RETRACTED ARTICLE

Received: 21 November 2012 / Accepted: 18 January 2013 / Published online: 29 January 2013
© Springer Science+Business Media New York 2013

Abstract Tumor necrosis factor receptor-associated factor 6 (TRAF6), which plays an important role in inflammation and immune response, is an essential adaptor protein for the NF-κB (nuclear factor κB) signaling pathway. Recent studies have shown that TRAF6 played an important role in tumorigenesis and invasion by suppressing NF-κB activation. However, up to now, the biologic role of TRAF6 in glioma has still remained unknown. To address the expression of TRAF6 in glioma cells, four glioma cell lines (U251, U-87MG, LN-18, and U373) and a non-cancerous human glial cell line SVG p12 were used to explore the protein expression of TRAF6 by Western blot. Our results indicated that TRAF6 expression was upregulated in human glioma cell lines, especially in metastatic cell lines. To investigate the role of TRAF6 in cell proliferation, apoptosis, invasion, and migration of glioma, we generated human glioma U-87MG cell lines in which TRAF6 was either overexpressed or depleted. Subsequently, the effects of TRAF6 on cell viability, cell cycle distribution, apoptosis, invasion, and migration in U-87MG cells were determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, flow cytometry analysis, transwell invasion assay, and wound healing assay. The results showed that knockdown of TRAF6 could decrease cell viability, suppress cell proliferation, invasion and migration, and promote cell apoptosis, whereas overexpression of TRAF6 displayed the opposite effects. In addition, the effects of TRAF6 on the expression of phosphor-NF-κB (p-p65), cyclin D1, caspase 3, and MMP-9 were also probed. Knockdown of TRAF6 could lower the expression of p-p65, cyclin D1, and MMP-9, and raise the expression of caspase 3. All these results suggested that TRAF6 might be involved in the potentiation of growth, proliferation, invasion, and migration of U-87MG cell, as well as inhibition of apoptosis of U-87MG cell by abrogating activation of NF-κB.

Keywords Glioma · TRAF6 · Proliferation · Apoptosis · Invasion

Z. Peng (✉) · Y. Shuangzhu
Department of Neurosurgery, The First Affiliated Hospital of Xinxiang Medical University, NO.88, Jian-kang Road, Xinxiang 453100, Henan, China
e-mail: zhangpeng_hn@163.com

J. Yongjie · L. Ying
Department of Gynaecology and Obstetrics, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang 453100, China

Z. Xinjun
Department of Urology, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang 453100, China

Introduction

Gliomas are the most common primary central nervous system tumors [1]. Malignant gliomas are not strictly focal lesions, but are characterized by the intracerebral dissemination of malignant cells along the myelinated axons and blood vessels and/or through the subarachnoid space [2]. Therefore, there is no obvious boundary between normal brain tissue and glioma which makes complete resection difficult [3, 4]. Despite the progress in brain tumor therapy, the prognosis of malignant glioma patients remains dismal [5]. Invasion and metastasis are the major causes of treatment failure and death from glioma. Consequently, innovative approaches that target the invasion and metastasis of glioma are urgently needed.

Nuclear factor kappa B (NF-κB), a transcription factor regulating a host of biologic events, plays an important role

in inflammation, immune response, cellular proliferation, apoptosis, tumorigenesis and invasion [6–10]. In the course of the activation of NF-κB, the inhibitor of NF-κB (IκBα) undergoes phosphorylation, ubiquitination and proteasome-mediated degradation, which leads to the nuclear translocation of the p50-p65 subunits of NF-κB followed by p65 phosphorylation, acetylation, and methylation, binding DNA, and gene transcription [11, 12]. However, excessive activation of NF-κB signaling pathway is often associated with cancer and various chronic diseases [13]. Therefore, NF-κB signaling pathway must be tightly regulated to properly perform its cellular functions which are essential for human health. Studies have demonstrated that constitutive activation of NF-κB could play an important role in the regulation of genes involved in tumorigenesis, invasion, and migration. In contrast, inhibiting NF-κB activation restrains the invasion and migration [14–18].

Tumor necrosis factor receptor-associated factor 6 (TRAF6), one member of tumor necrosis factor receptor-associated factor (TRAF) family, possesses a unique receptor-binding specificity that results in its crucial role as the signaling mediator for TNF receptor superfamily and interleukin-1 receptor/toll-like receptor superfamily-induced NF-κB activation [19–22]. Recent studies have reported that TRAF6 might play an important role in tumorigenesis, metastasis, and invasion by suppressing NF-κB activation [14]. However, so far, it has been unknown whether TRAF6 is involved in glioma occurence, migration, and invasion.

In this study, four glioma cell lines (U251, U-87MG, LN-18, and U373) and a non-cancerous human glial cell line SVG p12 were used to detect the expression of TRAF6 protein in glioma cell by Western blot. The effects of TRAF6 on cell viability, cell cycle distribution, apoptosis, and invasion within U-87MG cells were assayed by MTT method, flow cytometry analysis, and transwell invasion experiment. In addition, we analyzed the effects of TRAF6 on the expression of proteins p-p65, cyclin D1, caspase 3, and MMP-9 in U-87MG cells. These data might contribute to the prediction of glioma prognosis and the establishment of targeted therapies.

Materials and methods

Reagents

All cell culture components were purchased from Gibco-BRL (Gaithersburg, MD). U251, U-87MG, LN-18, U373, and SVG p12 cell lines were purchased from American type culture collection (ATCC; Rockville, MD, USA). Human TRAF6-shRNA constructs in retroviral untagged vector were purchased from OriGene Technologies (Rockville, MD). Homo TRAF6 (U78798.1) transfection-ready DNA and high performance transfection reagent were purchased from OriGene Technologies (Rockville, MD). Protein extraction buffer, Annexin V-FITC, propidium iodide (PI), crystal violet, and RNAse A were obtained from Sigma Chemical Co. (St. Louis, MO). Polyvinylidenedifluoride (PVDF) membranes were purchased from Millipore Inc. (Bedford, MA). The ECL chemiluminescence kit was purchased from Pierce (Rockford, IL). The transwell invasion chamber was obtained from Costar Corp. (Cambridge, MA). Matrigel was obtained from Collaborative Research, Inc. (Bedford, MA). The antibodies used in this study include: rabbit anti-human TRAF6 polyclonal antibody (LifeSpan BioSciences, Seattle,WA), phosphor-NF-κB p65 (Ser536) antibody (Cell Signaling Technology Inc., Beverly, MA), mouse anti-human cyclin D1 monoclonal antibody (BD Biosciences, San Jose, CA), goat anti-human caspase 3 polyclonal antibody (Novus Biologicals, Littleton, CO), rabbit anti-human MMP-9 polyclonal antibodies (Abnova Corp., Taipei, Taiwan), rabbit anti β-actin polyclonal antibody (Abbiotec Corp., San Diego, CA), horseradish peroxidase-conjugated goat anti-rabbit, and rabbit anti-mouse or rabbit anti-goat IgG polyclonal antibody (Invitrogen, Carlsbad, CA).

