Overexpression of *Fn14* in gliomas: tumor progression and poor prognosis

Dan Tan^{1,2,3,4}, Feng-Mei Pang^{1,2,3,4}, Dan Li^{1,2,3,4}, Longbo Zhang⁵, Jun Wu⁵, Zhao-Qian Liu^{1,2,3,4}, Xi Li^{*,1,2,3,4} & Han Yan^{**,6,7}

¹Department of Clinical Pharmacology, Xiangya Hospital, Central South University, Changsha, Hunan, 410008, PR China

²Institute of Clinical Pharmacology, Central South University, Changsha, Hunan, PR China

³Hunan Key Laboratory of Pharmacogenetics, Changsha, Hunan, 410078, PR China

⁴National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan, 410011, PR China

⁵Department of Neurosurgery, Xiangya Hospital, Center South University, Changsha, Hunan, 410008, PR China

⁶Institute of Clinical Pharmacy, Central South University, Changsha, Hunan, 410011, PR China

⁷Department of Pharmacy, The Second Xiangya Hospital, Central South University Changsha, Hunan, 410011, PR China

*Author for correspondence: lixili6931430@126.com

**Author for correspondence: yanhan501@csu.edu.cn

Aim: To confirm whether the expression level of *Fn14* could affect progression or prognosis of glioma patients. **Methods:** Glioma cohorts in The Cancer Genome Atlas, Gene Expression Omnibus and Chinese Glioma Genome Atlas databases were comprehensively analyzed. **Results:** Low-grade patients had lower expression level of *Fn14*, while patients with higher expression of *Fn14* tended to harbor shorter overall survival and disease-free survival. The expression level of *Fn14* was downregulated by *IDH1/IDH2* mutations while its gene body methylation was upregulated. After adjusting age, the expression level of *Fn14* was still significantly associated with overall survival and disease-free survival in low-grade gliomas. In a cell line data analysis, *Fn14* expression was positively correlated with temozolomide dosage. **Conclusion:** *Fn14* was an independent predictive biomarker for the progression and prognosis in low-grade gliomas.

First draft submitted: 3 November 2017; Accepted for publication: 3 January 2018; Published online: 9 May 2018

Keywords: *Fn14* • glioma • prognosis • progression

Gliomas are the most common CNS neoplasms with aggressive growth, high malignant degree and poor prognosis in human. Due to the blood–brain barrier, only a few of chemotherapeutic drugs can be used in the treatment of gliomas. Recently, concomitant radiochemotherapy combining with adjuvant chemotherapy based on temozolomide after resection of the tumor has become a standard therapeutic regimen of gliomas according to the National Comprehensive Cancer Network guidelines. However, the outcomes still varied greatly. As mentioned in several studies, the overall survival (OS) ranged from 1 to 15 years in low-grade gliomas (LGGs), and only a few patients had excellent curative effect [1–4]. Although some of the neuropathological and prognostic predicting biomarkers (such as *IDH1/IDH2* mutation, *1p/19q* co-deletion and *MGMT* promotor methylation [5–7]) had been identified, more investigations are needed to clarify the detailed mechanisms of prognosis variation in glioma treatment.

TNF, which contains 19 ligands, is one of the most important cytokines that are maintaining homeostasis of human organism through regulating cytokine production, cell survival and cell death after binding to the receptor [8]. Tumor necrosis factor like weak inducer of apoptosis (TWEAK), a type of transmembrane protein II, is a typical member of *TNF* superfamily [9,10]. When binding to its sole receptor, TWEAK affects cytological functions such as proliferation, differentiation, apoptosis, angiogenesis and inflammation [11,12]. FGF-inducible 14 (*Fn14*), also known as *TNF* receptor superfamily member 12A, is a transmembrane protein that was able to bind with TWEAK [13]. Previous studies found that *TWEAK* and *Fn14* participated in numerous cellular processes (such as cell proliferation, survival, migration, etc.) through activating a lot of intracellular signaling pathways (PI3K-AKT, NF- κ B, ERK and JNK) [14–17]. Both *Fn14* and *TWEAK* are expressed at low levels in most healthy normal tissues even in the brain [14–16]. However, *Fn14* but not *TWEAK* is overexpressed in gliomas [18]. *In vitro*

studies demonstrated that the overexpression of *Fn14* could promote the migration, invasion and survival of glioma cells [19–21]. Moreover, *Fn14* could regulate downstream pathways without TWEAK in glioma cells [22]. These results suggested that the expression of *Fn14* might be an independent factor affecting glioma development and progression.

Temozolomide is a DNA alkylating agent. Because of its small size and lipophilic properties, temozolomide can cross the blood–brain barrier so that it becomes one of the most commonly used drugs in glioma chemotherapy. In glioma cells, activating the TWEAK/*Fn14* signaling could promote their survival when exposing to temozolomide [19,23]. Thus, the expression level of *Fn14* is supposed to correlate with sensitivity of temozolomide.

To date, the expression pattern of *Fn14* in glioma patients was not well investigated. Only *in vitro* pertinent investigations on *Fn14* and gliomas were carried out. Meanwhile, one report found the high expression level of *Fn14* in glioblastoma (GBM) patients was associated with short-term survival [20]. Because the sample size of the study was small and the results have not been replicated in an independent cohort, the relationship between *Fn14* and prognosis of glioma treatment still needed to be confirmed. Accordingly, the comprehensive association study was performed.

