FXYD2 mRNA Expression Represents a New Independent Factor That Affects Survival of Glioma Patients and Predicts Chemosensitivity of Patients to Temozolomide

Fang Wang
Department of Encephalopathy, People's Hospital of Fujian University of Traditional Chinese Medicine & Fujian Provincial People's Hospital

Kaijia Zhou (zkj19770808@126.com)
Neuro-Oncology Surgery Department of Fujian Cancer Hospital & Fujian Medical University Cancer Hospital

Tao Jiang
Beijing Neurosurgical Institute, Capital Medical University

Hui Liang
Department of Encephalopathy, People's Hospital of Fujian University of Traditional Chinese Medicine & Fujian Provincial People's Hospital

Ming Zhang
Neuro-Oncology Surgery Department of Fujian Cancer Hospital & Fujian Medical University Cancer Hospital

Research Article

Keywords: FXYD2, mRNA, tumor, gliomas

DOI: https://doi.org/10.21203/rs.3.rs-140941/v1

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Abstract

Glioma is the most common primary intracranial tumor. Owing to the poor prognosis associated with high-grade gliomas, there is an urgent need to identify biomarkers related to prognosis and treatment sensitivity. Clinical features, FXYD2 mRNA expression levels, and survival data were analyzed for 1265 glioma samples from the Chinese Glioma Genome Map Project and two independent databases. The expression patterns for FXYD2 mRNA were compared using the chi-square test, and overall survival (OS) of glioma patients was evaluated according to FXYD2 mRNA expression levels. The factors affecting glioma survival were evaluated by Cox univariate and multivariate regression analysis. We found patients with primary oligodendroglioma, low World Health Organization (WHO) grade, low WHO molecular grade, isocitrate dehydrogenase (IDH) mutation, and combined deletion of 1p19q showed higher FXYD2 mRNA expression and longer survival times. Moreover, temozolomide (TMZ) chemotherapy was found to be an independent factor affecting survival in patients with high FXYD2 mRNA expression, but not in patients with low expression. So FXYD2 mRNA expression represents a new independent factor affecting the survival of glioma patients and may serve as an independent prognostic indicator to predict the sensitivity of gliomas to TMZ.

Introduction

Glioma, the most common primary malignant tumor of the brain [1], was classified in 2016 by the World Health Organization (WHO) based on co-deletion of the molecular markers, isocitrate dehydrogenase (IDH) and 1p19q [2]. Moreover, the prognosis of high-grade gliomas remains poor, particularly in patients with glioblastoma who have a 5-year survival rate of only 5% [1,3], even after administration of the standard three treatments: maximum surgical resection, radiotherapy, and chemotherapy. In fact, the average survival time for glioblastoma patients is only 14 months [4], which is caused not only by the difficulty associated with treating the heterogenous tumors [5] but also by the increasing level of resistance reported for temozolomide (TMZ), which renders this standard glioblastoma chemotherapeutic drug ineffective[6–9]. Meanwhile, the application of high-throughput technology for the molecular classification of gliomas as well as for screening differentially expressed genes and drug resistance genes has become a research hotspot to facilitate the development of corresponding targeted drugs.

Na/K-ATPase is an oligomeric transmembrane protein composed of α, β, and γ subunits that functions to maintain the dynamic membrane potential and is associated with many cellular functions as well as the pathogenesis of specific diseases [10]. Specifically, Na/K-ATPase upregulation has been reported in various cancers [11–15]. Meanwhile, inhibiting Na/K-ATPase activation and expression effectively inhibits cancer cell proliferation and survival [16–17]. FXYD2 (sodium/potassium-transporting ATPase subunit gamma) is the γ subunit of the Na/K-ATP enzyme and functions as a regulator of the enzyme activity [18]. Interestingly, a previous study reported that in ovarian clear cell carcinoma (CCC) patients, the expression level of FXYD2 was positively correlated with patient prognosis. Specifically, upregulated FXYD2 expression increased the sensitivity of ovarian CCC cells to the Na/K-ATPase inhibitor cardiotonic...
glycoside, thereby enhancing its therapeutic effect. However, the expression pattern and clinical significance of FXYD2 have not yet been reported in gliomas.

Here, through transcriptome sequencing, this study sought to establish the relationship between FXYD2 mRNA expression and the clinical features and survival data for glioma cases collected from the Chinese Glioma Genome Map Project as well as TCGA and REMBRANDT databases.