Methods

Cell culture and transfection

Human glioma cell lines, U251, U-87MG, LN-18, and U3738, were cultured in Dulbecco's modified eagle's medium. SVG p12 cells were cultured in EMEM medium containing 2 mM glutamine, 1 % nonessential amino acid (NEAA), 10 % fetal bovine serum (FBS), 50 U/ml penicillin, and 50 U/ml streptomycin. All glioma cell lines were maintained at 37 °C in a humidified atmosphere with 5 % CO_2.

On the day of transfection, cells at about 70–90 % confluency were changed to serum-free medium just before experiments. Transient transfections were performed using high performance transfection reagent following the manufacturer's recommendation (OriGene Technologies). The engineered stable cell lines were maintained by adding 0.8 μg/ml puromycin or 1 mg/ml G418 to the culture media for 2 weeks.

Western blot

A Protein Extraction Kit was used to extract total protein from cell lines, U251, U-87MG, LN-18, U373, SVG p12, and U-87MG infected with TRAF6 overexpression vector or TRAF6 knockdown vector, and the total protein was quantified using a BCA assay kit. Total protein was separated by SDS-PAGE and transferred to a PVDF membrane.

RETRACTED ARTICLE

The membrane was blocked for 1 h in TBS (10 mM Tris–HCl, pH 7.5, 150 mM NaCl) solution containing 5 % skimmed milk, then probed with primary antibody at 4 °C overnight, washed 3 × 5 min in TBST, and probed with corresponding secondary antibody at room temperature for 2 h. After washed with TBST, autoradiography was conducted with ECL chemiluminescence reagents. The relative expression of the target protein was valuated with the gray value ratio of target protein content to β-actin (target protein/β-actin) content.

Determination of U-87MG cell viability

The effect of TRAF6 on the viability of glioma cells U-87MG was determined by MTT assay. U-87MG cells, in which TRAF6 was either overexpressed or depleted, were seeded into 96-well plates at the density of 1×10^3 cells/well, and allowed to adhere overnight. 10 μl MTT (5 mg/ml) was added to the cells and incubated for another 4 h. Media was then removed and 150 μl DMSO was added and thoroughly mixed to dissolve the crystals. OD values were measured with microplate reader at 570- and 630-nm wavelength. The relative cell proliferation (%) was calculated by the equation as described in previous study [23] and the experiment was repeated three times.

Determination of U-87MG cell cycle

The effect of TRAF6 on cell cycle of glioma cells U-87MG was investigated with flow cytometry. Then, the cells were detached by trypsinization, washed twice in PBS, and fixed in 70 % cold ethanol overnight at −20 °C. The next day, after washing by citrate phosphate buffer, followed by PBS, U-87MG cells were incubated with RNase A solution (150 μg/ml) for 1 h at 37 °C. At last, U-87MG cells were incubated in PI solution (100 μg/ml in PBS) at room temperature for 30 min. The cell cycle was detected by flow cytometry. The experiment was performed in triplicate.

Determination of U-87MG cell apoptosis

U-87MG cell apoptosis was detected by flow cytometry according to the manufacturer's instructions. Briefly, U-87MG cells were harvested, washed twice with PBS, and resuspended in 195 μl Annexin V binding buffer. A volume of 5 μl Annexin V-FITC was added and gently mixed, and U-87MG cells were stained in the dark at room temperature for 10 min. Then, U-87MG cells were centrifuged at 1,000×*g* for 5 min, and gently resuspended in 190 μl of Annexin V binding buffer. At last, 10 μl propidium iodide staining solution was added and gently mixed, and U-87MG cells were kept on ice in the dark and immediately subjected to flow cytometry analysis. Cell Quest software was used to analyze the results and the experiment was performed three times.

Determination of U-87MG cell invasion capability

The invasive ability of U-87MG cells was calculated by the transwell invasion chamber test. The chamber was washed with serum-free medium, and then 20 μl matrigel (1 mg/ml) was added to evenly cover the surface of the polycarbonate membrane (8-μm pore size) to create the matrigel membrane. The chamber was divided into upper and lower chambers. For invasion assays, U-87MG cells (4×10^5) were plated in the top chambers of transwells in 200 μl serum-free DMEM, whereas the bottom chambers were filled with 600 μl DMEM medium containing 10 % FBS. After 48-h incubation, U-87MG cells were fixed by replacing the culture medium with 4 % formaldehyde. After fixed for 15 min at room temperature, the chambers were rinsed with PBS and stained with 1 % crystal violet for 10 min. After removing the cells from the top of the matrigel membrane by cotton swab, the remaining cells are the ones that have invaded the matrigel membrane. The invasive ability of U-87MG cells was calculated by the number of cells passing through a polycarbonate membrane. The results are presented as the mean ± SD, and the experiment was repeated three times.

Determination of U-87MG cell migration capability

Wound-healing assay was performed to evaluate U-87MG cell migration capability. Equal numbers of U-87MG cells from each group were seeded into six-well culture plates. A scratch wound was created in the center of the cell culture plate with a sterile plastic pipette tip when the cells reached 90 % confluence. Removing the debris by washing the cells with serum-free culture medium, cells boarding the wound were visualized and photographed under an inverted microscope 24 h after the wound was created. The distance cells migrated into the wounded area were calculated by subtracting the distance 24 h after wound-healing from the initial distance. A total of six areas were selected randomly from each well under a 40× objective, and the cells in three wells of each group were quantified in each experiment.

Gelatin zymography assay

Gelatinase activity was assayed to analyze the activity of MMP-9. Briefly, cells were homogenized in PBS followed by centrifugation at 1,000×*g* at 4 °C to remove the cellular debris. The supernatant was again centrifuged at 10,000×*g* at 4 °C and the resultant supernatant was subjected to gelatin zymography after estimation of protein by Bradford method.