Methods

Datasets

Two types of open access datasets were downloaded and analyzed. The first type contains six datasets involving the whole genome expression data and prognostic information of glioma treatment for each patient. The two datasets were obtained from The Cancer Genome Atlas (TCGA), Chinese Glioma Genome Atlas (CGGA) and Gene expression omnibus databases, and used for testing the association between *Fn14* expression level and OS or disease-free survival (DFS). Moreover, some datasets also contain whole genome methylation information and/or *IDH1*/*IDH2* mutation status which were used to confirm whether the expression of *Fn14* was regulated by *IDH* mutations. Another type was a tumor cell line dataset in Genomics of Drug Sensitivity in Cancer (GDSC) database. This dataset contains whole genome expression and half maximal inhibitory concentration (IC₅₀) data of temozolomide for each cell line, which were used for analyzing the association between the expression level of *Fn14* and temozolomide sensitivity *in vitro*. The basic characteristics of the selected datasets were listed in Supplementary Table 1.

TCGA-LGG dataset

TCGA-LGG dataset contains 515 WHO II or III patients, and most of whose whole exome somatic mutations data, whole genome RNA sequencing data, whole genome methylation level as well as clinical information are available. RNA sequencing was performed by using Illumina next generation sequencing platform. Illumina Infinium HumanMethylation450 (450 K) BeadChip was used to detect the whole genome methylation level, while whole exome sequencing based on Illumina platform was used to identify somatic mutations. Normalized whole genome expression and clinical information were downloaded from cBioPortal for Cancer Genomics [24,25], while the somatic mutation status and whole genome methylation data were obtained from Genomic Data Commons data portal (Release 7.0; <https://portal.gdc.cancer.gov/>).

TCGA-GBM dataset

TCGA-GBM dataset contains 596 GBM patients. Among them, 276 patients were employed to detect the whole exome somatic mutations by Illumina next generation sequencing platform. A total of 404 patients had been examined for the whole genome methylation information through Illumina (CA, USA) Infinium HumanMethylation27 or HumanMethylation450 BeadChip. A total of 568 patients were determined for the whole genome expression level by Affymetrix (CA, USA) HT Human Genome U133A, Agilent (CA, USA) Human Genome CGH Microarray Kit (244 K) and/or RNA sequencing. Similar to TCGA-LGG dataset, the normalized whole genome expression and the clinical information were downloaded from cBioPortal for cancer genomics, while the somatic mutation status and methylation data were obtained from Genomic Data Commons data portal.

CGGA glioma (CGGA-Batch1, CGGA-Batch2 & CGGA-RNAseq) datasets

CGGA-Batch1, CGGA-Batch2 and CGGA-RNAseq include 220, 86 and 326 diffuse glioma patients (WHO grade II–IV), respectively [26–28]. The patients' whole genome expression information in CGGA-Batch1 and CGGA-Batch2 datasets were detected by Agilent Whole Human Genome Oligo Microarray, while CGGA-RNAseq dataset

were collected by RNA sequencing. Both of CGGA-Batch1 and CGGA-Batch2 datasets have *IDH* mutation information. The *IDH* mutation status was obtained through pyrosequencing method. The normalized gene expression data, *IDH* mutation status and clinical information of these patients were downloaded from CGGA database (www.cgga.org.cn/).

Rembrandt repository of molecular brain neoplasia data (Rembrandt) dataset

Rembrandt dataset consists of more than 870 glioma patients (WHO grade I–IV), about 580 patients' whole genome expression level were detected by Affymetrix HT-Human Genome U133 Plus 2 microarray [29]. The raw gene expression data were downloaded from the Gene Expression Omnibus database, while the clinical information was obtained from the Georgetown Database of Cancer (<https://gdoc.georgetown.edu/>). The raw gene expression data were normalized by robust multiarray averaging (RMA) method [30].

GDSC cell line dataset

The GDSC cell line dataset includes more than 1000 cancer cell lines, 944 of which harbored IC_{50} values for temozolomide. After excluding the blood cancer cell lines and some source unknown cell lines, 779 solid tumor cell lines were selected for the subsequent analysis. Among these solid tumor cell lines, the whole genome expression information of 659 solid tumor cell lines has been detected through Affymetrix Human Genome U219 array. The RMA normalized basal expression profiles for these cell lines were downloaded from GDSC database (Release 6.1) (www.cancerrxgene.org/).

Statistical analysis

Analysis of variance was conducted to confirm the relationship between *Fn14* expression level and tumor grade. Fisher's least significant difference method was used for multiple comparisons. Cox regression analysis was used to test the association between *Fn14* expression level and OS or DFS. Linear regression analysis was performed to examine whether the expression level of *Fn14* was related to IC_{50} of temozolomide. To obtain Kaplan–Meier plots of *Fn14* expression, quartile method was used. Based on the expression value of *Fn14*, all patients were divided into four groups from lower to higher (0–25%, 25–50%, 50–75% and 75–100%). To confirm whether *Fn14* expression was affected by *IDH* mutations, the relationships among *Fn14* expression level, *Fn14* promoter methylation and *IDH* mutation status were analyzed. In addition, multivariate cox regression analysis was utilized to examine whether *Fn14* was an independent predictive biomarker. The covariates comprised in the cox regression model were age, tumor grade and *IDH* mutation status. As the expression values of *Fn14* were collected from different platform and normalized by different method. The expression data were standardly transformed (centered to a mean of 0 and then scaled the SD) before analysis. In this study, the LGGs include WHO grade II and III patients. This criterion of classification was reported by TCGA [6]. All of the statistical analyses were conducted by SPSS 20. Graphpad 6.0 and was utilized to generate the plots.