**Results**

**Clinical Features and FXYD2 mRNA Expression in 516 Glioma Patients in CGGA.**

Clinical features included sex, age, recurrence status, histopathology, WHO grade, IDH mutation status, 1p/19q co-deletion status, radiotherapy status, chemotherapy, and WHO classification (2016). The patients were divided into two groups according to a median age of 43 years. The 516 glioma patients were then classified as having low or high FXYD2 mRNA expression based on median expression values (Table 1). The median expression value was ≥ the median expression value and < median expression value (Table 1).
Table 1
Relationship between FXYD2 mRNA expression and clinical features in 516 glioma patients

| Parameter                        | Variable               | N    | FXYD2 mRNA expression | χ²   | P value |
|----------------------------------|------------------------|------|-----------------------|------|---------|
|                                  |                        |      | Low  | High |        |        |        |
|                                  |                        |      | %    | %    |        |        |        |
| Sex                              | Female                 | 225  | 115  | 51.1 | 110   | 48.9  | 0.197  | 0.657  |
|                                  | Male                   | 291  | 143  | 49.1 | 148   | 50.9  |        |        |
| Age*                             | < 43                   | 238  | 119  | 50.0 | 119   | 50.0  | 0.000  | 1.000  |
|                                  | ≥ 43                   | 278  | 139  | 50.0 | 139   | 50.0  |        |        |
| Progression status               | Primary                | 301  | 129  | 42.9 | 172   | 57.1  | 14.743 | 0.000  |
|                                  | Recurrent              | 215  | 129  | 60.0 | 86    | 40.0  |        |        |
| Histopathological types          | Oligodendroglioma      | 114  | 31   | 27.2 | 83    | 72.8  | 31.937 | 0.000  |
|                                  | Astrocytoma            | 218  | 117  | 53.7 | 101   | 46.3  |        |        |
|                                  | Glioblastoma           | 184  | 110  | 59.8 | 74    | 40.2  |        |        |
| WHO classification               | II                     | 134  | 60   | 44.8 | 74    | 55.2  | 10.951 | 0.004  |
|                                  | III                    | 198  | 88   | 44.4 | 110   | 55.6  |        |        |
|                                  | IV                     | 184  | 110  | 59.8 | 74    | 40.2  |        |        |
| IDH mutation status              | Wildtype               | 224  | 155  | 69.2 | 69    | 30.8  | 58.347 | 0.000  |
|                                  | Mutant                 | 292  | 103  | 35.3 | 189   | 64.7  |        |        |
| 1p/19q codeletion status         | Non-codel              | 401  | 231  | 57.6 | 170   | 42.4  | 41.636 | 0.000  |
|                                  | Codel                  | 115  | 27   | 23.5 | 88    | 76.5  |        |        |
| Radiotherapy                     | No                     | 108  | 54   | 50.0 | 54    | 50.0  | 0.000  | 1.000  |
|                                  | Yes                    | 408  | 204  | 50.0 | 204   | 50.0  |        |        |
| Chemotherapy                     | No                     | 124  | 60   | 48.4 | 64    | 51.6  | 0.170  | 0.680  |
|                                  | Yes                    | 392  | 198  | 50.5 | 194   | 49.5  |        |        |
| WHO classification (2016)        | IDH Mutant, 1p/19q     | 101  | 22   | 21.8 | 79    | 78.2  | 72.323 | 0.000  |
|                                  | Codel (LGG)            |      |      |      |       |       |        |        |

*The patients were divided into two groups according to the median age of 43 years.*
| Parameter           | Variable                                      | N  | FXYD2 mRNA expression | \(\chi^2\) | P value |
|---------------------|-----------------------------------------------|----|-----------------------|------------|---------|
|                     |                                               |    | Low  | High  |         |
| IDH Mutant, 1p/19q  | Non-codel (LGG)                               | 154| 68   | 44.2  | 86      | 55.8    |
| IDH Wildtype (LGG)  |                                               | 77 | 58   | 75.3  | 19      | 24.7    |
| IDH Mutant (GBM)    |                                               | 37 | 13   | 35.1  | 24      | 64.9    |
| IDH Wildtype (GBM)  |                                               | 147| 97   | 66.0  | 50      | 34.0    |

*The patients were divided into two groups according to the median age of 43 years.

