Pathol. Oncol. Res. (2012) 18:833–839
DOI 10.1007/s12253-012-9511-2

RESEARCH

Increased Expression of FAT10 is Correlated with Progression and Prognosis of Human Glioma

Jun Yuan · Yanyang Tu · Xinggang Mao · Shiming He · Liang Wang · Guoqiang Fu · Jianhai Zong · Yongsheng Zhang

Received: 27 November 2011 / Accepted: 20 February 2012 / Published online: 9 March 2012

Abstract FAT10, as a small ubiquitin-like modifier, plays an important role in various cellular processes, including mitosis, immune response, and apoptosis, the dys-regulation of which may arise tumorigenesis. Therefore, the aim of this study was to examine the expression of FAT10 at gene and protein levels in glioma samples with different WHO grades and its association with survival. One hundred and twenty-eight glioma specimens and 10 non-neoplastic brain tissues were collected. Immunohistochemistry assay, quantitative real-time PCR and Western blot analysis were carried out to investigate the expression of FAT10. Kaplan-Meier method and Cox's proportional hazards model were used in survival analysis. Immunohistochemistry showed that FAT10 protein was over-expressed in glioma tissues. FAT10 mRNA and protein levels were both higher in glioma compared to control on real-time PCR and Western blot analysis (both $P<0.001$). Additionally, its expression levels increase from grade I to grade IV glioma according to the results of real-time PCR, immunohistochemistry analysis and Western blot. Moreover, the survival rate of FAT10-positive patients was significantly lower than that of FAT10-negative patients ($P<0.001$). We further confirmed that the increased expression of FAT10 was a significant and independent prognostic indicator in glioma by multivariate analysis ($P<0.001$). Our data provides convincing evidence for the first time that the increased expression of FAT10 at gene and protein levels is correlated with poor outcome in patients with glioma. FAT10 may promote the aggressiveness of glioma and may be a potential prognosis predictor of glioma.

Keywords Glioma · FAT10 · Immunochemistry assay · Quantitative real-time PCR · Western blot analysis · Prognosis

Introduction

Human glioma is the most frequent neoplasm of the central nervous system [1]. According to the World Health Organization (WHO) guidelines [2], gliomas are histologically classified into four grades: pilocytic astrocytoma (grade I), low-grade diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III) and glioblastoma multiforme (GBM, grade IV). Both diagnostic technologies and therapeutic strategies have been greatly advanced, but glioma remains one of the deadliest human cancers. The 5-year survival rates of low-grade (grade I~II) and high-grade (grade III~IV) glioma patients in China are: 75.4 % and 18.2 %, respectively [3]. In particular, the median survival duration of patients with GBM ranges from 9 to 12 months [4]. There have been several prognostic factors for glioma patients, such as age, preoperative duration of symptoms, Karnofsky performance status (KPS) score, histologic grade, tumor

Jun Yuan and Yanyang Tu offer the equal contributions to this paper.

J. Yuan · Y. Zhang (✉)
Administrative Department, Tangdu Hospital,
Fourth Military Medical University,
Xi'an City 710038, People's Republic of China
e-mail: zhangys@fmmu.edu.cn

Y. Tu · G. Fu
Department of Emergency, Tangdu Hospital,
Fourth Military Medical University,
Xi'an City 710038, People's Republic of China

X. Mao
Department of Neurosurgery, 254th Hospital of PLA,
Tianjin City 300142, People's Republic of China

S. He · L. Wang · J. Zong
Department of Neurosurgery, Tangdu Hospital,
Fourth Military Medical University,
Xi'an City 710038, People's Republic of China

necrosis, surgical resection extent, use of postoperative radiation therapy and, probably, adjuvant chemotherapy. But these clinical parameters do not fully account for the observed variation in survival rates because of the heterogeneous of glioma patients [5]. Therefore, it is necessary to well understand the molecular mechanism of glioma development and to develop novel targeted approaches for management of this disease.

