Biochemical and Biophysical Research Communications 458 (2015) 476–482

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Over-expression of tetraspanin 8 in malignant glioma regulates tumor cell progression

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ARTICLE INFO

*Article history:*
Received 13 January 2015
Available online 11 February 2015

*Keywords:*
Malignant glioma
Tetraspanin 8
Proliferation
Migration
Temozolomide resistance
Focal adhesion kinase

ABSTRACT

Tumor cell invasion and proliferation remain the overwhelming causes of death for malignant glioma patients. To establish effective therapeutic methods, new targets implied in these processes have to be identified. Tetraspanin 8 (Tspn8) forms complexes with a large variety of trans-membrane and/or cytosolic proteins to regulate several important cellular functions. In the current study, we found that Tspn8 was over-expressed in multiple clinical malignant glioma tissues, and its expression level correlated with the grade of tumors. Tspn8 expression in malignant glioma cells (U251MG and U87MG lines) is important for cell proliferation and migration. siRNA-mediated knockdown of Tspn8 markedly reduced *in vitro* proliferation and migration of U251MG and U87MG cells. Meanwhile, Tspn8 silencing also increased the sensitivity of temozolomide (TMZ), and significantly increased U251MG or U87MG cell death and apoptosis by TMZ were achieved with Tspn8 knockdown. We observed that Tspn8 formed a complex with activated focal adhesion kinase (FAK) in both human malignant glioma tissues and in above glioma cells. This complexation appeared required for FAK activation, since Tspn8 knockdown inhibited FAK activation in U251MG and U87MG cells. These results provide evidence that Tspn8 contributes to the pathogenesis of glioblastoma probably by promoting proliferation, migration and TMZ-resistance of glioma cells. Therefore, targeting Tspn8 may provide a potential therapeutic intervention for malignant glioma.



## 1. Introduction

Malignant glioma is one of the most aggressive human malignancies, its overall survival is only 6–9 months [1]. A number of elements contribute to the poor prognosis of this devastating disease, such as late diagnosis, absence of specific diagnostic markers, resistance of traditional therapy, and the high proliferation or infiltration potential of the tumor cells [2,3]. Clinically, temozolomide (TMZ) is the only approved single chemo-treatment for malignant glioma, which only shows limit pro-surviving value [2]. Tumor cell invasion/infiltration and proliferation remain the overwhelming causes of death for malignant glioma cancer patients. To establish effective therapeutic methods, new targets implied in these processes have to be identified [4].

Tetraspanins are a family of four transmembrane proteins, which form complexes with a large variety of transmembrane and/or cytosolic proteins to regulate several important cellular functions [5,6,7]. Tetraspanins act as 'molecular facilitators' to modulate, stabilize or inhibit activities of associated molecules [6]. For example, tetraspanins regulate integrin compartmentalization, internalization, recycling and signaling to promote cell spreading, migration and cable formation [8]. They are also important component for cell adhesion and invasion [9,10]. Recent studies have discovered important roles of tetraspanins in progression of several tumor cells [11,12].

*Abbreviations:* IP, co-immunoprecipitation; FAK, focal adhesion kinase; FACS, fluorescence-activated cell sorting; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IHC, immunohistochemistry; TMZ, temozolomide; Tspn8, tetraspanin 8.

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http://dx.doi.org/10.1016/j.bbrc.2015.01.128
0006-291X/

Of all tetraspanins, Tspn8 expression is relatively restricted to a small number of tissues, such as colon and stomach epithelia. Studies have reported Tspn8 expression in several tumors including colon, liver, prostate, ovarian and cervical cancers [13]. Greco et al. showed that Tspn8 expression is associated with a poor prognosis in colorectal cancer [14]. Similar observations have been made for esophageal carcinoma [15], hepatocellular carcinoma [16] and melanoma [17]. A recent study by Ailane et al. showed that a mouse monoclonal antibody against Tspn8 inhibited colorectal cancer HT-29 *in vivo* growth in a nude mouse model [13]. In the current study, we found that Tspn8 is over-expressed in human malignant glioma tissues, its expression in malignant glioma cells (U251MG and U87MG lines) is important for cell migration, proliferation and TMZ-resistance.

## 2. Material and methods

### 2.1. Cell culture and reagents

Human glioma cell lines, U251MG and U87MG, were purchased from the Chinese Academy of Sciences Cell Bank. Glioma cells were maintained in a 37 °C, 5% $CO_2$ incubator in DMEM supplemented with 10% fetal bovine serum (FBS) and were routinely passaged at 2- to 3-day intervals. Temozolomide (TMZ) was purchased from Sigma–Aldrich Chemicals (Sigma, St. Louis, MO). Antibodies to focal adhesion kinase (FAK) (sc-271195), Tspn8 (sc-292058) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, sc-365062) were purchased from Santa Cruz Biotech (Santa Cruz, CA). Anti-FAK-Py397-phosphospecific antibody was obtained from Cellular Signaling Tech (Beverly, MA).