**Relationship Between FXYD2 mRNA Expression and Clinical Features in Glioma Patients.**

The relationship between clinical characteristics and FXYD2 mRNA expression was analyzed. FXYD2 mRNA expression was not associated with sex (P = 0.657), age (P = 1.000) or radiotherapy or chemotherapy status (P = 1.000, P = 0.680, respectively) in glioma patients. It was, however, significantly correlated with recurrence (P = 0.000), histopathology (P = 0.000), WHO grade (P = 0.004), IDH mutation (P = 0.000), 1p/19q co-deletion (P = 0.000), and WHO molecular grade (2016; P = 0.000). Patients with good prognostic indicators, such as primary, oligodendroglioma, low WHO grade, IDH mutation, 1p/19q co-deletion, and low WHO molecular grade had increased FXYD2 mRNA expression (Table 1).

**FXYD2 mRNA Expression Is Higher in Glioma Patients With Better Prognosis.**

FXYD2 mRNA expression in glioma patients with different clinical and molecular pathological features was compared using a scatter plot. The clinical features assessed included sex, age, recurrence (Fig. 1), histopathology, WHO grade (Fig. 2), IDH mutation, 1p/19q co-deletion status, and 2016 WHO molecular grade (Fig. 3). The patients were divided into two groups according to the median age of 43 years. The results showed that the expression of FXYD2 mRNA was higher in patients with a good prognosis, including those with primary glioma (P = 0.00031), oligodendroglioma (P = 5.6e-10), WHO low grade (P = 0.00011), IDH mutation (P = 2.5e-18), 1p/19q co-deletion (P = 5.3e-12), and low WHO molecular grade (2016) (P = 2.3e-20).

Moreover, the survival time for glioma patients with high FXYD2 mRNA expression was longer. Kaplan–Meier survival curves were used to explore the effect of FXYD2 mRNA expression on the total survival time of glioma patients. The results show that the survival time of patients with high expression of FXYD2 mRNA was longer than that of patients with low expression of WHOII (P = 0.000; Fig. 4A). After stratifying the data according to WHO grade, the same result was observed in patients with all grades of glioma: WHOII (P = 0.011; Fig. 4B), WHOIII (P = 0.000; Fig. 4C), and WHOIV (P = 0.043; Fig. 4D). The same results were also obtained for patients with primary initial gliomas (P = 0.000; Fig. 4E) and relapse (P = 0.000; Fig. 4F).

**FXYD2 mRNA Expression Can Predict the Survival and Prognosis of Glioma Patients.**
Subgroup analysis showed that different subgroups of glioma patients with high FXYD2 mRNA expression also had longer OS. Among them, low-grade glioma (P = 0.011), high-grade glioma (P = 0.000), oligodendroglioma (P = 0.004), astrocytoma (P = 0.000), \(IDH\) mutant type (P = 0.000), \(IDH\) wild type (P = 0.180), \(1p/19q\) co-deletion type (P = 0.033), and \(1p/19q\) non-co-deletion type (P = 0.000) (Fig. 5).

**FXYD2 mRNA Expression can Predict the Survival and Prognosis of Glioma Patients in Two Independent Databases.**

Using Kaplan–Meier survival curves, it was confirmed in two independent databases that glioma patients with high FXYD2 mRNA expression had a longer survival time than patients with low expression from TCGA (P = 0.000, Fig. 6A) and REMBRANDT database (P = 0.000, Fig. 6B). Further subgroup analysis on the two independent datasets showed that patients with low- or high-grade gliomas that had high FXYD2 mRNA expression also exhibited longer survival times (Fig. 6C–F).