The dys-regulation of cell cycle and apoptosis often lead to tumorigenesis. It has been demonstrated that ubiquitin and ubiquitin-related families of proteins are involved in these cellular processes [6]. FAT10, as a member of this family, is also known as diubiquitin [7]. It is an 18 kDa protein comprising 165 amino acid residues and contains two ubiquitin-like domains joined by a short linker [8]. FAT10 was originally discovered through the identification of expressed genes covering the HLA-F genomic locus. It is encoded by the major histocompatibility (MHC) class I locus [9]. FAT10 expression is induced by tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). A potential role of FAT10 in cell-cycle regulation has been suggested by its ability to bind to MAD2, a protein responsible for maintaining spindle integrity during mitosis [10]. Recent studies have reported that the FAT10 gene is upregulated in various cancers. For example, Ji et al. [11] demonstrated that the expression level of FAT10 protein was significantly higher in gastric cancer than in its adjacent and normal tissue. The FAT10 level in gastric cancer tissue was significantly correlated with lymph node metastasis, tumor, nodes, metastasis (TNM) staging and poor overall survival rate of patients. Lukasiak et al. [12] found that FAT10 mRNA was significantly overexpressed in cancers of liver and colon, which was consistent with the findings of Qing et al. [13] and Lee et al. [14]. All these previous studies have been implicated the potential roles of FAT10 in tumorigenesis. However, little is known about the expression level of FAT10 or its prognostic significance in human gliomas.

In order to gain further insight into the status of FAT10 in the progression of glioma, we used immunohistochemistry assay, quantitative real-time PCR and Western blot analysis to investigate the expression pattern of FAT10 in glioma specimens and normal control brain tissues. Next, we analyzed the relationship between FAT10 expression and the glioma stage as well as the survival of patients.

Materials and Methods

Patients and Tissue Samples

This study was approved by the Research Ethics Committee of the Institute for functional neurosurgery P.L.A, TangDu Hospital, Fourth Military Medical University, Xi'an, P.R. China. Written informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards.

A total of 128 formalin-fixed, paraffin-embedded specimens of gliomas resected between 2000 and 2010 were retrieved from the archives of the Pathology Department of Tangdu Hospital, Fourth Military Medical University, P. R. China. All the slides were re-evaluated according to WHO classifications [2] by two pathologists, with differences resolved by careful discussion. A total of 76 males and 52 females (1.46:1) were enrolled in this study, and the median age was 42 years (range, 12–71). Thirty-two of the 128 gliomas were classified as low-grade [18 pilocytic astrocytomas (WHO I) and 14 diffuse astrocytomas (WHO II)], and 96 were classified as high-grade gliomas [38 anaplasia astrocytomas (WHO III), and 58 primary glioblastomas (WHO IV)]. None of the patients had received chemotherapy or radiotherapy prior to surgery. The clinicopathological features and the treatment strategies of all the patients were indicated in Table 1. Paraffin and snap-frozen sections of nonneoplastic brain tissues from 10 patients with intractable epilepsy were also included as controls. Five years follow-up was performed, and all patients had complete follow-up until death. Overall survival time was calculated from the date of the initial surgical operation to death. Patients, who died of diseases not directly related to their gliomas or due to unexpected events, were excluded from this study. In addition, 20 glioma specimens [5 pilocytic astrocytomas (WHO I), 3 diffuse astrocytomas (WHO II), 3 anaplasia astrocytomas (WHO III), and 9 primary glioblastomas (WHO IV)] were snap-frozen in liquid nitrogen and stored at −80 °C following surgery for mRNA and protein isolation.

Immunohistochemistry Assay

Immunohistochemical assay was performed using the conventional immunoperoxidase technique according to the protocol of the Department of Neurosurgery, Institute for functional neurosurgery P.L.A, TangDu Hospital, Fourth Military Medical University, Xi'an, P.R. China. Briefly, following peroxidase blocking with 0.3 % H_2O_2/methanol for 30 min, specimens were blocked with phosphate-buffered saline (PBS) containing 5 % normal horse serum (Vector Laboratories Inc., Burlingame, CA, USA). All incubations with a rabbit polyclonal antibody to FAT10 (ENZO Life Science Int Inc, Plymoth Meeting, PA) at 1:50 dilution were carried out overnight at 4 °C. Then the specimens were briefly washed in PBS and incubated at room temperature with the anti-rabbit antibody and avidin-biotin peroxidase (Vector Laboratories Inc., Burlingame, CA, USA). The specimens were then washed in PBS and color-developed by diaminobenzidine solution (Dako Corporation,

Table 1 FAT10 expression in human glioma tissues with different clinical-pathological features