### 2.2. Cell death detection

Following treatments, cells were harvested with trypsin/EDTA, suspended in PBS, and mixed with an equal amount of 0.4% trypan blue stain (Invitrogen). Viable cells maintained membrane integrity and did not take up trypan blue. Cells with compromised cell membranes took up trypan blue, and were counted as dead. Cells were counted in five different fields, and the number of dead cells was calculated as percentage of the total cell population.

### 2.3. Colony formation assay

U251MG cells were trypsinized and plated (400 cells/well) in 6 well tissue culture plates. Cells were incubated in 5% $CO_2$ at 37 °C for a total of three weeks, and colonies were washed, fixed and stained with 0.005% crystal violet in methanol. Numbers of colonies were manually counted.

### 2.4. Apoptosis FACS analysis

After treatment, cells were washed in PBS, and fixed with ice-cold 70% ethanol overnight. Cells were then suspended in PBS containing RNase A (100 μg/mL), propidium iodide (50 μg/mL), Annexin V-FITC (50 μg/mL) and 0.1% Triton X-100, and incubated in the dark for 1 h. Fluorescence-activated cell sorting (FACS) was performed to quantify the apoptotic population showed by Annexin V staining.

### 2.5. Apoptosis ELISA assay

Apoptosis in U251MG/U87MG cells was also measured using the Cell Death Detection ELISA PLUS kit (Roche Diagnostics), which detects histone-complexed DNA fragments in cell cytoplasm [18]. Cells were seeded into a 96-well dish at a density of $1 \times 10^4$ cells/well. Following applied treatments, cell lysates were prepared following the manufacturer's procedure, and incubated with an anti-histone, biotin-conjugated antibody in a microplate with streptavidin-coated walls. After washing and incubation with peroxidase substrate, histone-complexed DNA fragments in the samples were quantified by reading the plate at 405 nm on a BenchMark™ Plus microplate spectrophotometer (Bio-Rad, Nanjing, China).

### 2.6. BrdU incorporation assay

U251MG cell ($1 \times 10^5$ cells/well) were seeded in 0.5 ml DMEM containing 10% FBS onto the 48-well tissue culture plates. 72 h after culture, the cell proliferation was assessed using BrdU incorporation though the BrdU ELISA colorimetric assay (Roche, Indianapolis, IN) according to the manufacturer's protocol. The ELISA OD value was recorded as indicator of cell proliferation. Each condition was tested in triplicate.

### 2.7. Western blot analysis

Cells were lysed in lysis buffer (30 mM Tris–HCl pH 8.0, 150 mM NaCl, 1% NP-40, 1 mM phenylmethylsulfonylfluoride and protease inhibitor cocktail) on ice for 30 min centrifuged at 18,000× *g* for 15 min at 4 °C, and the supernatants were collected as the cell lysates. Equal amounts of protein from each sample were separated by SDS-PAGE and transferred to PVDF membranes. Following incubation of indicated primary antibodies and secondary antibodies, specific bands were visualized using enhanced chemiluminescence (ECL) reagents for Western blot analysis (Amersham Pharmacia Biotech, Piscataway, NJ). Band intensity was quantified through total gray using the ImageJ software.

### 2.8. Co-immunoprecipitation (IP)

Aliquots of 1000 μg of proteins (in 1 ml of lysis buffer) of glioma tissues or in U251MG/U87MG cells were pre-cleared by incubation with 30 μl of protein A/G Sepharose (beads) (Sigma) for 1 h at 4 °C. The pre-cleared samples were incubated with the anti-Tspn8 (1 μg/ml) in lysis buffer overnight at 4 °C. Thereafter, 30 μl of protein A/G beads were added, and the samples were incubated for 2 h at 4 °C. The beads were washed five times with PBS (4 °C) and once with the lysis buffer, boiled, separated by 10% SDS-PAGE, and transferred onto a PVDF membrane followed by Western blotting analysis of p-FAK and FAK.

### 2.9. In vitro migration "transwell" assay

U251MG/U87MG cells ($1 \times 10^6$) were seeded into the upper compartment (Costar). Each polycarbonate filter had been coated with 10 μl of 0.5% Matrigel before the addition of cells. After 18 h of incubation at 37 °C in 5% $CO_2$, the cells on the underside of the chamber were fixed, stained (HE stain), and photographed. The five visual fields were photographed in every membrane, with manual counting of stained cells. All samples were run in triplicate.

### 2.10. Tspn8 gene knockdown with siRNA

U251MG/U87MG cells were seeded into six-well plates at $2 \times 10^5$ cells per well, grown for 24 h in complete medium and then transfected with ON-TARGET plus Smart pool siRNA specific to Tspn8 or scrambled siRNA-negative control (Dharmacon, Chicago, IL, USA) at a final concentration of 100 nM using Hyperfect transfection reagent (Qiagen, Courtaboeuf Cedex, France) according to the manufacturer's directions. Note that two different siRNAs

against non-overlapping mRNA sequence of Tspn8 were applied. To exam RNAi efficiency, cells were harvested 2 days after siRNA transfection and analyzed by Western blots as described above.