RETRACTED ARTICLE

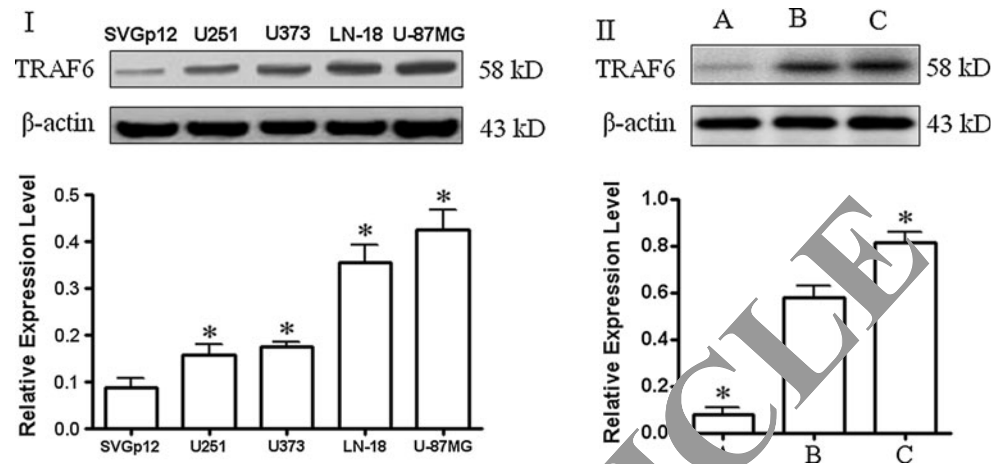


Fig. 1 **a** The expression of TRAF6 protein in glioma cell lines (* indicates $p < 0.05$ compared to SVG p12; **b** The expression of TRAF6 protein in U-87MG (*A* knockdown group, *B* blank group, *C* overexpression group * indicates $p < 0.05$ compared to blank group). The relative expression level of TRAF6 normalized by that of beta-actin. These data were analyzed by one-way ANOVA

RETRACTED ARTICLE

Equal amounts of protein samples (80 μg) were loaded in each lane in standard SDS loading buffer containing 0.1 % SDS without β-mercaptoethanol. Boiling was avoided because it caused aggregation and denaturation of proteins and then separated by SDS/PAGE on a 10 % (w/v) gel containing 0.1 % gelatin. The gel was washed twice in 2.5 % (w/v) Triton X-100 solution and incubated overnight at 37 °C in developing buffer (50 mmol/l Tris/HCl (pH 7.4), 10 mmol/l $CaCl_2$, 5 mmol/l $ZnCl_2$ and 0.05 % Brij-35), stained with 0.5 % Coomassie Blue, and then destained in a 40 % (v/v) methanol/10 % (v/v) acetic acid solution. Proteolytic activity was evidenced as clear bands against the blue background of the stained gelatin.

Statistical analysis

The data were analyzed by SPSS18.0 software package. The statistical methods, one-way ANOVA and Student's *t* test, were used to analyze the related data. All *p* values were two-sided and the results were considered to be statistically significant if $p < 0.05$.

Results

TRAF6 protein expression in glioma cell lines

To assess the expression of TRAF6 in glioma cells, four glioma cell lines (U251, U-87MG, LN-18, and U373) and a non-cancerous human glial cell line SVG p12 were cultured to examine the protein expression of TRAF6 by Western blot. The TRAF6 protein expression in glioma cell lines was significantly higher than that in non-cancerous glial cell line SVG p12 ($p < 0.05$). Among these glioma cell lines, U-87MG cells displayed the highest protein expression level of TRAF6. Previous studies have shown U-87MG cells are more aggressive in cell migration and invasion compared with the other three cell lines [24, 25]. Therefore, in the following studies, the U-87MG cells were used to study further unless specified otherwise. We generated human glioma U-87MG cell lines in which TRAF6 was either overexpressed or depleted. U-87MG cells were divided into three groups: overexpression group (infected with overexpression vector), knockdown group (infected with RNAi vector), and blank group (without any treatment). Western blot analysis showed that TRAF6 protein displayed significant upregulation in overexpression group and significant downregulation in knockdown group compared to blank group ($p < 0.01$). These data demonstrated that we successfully generated stable human glioma U-87MG cell lines in which TRAF6 was either overexpressed or depleted (Fig. 1b).

Effect of TRAF6 on U-87MG cell viability

As was stated above, U-87MG cells were divided equally into three groups, overexpression group, blank group and knockdown group. The same number of U-87MG cells from each group was inoculated and subjected to MTT assay. We found that U-87MG cells viability in overexpression group was significantly higher than that in blank group, and that U-87MG cell viability in knockdown group was significantly lower than that in blank group ($p < 0.05$) (Fig. 2). These results suggested that overexpression of TRAF6 might be related to the increase in U-87MG viability.

Effect of TRAF6 on cell cycle of U-87MG cells

Cell cycle analysis demonstrated that overexpression group had less U-87MG cells in G0/G1 phase than blank group ($p < 0.05$) and that knockdown group had more U-87MG cells in G0/G1 phase than blank group ($p < 0.05$). Furthermore, overexpression group had more U-87MG cells in S and G2

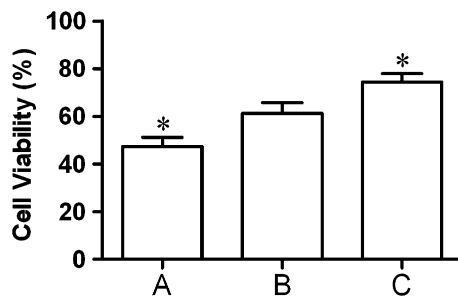


Fig. 2 The effect of the TRAF6 on U-87MG cell viability. (*A* knockdown group, *B* blank group, *C* overexpression group, * indicates $p < 0.05$ compared to blank group)

phase than blank group ($p < 0.05$), and knockdown group had less U-87MG cells in S and G2 phase than blank group ($p < 0.05$) (Fig. 3). These results indicated that downregulation of TRAF6 might lead to U-87MG cell cycle arrest in G0/G1 phase.

Effect of TRAF6 on apoptosis of U-87MG cells

Flow cytometry analysis of U-87MG cell apoptosis showed that the number of apoptotic cells in overexpression group was significantly lower than that in blank group, and that the number of apoptotic cells was significantly higher in knockdown group than that in blank group ($p < 0.05$) (Fig. 4). These data suggested that the overexpression of TRAF6 might inhibit U-87MG cell apoptosis, and that the inhibition of TRAF6 expression might promote U-87MG cell apoptosis.