Clinicopathological features	No. of cases	FAT10 (n, %)				
		−	+	++	+++	P
WHO grade						
I	18	12 (66.7)	6 (33.3)	0	0	<0.001
II	14	6 (42.9)	6 (42.9)	2 (4.2)	0	
III	38	4 (10.5)	18 (47.4)	14 (36.8)	2 (5.3)	
IV	58	0	0	15 (25.9)	43(74.1)	
Age						
<55	52	7 (13.5)	11 (21.2)	15 (28.8)	19 (36.5)	NS
≥55	76	15 (19.7)	19 (25.0)	16 (21.1)	26 (34.2)	
Gender						
Male	76	15 (19.7)	19 (25.0)	16 (21.1)	26 (34.2)	NS
Female	52	7 (13.5)	11 (21.2)	15 (28.8)	19 (36.5)	
KPS						
<80	78	2 (2.6)	15 (19.2)	23 (29.5)	38 (48.7)	0.008
≥80	50	20 (40.0)	15 (30.0)	8 (16.0)	7 (14.0)	

Carpinteria, CA, USA). After washing with water, specimens were counterstained with Meyer's hematoxylin (Sigma Chemical Co., St Louis, MO, USA). Non-neoplastic brain tissues were used as control tissues and non-immune IgG was also used as negative control antibody for immunohistochemical staining.

Stained sections were observed under a microscope. Immunostaining was scored by two independent experienced pathologists, who were blinded to the clinicopathologic parameters and clinical outcomes of the patients. An immunoreactivity score system was applied as described previously [15]. The extensional standard was: (1) the number of positively stained cells <5 % scored 0; 6–25 % scored 1; 26–50 % scored 2; 51–75 % scored 3; >75 % scored 4; (2) intensity of stain: colorless scored 0; pallide-flavens scored 1; yellow scored 2; brown scored 3. Multiply (1) and (2). The staining score was stratified as - (0 score, absent), + (1–4 score, weak), ++ (5–8 score, moderate) and +++ (9–12 score, strong) according to the proportion and intensity of positively stained cancer cells. Specimens were rescored if difference of scores from two pathologists was >3.

Quantitative Real-Time PCR

Total RNA purified from all 20 glioma tissues and 10 control brain tissues was prepared and reverse transcribed. Real-time monitoring of polymerase chain reactions (PCRs) was performed using the ABI 7900HT (Idaho Technology, Idaho Falls, ID, USA) and the SYBR green I dye (Biogene), which binds preferentially to double-stranded DNA. Fluorescence signals, which are proportional to the concentration of the PCR product, are measured at the end of each cycle and immediately displayed on a computer screen, permitting realtime monitoring of the PCR. The reaction is characterized by the point during cycling when amplification of PCR products is first detected, rather than the amount of PCR product accumulated after a fixed number of cycles. The higher the starting quantity of the template, the earlier a significant increase in fluorescence is observed. The threshold cycle is defined as the fractional cycle number at which fluorescence passes a fixed threshold above the baseline. The primers 5′-AAT GCT TCC TGC CTC TGT GT-3′ and 5′-GCC GTA ATC TGC CAT CAT CT-3′ were used to amplify 478-bp transcripts of FAT10 and the primers 5′-GGT GGC TTT TAG GAT GGC AAG-3′ and 5′-ACT GGA ACG GTG AAG GTG ACA G-3′ were used to amplify 161-bp transcripts of β-actin. All primers were synthesized by Sangon Co. (Shanghai, China). The PCR profile consisted of an initial melting step of 1 min at 94 °C, followed by 38 cycles of 15 s at 94 °C, 15 s at 56 °C and 45 s at 72 °C, and a final elongation step of 10 min at 72 °C.

Fluorescence data were converted into cycle threshold measurements using the SDS system software and exported to Microsoft Excel. FAT10 mRNA levels were compared to β-actin. Thermal dissociation plots were examined for biphasic melting curves, indicative of whether primer-dimers or other nonspecific products could be contributing to the amplification signal.

Western Blot Analysis

Twenty glioma tissues and 10 control brain tissues were homogenized in lysis buffer [PBS, 1 % nonidet P-40 (NP-40), 0.5 % sodium deoxycholate, 0.1 % sodium dodecyl sulfate (SDS), 100 μg/ml aprotinin, 100 μg/ml phenylmethylsulfonyl fluoride (PMSF), Sodium orthovanadate] at 4 °C throughout all procedures, and sonicated for 70 s, then add