**FXYD2 mRNA Expression is an Independent Factor Affecting the Survival of Glioma Patients.**

Univariate Cox analysis was used to identify the factors affecting the survival of glioma patients, including sex, age, recurrence, histopathology, WHO grade, \(IDH\) mutation status, \(1p/19q\) co-deletion status, radiotherapy or chemotherapy status, and FXYD2 mRNA expression. Multivariate Cox analysis showed that high FXYD2 mRNA expression (HR: 0.751, 95% CI 0.583–0.967, P = 0.026), \(IDH\) mutation (HR: 0.714, 95% CI: 0.532–0.959, P = 0.025), \(1p/19q\) co-deletion (HR: 0.379, 95% CI: 0.253–0.567, P = 0.000), and chemotherapy status (HR: 0.615, 95% CI: 0.452–0.835, P = 0.002) were independent factors associated with longer patient survival. Meanwhile, age (HR: 1.015, 95% CI: 1.005–1.024, P = 0.002), relapse (HR: 2.081, 95% CI: 1.650–2.625, P = 0.000), and WHO grade (HR: 2.754, 95% CI: 1.972–3.847, P = 0.000) represented independent factors associated with poor survival (Table 2).
Table 2
Correlation analysis between FXYD2 mRNA expression and overall survival among 516 glioma patients in CGGA. HR = hazard ratio; CI = confidence interval.

| Parameter                  | Univariate Cox Regression | Multivariate Cox Regression |
|---------------------------|---------------------------|----------------------------|
|                           | P value | HR    | 95% CI (low-up) | P value | HR    | 95% CI (low-up) |
| Sex                       | 0.447   | 1.091 | 0.871–1.366     |         |       |                |
| Age                       | 0.000   | 1.027 | 1.017–1.036     | 0.002   | 1.015 | 1.005–1.024     |
| Recurrent                 | 0.000   | 2.117 | 1.690–2.651     | 0.000   | 2.081 | 1.650–2.625     |
| Histopathological types   | 0.000   | 1.827 | 1.657–2.015     | 0.225   | 0.876 | 0.708–1.085     |
| WHO classification        | 0.000   | 2.804 | 2.377–3.308     | 0.000   | 2.754 | 1.972–3.847     |
| IDH mutation status       | 0.000   | 0.321 | 0.256–0.403     | 0.025   | 0.714 | 0.532–0.959     |
| 1p/19q codeletion status  | 0.000   | 0.282 | 0.199–0.400     | 0.000   | 0.379 | 0.253–0.567     |
| Radiotherapy              | 0.467   | 1.109 | 0.839–1.465     | 0.221   | 0.824 | 0.604–1.123     |
| Chemotherapy              | 0.230   | 1.176 | 0.903–1.533     | 0.002   | 0.615 | 0.452–0.835     |
| FXYD2 mRNA expression     | 0.000   | 0.469 | 0.374–0.589     | 0.026   | 0.751 | 0.583–0.967     |

**Increased FXYD2 mRNA Expression Can Predict the Chemosensitivity of Glioma Patients.**

According to the median expression of FXYD2 mRNA, the patients were divided into two groups: low or high expression. Univariate Cox analysis was used to investigate the related factors affecting the survival time of glioma patients, including sex, age, recurrence, histopathology, WHO grade, IDH mutation status, 1p/19q co-deletion status, radiotherapy, and chemotherapy status. The results of multivariate Cox analysis showed that in the group with high FXYD2 mRNA expression, chemotherapy status (HR: 0.427, 95% CI: 0.262–0.695, P = 0.001), IDH mutation status (HR: 0.498, 95% CI 0.316–0.783, P = 0.003), and 1p/19q co-deletion (HR: 0.405, 95% confidence interval 0.229–0.716, P = 0.002) were independent factors associated with longer survival. Meanwhile, age (HR value: 1.016, 95% CI: 1.000–1.031, P = 0.043), relapse (HR value: 2.669, 95% CI: 1.832–3.889, P = 0.000), WHO grade (HR value: 3.749, 95% CI: 2.109–6.663, P = 0.000) were independent factors associated with poor survival (Table 3). However, in the group with low FXYD2 mRNA expression, chemotherapy status was not an independent factor affecting the survival of patients with glioma (univariate Cox analysis P = 0.132, multivariate Cox analysis P = 0.192; Table 3).
Table 3
Correlation analysis between chemotherapy and overall survival among glioma patients with high and low FXYD2 mRNA expression in CGGA. HR = hazard ratio; CI = confidence interval.