Results

The expression level of *Fn14* associated with tumor grade

Analysis of variance (ANOVA) analysis results showed that, the expression level of *Fn14* was significantly associated with tumor grade in multiple glioma cohorts. In TCGA-LGG dataset, *Fn14* was significantly overexpressed in grade III patients than that in grade II patients ($p < 1.00 \times 10^{-6}$). Consistently, results were found in CGGA-Batch1, CGGA-Batch2, CGGA-RNAseq and Rembrandt datasets ($p < 1.00 \times 10^{-6}$, respectively). In all of these datasets, *Fn14* expression was observed to have a positive association with histological grades. Figure 1 displays the expression pattern of *Fn14* in TCGA, CGGA and Rembrandt datasets.

The expression level of *Fn14* associated with OS

After cox regression analysis, the expression level of *Fn14* was found significantly associated with OS in all glioma cohorts (Table 1). In TCGA-LGG dataset, patients with lower expression level of *Fn14* showed significantly longer OS (Figure 2A) ($p < 1.00 \times 10^{-6}$; hazard ratio [HR]: 1.486; 95% CI: 1.363–1.620) than those with higher expression level. Similar results were found in TCGA-GBM, CGGA and Rembrandt datasets. In TCGA-GBM dataset, the expression level of *Fn14* was significantly or marginal significantly associated with OS based on the data generating from different platforms (Affymetrix array: $p = 0.001$; HR: 1.201; 95% CI: 1.078–1.339, Agilent array: $p = 0.008$; HR: 1.194; 95% CI: 1.047–1.362, RNAseq: $p = 0.013$; HR: 1.290; 95% CI: 1.054–1.578). In CGGA

Figure 1. The expression pattern of *Fn14* in The Cancer Genome Atlas, Chinese Glioma Genome Atlas and Rembrandt datasets. (A–E) *Fn14* expression was in positive association with histological grades in glioma patients. CGGA: Chinese Glioma Genome Atlas; LGG: Low grade glioma; TCGA: The Cancer Genome Atlas.

datasets, patients with lower expression level of *Fn14* tend to have longer OS (CGGA-Batch1: $p < 1.00 \times 10^{-6}$; HR: 1.528; 95% CI: 1.358–1.719, CGGA-Batch2: $p < 1.00 \times 10^{-6}$; HR: 1.560; 95% CI: 1.322–1.840, CGGA-RNAseq: $p < 1.00 \times 10^{-6}$; HR: 1.714; 95% CI: 1.535–1.914). In Rembrandt dataset, patients with higher expression level of *Fn14* harbored higher mortality risk ($p < 1.00 \times 10^{-6}$; HR: 1.404; 95% CI: 1.276–1.545). Figure 2 displays the survival curve of OS according to different *Fn14* expression levels.

Figure 2. The survival curves of overall survival in different datasets based on expression level of *Fn14*. 0%–25%, 25%–50%, 50%–75%, 75%–100% represent the lowest to highest expression level of *Fn14*; (A–H) Patients with higher *Fn14* expression presented with shorter OS than those of lower *Fn14* expression.

CGGA: Chinese Glioma Genome Atlas; GBM: Glioblastoma; LGG: Low grade glioma; OS: Overall survival; TCGA: The Cancer Genome Atlas.

Table 1. Association analysis results between *Fn14* expression and overall survival.

Dataset	n	OS-H [†] (mean ± SD)	OS-L [‡] (mean ± SD)	HR (95% CI)	p-value	Adjusted HR (95% CI)	Adjusted p-value
TCGA-LGG	510	29.972 ± 31.967	33.306 ± 30.532	1.486 (1.363–1.620)	<1.00 × 10 ⁻⁶	1.327 (1.185–1.487) [§]	1.00 × 10 ⁻⁶
TCGA-GBM	Affymetrix 392	19.032 ± 17.021	19.892 ± 18.678	1.201 (1.078–1.339)	0.001	1.045 (0.867–1.260) [#]	0.645
	Agilent 294	17.603 ± 13.971	18.227 ± 15.950	1.194 (1.047–1.362)	0.008	1.024 (0.835–1.256) [#]	0.820
	RNAseq 111	15.010 ± 11.809	17.551 ± 14.861	1.290 (1.054–1.578)	0.013	1.170 (0.935–1.464) [#]	0.170
CGGA-Batch1	218	18.066 ± 9.621	27.747 ± 10.234	1.528 (1.358–1.719)	<1.00 × 10 ⁻⁶	1.086 (0.925–1.274) [§]	0.312
CGGA-Batch2	86	15.064 ± 11.469	33.499 ± 19.153	1.560 (1.322–1.840)	<1.00 × 10 ⁻⁶	1.265 (0.997–1.605) [§]	0.053
CGGA-RNAseq	312	18.893 ± 17.940	52.209 ± 33.119	1.714 (1.535–1.914)	<1.00 × 10 ⁻⁶	1.321 (1.149–1.519) [¶]	9.20 × 10 ⁻⁵
Rembrandt	450	24.636 ± 29.948	46.136 ± 42.127	1.404 (1.276–1.545)	<1.00 × 10 ⁻⁶	1.081 (0.934–1.250) [¶]	0.295

[†]*Fn14* high expression.[‡]*Fn14* low expression.[§]The HR and p-values were adjusted by age, tumor grade and *IDH1*/*IDH2* mutation status.[#]The HR and p-values were adjusted by age and *IDH1*/*IDH2* mutation status.[¶]The HR and p-values were adjusted by age and tumor grade.