300 μg PMSF per gram of tissue and incubate on ice for 30 min, followed by centrifugation at 15,000 rpm for 20 min at 4 °C. The protein content was determined according to Bradford's method (Bradford 1976), with bovine serum albumin used as a standard. Protein samples (30 μg) were boiled with 2 × sample buffer containing 5 % β-mercaptoethanol for 5 min, separated by size on 15 % polyacrylamide gel under SDS denaturing conditions, and transferred to a nitrocellucose membrane at 90 V for 2 h. The nitrocellulose membranes were stained with ponceau S to assess the efficiency of transfer. Non-specifi c binding was blocked by incubation in block buffer (5 % non-fat dry milk, 0.05 % Tween-20, 1 × tris-Cl-buffered saline) overnight at 4 °C, The membranes were hybridized with a rabbit polyclonal antibody to FAT10 (ENZO Life Science Int Inc, Plymoth Meeting, PA), then incubated with a horseradish peroxidase-labeled goat anti-mouse IgG (1: 500). The bound secondary antibody was detected by enhanced chemiluminescence (Amersham Life Science, Little Chalfont, UK). Housekeeping protein β-actin was used as a loading control. Positive immunoreactive bands were quantified densitometrically (Leica Q500IW image analysis system) and expressed as ratio of FAT10 to β-actin in optical density units.

Statistical Analysis

All computations were carried out using the software of SPSS version13.0 for Windows (SPSS Inc, IL, USA). The rank sum test was used to analyze the ranked data. The measurement data were analyzed by one-way ANOVA. Randomized block design ANOVA was used to analyze the statistical difference among different tissue types. In the analysis of glioma morbidity for all patients, we used the Kaplan-Meier estimator and univariate Cox regression analysis to assess the marginal effect of each factor. The differences between groups were tested by log-rank analyses. The joint effect of different factors was assessed using multivariate Cox regression. A Spearman's analysis was carried out to analyze the correlation between FAT10 mRNA and protein expression levels. Differences were considered statistically significant when p was less than 0.05.

Results

FAT10 Protein Levels in Glioma Tissues by Immunohistochemistry Assay and Survival Analysis

Immunohistochemistry analysis was performed to detect the expression of FAT10 protein in 10 nonneoplastic brain tissues and 128 glioma specimens. As the results, positive staining for FAT10 was mainly observed in the nuclei of tumor cells in glioma tissues. The representative photographs were shown in Fig. 1a and b. Among the glioma specimens, 106 (82.8 %) glioma specimens were positively stained, and 22 (17.2 %) glioma specimens were negatively stained. In contrast, its immunoreactivities in non-neoplastic brain sections ranged from undetectable to low (Fig. 1c). According to the statistical analysis, the expression level of FAT10 protein in glioma tissues was significantly higher ($P<0.001$) than that in non-neoplastic brain tissues.

In addition, FAT10 expression was not significantly affected by the gender and age (both $P>0.05$) of the patients. In contrast, the FAT10 expression was the closely correlated with WHO grade (Table 1; $P<0.001$), as well as Karnofsky performance Status (KPS) (Table 1; $P=0.008$).

Moreover, we reviewed clinical information of these FAT10-positive or -negative glioma patients. During the follow-up period, 100 of the 128 glioma patients (78.1 %) had died (18 from the FAT10-negative group and 82 from the FAT10-positive group). As determined by the log-rank test, the survival rate of patients with positive FAT10 staining was lower than those without FAT10 staining ($P<0.001$; Fig. 2a). The median survival time of patients with negative expression of FAT10 could not be estimated by statistical analysis because all patients survived better than the overall median level, and those patients with strong positive (+++), moderate positive (++) and weak positive (+) of FAT10 were 8.8±1.3 months, 12.1±1.6 months and 23.0±2.5 months (log-rank test: $P<0.001$).

Furthermore, Fig. 2b shows the post-operative survival curve of patients with glioma and FAT10 expression after adjusting for age, gender, WHO grade and KPS. By multivariate analysis, the loss of FAT10 expression was a significant

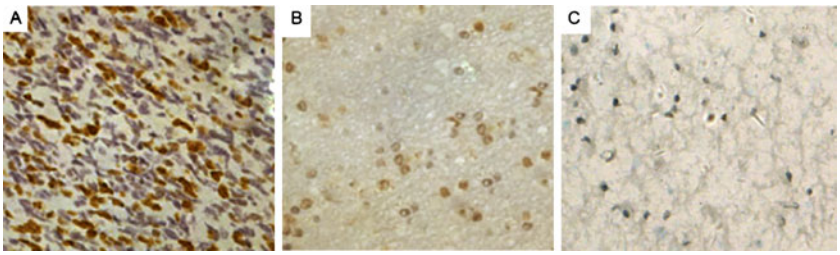

Fig. 1 Immunohistochemical staining of FAT10 protein in tumor cells of GBM (a) and astrocytoma (b) (Original magnification × 400). Staining for this antigen is described in "Materials and Methods". Positive staining of FAT10 is seen in the nuclei of tumors cells and is more abundant in the high- (**a**) than the low-grade (**b**) tumors. Negative expression of FAT10 (**c**) was observed in non-neoplastic brain tissues