| Parameter                        | Univariate Cox Regression | Multivariate Cox Regression |
|----------------------------------|---------------------------|----------------------------|
|                                  | P value | HR     | 95% CI (low-up) | P value | HR     | 95% CI (low-up) |
| High                             |         |        |                |         |        |                |
| Sex                              | 0.002   | 1.792  | 1.231–2.607    | 0.096   | 1.418  | 0.940–2.138    |
| Age                              | 0.000   | 1.031  | 1.015–1.047    | 0.043   | 1.016  | 1.000–1.031    |
| Recurrent                        | 0.000   | 2.041  | 1.432–2.910    | 0.000   | 2.669  | 1.832–3.889    |
| Histopathological types          | 0.000   | 2.037  | 1.754–2.365    | 0.472   | 0.879  | 0.618–1.250    |
| WHO classification               | 0.000   | 3.589  | 2.727–4.723    | 0.000   | 3.749  | 2.109–6.663    |
| IDH mutation status              | 0.000   | 0.261  | 0.181–0.375    | 0.003   | 0.498  | 0.316–0.783    |
| 1p/19q codeletion status         | 0.000   | 0.309  | 0.199–0.480    | 0.002   | 0.405  | 0.229–0.716    |
| Radiotherapy                     | 0.256   | 1.300  | 0.827–2.043    | 0.359   | 0.781  | 0.461–1.324    |
| Chemotherapy                     | 0.909   | 1.024  | 0.682–1.537    | 0.001   | 0.427  | 0.262–0.695    |
| Low                              |         |        |                |         |        |                |
| Sex                              | 0.101   | 0.786  | 0.589–1.048    |         |        |                |
| Age                              | 0.000   | 1.023  | 1.011–1.035    | 0.028   | 1.013  | 1.001–1.025    |
| Recurrent                        | 0.000   | 1.880  | 1.399–2.525    | 0.000   | 1.856  | 1.374–2.508    |
| Histopathological types          | 0.000   | 1.583  | 1.387–1.807    | 0.236   | 0.849  | 0.648–1.113    |
| WHO classification               | 0.000   | 2.222  | 1.818–2.716    | 0.000   | 2.472  | 1.630–3.750    |
| IDH mutation status              | 0.000   | 0.472  | 0.347–0.642    | 0.398   | 0.851  | 0.586–1.237    |
| 1p/19q codeletion status         | 0.001   | 0.363  | 0.197–0.668    | 0.009   | 0.426  | 0.225–0.808    |
| Radiotherapy                     | 0.861   | 0.969  | 0.679–1.382    | 0.253   | 0.797  | 0.541–1.176    |
| Chemotherapy                     | 0.132   | 1.310  | 0.922–1.862    | 0.192   | 0.764  | 0.510–1.145    |

Discussion

The FXYD2 gene is located on chromosome 11q23 [21], while the FXYD2 protein is the r subunit of the Na-K-ATP enzyme. FXYD2 has been shown to reduce the Na ion affinity of Na-K-ATP [22], resulting in
subsequent inhibition of cell proliferation [23]. However, the expression and application value of FXYD2 mRNA in gliomas have not been previously reported.

This study revealed that the expression of FXYD2 mRNA is related to the degree of malignancy of gliomas. Specifically, higher degree malignancies are associated with lower FXYD2 mRNA expression, suggesting that FXYD2 mRNA expression can be used as a predictive biomarker for the degree of malignancy of gliomas. Moreover, FXYD2 mRNA expression was found to be related to the survival time of glioma patients with lower expression associated with shorter survival time, suggesting that it can also be used to predict patient survival prognosis. FXYD2 mRNA expression was also related to the chemosensitivity of glioma patients to TMZ. Meanwhile, TMZ represents an independent factor affecting the survival of glioma patients with high expression of FXYD2 mRNA, but not patients with low expression. Hence, we postulate that the expression of FXYD2 mRNA can be used to predict the chemosensitivity to TMZ. Specifically, patients with high FXYD2 mRNA expression will be more likely to respond to TMZ therapy, thereby prolonging survival time, while those with low expression will not benefit from this therapy. These results were similar to those reported by Hsu I-Ling et al [24] who found that, compared with ovarian cancer cells expressing low levels of FXYD2, those with high expression were more sensitive to cardiosides, while cardiotonic glycosides can effectively inhibit the growth of ovarian cancer cells.