CGGA: Chinese Glioma Genome Atlas; DFS: Disease-free survival; GBM: Glioblastoma; HR: Hazard ratio; LGG: Low grade glioma; OS: Overall survival; SD: Standard deviation; TCGA: The Cancer Genome Atlas.

Table 2. Association analysis results between *Fn14* expression and disease-free survival.

Dataset	n	DFS-H [†] (mean ± SD)	DFS-L [‡] (mean ± SD)	HR (95% CI)	p-value	Adjusted HR (95% CI)	Adjusted p-value
TCGA-LGG	474	24.551 ± 26.128	28.017 ± 25.501	1.715 (1.518–1.937)	<1.00 × 10 ⁻⁶	1.487 (1.241–1.783) [§]	1.70 × 10 ⁻⁵
TCGA-GBM	Affymetrix 393	9.396 ± 9.413	11.756 ± 14.941	1.248 (1.122–1.389)	4.50 × 10 ⁻⁵	1.025 (0.857–1.225) [#]	0.789
	Agilent 295	8.579 ± 8.038	10.397 ± 11.553	1.207 (1.064–1.369)	0.003	0.984 (0.814–1.189) [#]	0.867
	RNAseq 111	7.790 ± 8.437	11.426 ± 13.493	1.388 (1.140–1.690)	0.001	1.276 (1.028–1.584) [#]	0.027
CGGA-RNAseq	312	13.121 ± 15.646	39.865 ± 29.007	1.679 (1.508–1.869)	<1.00 × 10 ⁻⁶	1.291 (1.126–1.481) [¶]	2.58 × 10 ⁻⁴

[†]*Fn14* high expression.[‡]*Fn14* low expression.[§]The HR and p-values were adjusted by age, tumor grade and *IDH1*/*IDH2* mutation status.[#]The HR and p-values were adjusted by age and *IDH1*/*IDH2* mutation status.[¶]The HR and p-values were adjusted by age and tumor grade.

CGGA: Chinese Glioma Genome Atlas; DFS: Disease-free survival; GBM: Glioblastoma; HR: Hazard ratio; LGG: Low grade glioma; OS: Overall survival; SD: Standard deviation; TCGA: The Cancer Genome Atlas.

The expression level of *Fn14* associated with DFS

Since TCGA-LGG, TCGA-GBM and CGGA-RNAseq datasets possess DFS information, the relationship between *Fn14* expression and DFS was further analyzed. Cox regression analysis results showed that the expression level of *Fn14* was significantly associated with DFS in all of the above three datasets (Table 2). In TCGA-LGG dataset, lower expression level of *Fn14* tended to harbor longer DFS ($p < 1.00 \times 10^{-6}$; HR: 1.715; 95% CI: 1.518–1.937). The same results were found in both of TCGA-GBM (Affymetrix array: $p = 4.50 \times 10^{-5}$; HR: 1.248; 95% CI: 1.122–1.389, Agilent array: $p = 0.003$; HR: 1.207; 95% CI: 1.064–1.369, RNAseq: $p = 0.001$; HR: 1.388; 95% CI: 1.140–1.690) and CGGA-RNAseq ($p < 1.00 \times 10^{-6}$; HR: 1.679; 95% CI: 1.508–1.869) datasets. Figure 3 displays the survival curve of DFS according to different *Fn14* expression level.

Fn14 expression & methylation regulated by *IDH* mutations

In TCGA-LGG dataset, patients with *IDH1* and/or *IDH2* mutations had significantly lower *Fn14* expression level than those with *IDH1* or *IDH2* wild-type ($p < 1.00 \times 10^{-6}$; $r = -0.435$). The same trend was found in TCGA-GBM, CGGA-Batch1 and CGGA-Batch2 datasets. In all of these datasets, *IDH* mutations significantly downregulated the expression level of *Fn14* (TCGA-GBM Affymetrix array: $p < 1.00 \times 10^{-6}$; $r = -0.396$, TCGA-GBM Agilent array: $p < 1.00 \times 10^{-6}$; $r = -0.360$, TCGA-GBM RNAseq: $p = 0.005$; $r = -0.271$, CGGA-Batch1: $p < 1.00 \times 10^{-6}$; $r = -0.442$, CGGA-Batch2: $p = 2.00 \times 10^{-6}$; $r = -0.498$). As whole genome methylation data are available in TCGA dataset, relationships between *IDH* mutations and *Fn14* methylation were detected. In Illumina methylation array, 12 probes mapped to *Fn14*, and nine of them harbored detection values (β -value) in TCGA datasets. The association analysis results between the nine probes and *IDH* mutations are listed in Table 4. The methylation level of three probes (cg00510447, cg20195987 and cg26808293) were found significantly upregulated by *IDH* mutations with

Figure 3. The survival curves of disease-free survival in different datasets based on expression level of *Fn14*. 0–25%, 25–50%, 50–75% and 75–100% represent the lowest to highest expression level of *Fn14*; (A–E) Patients with higher *Fn14* expression presented with shorter DFS than those of lower *Fn14* expression.

CGGA: Chinese Glioma Genome Atlas; DFS: Disease-free survival; GBM: Glioblastoma; LGG: Low grade glioma; TCGA: The Cancer Genome Atlas.

β difference ≥ 0.2 . All of them were negatively correlated with *Fn14* mRNA expression in both of TCGA-LGG and TCGA-GBM datasets with correlation coefficient ≤ -0.316 . Interestingly, all of these three probes were locating external of the promoter of *Fn14*.