Effect of TRAF6 on U-87MG cell invasion and migration

The invasive ability of U-87MG was evaluated based on the number of U-87MG cells passing through the polycarbonate membrane of Transwell invasion chamber. The results showed that the number of U-87MG cells passing through the polycarbonate membrane in overexpression group was significantly higher than that in blank group, and that the number of U-87MG cells passing through the polycarbonate membrane in konckdown group was significantly lower than that in blank group ($p < 0.05$) (Fig. 5).

Wound-healing assay was performed to evaluate U-87MG cell migration capability. The results showed that the migration capability of U-87MG cells in overexpression group was significantly higher than that in blank group, and that the migration capability of U-87MG cells in knockdown group was significantly lower than that in blank group ($p < 0.05$) (Fig. 6). These data indicated that the inhibition of TRAF6 expression might suppress the ability of invasion and migration of U-87MG cells.

Effect of TRAF6 on the expression of p-p65, cyclin D1, caspase-3, and MMP-9

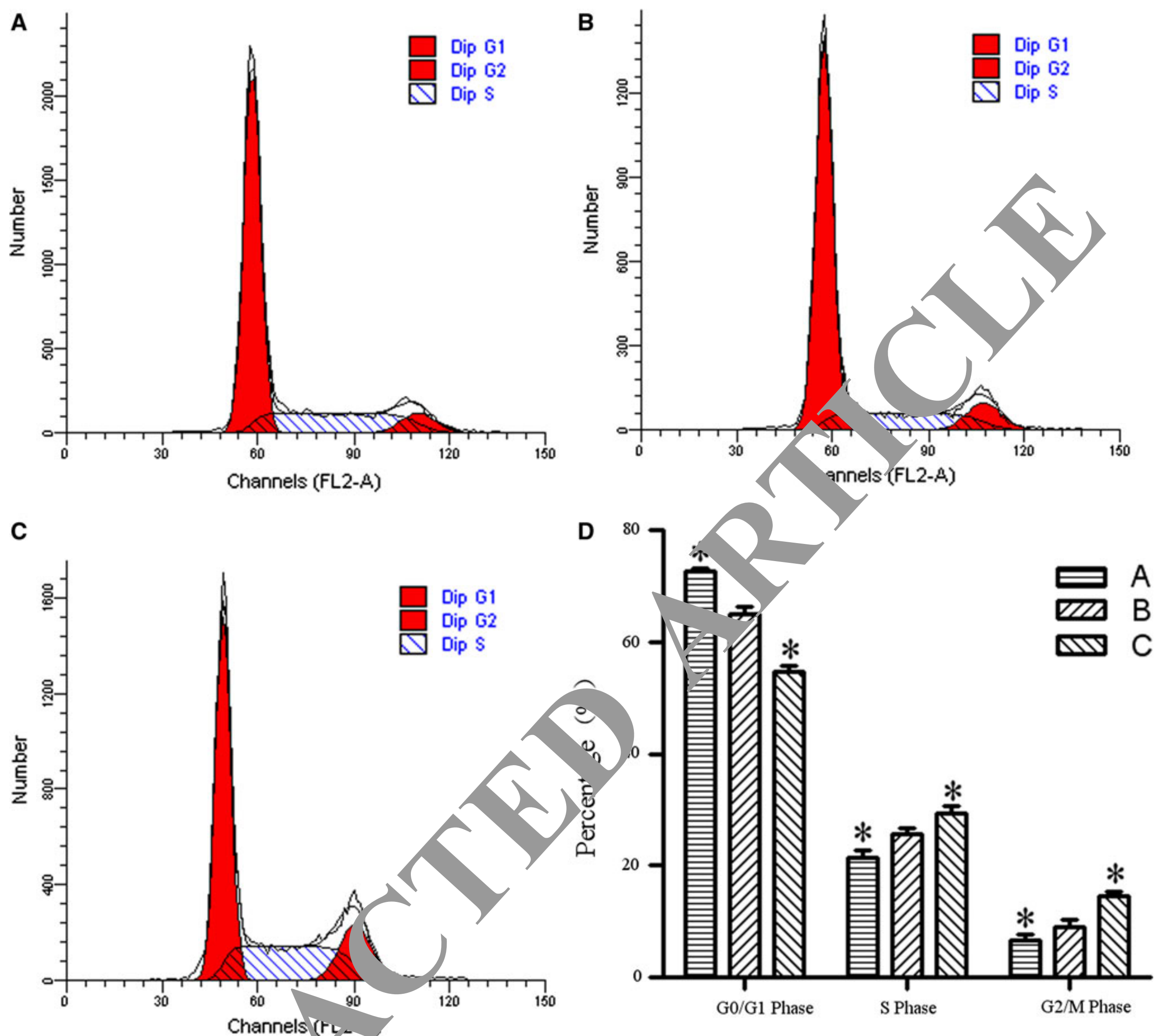
Western blot analysis indicated that the protein expressions of p-p65, cyclin D1, and MMP-9 were at a higher level in overexpression group than those in blank group, and that they were at a lower level in knockdown group than those in blank group ($p < 0.05$), while caspase-3 expression displayed a lower level in overexpression group than that in blank group and displayed a higher level in knockdown group than that in blank group ($p < 0.05$) (Fig. 7). At the same time, MMP-9 levels were analyzed by gelatin zymography. The results showed that TRAF6 improved MMP-9 expression which is consistent with enzyme activity in gelatin zymography assay (Fig. 8). These results indicated that the upregulation of TRAF6 might be associated with the upregulation of p-p65, cyclin D1, and MMP-9, and the downregulation of caspase-3.

Discussion

The adapter protein TRAF6 is critical for mediating signal transduction from members of the IL-1R/TLR and TNFR superfamilies [26]. Recently, it has been found that TRAF6 could promote NF-κB activation [27, 28]. Studies have also demonstrated that constitutive activation of NF-κB could play a vital role in tumorigenesis, migration, and invasion [29–31]. In lung cancer and osteosarcoma, TRAF6 was reported to enhance tumor incidence and invasion ability [32–35]. However, whether TRAF6 is involved in glioma incidence, migration, and invasion remains elusive.

