High TROAP Expression Correlates With Shorter Survival in Patients With Glioma: A Study Based on Multiple Data Fusion Analysis

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Research Article

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Abstract

Trophin-associated protein (TROAP) was originally identified to mediate the embryo transfer process and participate in the regulation of microtubules but was later found to be associated with the biological behavior of various types of cancers. However, there is limited information about the role of TROAP in glioma. In this study, thousands of glioma samples were obtained from multiple independent datasets to detect changes in TROAP mRNA and protein expression levels in glioma, we found that compared with normal brain tissues, the expression of TROAP in glioma was significantly increased at both levels. Then, the correlations between TROAP and clinical characteristics and prognosis in glioma were revealed through a series of bioinformatics analysis methods. The overexpression of TROAP was an independent risk factor for glioma and was associated with a reduced overall survival rate of glioma patients. In addition, TROAP had value for determining the prognosis of patients, especially patients with WHO grade III glioma. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used to verify the expression level of TROAP in glioma cell lines. Subsequently, GSEA identified homologous recombination, cell cycle and p53 signalling pathways as differentially enriched with the high TROAP expression phenotype. Finally, four drugs that may inhibit TROAP expression and have potential therapeutic value for glioma were screened out through CMap website: bezafibrate, clobetasol, scriptaid, and thioguanosine. In conclusion, TROAP, as a new oncogene, leads to poor prognosis of glioma patients, and as a highly specific biomarker, provides the possibility for individual clinical treatment of glioma patients.

Introduction

Glioma is the most common and malignant type of primary brain tumor [1]. Four to eleven per 100,000 persons are diagnosed with glioma each year worldwide, and glioma is more common in highly developed, industrialized countries. Gliomas are termed low grade (WHO grades I and II) or high grade (WHO grades III and IV), depending on the molecular characteristics of the glioma cells [2, 3]. Glioblastoma (WHO grade IV) is the deadliest glioma, with a median survival of only 14-17 months in the era of safe maximum resection and temozolomide chemoradiotherapy [4, 5]. In addition, glioma has a strong ability to infiltrate deep into surrounding normal brain tissues, which often leads to tumors that cannot be completely removed, resulting in a high recurrence rate after surgery [6]. The grim reality of glioma exists because we have not fully clarified its pathogenesis. With advances in science and technology in recent years, humans have realized that the occurrence and development of each type of tumor is a multigene, multistage process. Of course, gliomas are no exception. A variety of tumor-specific molecular changes have been closely related to glioma, especially IDH mutation, TP53 mutation, 1p/19q co-deletion and amplification of epidermal growth factor receptor variant III (EGFRvIII), which have been proven to be indicators in the diagnosis and prognosis of glioma [7-10]. However, these biomarkers cannot fully reveal the pathological processes of gliomas because the development of tumors is multifaceted and influenced by many factors. Hence, it is necessary to identify new biomarkers to gain an in-depth understanding of the pathological mechanism of glioma and then provide more options for the diagnosis and treatment of glioma patients.

TROAP, officially known as tasting, was initially identified in the cytoplasm as a cell adhesion molecule that forms a complex with trophoblast protein and bystin to mediate trophoblast cells initially attached to the endometrial epithelium during early embryo implantation [11]. Given that embryo transfer is similar to the metastasis and invasion mechanisms of malignant tumor cells, we speculate that TROAP may also participate in the metastasis and invasion of tumor cells. Interestingly, after reviewing the literature, we found that overexpression of TROAP has been reported to be positively linked with the poor prognosis of ovarian cancer. Thus, TROAP is considered to be a reliable prognostic marker for ovarian cancer [12]. Some studies have also shown that the downregulation of TROAP can significantly inhibit the transition from G1 to S phase and the invasion and migration of breast cancer cells in vitro [13]. Some reports revealed that abnormal expression of TROAP has the ability to enhance the malignancy and development of hepatocellular carcinoma, colorectal cancer and other tumors [14, 15]. However, the relationship between TROAP and the prognosis and clinical characteristics of glioma has not been elucidated.