### 2.11. Patient's pancreatic cancer tissues isolation and preparation

Surgery isolated fresh malignant glioma tissues of different grade (1–4) and their surrounding normal tissues were homogenized and lysed, proteins were isolated, and expression and association of list proteins in lysed tissues were examined by western blots and Co-IP respectively. The study was approved by the institutional review board of all authors' affiliations, and written informed consent was obtained from each participating patient. All clinical investigations were conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). The privacy rights of human subjects are always observed.

### 2.12. Immunohistochemistry (IHC)

The staining was performed on cryostat sections (3 μm) of indicated tissues according to standard methods. Slides were incubated in the appropriate dilutions of primary antibody (anti-Tspn8, 1:100) and subsequently stained them with horseradish peroxidase (HRP)-coupled secondary antibody (Santa Cruz). We visualized peroxidase activity using 3-amino-9-ethyl-carbazol (AEC) and counterstained tissues with MAYER'S hemalaun solution (Merck).

### 2.13. Statistical analysis

All statistics were calculated using SPSS Graduate Pack 11.0 statistical software (SPSS, Chicago, IL). Descriptive statistics including mean and SD along with one-way ANOVAs were used to determine significant differences. $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Tspn8 is over-expressed in human malignant gliomas

First, we examined the expression of Tspn8 in human malignant glioma tissues. As shown in Fig. 1A, in all human malignant glioma tissues ("T"), expression of Tspn8 was significantly higher than that in surrounding normal tissues ("N"). Tspn8 protein expression in tumor tissues was 2–3 times higher than that in normal tissues (Fig. 1B, each contained 12 tissues per group). Meanwhile, Tspn8 level was closely correlated the grade of the tumor, and gliomas of Grade 3–4 showed significant higher expression of Tspn8 than

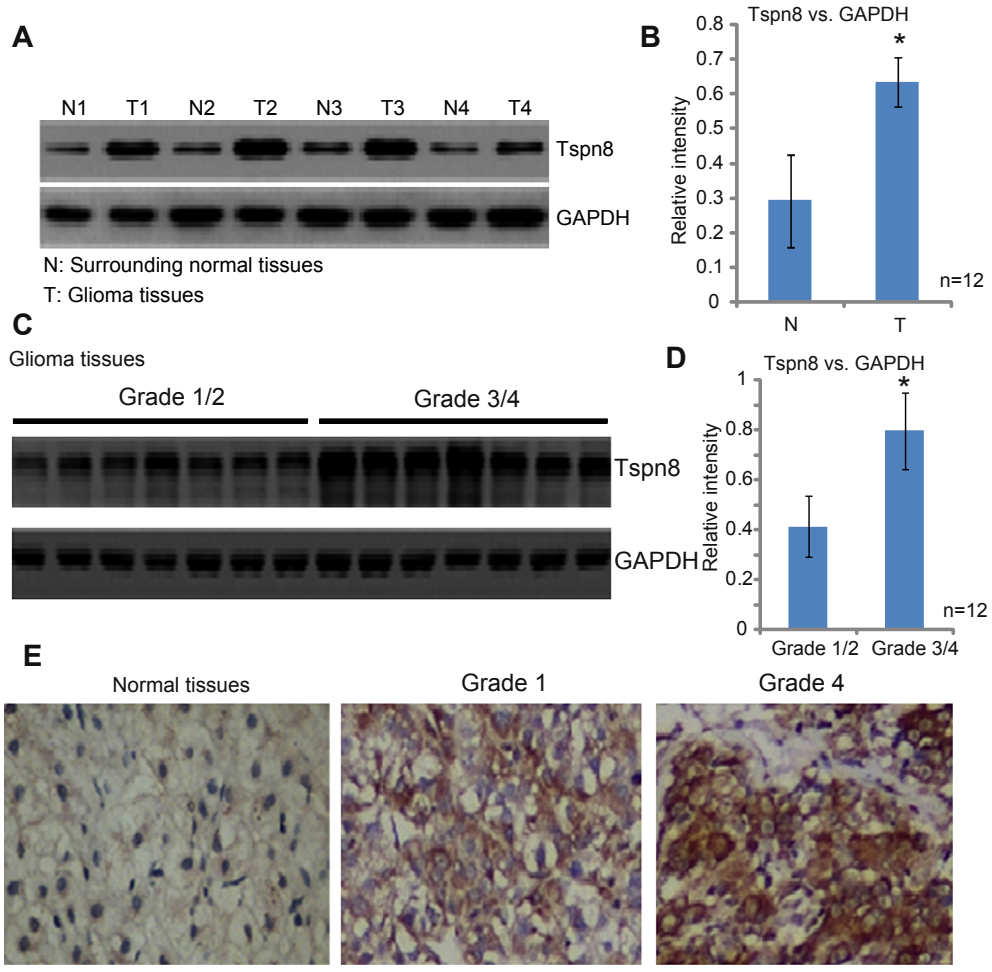


**Fig. 1.** Tspn8 is over-expressed in human malignant gliomas. Tspn8 and GAPDH (the loading control) expression in different human malignant glioma tissues ("T") or in surrounded normal tissues ("N") was tested by Western blots (A and C), relative Tspn8 expression (vs. GAPDH, 12 clinical tissues for each group) was quantified (B and D). Representative IHC images showed Tspn8 protein expression in human malignant glioma tissues or in normal tissue (E). Data were presented as mean ± SD. $^*p < 0.05$ vs. surrounding normal tissue group (B). $^*p < 0.05$ vs. low-grade tumors (D).