In this study, we found that TRAF6 displayed higher expression level in glioma cell lines (U251, U-87MG, LN-18, and U373) compared to its expression level in non-cancerous glial cell line SVG p12. The expression model of TRAF6 in glioma cell lines was consistent with that in lung cancer cell lines, which showed that TRAF6 was significantly upregulated in lung cancer cell lines [32]. We therefore speculated that TRAF6 might positively regulate glioma cell proliferation, migration, and invasion. In order to explore the role of TRAF6 in glioma cells, we constructed a human glioma U-87MG cell model in which TRAF6 was either overexpressed or depleted. Based on the established glioma U-87MG cell models, we explored the effect of TRAF6 on cell cycle, apoptosis, and invasion. The results indicated that the down-regulation of TRAF6 could inhibit U-87MG cell proliferation and lead to cell cycle arrest in G1 phase, which was possibly due to a reduction of growth-promoting factors or an increase of

RETRACTED ARTICLE



RETRACTED ARTICLE

Fig. 3 The effect of TRAF6 on cell cycle of U-87MG cells (*A* knockdown group, *B* blank group, *C* overexpression group, *D* the relative content of various phases of the cell cycle. * indicates $p < 0.05$ compared to blank group)

growth-inhibitory factors in the downstream of the TRAF6 signal pathway. Therefore, the positive cell cycle regulator cyclin D1 was examined in our study. The results showed that cyclin D1 was suppressed in knockdown group. However, the exact mechanism still requires further study. Our results also showed that downregulation of TRAF6 could promote U-87MG cell apoptosis. For this reason, we examined the expression of the proapoptotic protein caspase-3 and found that caspase-3 was upregulated in knockdown group. These results implied that overexpression of TRAF6 may improve the growth and proliferation of U-87MG cell and suppress the apoptosis of U-87MG cells.

Our study also demonstrated that U-87MG cells had the highest level in TRAF6 protein expression and were more aggressive in cell migration and invasion compared with other glioma cell lines [24, 25]. These data suggested that TRAF6 might be involved in invasion and metastasis of glioma cells. Therefore, we investigated the effect of TRAF6 on the invasion ability of U-87MG cells in vitro. Our results suggested that TRAF6 played a stimulative role in U-87MG cell invasion. These data well documented that TRAF6 might be involved in invasion and metastasis-related molecular pathways. TRAF6 could improve the activation of NF-κB signaling pathway [27, 28]. NF-κB activation could deliver significant improvements in tumorigenesis, migration, and

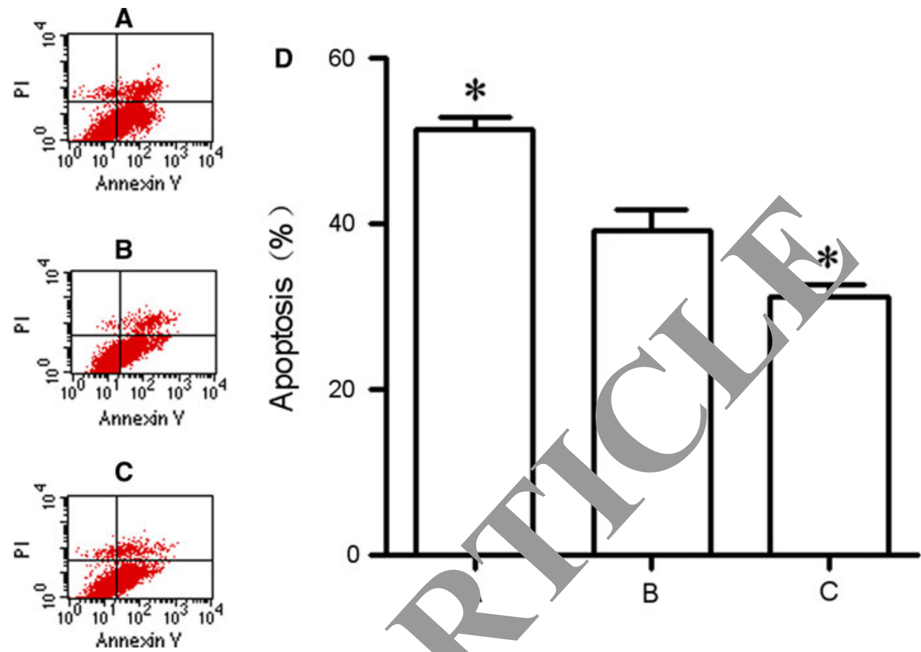


Fig. 4 The effect of TRAF6 on apoptosis of U-87MG cells (*A* knockdown group, *B* blank group, *C* overexpression group, *D* the relative content of the apoptosis cell, * indicates $p < 0.05$ compared to blank group)