To explore the relationship between TROAP and the prognosis of glioma, this study first collected thousands of tissue samples and clinicopathological data through a variety of databases, GEPIA, TCGA, HPA, CGGA and GEO. The above databases not only contain the information of different races but also use a variety of detection technologies, such as gene sequencing, gene microarray, and even experimental immunohistochemistry. Therefore, this study comprehensively revealed the pathological
mechanism by which abnormally high expression of TROAP leads to poor prognosis in glioma patients from multiple levels and further identified small molecule compounds with the potential to inhibit TROAP based on omics studies and large datasets. Given what is known about TROAP, we have sufficient reason to speculate that this study will reveal pathological mechanisms of glioma by confirming the function of TROAP. In addition, this research provides valuable biomarkers for the individualized treatment of glioma to prolong the survival time of glioma patients.

Materials And Methods

Data collection

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html) is an online public database for the analysis of a variety of human tumor tissue samples and corresponding normal tissue samples established by Peking University[16]. We searched and obtained TROAP data from a variety of human tissues, including 163 glioblastoma, 518 low-grade glioma and 207 normal brain tissues. Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) is a large public genetic database for storing high-throughput microarray and next-generation sequencing functional genomic datasets submitted by the research community[17]. We downloaded two microarray datasets, GSE50161 and GSE116520, from the GEO database. In addition, we collected samples from 1669 glioma patients, including 748 samples from the China Glioma Atlas (CGGA)-RNA sequencing project, 268 samples from the CGGA microarray project, and 653 samples from The Cancer Genome Atlas (TCGA)-RNA sequencing project. Table S1, Table S2 and Table S3 provide the clinical information of the patients corresponding to the three datasets. The Human Protein Atlas (HPA) (https://www.proteinatlas.org/) is a tool that is used to provide protein immunohistochemistry (IHC) data and is widely used in the study of protein localization and expression in human tissues and cells[18]. The expression of TROAP protein in glioma as analysed by immunohistochemistry was used to divide the samples into a normal group, low-grade group and high-grade group.

GSEA analysis of TROAP

Gene set enrichment analysis (GSEA) is an analysis method for whole-genome expression profiling data that compares genes with a predefined set of genes[19]. We used GSEA 4.0. software to analyses TROAP-related cell signaling pathways. Each analysis included 1,000 permutations. We then subjected the dataset to enrichment analysis using the KEGG database.

CMap predicts potential therapeutic drugs

Connectivity Map (CMap) (https://portals.broadinstitute.org/CMap/) is an online website that details the interactions among diseases, gene expression models, and small molecule compounds. Researchers often use it to obtain small molecule compounds that have potential therapeutic effects in certain diseases[20]. R language was used to screen out differentially expressed genes related to TROAP. Then, 20 differentially expressed genes (10 upregulated and 10 downregulated) were selected and uploaded to the CMap network tool, and potential drugs for treating glioma were explored.

Cell culture and Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) analysis

Table 1. Sequences of primers used for RT-qPCR analysis.

| Gene    | Primer sequence (5′-3′)          |
|---------|---------------------------------|
| TROAP-F | CAGGCCTTTCCCACCTGTGA           |
| TROAP-R | CCCCAATCTTTGTGATGTCTC           |
| GAPDH-F | CAAGGTCATCCATGACAATTCT        |
| GAPDH-R | GCCACCACCTGGTGCTG             |
Note: F: forward; R: reverse.