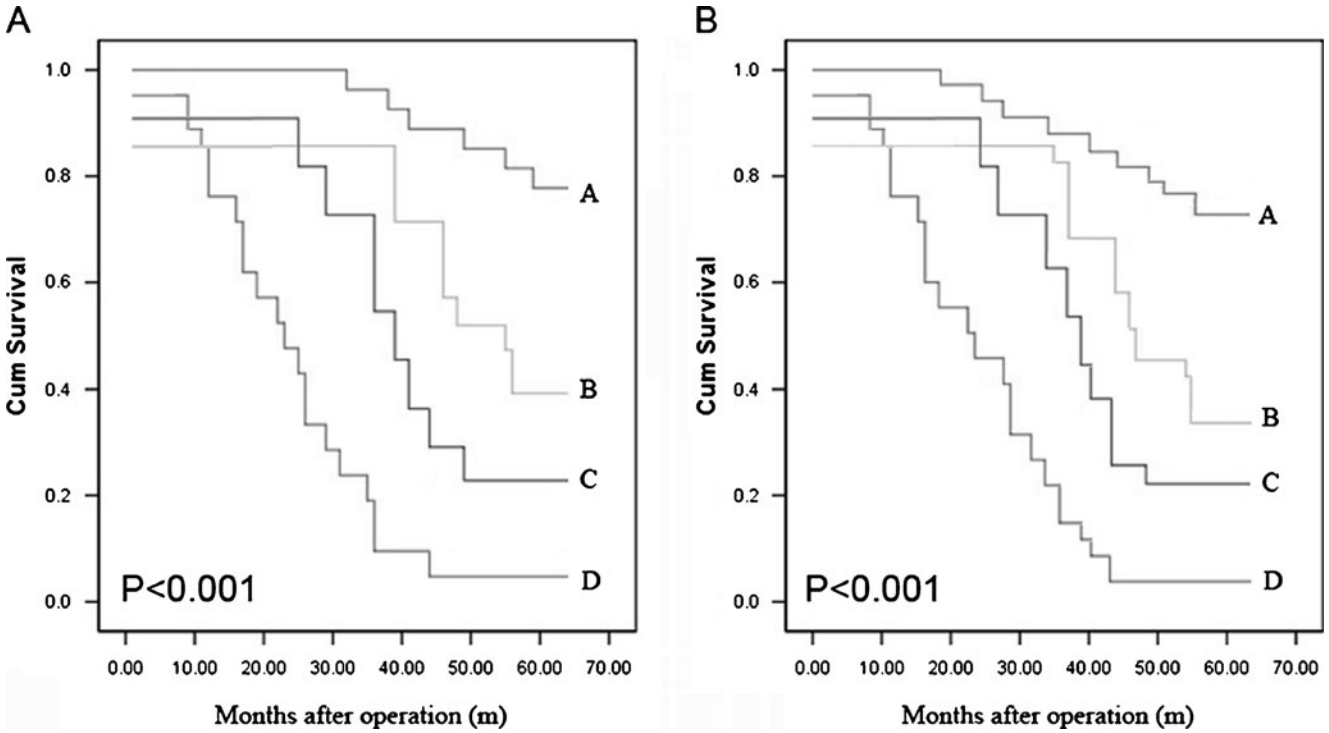

Fig. 2 Postoperative survival curves for patterns of patients with glioma and FAT10 expression. **a** Kaplan-Meier postoperative survival curve for patterns of patients with glioma and FAT10 expression. **b** Cox proportional hazards model after adjusting for age, gender and grade. FAT10 might be an independent predictor of survival, without consideration of age, gender or grade. 'A' refers to FAT10 negative expression group; 'B' refers to FAT10 weak positive (+) expression group; 'C' refers to FAT10 moderate positive (++) expression group; 'D' refers to FAT10 strong positive (+++) expression group

and independent prognostic indicator for patients with glioma besides age, WHO grade and KPS. The Cox proportional hazards model showed that FAT10 over-expression was associated with poor overall survival.