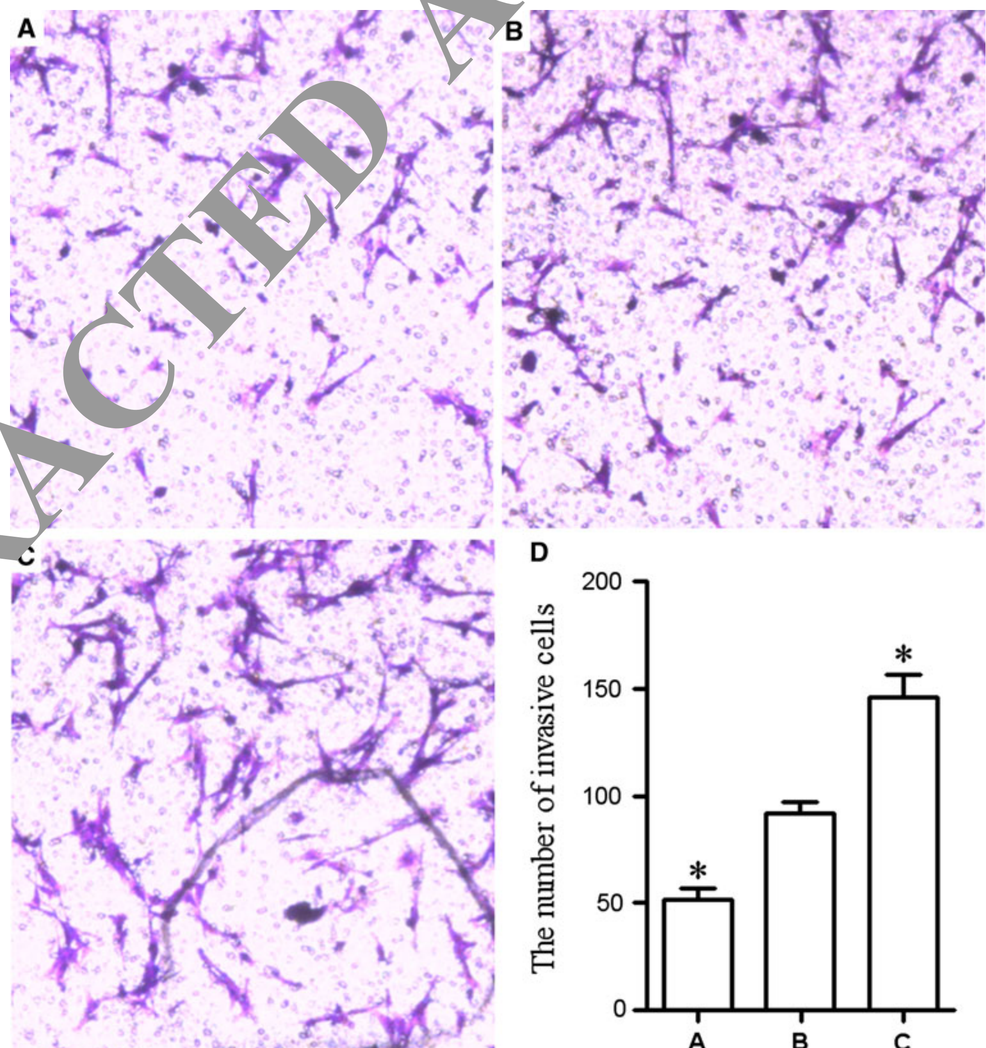


Fig. 5 The effect of TRAF6 on invasion ability of U-87MG cells. **a–c** The crystal violet staining of the U-87MG cells that passed through the polycarbonate membrane (*A* knockdown group, *B* blank group, *C* overexpression group), **d** the number of cells passed through transwell invasion chamber (* indicates $p < 0.05$ compared to blank group)

RETRACTED ARTICLE

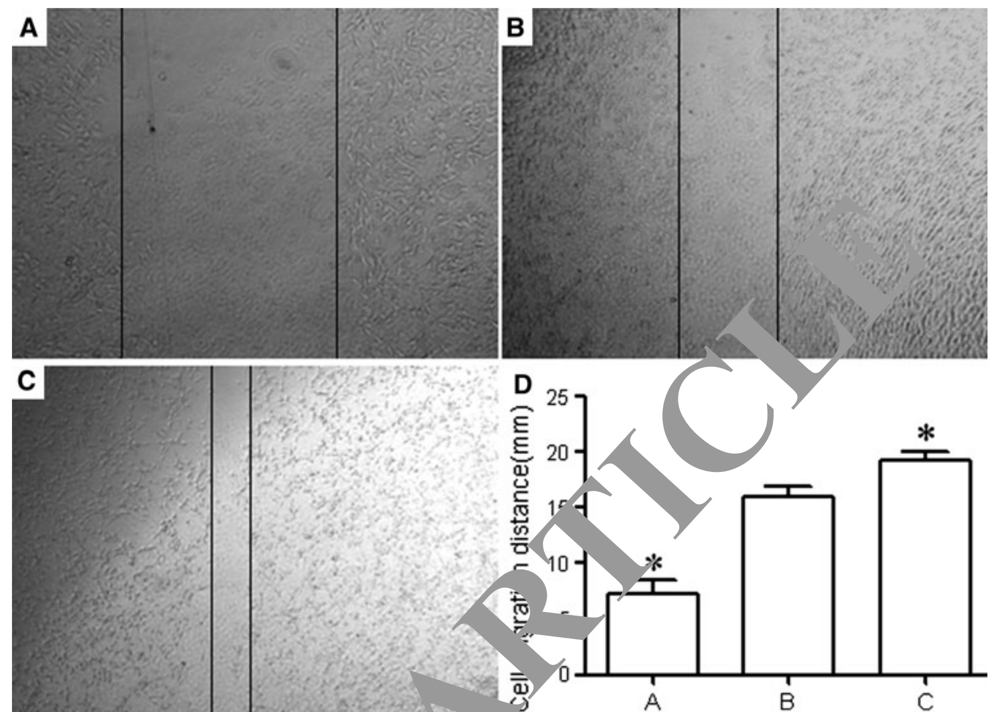


Fig. 6 The effect of TRAF6 on migration ability of U-87MG cells. **a–c** The migration distance of the U-87MG (*A* knockdown group, *B* blank group, *C* overexpression group), **d** statistical analysis of cell migration distances (* indicates $p < 0.05$ compared to blank group)