tumors of grade 1–2 (Fig. 1C), and data of 12 glioma tissues per group were combined for quantification (Fig. 1D). Representative IHC images in Fig. 1E further confirmed Tspn8 over-expression in human malignant gliomas, especially in the high grade one (Fig. 1E). Together, we show that Tspn8 expression is elevated in human malignant glioma tissues.

### 3.2. siRNA-knockdown of Tspn8 inhibits glioma cell proliferation and migration in vitro

Above results clearly demonstrated that Tspn8 is over-expressed in human glioma tissues, and its expression level corrects with the progression (grade) of the tumor. To study the potential role of

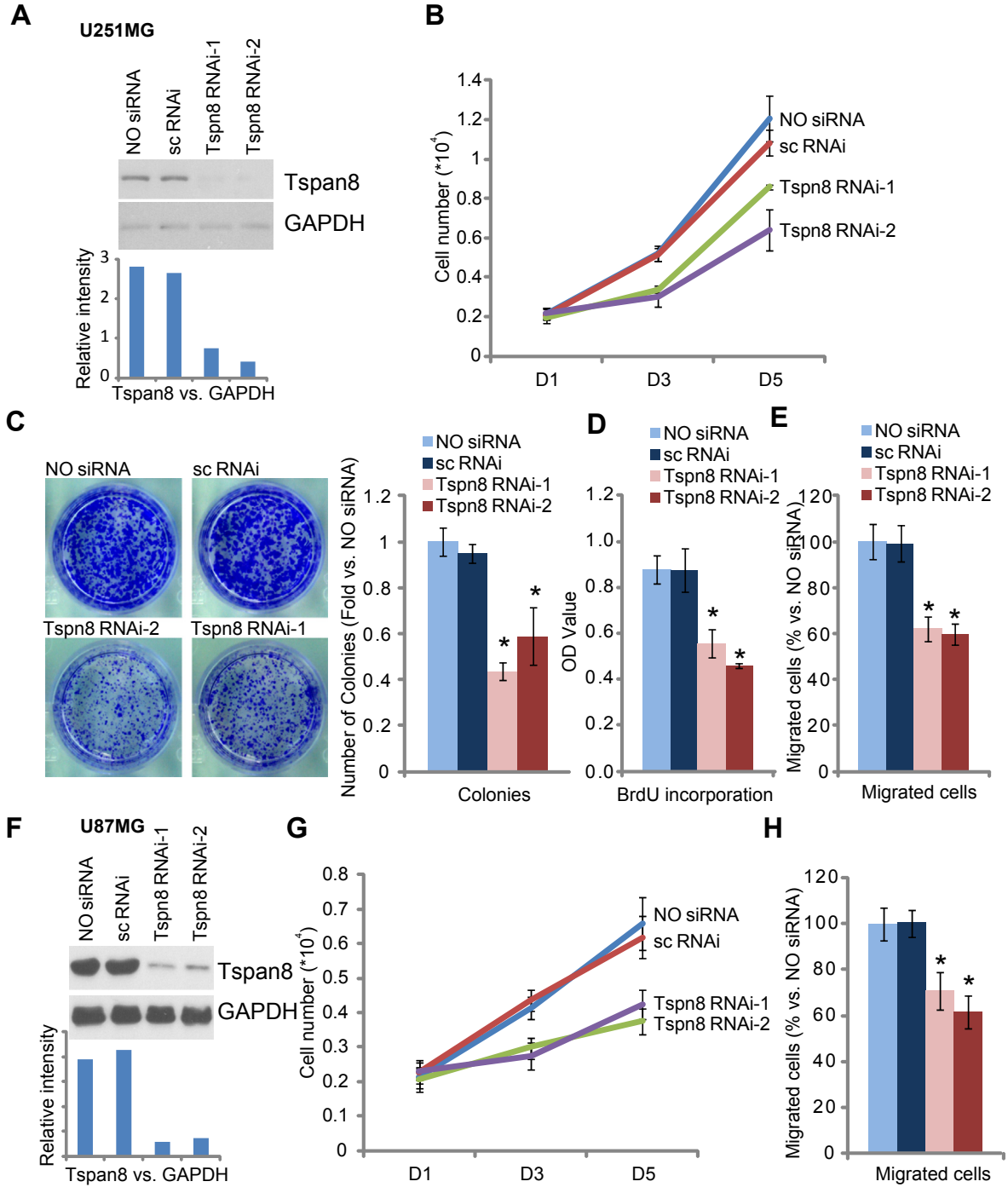


**Fig. 2.** siRNA-knockdown of Tspn8 inhibits glioma cell proliferation and migration *in vitro*. Tspn8 and GAPDH expression in un-transfected control U251MG/U87MG cells ("NO siRNA") or U251MG/U87MG cells transfected with indicated siRNA (100 nM each, 48 h) was tested by Western blots (A and F); Relative Tspn8 expression (vs. GAPDH) was quantified (A and F). Same amount of above U251MG/U87MG cells ($0.2 \times 10^4$ each), with or without indicated siRNA, were maintained in growth medium, cells were further cultured for 3 and 5 days, cell number was counted (B and G). Above U251MG cells were also subjected to colony formation assay (C, 3 weeks) or BrdU incorporation assay (D, 72 h) to test cell proliferation. Representative colony formation images were shown (C). *In vitro* migration of the U251MG/U87MG cells with or without indicated siRNA was also tested by transwell assay described (E and H). "sc-RNAi" stands for scramble control siRNA. Experiments in this and all following figures were repeated three times, with similar results obtained. Data were presented as mean ± SD. $^*p < 0.05$ vs. sc-RNAi group.