Currently, the underlying mechanism associated with the effects of FXYD2 in tumors is unclear. However, the Na-K-ATPase serves as the transport system for Na and K ions on the cell membrane [25], which serves to maintain the Na/K ion concentration gradient inside and outside of the cell. These gradients are essential for maintaining cell volume and membrane potential and also guarantee the maintenance of intracellular homeostasis[25]. They also provide nutrients to the cells and regulate the concentration of intracellular pH and calcium ions. Meanwhile, increased expression of FXYD2 was found to decrease the activity of Na/K-ATPase, causing us to speculate that the increase in FXYD2 decreased Na/K-ATPase activity, thereby disrupting the ion homeostasis inside and outside of the tumor cell membrane while also decreasing the nutritional supply to tumor cells and subsequently inhibiting tumor cell proliferation causing tumor cells to become more unstable and vulnerable to chemotherapeutic drugs. However, these hypotheses must be verified by further investigations.

Moreover, another study has reported that the body senses mechanical pain abnormalities caused by peripheral inflammation through FXYD2 in neurons [26]. Following peripheral tissue inflammation, the interaction between FXYD2 and the α subunit of Na/K-ATPase is enhanced, causing downregulation of Na/K-ATPase activity, while increasing neuronal membrane potential depolarization and excitability. The body then senses peripheral inflammatory stimulation signals, resulting in corresponding inflammatory stress and clearance responses. We, therefore, speculate that FXYD2 also exerts anti-tumor effects by enhancing the inflammatory response of the body toward tumors. However, this hypothesis also requires further verification.

Methods
Data Collection. Data, including clinical information (sex, age), histopathology, WHO grade, molecular pathology (IDH mutation, 1p/19q deletion), and follow-up information (survival time), of 516 glioma patients were collected from the Chinese glioma Genome Map Project (CGGA). All patients were classified according to the World Health Organization (WHO) molecular classification in 2016\(^2\). The overall survival (OS) rate of clinical end-point events was calculated from the initial pathological diagnosis to death or last follow-up. This study was approved by the Institutional Ethics Committee of Beijing Tiantan Hospital (KYSB 2015–017), and all patients provided written informed consent.

mRNA Sequencing.

mRNA Transcriptome Sequencing. According to the manufacturer’s instructions, total RNA was extracted with an RNeasy Mini Kit (Qiagen). Pestle and QIAshredder (Qiagen) were used to crush and homogenate the frozen tissue. The RNA integrity was assessed via electrophoresis using the 2100 bioanalyzer (Agilent Technologies), and only high-quality samples with RNA integrity numbers (RIN) $\geq 6.8$ were used to construct the sequencing library. Briefly, 1 $\mu$g of total RNA was used in conjunction with the TruSeq RNA library preparation kit (Illumina). With the exception of SuperScript III reverse transcriptase (Invitrogen) used the synthesis of the first strand of cDNA, all other operations were low-throughput. Following PCR amplification, and purification of the junction fragments, the DNA concentration of the junction was determined by quantitative PCR (biological system 7500) with QP1 5$^\prime$-AATGATACGGCGACCACCGA-3$^\prime$ primers and QP2 5$^\prime$-CAAGCAGAAGACGGCATACGAGA-3$^\prime$ primers. The length of the DNA fragment was measured using a 2100 bioanalyzer, and the median size of the inserted fragment was 200 bp. The RNA-seq library was sequenced using the Illumina HiSeq 2000 Universe 2500 Universe 4000 sequencing system. The library adopts a paired end strategy, with reading lengths of 101 bp, 125 bp, or 150 bp. Base invocation was performed using the Illumina Casava v1.8.2 pipeline.

Mapping and Quantification. STAR (v2.5.2b, Dobin et al., 2012) and RSEM (v1.2.31, Li et al., 2011) software were used for RNA-seq mapping and quantification. These reads were then compared with the Human Genome reference (GENCODE v19, hg19) for STAR, after which RSEM was used to calculate the sequencing reads for each GENCODE gene. The expression levels of different samples were combined into an FPKM matrix (fragments per million fragments per kilobase transcriptome). Only when the expression level was $> 0$ in half the samples was a gene defined as expressed. Finally, we retained only the expressed genes in the mRNA expression profile.

RNA-Seq Comparison Workflow. STAR (v2.5.2b) was used to compare the mRNA profiles. For each RNA-seq sample, STAR compares each read group with the human reference genome (GENCODE v19, hg19), and then merges the alignment results. This workflow generates a BAM file that contains both aligned and unaligned reads (against data). All experimental methods were carried out in accordance with the relevant guidelines and regulations that were previously reported [19]. Preparation, sequencing, and data analysis of the RNA-seq library were the same as that described previously[20].
**Verification Group Data Collection.** The clinical, histopathological, and survival follow-up data, as well as FXYD2 mRNA sequencing data, for glioma patients were collected from two open independent datasets. Among them, 481 cases were from the cancer genome map database (TCGA, http://cancergenome.nih.gov), and 268 were from the molecular brain tumor database (REMBRANDT, http://caintegrator-info.nci.nih.gov).