Human glioma cell lines (T98, LN229, A172 and U251) and human-derived astrocyte (HA) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were grown in incubators at 37°C and 5% carbon dioxide and cultured using DMEM medium (Hyclone, USA) plus 10% FBS (Thermo Fisher Scientific, USA). To detect the expression level of four glioma cell lines (T98, LN229, A172 and U251) in \( TROAP \). First, total RNA was extracted from T98, LN229, A172, U251 and HA using Tri-Reagent (Sigma, USA). The quality and quantity assessments of total RNA were determined using NanoDrop One spectrophotometer (Thermo Fisher Scientific, USA), measuring 260/280 nm absorbance values. Subsequently, the cDNA was reverse transcribed from the total RNA using the Transcriptor First Strand cDNA Synthesis kit (Novoprotein Scientific Inc, Shanghai, China). RT-qPCR was performed according to the guidelines for FastStart Universal SYBR Green Master (ROX) (Novoprotein Scientific Inc, Shanghai, China). Results were quantified using QuantStudio software (Thermo Fisher Scientific, USA) following the manufacturer's instructions. GADPH was regarded as an internal reference. The primer sequences used in this research are listed in Table 1. Relative expression levels were determined using the \( 2^{-\Delta\Delta CT} \) method. The expression level of \( TROAP \) was detected by the \( "2^{-\Delta\Delta CT}" \) method. Statistical difference was analyzed by unpaired T test, and when p-value was < 0.05, the results were statistically significant.

**Statistical analysis**

R (v.3.6.1 version) was used for processing data and performing statistical analysis. The expression of \( TROAP \) in glioma and normal brain tissue was measured by the Wilcoxon test. The overall survival of the \( TROAP \) high expression and low expression groups was analysed by Cox regression and the Kaplan–Meier method, and the survival curves of clinical patients were drawn. Wilcoxon or Kruskal tests were utilized to explore the relevance between clinical features and \( TROAP \) expression in glioma patients. All P-values were two-tailed and considered statistically significant when less than 0.05.

**Results**

**Abnormal overexpression of \( TROAP \) in glioma**

The GEPIA database contains gene expression data for a variety of tumors. Analysis GEPIA data revealed that \( TROAP \) expression was increased in glioblastoma multiforme (GBM), lower-grade glioma (LGG), colon adenocarcinoma (COAD) and stomach adenocarcinoma (STAD) tissues compared with non-tumor tissues (Figure 1a). In addition, we further downloaded the GSE50161 and GSE116520 glioma datasets from the GEO database. In the GSE50161 dataset, 34 glioma tissue specimens were compared with 13 normal brain tissue specimens (Figure 1b). In the GSE116520 dataset, 34 glioma tissue specimens were compared with 8 normal brain tissue specimens (Figure 1c). Through the above research on glioma and normal tissue data from the GEPIA and GEO databases, we found that the expression level of \( TROAP \) in glioma was indeed higher than that in the corresponding non-tumor tissues. To verify these analysis results, we further detected the expression level of \( TROAP \) in four glioma cell lines (T98, LN229, A172 and U251) and human astrocyte (HA) by RT-qPCR. The result demonstrated that \( TROAP \) was highly overexpressed in glioma cell lines compared with HA (Figure 1d).

**Increased expression of \( TROAP \) is associated with a reduction in the overall survival (OS) of glioma patients**

Additionally, we retrieved three groups of data, namely, CGGA RNA-seq, CGGA microarray and TCGA RNA-seq data, to further explore the role of \( TROAP \) in the pathological processes of glioma and then plotted the survival curves of the three groups of patients via Cox regression and Kaplan-Meier analyses. High expression of \( TROAP \) in the CGGA-RNA sequencing, CGGA microarray and TCGA-RNA sequencing datasets was associated with a significant decrease in patient OS (Figure. 2a, b, and c). These results suggested that high \( TROAP \) expression may be associated with the OS of glioma, but whether this gene can serve as an independent risk factor for glioma remains to be further verified.

\( TROAP \) is an independent risk factor for glioma patients.