Fn14 expression in low grade glioma

To confirm whether the expression level of *Fn14* is an independent factor for glioma prognosis, multiple cox regression analysis was applied. Age, tumor grade and *IDH* mutations status were included in the cox regression model as covariates. After adjusting for age, tumor grade and *IDH* mutation status, *Fn14* expression level is

Table 3. Multivariate analysis of factors correlated with overall survival and disease-free survival in low-grade gliomas.

Sample	Factors	OS		DFS	
		HR (95% CI)	p-value	HR (95% CI)	p-value
TCGA-LGG	Age (years)	1.049 (1.033–1.066)	$<1.00 \times 10^{-6}$	1.015 (1.002–1.029)	0.020
	IDH mutations [†]	0.324 (0.210–0.500)	$<1.00 \times 10^{-6}$	0.267 (0.177–0.403)	$<1.00 \times 10^{-6}$
	Tumor grade [‡]	0.518 (0.340–0.787)	0.002	0.931 (0.661–1.311)	0.683
	Fn14 expression	1.327 (1.185–1.487)	1.00×10^{-6}	1.487 (1.241–1.783)	1.70×10^{-5}
CGGA-Batch1-LGG	Age (years)	1.019 (0.981–1.059)	0.332	NA	NA
	IDH mutations [†]	0.921 (0.328–2.588)	0.876	NA	NA
	Tumor grade [‡]	0.327 (0.105–1.021)	0.054	NA	NA
	Fn14 expression	1.468 (1.088–1.981)	0.012	NA	NA
CGGA-Batch2-LGG	Age (years)	1.052 (0.978–1.133)	0.175	NA	NA
	IDH mutations [†]	0.541 (0.141–2.083)	0.372	NA	NA
	Tumor grade [‡]	0.645 (0.158–2.626)	0.540	NA	NA
	Fn14 expression	1.422 (1.009–2.004)	0.044	NA	NA

[†]IDH1 or IDH2 mutation versus wild-type.[‡]WHO grade II versus III.

CGGA: Chinese Glioma Genome Atlas; DFS: Disease-free survival; HR: Hazard ratio; LGG: Low grade glioma; NA: Not available; OS: Overall survival; TCGA: The Cancer Genome Atlas.

Table 4. Correlation analysis results between *Fn14* expression and *Fn14* methylation.

Probe	Location	TCGA-LGG				TCGA-GBM			
		IDH (M:W) [†]	β-difference	p-value	r [‡]	IDH (M:W) [†]	β-difference	p-value	r [‡]
cg02105042	TSS1500	414:95	0.073	$<1.00 \times 10^{-6}$	-0.174**	7:138	0.082	0.062	0.163
cg05336707	1stExon	414:95	0.001	0.918	-0.078	7:138	0.007	0.170	-0.341*
cg06097320	TSS200	414:95	-0.006	0.353	-0.131**	7:138	0.002	0.747	-0.187
cg08798492	TSS200	414:95	-0.009	0.111	-0.071	7:138	0.005	0.270	-0.175
cg15460516	TSS200	414:95	-0.009	0.171	-0.136**	25:313	-0.006	0.146	-0.114
cg00510447	Body	414:95	0.515	$<1.00 \times 10^{-6}$	-0.514**	7:138	0.599	$<1.00 \times 10^{-6}$	-0.521**
cg06209210	Body	414:95	0.230	$<1.00 \times 10^{-6}$	-0.316**	7:138	0.185	$<1.00 \times 10^{-6}$	-0.395**
cg20195987	Body	414:95	0.424	$<1.00 \times 10^{-6}$	-0.382**	7:138	0.520	$<1.00 \times 10^{-6}$	-0.494**
cg26808293	3'UTR	414:95	0.353	$<1.00 \times 10^{-6}$	-0.406**	7:138	0.233	$<1.00 \times 10^{-6}$	-0.488**

[†]M means IDH mutation, W means IDH wild-type.[‡]Pearson correlation coefficient between methylation and *Fn14* expression.

*p < 0.05; **p < 0.01.

GBM: Glioblastoma; LGG: Low grade glioma; TCGA: The Cancer Genome Atlas.

significantly associated with glioma prognosis in all low grade glioma cohorts. In TCGA-LGG dataset, patients with lower expression level of *Fn14* tend to have longer OS ($p = 1.00 \times 10^{-6}$; HR: 1.327; 95% CI: 1.185–1.487) and DFS ($p = 1.70 \times 10^{-5}$; HR: 1.487; 95% CI: 1.241–1.783). Similar results were found in both of CGGA-Batch1-LGG and CGGA-Batch2-LGG datasets. Higher expression of *Fn14* heralded shorter OS (CGGA-Batch1-LGG: $p = 0.012$; HR: 1.468; 95% CI: 1.088–1.981, CGGA-Batch2: $p = 0.044$; HR: 1.422; 95% CI: 1.009–2.004). The detailed results are listed in Tables 1–3. Moreover, the data in IDH mutation or wild-type patients were analyzed, respectively. In accordance with multiple regression results, *Fn14* was significantly associated with prognosis in the absence of IDH mutations in LGG patients (Supplementary Table 2).

Expression level of *Fn14* related to temozolomide sensitivity

In GDSC dataset, the expression level of *Fn14* was found significantly associated with temozolomide IC₅₀ with $p < 1.00 \times 10^{-6}$. The expression level of *Fn14* is positively correlated with temozolomide IC₅₀ values ($r = 0.224$) which indicates that high expression of *Fn14* would lead to temozolomide resistant. Figure 4 is a scatter chart for temozolomide IC₅₀ values and *Fn14* expression values.