Quantitative Analysis of FAT10 Protein Expression Based on WHO Grade in Gliomas

As the results of Western blot analysis, we found that FAT10 protein expression tended to increase from the glioma to the normal tissue (Fig. 3a, c). We also investigated whether the expression of FAT10 correlated with the WHO grade. FAT10 expression was highest in grade IV and lowest in grade I (Fig. 3b, c), which was consistent with the findings of the immunohistochemistry analysis and indicated a close correlation of FAT10 protein expression with WHO grade.

Quantitative Analysis of FAT10 Gene Expression in Glioma

We determined the mRNA expression of FAT10 normalized to β-actin by real-time PCR. As shown in Fig. 4, there was a conspicuous increase in the expression of FAT10 mRNA from the control brain tissues to glioma tissues ($P<0.001$). We further analyzed the expression of FAT10 mRNA based on WHO grade. Interestingly, FAT10 mRNA expression increased with advancement of WHO grade I to grade IV ($P<0.001$). There was a significant positive correlation between the expression of FAT10 mRNA and protein expression levels from the same glioma tissues (rs=0.86, $P<0.001$).

Discussion

In the present study, we investigated the expression of FAT10 in 128 cases of human glioma and compared the expression with tumor grade and survival rates of patients. Our data demonstrated that FAT10 gene and protein were both increased in glioma compared to non-neoplastic brain tissue. We found an increased trend of both FAT10 protein level and mRNA level from WHO grade I to WHO grade IV glioma. These results suggest that the transcriptional and translational activation of human FAT10 might participate in the tumorigenesis and progression of glioma.

The basic functions of FAT10 can be divided into two perspectives. Firstly, it plays a role in immune regulation. FAT10 gene is expressed in dendritic cells and mature B cells, and can be synergistically induced by the IFN-γ and TNF-α cytokines [16]. Secondly, it plays a role in cell-cycle and apoptosis regulation, which attracts the attention of

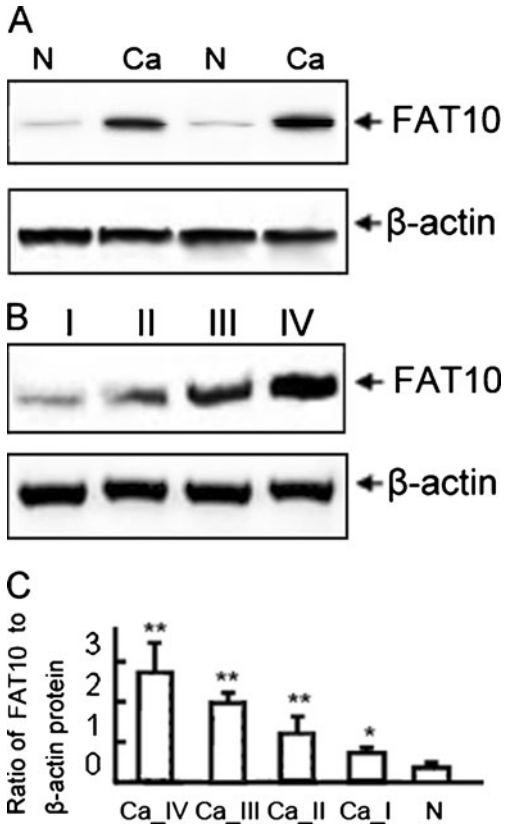

Fig. 3 Expression of FAT10 protein in glioma and non-neoplastic brain tissues by Western blot analysis. **a** FAT10 expression levels in glioma and non-neoplastic brain tissues. **b** FAT10 expression levels in glioma with different WHO grades. **c** FAT10 expression levels in non-neoplastic brain tissues and glioma with different WHO grades. 'N' refers to non-neoplastic brain tissues; 'Ca' refers to glioma tissues; 'Ca_ I'~'Ca_ IV' refer to glioma tissues with WHO grade I~IV. β-actin was used as a control for equal protein loading. Values are means ± SD. '*', $p<0.05$, comparison with non-neoplastic brain tissues; '**', $p<0.001$, comparison with non-neoplastic brain tissues