Next, prognostic factors for OS were analysed by the Cox regression model. In the CGGA RNA-seq data cohort, enhanced \( TROAP \) expression in glioma was considerably linked with poor prognosis (hazard ratio [HR]= 1.605), old age (HR= 1.624), advanced
histology (HR= 4.487), PRS type (HR= 2.123), high grade (HR= 2.883), chemotherapy (HR= 1.647), 1p19q co-deletion (HR= 0.231), and IDH mutation (HR= 0.317) (Figure 3a). In the CGGA microarray dataset, increased TROAP expression in glioma was significantly associated with poor prognosis (HR= 1.929), TCGA subtype (HR= 0.632), high grade (HR= 2.567), old age (HR= 1.736), chemotherapy (HR= 1.530), advanced histology (HR= 4.437), radiotherapy (HR= 0.459), PRS type (HR= 2.042), high grade (HR= 2.567) and IDH mutation (HR=0.423) (Figure 3c). In the TCGA RNA-seq dataset, overexpression of TROAP in glioma was significantly related to poor prognosis (HR= 1.128), old age (HR=1.072) and high grade (HR=4.634) (Figure 3e). These results reveal that high expression of TROAP might be a significant factor leading to unfavorable clinical outcomes.

Subsequently, we performed multivariate analysis with the Cox regression model. In the CGGA RNA-seq dataset, enhanced TROAP expression in glioma was closely linked with poor prognosis (HR = 1.217), chemotherapy (HR= 0.680), PRS type (HR = 1.994), old age (HR = 1.269), IDH mutation (HR=0.600), high grade (HR = 2.378), and 1p19q co-deletion (HR = 0.424) (Figure 3b). In the CGGA microarray, overexpression of TROAP in glioma was significantly linked with poor prognosis (HR = 1.420), radiotherapy (HR= 0.583), PRS type (HR=1.501) and high grade (HR = 2.666) (Figure 3d). In TCGA RNA-seq dataset, enhanced TROAP expression in glioma was importantly related to poor prognosis (HR = 1.060), high grade (HR = 2.888), and old age (HR = 1.048) (Figure 3f). These results reveal that high expression of TROAP might be a significant factor leading to unfavorable clinical outcomes.

**Clinical diagnostic value of TROAP**

Cox regression and the Kaplan-Meier method were used to plot ROC curves for the three datasets mentioned above to validate the clinical prognostic value of overexpression of TROAP in glioma. Overexpression of TROAP was confirmed to have diagnostic value (Figure 4a, b, c). The area under the curve was larger than 0.7 at 1,3,5 years, which indicated that high TROAP expression had modest prognostic value.

**Correlations between TROAP expression and clinical characteristics in glioma patients**

Software was used to carry out Wilcox or Kruskal tests to analyse the relationship between TROAP expression and different clinical characteristics from glioma samples from the three datasets (Figure 5a–g). As depicted in Figure 5a and c, the expression level of TROAP was significantly positively correlated with WHO grade and age in the CGGA RNA-seq, CGGA microarray and TCGA RNA-seq cohorts (p <0.005). As shown in Figure 5b and d, the expression level of TROAP was significantly correlated with 1p19q codeletion status and chemotherapy status in the CGGA RNA-seq cohort (p <0.001). In addition, Figure 5e, f and g demonstrate that the expression level of TROAP was significantly correlated with IDH mutation status, PRS-type and histology in the CGGA RNA-seq and CGGA microarray cohorts. These studies indicated that the expression level of TROAP was significantly associated with multiple clinical features related to the prognosis of glioma.