Figure 4. The scatter plot of *Fn14* expression and temozolomide IC_{50} .

Discussion

In this study, we tested the association between the expression level of *Fn14* and tumor grade, as well as prognosis in glioma patients. Study results showed that patients with higher expression level of *Fn14* tend to have higher tumor grade, shorter OS and DFS than those with lower expression level of *Fn14*. Cell line data analysis results indicated that the high expression level of *Fn14* might lead to resistance to temozolomide. Our results indicated that the expression level of *Fn14* might be used for predicting tumor progression, prognosis and sensitivity of temozolomide in glioma treatment.

Major reasons for poor prognosis of glioma mainly due to the tumor cells invasion and resistant to radiotherapy or chemotherapy. Glioma cells always invade surrounding normal tissue which leads doctors hard to resect tumor thoroughly and thus result in incurable or relapse in glioma [31]. In addition, resistant to radiotherapy or chemotherapy also causes the treatment failure in gliomas. Interestingly, *Fn14* has been reported to correlate with both glioma cell invasion and resistance to temozolomide. Previous study found that *Fn14* mRNA and protein expression were upregulated in migration activated glioma cells [32]. Overexpression of *Fn14* in glioma cell lines could promote cell migration and invasion [20]. The key approach for *Fn14* affecting glioma cell invasion and migration might be through activation of NF- κ B pathway and upregulating BCL-XL/BCL-W expression in glioma cells [19,20]. Activation of TWEAK–*Fn14* signaling also lead to temozolomide resistance in glioma cells. The main mechanism might be through upregulating the expression of SGEF which could bind with BRCA1 to promote glioma cells escaping from temozolomide induced cell apoptosis [33]. Moreover, TWEAK–*Fn14* complex could active PI3K–AKT signaling which had been found to be related with invasion and temozolomide sensitivity in multiple cancer cell lines [14,34–37]. These results demonstrated that overexpression of *Fn14* might result in poor outcomes in glioma. Consistent results were found in our study. We found that the high expression of *Fn14* was associated with poor prognosis in glioma patients and high IC_{50} of temozolomide in solid tumor cell lines.

IDH mutation status is a recognized factor for glioma prognosis prediction. Patients with *IDH1* or *IDH2* mutations have longer OS and better temozolomide response than those without [6,38–40]. However, the detailed mechanism for *IDH* mutation affecting the outcome of glioma patients is still unclear. Recently, it was evidenced that *IDH* mutations could improve temozolomide sensitivity through impairment of PARP1-mediated DNA repair [41]. In this study, we found *IDH* mutations might downregulate *Fn14* expression through upregulating the methylation level of some CG sites in *Fn14* coding gene. This result suggested a new possible way to explain how *IDH* mutations improve glioma prognosis and temozolomide response: *IDH* mutations might reduce glioma cell invasion and migration, and improve sensitivity to temozolomide through downregulation of *Fn14*. In future study, mechanism experiments are needed to prove this hypothesis.

In the study, the most relevant probe of *Fn14* expression was cg00510447 which locates in exon 3 of *Fn14*, while the β -values of the probes in gene promoter were low correlated with gene expression. These results suggested that the gene body methylation might influence *Fn14* expression. Previous studies found that gene body methylation can be positively or negatively correlated with gene expression [42,43]. However, the detailed regulatory mechanism is not clear at present. As a result, *Fn14* may be a good research object to clarify the mechanism between gene body methylation and gene expression.

As only a small number of glioma cell lines are in the GDSC dataset, we used all solid tumor cell lines to analyze the relationship between *Fn14* expression and temozolomide IC_{50} . This process might lead to the false positive

results. To validate the results, cellular and animal experiments are needed. Moreover, in this study, we found the expression of *Fn14* was significantly associated with tumor grade and prognosis of glioma patients, which had been validated in multiple cohorts. However, all of the results come from retrospective studies. To validate the prediction value of *Fn14* expression in glioma treatment, prospective studies are required in the future.

Conclusion & future perspective

We first confirm that the expression level of *Fn14* was associated with tumor grade, OS and DFS in multiple glioma cohorts. After adjusting age, tumor grade and *IDH* mutation stats, *Fn14* still associated significantly with OS and DFS in low-grade gliomas. In cell line data, *Fn14* expression was also correlated with IC₅₀ of temozolomide. Further analysis found that *Fn14* expression was downregulated by *IDH* mutations while *Fn14* methylation level was upregulated. These results indicated that the expression level of *Fn14* was a potential prediction biomarker in treatment for low-grade gliomas.

Summary points

- *Fn14* is a transmembrane protein that correlates with multiple intracellular processes *in vitro*.
- Whether *Fn14* could affect the glioma progression or prognosis in clinical are unclear yet.
- *Fn14* expression was significantly associated with tumor grade in gliomas.
- Glioma patients with higher *Fn14* expression tended to harbor shorter overall survival and disease-free survival.
- *Fn14* expression level was downregulated by *IDH1/IDH2* mutations.
- *IDH1/IDH2* mutations upregulated *Fn14* gene body methylation.
- *Fn14* expression was positively correlated with temozolomide IC₅₀.
- *Fn14* was an independent predictive biomarker for the progression and prognosis of gliomas.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: <https://www.futuremedicine.com/doi/suppl/10.2217/fo-2017-0598>

Authors' contributions

D Tan wrote the paper and analyzed the data. FM Pang and D Li prepared the analysis data. L Zhang and J Wu revised the whole paper. ZQ Liu revised the method and discussion. X Li and H Yan contributed to the study design, data analysis and final approval of the version to be published.