**Statistical Analysis.** R software 3.3.2 and SPSS software 25.0 were used to perform all statistical analyses and to generate box scatter plots and survival curves. The normally distributed data were expressed as mean ± standard deviation (x ± s). Student’s t-tests, one-way ANOVA, and LSD-t pairwise comparisons were used to compare FXYD2 mRNA expression in different groups. Kaplan–Meier curve and log rank test were used to analyze the OS of patients in different groups. Univariate and multivariate Cox regression analyses were used to analyze the factors affecting the survival time of glioma patients. All statistical analyses were bilateral, and results were considered statistically significant at P < 0.05.

**Conclusions**

This study revealed that the expression of FXYD2 mRNA in gliomas can predict the degree of malignancy and survival time of patients. At the same time, FXYD2 mRNA expression can predict the chemosensitivity of glioma patients to TMZ. However, considering that our study is limited to mRNA, the transcriptional regulation, protein translation, as well as underlying regulatory mechanisms and pathways of FXYD2 remain unclear and require further investigation.

**Declarations**

**Acknowledgements**

This work was supported by the National Natural Science Foundation of China (NSFC)/Research Grants Council (RGC) Joint Research Scheme (81761168038, 2018.01-2021.12), and the Beijing Municipal Administration of Hospitals’ Mission Plan (SML20180501, 2018.03-2022.02).

**Author contributions statement**

Fang Wang performed the experiments and analysis, collected and interpreted the data, and wrote the manuscript. Kaijia Zhou, Tao Jiang, Hui Liang, and Ming Zhang collected the data. Kaijia Zhou designed the study and edited the manuscript. All authors reviewed the manuscript.

**Competing Interests**

The authors declare that manuscript, or any part of it, has not been previously published or submitted concurrently to any other journal.

**References**
1. Jiang, T. *et al.* CGCG clinical practice guidelines for the management of adult diffuse gliomas. *Cancer Lett* **375**, 263-273, doi:10.1016/j.canlet.2020.10.050 (2021).

2. Louis, D.N. *et al.* The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol* **131**, 803-820, doi:10.1007/s00401-016-1545-1 (2020).

3. Jiang, T. *et al.* Clinical practice guidelines for the management of adult diffuse gliomas. *Cancer Lett* **499**, 60-72, doi:10.1016/j.canlet.2020.10.050 (2020).

4. Lin, T. K. *et al.* The long non-coding RNA LOC441204 enhances cell growth in human glioma. *Sci Rep* **7**, 5603, doi:10.1038/s41598-017-05688-0 (2017).

5. Zhao, C. B. *et al.* MicroRNA-128-3p Enhances the Chemosensitivity of Temozolomide in Glioblastoma by Targeting c-Met and EMT. *Sci Rep* **10**, 9471, doi:10.1038/s41598-020-65331-3 (2020).

6. Choi, S., Yu, Y., Grimmer M. R., Wahl, M., Chang, S. M. & Costello, J. F. Temozolomide-associated hypermutation in gliomas. *Neuro-oncology* **20**, 1300-1309, doi:10.1093/neuonc/noy016 (2018).

7. Wang, J. *et al.* Clonal evolution of glioblastoma under therapy. *Genet* **48**, 768-776, doi:10.1038/ng.3590 (2016).

8. Muscat, A. M. *et al.* The evolutionary pattern of mutations in glioblastoma reveals therapy-mediated selection. *Oncotarge* **9**, 7844-7858, doi:10.18632/oncotarget.23541(2018).

9. Handing, J. W. & Criss, A. K. The lipooligosaccharide-modifying enzyme LptA enhances gonococcal defence against human neutrophils. *Cell Microbio* **17**, 910-921, doi:10.1111/cmi.12411 (2015).

10. Rose, A. M. & Valdes, R. J. Understanding the sodium pump and its relevance to disease. *Clin Chem* **40**, 1674–1685, (1994).