**Identification of the signalling pathways affected by TROAP in glioma by GSEA.**

**Table 2. The gene set enriches the high TROAP expression phenotype.**

| Gene set name                           | CGGA RNA-seq | CGGA microarray | TCGA RNA-seq |
|-----------------------------------------|--------------|----------------|--------------|
|                                         | NES          | NOM p-value    | FDR q-value  | NES          | NOM p-value    | FDR q-value  | NES          | NOM p-value    | FDR q-value  |
| KEGG_CELL_CYCLE                        | 2.033        | 0              | 0.018        | 1.955        | 0              | 0.056        | 2.157        | 0              | 0.007        |
| KEGG_HOMOLOGOUS_RECOMBINATION          | 1.891        | 0              | 0.061        | 1.922        | 0              | 0.044        | 1.972        | 0              | 0.016        |
| KEGG_P53_SIGNALING_PATHWAY             | 1.986        | 0              | 0.021        | 1.736        | 0.014          | 0.228        | 2.098        | 0              | 0.008        |

NES: normalized enrichment score; NOM: nominal; FDR: false discovery rate. Gene sets with NOM P-
value <0.05 and FDR q-value <0.25 were considered as significantly enriched.

By gene set enrichment analysis using low and high expression datasets, the signaling pathways affected by TROAP in glioma were identified, with significantly enriched pathways subjected to analysis via MSigDB (c2.cp.biocarta and h.all. v6.1. symbols). As shown in Table 2 and Figure 6a, b and c, the homologous recombination, cell cycle and p53 signaling pathways were identified in the high TROAP expression group. Therefore, TROAP may play a potential role in the carcinogenesis and progression of glioma.

**TROAP protein expression was increased in glioma tissues.**

We downloaded six immunohistochemistry (IHC) datasets (2 normal tissues, 2 low grade tissues, and 2 high grade tissues) generated with HPA044102 from the HPA online website and divided them into a male group (Figure 7a, b and c) and a female group (Figure 7d, e and f) to verify the differences in TROAP protein expression between normal tissues, low-grade tissues and high-grade tissues. The results showed that TROAP expression was upregulated in glioma tissues compared with non-tumor tissues.

The higher the grade of the tissue, the more protein was expressed.

**Table 3. Four small molecule compounds identified as potential drugs for glioma treatment in CMap analysis.**

| CMap name   | Mean  | N  | Enrichment | P-value |
|-------------|-------|----|------------|---------|
| Bezafibrate | -0.599| 4  | -0.833     | 0.00143 |
| Clobetasol  | -0.668| 3  | -0.903     | 0.00172 |

CMap: connectivity map

Identification of four potential therapeutic drugs for the treatment of glioma based on CMap analysis.

To identify small molecule drugs that can inhibit the expression of TROAP to improve the OS of glioma patients, we screened the identified differential genes using R language, selected the top 10 positive and 10 negative genes according to their correlation values, and made a Venn diagram of the relationship between them (Figure 8a and b). Next, we uploaded these genes to CMap, which predicted potential drugs. Bezafibrate, clobetasol, scriptaid and thioguanosine were identified, as shown in Table 3. In addition, the two-dimensional and three-dimensional structures of these drugs were obtained from PubChem (Figure 9a, b, c and d).

**Discussion**

Accumulating evidence have demonstrated that TROAP plays a key role in various malignancies, and the dysregulation of this gene can facilitate the occurrence, invasion, and metastasis of tumor cells in different ways. For example, Jing et al. revealed that knockdown of TROAP considerably inhibited cell proliferation, the G1 to S cell cycle transition, and the migration and invasion ability of gastric cancer cells. In other words, TROAP is overexpressed in GC and plays an oncogenic role in gastric cancer[21]. Moreover, Chen et al. identified TROAP as an enhancer of cell invasion and a prognostic factor for lung adenocarcinoma[22]. Nevertheless, there is a paucity of information regarding TROAP in glioma. Therefore, the goal of our study was to reveal the role of abnormal TROAP expression in glioma tissues and to propose novel ideas for the diagnosis and prognostication of glioma.