Tspn8 in glioma cells, siRNA strategy was then applied. Two non-overlapping Tspn8 siRNAs, each corresponding to non-overlapping sequence of Tspn8 mRNA, were transfected into U251MG cells to selectively knockdown Tspn8. Western blot results in Fig. 2A demonstrated that both siRNAs (-1 and -2) dramatically downregulated Tspn8 expression in U251MG cells, with the knockdown efficiency over 80% (Fig. 2A, quantification). U251MG cell proliferation, tested by simple cell counting (Fig. 2B), clonogenicity assay (Fig. 2C) and BrdU incorporation assay (Fig. 2D), was significantly inhibited by the two Tspn8 RNAis. Transwell assay results in Fig. 2E showed that U251MG cell *in vitro* migration was also inhibited with Tspn8 siRNA knockdown. Note that Transwell assay was performed for only 18 h, when no significant cell proliferation inhibition was observed (Data not shown). Similar results were also repeated in U87MG cells, where Tspn8 siRNAs (Fig. 2F) dramatically inhibited cell growth (Fig. 2G) and *in vitro* migration (Fig. 2H).

### 3.3. siRNA-knockdown of Tspn8 increases TMZ sensitivity

The efficiency of the current standard TMZ or TMZ-based regimens is moderate, probably due to pre-existing or acquired resistance of the glioma cells. The majority of phase III trials exploring TMZ-based combinations have been failed [4]. We next tested whether Tspn8 played a role in TMZ resistance. As shown in Fig. 3A and B, TMZ (100 μM) alone only induced minor cell death (less than 20%, tested by trypan blue staining assay) and apoptosis (less than 10%, tested by Annexin V FACS assay) in U251MG cells (Fig. 3A and B), which were both significantly aggravated by Tspn8 silencing (Fig. 3A and B). Histone-DNA apoptosis ELISA assay further confirmed the apoptosis-sensitization effect by Tspn8 knockdown in U251MG cells (Fig. 3C). We also repeated the experiments in U87MG cells, Tspn8 siRNAs (-1/-2) facilitated TMZ-induced cell death (Fig. 3D, trypan blue assay), and cell apoptosis (Fig. 3F, histone-DNA apoptosis ELISA assay). These results suggest that