11. Lefranc, F. *et al.* Targeting the alpha 1 subunit of the sodium pump to combat glioblastoma cells. *Neurosurgery* **62**, 211–212, doi:10.1227/01.NEU.0000311080.43024.0E (2008).

12. Mijatovic, T. *et al.* The alpha1 subunit of the sodium pump could represent a novel target to combat non-small cell lung cancers. *J Patho* **212**, 170–179, doi:10.1002/path.2172 (2007).

13. Sakai, H. *et al.* Up-regulation of Na(+), K(+)-ATPase alpha 3-isofrom and down-regulation of the alpha1-isofrom in human colorectal cancer. *FEBS Lett* **563**, 151–154, doi:10.1016/S0014-5793(04)00292-3 (2004).

14. Mathieu, V. *et al.* The sodium pump alpha1 sub-unit: a disease progression-related target for metastatic melanoma treatment. *J Cell Mol Med* **13**, 3960–3972, doi:10.1111/j.1582-4934.2009.00708.x (2009).

15. Shibuya, K. *et al.* Increase in ouabain-sensitive K+-ATPase activity in hepatocellular carcinoma by overexpression of Na+, K+-ATPase alpha 3-isofrom. *Eur J Pharmacol* **638**, 42–46, doi:10.1016/j.ejphar.2010.04.029 (2010).

16. Arrebola, F., Zabiti, S., Cañizares F. J., Cubero M. A., Crespo, P. V. & Fernández, S. Changes in intracellular sodium, chlorine, and potassium concentrations in staurosporine-induced apoptosis. *J Cell Physiol* **204**, 500-507, doi:10.1002/jcp.20306 (2005).
17. Tian, J. et al. Changes in sodium pump expression dictate the effects of ouabain on cell growth. *J Biol Chem* 284, 14921–14929, doi:10.1074/jbc.M808355200 (2009).

18. Minor, N. T., Sha, Q., Nichols, C. G. & Mercer, R. W. The gamma subunit of the Na,K-ATPase induces cation channel activity. *Proc Natl Acad Sci USA* 95, 6521–6525, doi:10.1073/pnas.95.11.6521 (1998).

19. Bao, Z. S. et al. RNA-seq of 272 gliomas revealed a novel, recurrent PTPRZ1-MET fusion transcript in secondary glioblastomas. *Genome Res* 24, 1765-1773, doi:10.1101/gr.165126.113 (2014).

20. Zhao, Z., Meng, F. L., Wang, W., Wang, Z., Zhang, C. B. & Jiang, T. Comprehensive RNA-seq transcriptomic profiling in the malignant progression of gliomas. *Sci Data* 4, 170024, doi:10.1038/sdata.2017.24 (2017).

21. Sweadner, K. J., Wetzel, R. K. & Arystarkhova, E. Genomic organization of the human FXYD2 gene encoding the of the Na,K-ATPase. *Biochem Biophys Res Commun* 279, 196–201, doi:10.1006/bbrc.2000.3907 (2000).

22. Béguin, P., Crambert, G., Guennoun, S., Garty, H., Horisberger, J. D. & Geering, K. CHIF, a member of the FXYD protein family, is a regulator of Na,K-ATPase distinct from the gamma-subunit. *EMBO J* 20, 3993-4002, doi:10.1093/emboj/20.15.3993 (2001).

23. Arystarkhova, Elena., Donnet, Claudia., Asinovski, N. K. & Sweadner, K. J. Differential regulation of renal Na,K-ATPase by splice variants of the gamma subunit. *J Biol Chem* 277, 10162-10172, doi:10.1074/jbc.M111552200 (2002).

24. Hsu, L. L. et al. Targeting FXYD2 by cardiac glycosides potently blocks tumor growth in ovarian clear cell carcinoma. *Oncotarget* 7, 62925-62938, doi:10.18632/oncotarget.7497 (2016).

25. Delprat, B., Bibert, S. & Geering, K. FXYD proteins: novel regulators of Na,K-ATPase. *Med Sci (Paris)* 22, 633-638, doi:10.1051/medsci/20062267633 (2006).

26. Wang, F. et al. FXYD2, a γ subunit of Na\(^+\), K\(^+\)-ATPase, maintains persistent mechanical allodynia induced by inflammation. *Cell Res* 25, 318-334, doi:10.1038/cr.2015.12 (2015).