Financial & competing interests disclosure

This project was supported by Chinese National Science Foundation (No. 81403017), and National key research and development program (No. 2016YFC0905000, No. 2016YFC0905001, No. 2016YFC0905003, No. 2016YFC1201805), Hunan Provincial Natural Science Foundation of China (2017JJ3480), and Central South University. Postgraduate Independent Exploration and Innovation Project (2017zzts871). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The clinical data of this research were obtained from the TCGA, CGGA and rembrandt plans and so on, the clinical studies of these plans passed relevant ethical approval and informed consent were obtained from the patients for the participation.

Open access

This work is licensed under the Attribution-NonCommercial-NoDerivatives 4.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Van Den Bent MJ. Practice changing mature results of RTOG study 9802: another positive PCV trial makes adjuvant chemotherapy part of standard of care in low-grade glioma. *Neuro. Oncol.* 16(12), 1570–1574 (2014).
2. Van Den Bent MJ, Brandes AA, Taphoorn MJ *et al.* Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. *J. Clin. Oncol.* 31(3), 344–350 (2013).
3. Jiang T, Mao Y, Ma W *et al.* CGCG clinical practice guidelines for the management of adult diffuse gliomas. *Cancer Lett.* 375(2), 263–273 (2016).
- **Tells us about the overall background of adult diffuse gliomas.**
4. Li C, Wang Z, Tang X, Zeng L, Fan X, Li Z. Molecular mechanisms and potential prognostic effects of REST and REST4 in glioma (review). *Mol. Med. Rep.* 16(4), 3707–3712 (2017).
5. Brennan CW, Verhaak RG, McKenna A *et al.* The somatic genomic landscape of glioblastoma. *Cell* 155(2), 462–477 (2013).
6. Cancer Genome Atlas Research Network, Brat DJ, Verhaak RG. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N. Engl. J. Med.* 372(26), 2481–2498 (2015).
- **Provides us with some knowledge about IDH mutations and hypermethylation in gliomas.**
7. Mao CX, Yin JY, Zhang Y *et al.* The molecular classification of astrocytic tumors. *Oncotarget* 8(56), 96340–96350 (2017).
8. Annibaldi A, Meier P. Checkpoints in TNF-induced cell death: implications in inflammation and cancer. *Trends Mol. Med.* 24(1), 49–65 (2017).
9. Novoyatleva T, Sajjad A, Engel FB. TWEAK–Fn14 cytokine-receptor axis: a new player of myocardial remodeling and cardiac failure. *Front. Immunol.* 5, 50 (2014).
10. Wang X, Xiao S, Xia Y. Tumor necrosis factor receptor mediates fibroblast growth factor-inducible 14 signaling. *Cell. Physiol. Biochem. Int. J. Exper. Cell. Physiol. Biochem. Pharmacol.* 43(2), 579–588 (2017).
11. Zimmermann M, Koreck A, Meyer N *et al.* TNF-like weak inducer of apoptosis (TWEAK) and TNF- α cooperate in the induction of keratinocyte apoptosis. *J. Allergy Clin. Immunol.* 127(1), 200–207 (2011).
12. Lu JJ, Wang Q, Xie LH, Zhang Q, Sun SH. Tumor necrosis factor-like weak inducer of apoptosis regulates quadriceps muscle atrophy and fiber-type alteration in a rat model of chronic obstructive pulmonary disease. *Tobacco Induced Dis.* 15, 43 (2017).
13. Wiley SR, Cassiano L, Lofton T *et al.* A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. *Immunity* 15(5), 837–846 (2001).
14. Winkles JA. The TWEAK–Fn14 cytokine-receptor axis: discovery, biology and therapeutic targeting. *Nat. Rev. Drug Discov.* 7(5), 411–425 (2008).
- **Provides some important background information of TWEAK and Fn14.**
15. Burkly LC, Michaelson JS, Zheng TS. TWEAK/Fn14 pathway: an immunological switch for shaping tissue responses. *Immunol. Rev.* 244(1), 99–114 (2011).
- **Provides some information of TWEAK/Fn14 pathway.**
16. Burkly LC. TWEAK/Fn14 axis: the current paradigm of tissue injury-inducible function in the midst of complexities. *Semin. Immunol.* 26(3), 229–236 (2014).
17. Xu J, He J, He H, Peng R, Xi J. TWEAK–Fn14 influences neurogenesis status via modulating NF- κ B in mice with spinal cord injury. *Mol. Neurobiol.* 54(9), 7497–7506 (2016).
18. Perez JG, Tran NL, Rosenblum MG *et al.* The TWEAK receptor Fn14 is a potential cell surface portal for targeted delivery of glioblastoma therapeutics. *Oncogene* 35(17), 2145–2155 (2016).
- **We can know that Fn14 is a potential target of glioblastoma therapeutics from this article and we verified some of its results.**
19. Tran NL, McDonough WS, Savitch BA, Sawyer TF, Winkles JA, Berens ME. The tumor necrosis factor-like weak inducer of apoptosis (TWEAK)-fibroblast growth factor-inducible 14 (Fn14) signaling system regulates glioma cell survival via NF- κ B pathway activation and BCL-XL/BCL-W expression. *J. Biol. Chem.* 280(5), 3483–3492 (2005).
- **Demonstrates that TWEAK/Fn14 pathway can regulate glioma cell survival via NF- κ B pathway.**
20. Tran NL, McDonough WS, Savitch BA *et al.* Increased fibroblast growth factor-inducible 14 expression levels promote glioma cell invasion via Rac1 and nuclear factor- κ B and correlate with poor patient outcome. *Cancer Res.* 66(19), 9535–9542 (2006).
21. Fortin SP, Ennis MJ, Schumacher CA *et al.* Cdc42 and the guanine nucleotide exchange factors Ect2 and trio mediate Fn14-induced migration and invasion of glioblastoma cells. *Mol. Cancer Res.* 10(7), 958–968 (2012).
22. Brown SA, Cheng E, Williams MS, Winkles JA. TWEAK-independent Fn14 self-association and NF- κ B activation is mediated by the C-terminal region of the Fn14 cytoplasmic domain. *PLoS ONE* 8(6), e65248 (2013).
23. Fortin SP, Ennis MJ, Savitch BA *et al.* Tumor necrosis factor-like weak inducer of apoptosis stimulation of glioma cell survival is dependent on Akt2 function. *Mol. Cancer Res.* 7(11), 1871–1881 (2009).
24. Gao J, Aksoy BA, Dogrusoz U *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science Signaling* 6(269), p11 (2013).