cancer biologists. FAT10 belongs to the ubiquitin-like modifier family, the members of which have been involved in cell-cycle regulation and apoptosis. It comprises two ubiquitin-like domains fused in tandem and is an important player in a variety of fundamental cellular processes including signal transduction, protein translocation, cell-cycle regulation and apoptosis [17]. Recent studies have been observed the over-expression of the FAT10 gene in several epithelial cancers, such as hepatocellular carcinoma, gastrointestinal and gynecological cancers [12–14]. The high FAT10 expression can increase genome instability by reducing kinetochore localization of MAD2 during the prometaphase stage of cell-cycle. Indeed, MAD2 dys-regulation has been shown to cause increased tumor incidence in mice [18]. In the present study, we also found that the expression of FAT10 at gene and protein levels were both increased in human glioma tissues. Additionally, the sub-cellular localization of FAT10 protein in tumor cells of glioma tissues was nuclei according to our observation, which was the same as the previous studies on other cancers [12–14]. However, there are some earlier reports that have indicated that FAT10 protein is localized to the cytoplasm of γ-interferon-induced B lymphoblastoid cells and murine fibroblast cells [19]. The possible reason for this difference might be there were no proper subcellular markers used as controls in their experiments [14]. Notably, the nuclear localization of FAT10 in tumor cells of glioma tissues may be suggestive of a role of FAT10 in the regulation of cell division and cell-cycle. The up-regulation of FAT10 protein and mRNA in glioma tissues also may be suggestive of a role of FAT10 in the process of glioma tumorigenesis.

We further analyzed the correlation of FAT10 expression and survival rates of patients. Our data indicated that nearly 83 % of glioma cases showed positive staining for FAT10. The survival rate of patients with positive FAT10 staining was lower than those without. Kaplan-Meier analysis of the

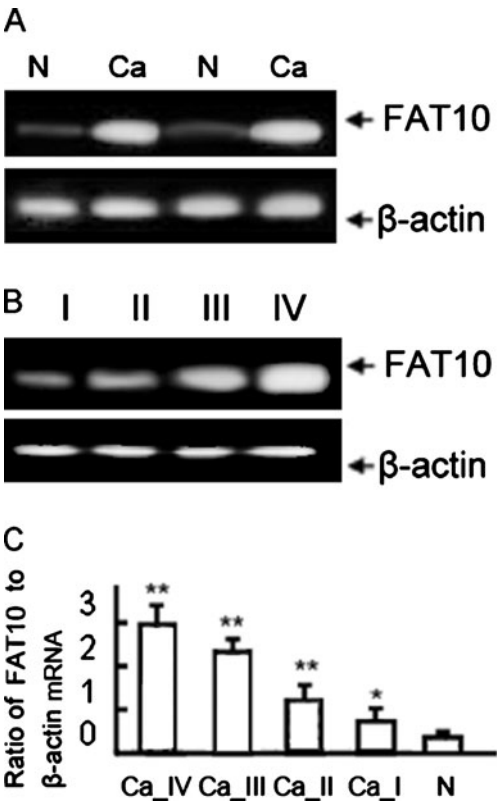

Fig. 4 Expression of FAT10 mRNA in glioma and non-neoplastic brain tissues by quantitative real-time PCR analysis. **a** FAT10 mRNA expression levels in glioma and non-neoplastic brain tissues. **b** FAT10 mRNA expression levels in glioma with different WHO grades. **c** FAT10 mRNA expression levels in non-neoplastic brain tissues and glioma with different WHO grades. 'N' refers to non-neoplastic brain tissues; 'Ca' refers to glioma tissues; 'Ca_ I'~'Ca_ IV' refer to glioma tissues with WHO grade I~IV. β-actin was used as a control for equal protein loading. Values are means ± SD. '*', $p<0.05$, comparison with non-neoplastic brain tissues; '**', $p<0.001$, comparison with non-neoplastic brain tissues

survival curves showed a significantly worse overall survival for patients whose tumors had high FAT10 levels, indicating that high FAT10 protein level is a marker of poor prognosis for patients with glioma, which was the same as the previous finding in gastric cancer [11]. Moreover, multivariate analysis showed FAT10 positive expression to be a marker of worse outcome independent of the known clinical prognostic indicators such as age, KPS and grade. Finally, there were a large number of samples (n=128) detected by immunohistochemistry, real-time PCR and Western blot analysis in our study. Thus, a large sample size, a good methodology and a detailed clinical follow-up in this study make our results more reliable.

In conclusion, our data provides convincing evidence for the first time that the increased expression of FAT10 at gene and protein levels is correlated with the advanced clinicopathological features and poor outcome in patients with gliomas. FAT10 may promote the aggressiveness of glioma and may be a potential prognosis predictor of glioma.