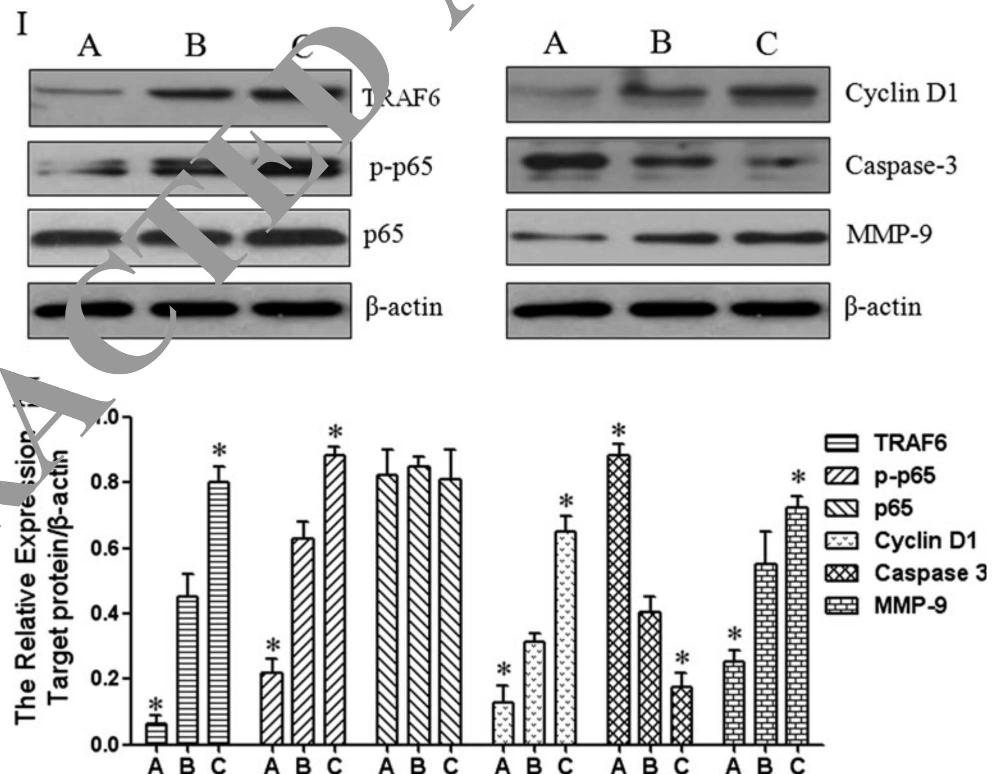


Fig. 7 The effects of TRAF6 on the expression of p-p65, p65, cyclin D1, caspase-3, and MMP-9. **a** p-p65, p65, cyclin D1, caspase-3, and MMP-9 protein expression in U-87MG cells, **b** relative expression of p-p65, p65, cyclin D1, caspase-3, and MMP-9 in U-87MG cells. (*A* knockdown group, *B* blank group, *C* overexpression group, * indicates $p < 0.05$ compared to blank group)

RETRACTED ARTICLE

invasion [29–31]. Therefore, in this study, we examined phosphor-NF-κB (p-p65), a key factor for the activation of NF-κB. NF-κB was a key transcription factor for the production of MMP-9 [36], which was believed to play a critical role in tumor invasion and metastasis [37–39]. Our results demonstrated that p-p65 and MMP-9 both displayed higher expression level in overexpression group and vice versa in knockdown group. Based on these data, we supposed that the inhibition effect of TRAF6 on tumor cell invasion were exerted possibly through downregulation of p-p65 and

RETRACTED ARTICLE

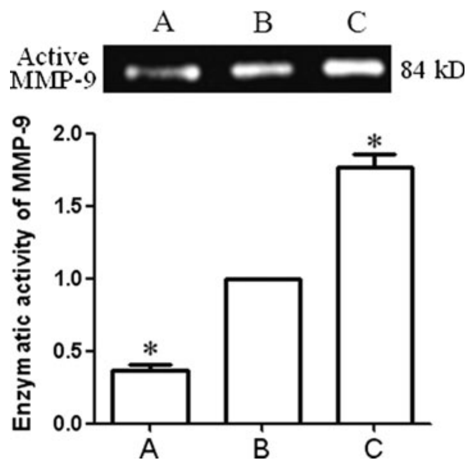


Fig. 8 The enzyme activity of MMP-9 was analyzed by gelatin zymography. (A: knockdown group, B: blank group, C: overexpression group, * indicates $p < 0.05$ compared to blank group)

MMP-9 in U-87MG cells. Of course, further investigation is warranted to dissect the exact mechanism.

In view of the above, we inferred that TRAF6 might be involved in the improvements of proliferation and invasion of U-87MG cells, as well as inhibition of apoptosis of U-87MG cells. However, further research is still needed to provide a good understanding of its function and mechanism.

References

1. Farias-Eisner G, Bank AM, Hwang BY, Appelboom G, Piazza MA, Bruce SS, Sander Connolly E (2012) Glioblastoma biomarkers from bench to bedside: advances and challenges. Br J Neurosurg 26:189–194
2. Walecki J, Tarasow E, Kubas B, Czemicki Z, Lewko J, Podgorski J, Sokol M, Grieb P (2003) Hydrogen-1 MR spectroscopy of the peritumoral zone in patients with cerebral glioma: assessment of the value of the method. Acad Radiol 10:145–153
3. Ferguson SD (2011) Malignant gliomas: diagnosis and treatment. Dis Mon 57:558–569
4. Rainov NG, Heidecke V (2011) Clinical development of experimental therapies for malignant glioma. Sultan Qaboos Univ Med J 11:5–28
5. Yamanaka R, Kajiwara K (2012) Dendritic cell vaccines. Adv Exp Med Biol 746:187–200
6. Jin HR, Jin SZ, Cai XF, Li D, Wu X, Nan JX, Lee JJ, Jin X (2012) Cryptopleurine targets NF-kappaB pathway, leading to inhibition of gene products associated with cell survival, proliferation, invasion, and angiogenesis. PLoS One 7:e40355
7. Liu HQ, Hu XY, Lu T, Cheng YN, Young CY, Yuan HQ, Lou HX (2012) Retigeric acid B exhibits antitumor activity through suppression of nuclear factor-kappaB signaling in prostate cancer cells in vitro and in vivo. PLoS One 7:e38000
8. Kang K, Lim JS (2012) Induction of functional changes of dendritic cells by silica nanoparticles. Immune Netw 12:104–112
9. Prasad S, Yadav VR, Sung B, Reuter S, Kannappan R, Deorukhkar A, Diagaradjane P, Wei C, Baladandayuthapani V, Krishnan S et al (2012) Ursolic acid inhibits growth and metastasis of human colorectal cancer in an orthotopic nude mouse model by targeting multiple cell signaling pathways: chemosensitization with capecitabine. Clin Cancer Res 18:4942–4953
10. Ge Y, Xu Y, Sun W, Man Z, Zhu L, Xia X, Zhao L, Zhao Y, Wang X (2012) The molecular mechanisms of the effect of dexamethasone and cyclosporin A on TLR4/NF-kappaB signaling pathway activation in oral lichen planus. Gene 508:157–164
11. Jia L, Gopinathan G, Sukumar JT, Gribben JG (2012) Blocking autophagy prevents bortezomib-induced NF-kappaB activation by reducing I-kappaBalpha degradation in lymphoma cells. PLoS One 7:e32584
12. Gupta SC, Sundaram C, Reuter S, Aggarwal BB (2010) Inhibiting NF-kappaB activation by small molecules as a therapeutic strategy. Biochim Biophys Acta 1799:775–787
13. Tsuchiya Y, Asano T, Nakayama K, Kato T Jr, Karin M, Kamata H (2010) Nuclear IKKbeta is an adaptor protein for IkappaBalpha ubiquitination and degradation in UV-induced NF-kappaB activation. Mol Cell 39:570–582
14. Tao T, Cheng C, Ji Y, Xu G, Zhang J, Zhang L, Shen A (2012) Numbl inhibits glioma cell migration and invasion by suppressing TRAF5-mediated NF-kappaB activation. Mol Biol Cell 23:2635–2644
15. Tran NL, McDonough WS, Savitch BA, Fortin SP, Winkles JA, Symons M, Nakada M, Cunliffe HE, Hostetter G, Hoelzinger DB et al (2006) Increased fibroblast growth factor-inducible 14 expression levels promote glioma cell invasion via Rac1 and nuclear factor-kappaB and correlate with poor patient outcome. Cancer Res 66:9535–9542
16. Sarkar D, Park ES, Emdad L, Lee SG, Su ZZ, Fisher PB (2008) Molecular basis of nuclear factor-kappaB activation by astrocyte elevated gene-1. Cancer Res 68:1478–1484
17. Sung B, Pandey MK, Nakajima Y, Nishida H, Konishi T, Chaturvedi MM, Aggarwal BB (2008) Identification of a novel blocker of IkappaBalpha kinase activation that enhances apoptosis and inhibits proliferation and invasion by suppressing nuclear factor-kappaB. Mol Cancer Ther 7:191–201
18. Ichikawa H, Takada Y, Murakami A, Aggarwal BB (2005) Identification of a novel blocker of I kappa B alpha kinase that enhances cellular apoptosis and inhibits cellular invasion through suppression of NF-kappa B-regulated gene products. J Immunol 174:7383–7392
19. Schneider M, Zimmermann AG, Roberts RA, Zhang L, Swanson KV, Wen H, Davis BK, Allen IC, Holl EK, Ye Z et al (2012) The innate immune sensor NLRC3 attenuates Toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-kappaB. Nat Immunol 13:823–831
20. Maruyama K, Kawagoe T, Kondo T, Akira S, Takeuchi O (2012) TRAF family member-associated NF-kappaB activator (TANK) is a negative regulator of osteoclastogenesis and bone formation. J Biol Chem 287:29114–29124
21. Zhou F, Zhang X, van Dam H, Ten Dijke P, Huang H, Zhang L (2012) Ubiquitin-specific protease 4 mitigates toll-like/interleukin-1 receptor signaling and regulates innate immune activation. J Biol Chem 287:11002–11010
22. Hamidi A, von Bulow V, Hamidi R, Winssinger N, Barluenga S, Heldin CH, Landstrom M (2012) Polyubiquitination of transforming growth factor beta (TGFbeta)-associated kinase 1 mediates nuclear factor-kappaB activation in response to different inflammatory stimuli. J Biol Chem 287:123–133
23. Wang HJ, Ruan HJ, He XJ, Ma YY, Jiang XT, Xia YJ, Ye ZY, Tao HQ (2010) MicroRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion. Eur J Cancer 46:2295–2303
24. Jacobs W, Mikkelsen T, Smith R, Nelson K, Rosenblum ML, Kohn EC (1997) Inhibitory effects of CAI in glioblastoma growth and invasion. J Neurooncol 32:93–101