25. Cerami E, Gao J, Dogrusoz U *et al.* The cBio Cancer Genomics Portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* 2(5), 401–404 (2012).
26. Yan W, Zhang W, You G *et al.* Molecular classification of gliomas based on whole genome gene expression: a systematic report of 225 samples from the Chinese Glioma Cooperative Group. *Neuro Oncol.* 14(12), 1432–1440 (2012).
27. Sun Y, Zhang W, Chen D *et al.* A glioma classification scheme based on coexpression modules of EGFR and PDGFRA. *Proc. Natl Acad. Sci. USA* 111(9), 3538–3543 (2014).
28. Bao ZS, Chen HM, Yang MY *et al.* RNA-seq of 272 gliomas revealed a novel, recurrent PTPRZ1-MET fusion transcript in secondary glioblastomas. *Genome Res.* 24(11), 1765–1773 (2014).
29. Madhavan S, Zenklusen J-C, Kotliarov Y, Sahni H, Fine HA, Buetow K. Rembrandt: helping personalized medicine become a reality through integrative translational research. *Mol. Cancer Res.* 7(2), 157–167 (2009).
30. Irizarry RA, Hobbs B, Collin F *et al.* Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 4(2), 249–264 (2003).
31. Cuddapah VA, Robel S, Watkins S, Sontheimer H. A neurocentric perspective on glioma invasion. *Nat. Rev. Neurosci.* 15(7), 455–465 (2014).
32. Tran NL, McDonough WS, Donohue PJ *et al.* The human Fn14 receptor gene is up-regulated in migrating glioma cells *in vitro* and overexpressed in advanced glial tumors. *Am. J. Pathol.* 162(4), 1313–1321 (2003).
33. Ensign SPF, Roos A, Mathews IT *et al.* SGEF is regulated via TWEAK/Fn14/NF- κ B signaling and promotes survival by modulation of the DNA repair response to temozolomide. *Mol. Cancer Res.* 14(3), 302–312 (2016).
- **Provides us some ideas about Fn14's effect in response to temozolomide.**
34. Yang J, Gong X, Ouyang L, He W, Xiao R, Tan L. PREX2 promotes the proliferation, invasion and migration of pancreatic cancer cells by modulating the PI3K signaling pathway. *Oncol. Lett.* 12(2), 1139–1143 (2016).
35. Vara JF, Casado E, De Castro J, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt signalling pathway and cancer. *Cancer Treat. Rev.* 30(2), 193–204 (2004).
36. Sinnberg T, Lasithiotakis K, Niessner H *et al.* Inhibition of PI3K-AKT-mTOR signaling sensitizes melanoma cells to cisplatin and temozolomide. *J. Investigat. Dermatol.* 129(6), 1500–1515 (2009).
37. Choi EJ, Cho BJ, Lee DJ *et al.* Enhanced cytotoxic effect of radiation and temozolomide in malignant glioma cells: targeting PI3K-AKT-mTOR signaling, HSP90 and histone deacetylases. *BMC Cancer* 14, 17 (2014).
38. Verhaak RG. Moving the needle: optimizing classification for glioma. *Science Translational Med.* 8(350), 350fs314 (2016).
39. Houillier C, Wang X, Kaloshi G *et al.* IDH1 or IDH2 mutations predict longer survival and response to temozolomide in low-grade gliomas. *Neurology* 75(17), 1560–1566 (2010).
40. Songtao Q, Lei Y, Si G *et al.* IDH mutations predict longer survival and response to temozolomide in secondary glioblastoma. *Cancer Sci.* 103(2), 269–273 (2012).
41. Lu Y, Kwintkiewicz J, Liu Y *et al.* Chemosensitivity of IDH1 mutant gliomas due to an impairment in PARP1-mediated DNA repair. *Cancer Res.* 77(7), 1709–1718 (2017).
42. Yang X, Han H, De Carvalho DD, Lay FD, Jones PA, Liang G. Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell* 26(4), 577–590 (2014).
- **Tells us about the relationship between gene body methylation and gene expression.**
43. Kulis M, Heath S, Bibikova M *et al.* Epigenomic analysis detects widespread gene-body DNA hypomethylation in chronic lymphocytic leukemia. *Nat. Genetics* 44(11), 1236–1242 (2012).