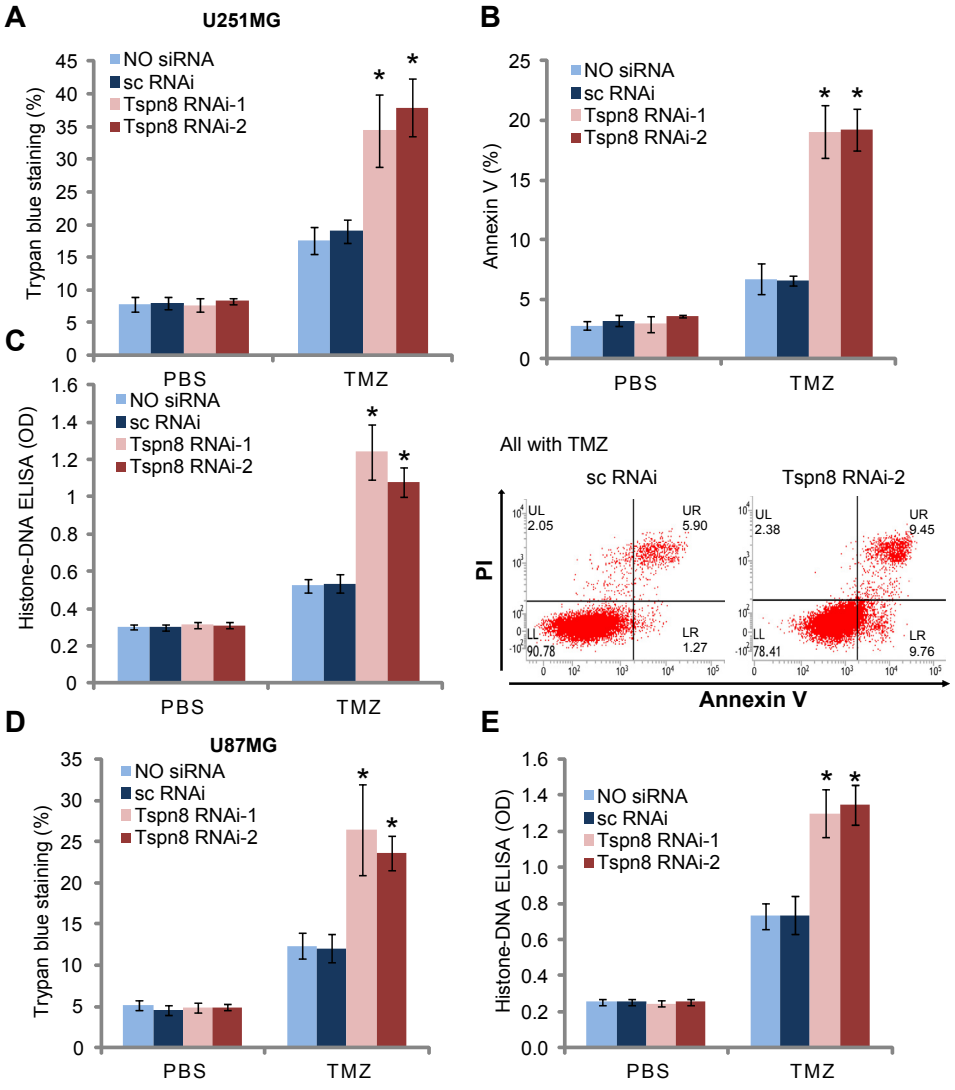


**Fig. 3.** siRNA-knockdown of Tspn8 facilitates TMZ-induced cytotoxicity in glioma cells. Un-transfected control U251MG/U87MG cells ("NO siRNA") as well as U251MG/U87MG cells transfected with scramble siRNA (sc-RNAi) or Tspn8 siRNA (-1 or -2) (100 nM each, 48 h) were treated with TMZ (100 μM), cells were further cultured, cell death was tested by trypan blue staining assay (A and D, 72 h); Cell apoptosis was tested by Annexin V FACS assay (B, for U251MG cells, 48 h) or Histone-DNA ELISA plus assay (C and F, 48 h). Representative Annexin V FACS images for U251MG cells were shown, all with TMZ treatment (B). Data were presented as mean ± SD. "sc-RNAi" stands for scramble control siRNA. $^{*}p < 0.05$ vs. sc-RNAi group.

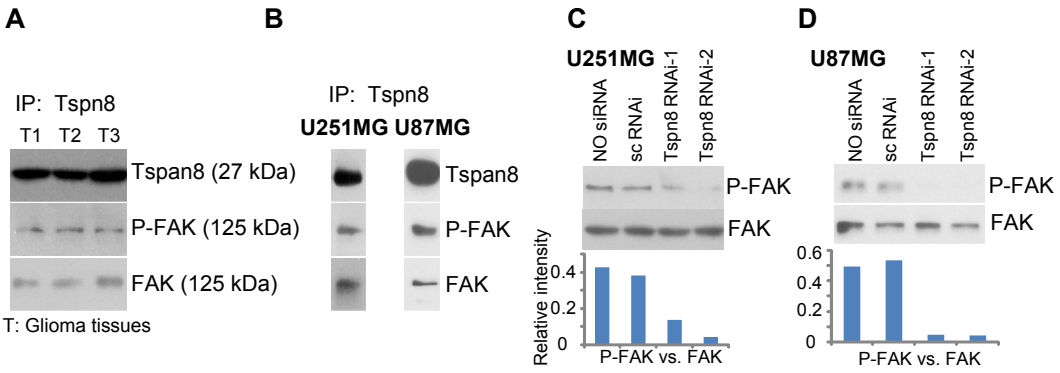


**Fig. 4.** Tspn8 forms a complex with FAK, required for FAK activation. Tspn8 and FAK (both regular and phosphorylated) association in three human glioma tissues (A) and in U251MG/U87MG cells (B) was tested by Co-IP assay. Expression of FAK (both regular and phosphorylated) in un-transfected control U251MG/U87MG cells ("NO siRNA") as well as U251MG/U87MG cells transfected with scramble siRNA (sc-RNAi) or Tspn8 siRNA (-1 or -2) (100 nM each, 48 h) was shown (C and D), FAK phosphorylation was quantified (C and D).

Tspn8 might be important for TMZ resistance in glioma cells, and knockdown of Tspn8 could increase TMZ sensitivity.