25. Meng QH, Zhou LX, Luo JL, Cao JP, Tong J, Fan SJ (2005) Effect of 7-hydroxystaurosporine on glioblastoma cell invasion and migration. Acta Pharmacol Sin 26:492–499
26. Walsh MC, Kim GK, Maurizio PL, Molnar EE, Choi Y (2008) TRAF6 autoubiquitination-independent activation of the NFkappaB and MAPK pathways in response to IL-1 and RANKL. PLoS One 3:e4064
27. Inubushi T, Kawazoe A, Miyauchi M, Kudo Y, Ao M, Ishikado A, Makino T, Takata T (2012) Molecular mechanisms of the inhibitory effects of bovine lactoferrin on lipopolysaccharide-mediated osteoclastogenesis. J Biol Chem 287:23527–23536
28. Hartupee J, Li X, Hamilton T (2008) Interleukin 1alpha-induced NFkappaB activation and chemokine mRNA stabilization diverge at IRAK1. J Biol Chem 283:15689–15693
29. Lim KH, Yang Y, Staudt LM (2012) Pathogenetic importance and therapeutic implications of NF-kappaB in lymphoid malignancies. Immunol Rev 246:359–378
30. Baldwin AS (2012) Regulation of cell death and autophagy by IKK and NF-kappaB: critical mechanisms in immune function and cancer. Immunol Rev 246:327–345
31. Ling J, Kumar R (2012) Crosstalk between NF-kB and glucocorticoid signaling: a potential target of breast cancer therapy. Cancer Lett 322:119–126
32. Zhong L, Cao F, You Q (2012) Effect of TRAF6 on the biological behavior of human lung adenocarcinoma cell. Tumour Biol. doi:10.1007/s13277-012-0543-8
33. Liu H, Zhang T, Ye J, Li H, Huang J, Li X, Wu B, Huang X, Hou J (2012) TNF receptor-associated factor 6 in advanced non-small cell lung cancer: clinical and prognostic implications. J Cancer Res Clin Oncol 138:1853–1863
34. Chaudhry SI, Hooper S, Nye E, Williamson P, Harrington K, Sahai E (2012) Autocrine IL-1beta-TRAF6 signalling promotes squamous cell carcinoma invasion through paracrine TNFalpha signalling to carcinoma-associated fibroblasts. Oncogene. doi: 10.1038/onc.2012.91
35. Meng Q, Zheng M, Liu H, Song C, Zhang W, Yan [illegible], Qin L, Liu X (2012) TRAF6 regulates proliferation, apoptosis, and invasion of osteosarcoma cell. Mol Cell Biochem 371:[illegible]
36. Li YF, Xu XB, Chen XH, Wei G, He B, Wang [illegible] (2012) The nuclear factor-kappaB pathway is involved in matrix metalloproteinase-9 expression in RU486-induced endometrium breakdown in mice. Hum Reprod 27:[illegible]
37. Cock-Rada AM, Medjkane S, Janski N, Yousfi N, Perichon M, Chaussepied M, Chluba J, Langsley G, Weitzman JB (2012) SMYD3 promotes cancer invasion by epigenetic upregulation of the metalloproteinase MMP-9. Cancer Res 72:810–820
38. Yang L, Zeng W, Li D, [illegible] R (2009) Inhibition of cell proliferation, migration and invasion by DNAzyme targeting MMP-9 in A549 cells. Oncol Rep 22:121–126
39. Jee BK, Park KM, Surendran S, Lee WK, Han CW, Kim YS, Lim Y (2006) KAI1/CD82 suppresses tumor invasion by MMP9 inactivation via [illegible] regulation in the H1299 human lung carcinoma cell line. Biochem Biophys Res Commun 342:655–661

RETRACTED ARTICLE