Acknowledgments This work was funded by Natural Science Foundation of China (NO. 81101736).

Conflict of interest None

References

1. Ohgaki H, Kleihues P (2005) Epidemiology and etiology of gliomas. Acta Neuropathol 109:93
2. Louis DN, Ohgaki H, Wiestler OD (2007) The 2007 WHO classifcation of tumors of the central nervous system. Acta Neuropathol 114:97–109
3. Mu YG, Chen MZ, Chen ZP, Zhou WN, Zhang XH, Sai K (2004) Microsurgical technique of brain glioma—a report of 183 cases. Ai Zheng 23:1317–1321
4. Chang CY, Li MC, Liao SL, Huang YL, Shen CC, Pan HC (2005) Prognostic and clinical implication of IL-6 expression in glioblastoma multiforme. J Clin Neurosci 12:930–933
5. Lin H, Wang Y, Zhang X, Liu B, Zhang W, Cheng J (2011) Prognostic significance of kappaB-Ras1 expression in gliomas. Med Oncol In press
6. Jentsch S, Pyrowolakis G (2000) Ubiquitin and its kin: how close are the family ties? Trends Cell Biol 10:335–342
7. Li T, Santockyte R, Yu S, Shen RF, Tekle E, Lee CG, Yang DC, Chock PB (2011) FAT10 modifies p53 and upregulates its transcriptional activity. Arch Biochem Biophys 509:164–169
8. Canaan A, Yu X, Booth CJ, Lian J, Lazar I, Gamfi SL, Castille K, Kohya N, Nakayama Y, Liu YC, Eynon E, Flavell R, Weissman SM (2006) FAT10/diubiquitin-like protein-deficient mice exhibit minimal phenotypic differences. Mol Cell Biol 26:5180–5189
9. Hipp MS, Kalveram B, Raasi S, Groettrup M, Schmidtke G (2005) FAT10, a ubiquitin-independent signal for proteasomal degradation. Mol Cell Biol 25:3483–3491
10. Raasi S, Schmidtke G, Groettrup M (2001) The ubiquitin-like protein FAT10 forms covalent conjugates and induces apoptosis. J Biol Chem 276:35334–35343
11. Ji F, Jin X, Jiao CH, Xu QW, Wang ZW, Chen YL (2009) FAT10 level in human gastric cancer and its relation with mutant p53 level, lymph node metastasis and TNM staging. World J Gastroenterol 15:2228–2233
12. Lukasiak S, Schiller C, Oehlschlaeger P, Schmidtke G, Krause P, Legler DF, Autschbach F, Schirmacher P, Breuhahn K, Groettrup M (2008) Proinflammatory cytokines cause FAT10 upregulation in cancers of liver and colon. Oncogene 27:6068–6074
13. Qing X, French BA, Oliva J, French SW (2011) Increased expression of FAT10 in colon benign, premalignant and malignant epithelial neoplasms. Exp Mol Pathol 90:51–54
14. Lee CG, Ren J, Cheong IS, Ban KH, Ooi LL, Yong Tan S, Kan A, Nuchprayoon I, Jin R, Lee KH, Choti M, Lee LA (2003) Expression of the FAT10 gene is highly upregulated in hepatocellular carcinoma and other gastrointestinal and gynecological cancers. Oncogene 22:2592–2603
15. Brown RS, Wahl RL (1993) Overexpression of Glut-1 glucose transporter in human breast cancer: an immunohistochemical study. Cancer 72:2979–2985
16. Ren J, Wang Y, Gao Y, Mehta SB, Lee CG (2011) FAT10 mediates the effect of TNF-α in inducing chromosomal instability. J Cell Sci 124:3665–3675
17. Zhang DW, Jeang KT, Lee CG (2006) p53 negatively regulates the expression of FAT10, a gene upregulated in various cancers. Oncogene 25:2318–2327
18. Ren J, Kan A, Leong SH, Ooi LL, Jeang KT, Chong SS, Kon OL, Lee CG (2006) FAT10 plays a role in the regulation of chromosomal stability. J Biol Chem 281:11413–11421
19. Liu YC, Pan J, Zhang C, Fan W, Collinge M, Bender JR, Weissman SM (1999) A MHC-encoded ubiquitin-like protein (FAT10) binds noncovalently to the spindle assembly checkpoint protein MAD2. Proc Natl Acad Sci U S A 96:4313–4318