### 3.4. *Tspn8 forms a complex with FAK, required for FAK activation*

Above results indicate that Tspn8 is important for U251MG/U87MG cell progression. Thus, we studied the potential underlying mechanisms. Focal adhesion kinase (FAK) is an important component of the integrin-mediated signal transduction pathway and plays a vital role in modulating cell migration and proliferation [19,20,21]. FAK is activated through tyrosine phosphorylation. Its phosphorylation on tyrosine-397 is necessary for FAK activation and FAK-induced cell adhesion, migration and proliferation [19,20,21]. Co-IP results showed that Tspn8 associated with regular- and activated- FAK in human glioma tissues (Fig. 4A) as well as in U251MG cells and U87MG cells (Fig. 4B). This association appeared important for FAK activation, as knockdown of Tspn8 by siRNA inhibited phosphorylation of FAK at tyrosine-397 in U251MG (Fig. 4C) and U87MG cells (Fig. 4D). Thus, these results show that Tspn8 forms a complex with FAK, which is required for FAK activation.

## 4. Discussions

Tspn8, also known as C0-029, was first identified as a tumor-associated antigen present at high levels in several types of human carcinoma and sarcoma [11,22]. Its overexpression correlates with progression of several cancers, thus representing a marker of poor prognosis [11,22]. In this current study, we propose that Tspn8 might be an important oncogene for malignant glioma; Our evidences include that Tspn8 is over-expressed in multiple clinical malignant glioma tissues, and its expression is low in surrounding normal brain tissues. Second, the expression level of this molecular is correlated with the grade of tumors. Third, Tspn8 expression in malignant glioma cells (U251MG and U87MG lines) regulates cell proliferation and migration, and knocking down of Tspn8 by targeted siRNAs suppressed U251MG/U87MG proliferation and *in vitro* migration. Fourth, Tspn8 silencing significantly increased TMZ sensitivity in malignant glioma cells. Fifth, Tspn8 forms a complex with activated FAK in glioma tissues or cells, knocking down of Tspn8 inhibited activation of FAK in U251MG and U87MG cells. All these evidences point out an important role of Tspn8 of malignant glioma progression.

Very little is known about the mechanisms of Tspn8-mediated cell migration. It has been suggested that Tspn8 promotes cell motility and proliferation of metastatic cancer cell lines, mostly through its association with α6β4 integrins and CD-151 [23,24]. Others showed that Tspn8 association with α3β1 and α6β1 integrins regulates spread of tumor cells [25,26]. An interesting finding in this study is that Tspn8 was in the complex with activated FAK in both human malignant glioma tissues and in U251MG and U87MG glioma cells, and siRNA knockdown of Tspn8 reduced FAK activation in U251MG and U87MG cells. Thus, Tspn8 mediates malignant behaviors of glioma cells probably through regulating FAK activation.

Significantly, we found that Tspn8, besides regulating cell migration, was also important for glioma cell proliferation. Our observations are in line with very recent studies showing that required of Tspn8 for proliferation of carcinoma cells [13,17]. Another important finding of this study is that Tspn8 also played a role in TMZ resistance. Although the detailed mechanisms need further characterizations, these studies should provide valuable information for possibly improving TMZ activity in gliomas. In summary, the present findings highlight several new aspects of the role of Tspn8 in the glioma cell progression. Our results suggest that Tspn8 expression might be critical for glioma cell migration, proliferation as well as TMZ resistance. Although much is yet to be learned regarding the clinical relevance of its function, we suggest that Tspn8 could be a promising new therapeutic target in anti-glioma therapy.

## Conflict of interests

No conflict of interests were stated.

## Acknowledgments

Financial disclosure: This work was supported by National Natural Science of China (81441072), the fund of Shanghai Municipal Health Bureau (20124324) and the fund of Shanghai Charitable Cancer Research Center. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.01.128.

## References

[1] R. Siegel, J. Ma, Z. Zou, A. Jemal, Cancer statistics, 2014, CA Cancer J. Clin. 64 (2014) 9–29.