In this study, we used the convenience and practicality of databases containing high-throughput sequencing data to obtain information from thousands of glioma samples from CGGA, TCGA, and GEO for analysis and research. As seen in figure. 1, TROAP showed significantly higher expression level in glioma than normal brain tissues in the data from GEO and GEPIA. This finding is in agreement with some literature reports. The expression level of TROAP in liver cancer is significantly higher than that in non-tumor tissues, and high expression of TROAP is closely related to clinical stage and survival status[23]. Subsequently,
Kaplan-Meier curves for OS demonstrated that higher expression of \textit{TROAP} was related to worse outcomes in glioma patients. In addition, univariate and multivariate Cox analyses suggested that \textit{TROAP} mRNA expression might be a valuable biomarker for determining glioma prognosis, and ROC analysis established the prognostic value of \textit{TROAP} expression in glioma. \textit{TROAP}, a soluble cytoplasmic protein, has been reported to regulate prostate cancer progression via the WNT3 and surviving signaling pathways, and higher expression of \textit{TROAP} can lead to shorter overall survival in prostate cancer patients[24]. Similar results have also been found by Chang et al., who revealed that overexpression of \textit{TROAP} leads, at least to some extent, to great aggressiveness and unfavorable clinical outcomes of human gallbladder cancer by modulating the expression of integrin 3, mmp-7, mmp-9, and ets-1 expression[25]. These results support that \textit{TROAP}, as a prognostic factor, plays an essential role in the development of glioma.

Furthermore, our GSEA results indicated that high \textit{TROAP} expression was associated with enrichment in the homologous recombination (HR), cell cycle and p53 signaling pathways. Recent studies have shown that homologous recombination is directly and indirectly involved in tumor therapy, affecting the occurrence and development of tumors[26-28]. Artesunate (ART) can increase the lethality of temozolomide (TMZ) by inhibiting homologous recombination and senescence to induce increased glioma cell apoptosis[29]. In addition, targeting ataxia-telangiectasia mutated (ATM)-mediated HR pathway activation increased the sensitivity of glioma to ionizing radiation[30]. Abnormal regulation of the cell cycle is one of the main mechanisms of unlimited proliferation of malignant glioma cells. For example, the amplification and overexpression of cyclin D1 are common in glioblastoma cells and induce continuous activation of cyclin-dependent kinases such as CDK2, CDK4 and CDK6, resulting in continuous proliferation of glioblastoma cells[31, 32]. P53, a well-known \textit{tumour-inhibiting factor}, participates in regulating a variety of cell processes, such as cell growth, metabolism and apoptosis[33, 34]. Some studies have shown that mutations in P53 significantly promote the growth and migration of glioma cells[35]. In addition, P53 plays a key role in mediating the radiation resistance of glioma[36]. In general, these signaling pathways exacerbate glioma pathogenesis, implying that \textit{TROAP} may influence the growth, invasion and migration of glioma.

In order to improve the prognosis of patients with glioma, we used CMap to obtain four small molecule compounds with potential therapeutic effects on glioma. Recently, a study uncovered that bezafibrate can serves as a drug for lung adenocarcinoma in vitro assays, in which inhibited proliferation and caused cell cycle arrest [37]. Giacinti et al. revealed that scriptaid has strong antitumour activity in breast cancer cell lines[38]. Thioguanosine, as a purine analogue, has been reported to inhibit ribosomal RNA maturation in liver cancer cells[39]. Interestingly, Clobetasol, which is used to fight skin inflammation, has also been shown to have antitumour effects[40]. Combined with the above results, we speculate that bezafibrate, scriptaid, thioguanosine and clobetasol may also have potential therapeutic effect on glioma, which may help to improve the survival and prognosis of glioma patients.