[2] F. Lefranc, M. Rynkowski, O. DeWitte, R. Kiss, Present and potential future adjuvant issues in high-grade astrocytic glioma treatment, Adv. Tech. Stand Neurosurg. 34 (2009) 3–35.
[3] Y. Wang, T. Jiang, Understanding high grade glioma: molecular mechanism, therapy and comprehensive management, Cancer Lett. 331 (2013) 139–146.
[4] M. Westphal, K. Lamszus, The neurobiology of gliomas: from cell biology to the development of therapeutic approaches, Nat. Rev. Neurosci. 12 (2011) 495–508.
[5] S. Charrin, F. le Naour, O. Silvie, P.E. Milhiet, C. Boucheix, E. Rubinstein, Lateral organization of membrane proteins: tetraspanins spin their web, Biochem. J. 420 (2009) 133–154.
[6] S. Levy, T. Shoham, The tetraspanin web modulates immune-signalling complexes, Nat. Rev. Immunol. 5 (2005) 136–148.
[7] S. Yue, W. Mu, M. Zoller, Tspan8 and CD151 promote metastasis by distinct mechanisms, Eur. J. Cancer 49 (2013) 2934–2948.
[8] F. Berditchevski, Complexes of tetraspanins with integrins: more than meets the eye, J. Cell. Sci. 114 (2001) 4143–4151.
[9] N.E. Winterwood, A. Varzavand, M.N. Meland, L.K. Ashman, C.S. Stipp, A critical role for tetraspanin CD151 in alpha3beta1 and alpha6beta4 integrin-dependent tumor cell functions on laminin-5, Mol. Biol. Cell. 17 (2006) 2707–2721.
[10] M. Hasegawa, M. Furuya, Y. Kasuya, M. Nishiyama, T. Sugiura, T. Nikaido, Y. Momota, M. Ichinose, S. Kimura, CD151 dynamics in carcinoma-stroma interaction: integrin expression, adhesion strength and proteolytic activity, Lab. Invest 87 (2007) 882–892.
[11] M. Sala-Valdes, N. Ailane, C. Greco, E. Rubinstein, C. Boucheix, Targeting tetraspanins in cancer, Expert Opin. Ther. Targets 16 (2012) 985–997.
[12] S. Veenbergen, A.B. van Spriel, Tetraspanins in the immune response against cancer, Immunol. Lett. 138 (2011) 129–136.
[13] N. Ailane, C. Greco, Y. Zhu, M. Sala-Valdes, M. Billard, I. Casal, O. Bawa, P. Opolon, E. Rubinstein, C. Boucheix, Effect of an anti-human Co-029/tspan8 mouse monoclonal antibody on tumor growth in a nude mouse model, Front. Physiol. 5 (2014) 364.
[14] C. Greco, M.P. Bralet, N. Ailane, A. Dubart-Kupperschmitt, E. Rubinstein, F. Le Naour, C. Boucheix, E-cadherin/p120-catenin and tetraspanin Co-029 cooperate for cell motility control in human colon carcinoma, Cancer Res. 70 (2010) 7674–7683.
[15] Z. Zhou, Y.L. Ran, H. Hu, J. Pan, Z.F. Li, L.Z. Chen, L.C. Sun, L. Peng, X.L. Zhao, L. Yu, L.X. Sun, Z.H. Yang, TM4SF3 promotes esophageal carcinoma metastasis via upregulating ADAM12m expression, Clin. Exp. Metastasis 25 (2008) 537–548.
[16] K. Kanetaka, M. Sakamoto, Y. Yamamoto, S. Yamasaki, F. Lanza, T. Kanematsu, S. Hirohashi, Overexpression of tetraspanin CO-029 in hepatocellular carcinoma, J. Hepatol. 35 (2001) 637–642.
[17] O. Berthier-Vergnes, M.E. Kharbili, A. de la Fouchardiere, T. Pointecouteau, P. Verrando, A. Wierinckx, J. Lachuer, F. Le Naour, J. Lamartine, Gene expression profiles of human melanoma cells with different invasive potential reveal TSPAN8 as a novel mediator of invasion, Br. J. Cancer 104 (2011) 155–165.
[18] P. Salgame, A.S. Varadhachary, L.L. Primiano, J.E. Fincke, S. Muller, M. Monestier, An ELISA for detection of apoptosis, Nucleic Acids Res. 25 (1997) 680–681.
[19] J.T. Parsons, Focal adhesion kinase: the first ten years, J. Cell. Sci. 116 (2003) 1409–1416.
[20] A.P. Gilmore, L.H. Romer, Inhibition of focal adhesion kinase (FAK) signaling in focal adhesions decreases cell motility and proliferation, Mol. Biol. Cell. 7 (1996) 1209–1224.
[21] S.K. Mitra, D.A. Hanson, D.D. Schlaepfer, Focal adhesion kinase: in command and control of cell motility, Nat. Rev. Mol. Cell. Biol. 6 (2005) 56–68.
[22] C. Boucheix, G.H. Duc, C. Jasmin, E. Rubinstein, Tetraspanins and malignancy, Expert Rev. Mol. Med. 2001 (2001) 1–17.
[23] S. Gesierich, C. Paret, D. Hildebrand, J. Weitz, K. Zgraggen, F.H. Schmitz-Winnenthal, V. Horejsi, O. Yoshie, D. Herlyn, L.K. Ashman, M. Zoller, Colocalization of the tetraspanins, CO-029 and CD151, with integrins in human pancreatic adenocarcinoma: impact on cell motility, Clin. Cancer Res. 11 (2005) 2840–2852.
[24] M. Herlevsen, D.S. Schmidt, K. Miyazaki, M. Zoller, The association of the tetraspanin D6.1A with the alpha6beta4 integrin supports cell motility and liver metastasis formation, J. Cell. Sci. 116 (2003) 4373–4390.
[25] C. Claas, S. Seiter, A. Claas, L. Savelyeva, M. Schwab, M. Zoller, Association between the rat homologue of CO-029, a metastasis-associated tetraspanin molecule and consumption coagulopathy, J. Cell. Biol. 141 (1998) 267–280.
[26] K. Kanetaka, M. Sakamoto, Y. Yamamoto, M. Takamura, T. Kanematsu, S. Hirohashi, Possible involvement of tetraspanin CO-029 in hematogenous intrahepatic metastasis of liver cancer cells, J. Gastroenterol. Hepatol. 18 (2003) 1309–1314.