Although the combined analysis of thousands of glioma samples from multiple datasets was used to help us understand the role of abnormal \textit{TROAP} expression in glioma, which should reduce the statistical bias from race, there are still some limitations. First, the data obtained from the CGGA RNA-seq, TCGA microarray and TCGA RNA-seq datasets are from glioma, and the datasets did not contain normal brain tissues as controls. To compensate for this defect, we identified and employed data from normal brain tissues and glioma tissues from the GEPIA and GEO datasets. Second, ideally, the detailed treatment strategies of patients in the datasets we used should be included in our analysis, but because our data were all from public databases, the detailed treatment strategies for each patient were not available. However, our statistical methods and processing methods were effective and robust: not only can the large amount of tissue sample data ensure the reliability of the research results, the ethnic diversity of the tissue data can also help us understand the influence of \textit{TROAP} on the prognosis of glioma patients in all ethnic groups. In addition, we used multiple data types, including gene microarray, gene sequencing, and immunohistochemistry data, as well as other means to verify the expression level of \textit{TROAP} in tumor tissues.

\section*{Conclusion}

In summary, our findings reveal that elevated expression of \textit{TROAP} plays a critical role in glioma prognosis, and \textit{TROAP} could be a candidate marker for glioma diagnosis and treatment. To the best of our knowledge, multiple data fusion analysis to study the
role of TROAP in human glioma are relatively rare, and we believe that our efforts will lay the foundation for future research in glioma.

**Abbreviations**

TCGA: The Cancer Genome Atlas  
CGGA: Chinese Glioma Genome Atlas  
GEO: Gene Expression Omnibus  
HPA: The Human Protein Atlas  
IHC: immunohistochemical  
GSEA: Gene set enrichment analysis  
GBM: glioblastoma  
TROAP: Trophinin-associated protein

**Declarations**

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**Availability of data and materials:** Not applicable

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**Authors’ contributions:** Liu ZD, Lian XY, Liu RZ and Cheng XB contributed equally to this work; Liu ZD and Gao YZ designed the research; Wang JL, Zhang W, Liu BF and Ren ZS performed the research; Zhang MJ, Lian XY and Wang HB analyzed the data; Lian XY and Liu ZD wrote the paper.

**Ethics approval and consent to participate:** The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board. Informed consent was obtained from all subjects involved in the study.

**Consent for publication:** Not applicable

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**Figures**
Figure 1

The expression of TROAP in glioma patients. (a) Different expression levels of TROAP in various human tumor tissue samples, the color of the tumor name represents the level of TROAP expression in the tumor: the red and green denote higher and lower expression, respectively. (b) 34 glioma tissue specimens were compared with 13 normal brain tissue specimens in the data set of GSE50161, and high expression of TROAP in glioma. (c) 34 glioma tissue specimens were compared with 8 normal brain tissue specimens in the data set of GSE116520, and the expression level of TROAP in glioma tissues was significantly increased. RT-qPCR in glioma cell lines (T98, LN229, A172 and U251) and human-derived astrocyte (HA). Statistical difference was determined by one-way ANOVA, ***p<0.001, ****p<0.001.
**Figure 2**

The relationship between the difference of the expression level of ESPL1 and Overall Survival (OS): (a) CGGA RNA-seq data set. (b) CGGA microarray data set. (c) TCGA RNA-seq data set.

**Table 1**

|   | Prostate | Hazard Ratio   |
|---|----------|----------------|
| a | TROAP    | 1.00(1.500-1.716) |
|   | PRS_type | 2.12(1.818-2.478) |
|   | Histology| 4.48(2.605-5.448) |
|   | Grade    | 2.93(2.026-3.231) |
|   | Gender   | 1.044(0.866-1.258) |
|   | Age      | 1.041(1.045-1.060) |
|   | Radio    | 0.89(0.720-1.015) |
|   | Chemo    | 1.024(1.003-1.004) |
|   | IDH1 Mutation| 0.317(0.302-0.334) |
|   | tp1312c codetection| 0.231(0.106-0.315) |

**Figure 3**

The relationship between the difference of the expression level of ESPL1 and Overall Survival (OS): (a) TCGA RNA-seq data set. (b) TCGA microarray data set. (c) TCGA RNA-seq data set.
Univariate analysis and Multivariate analysis with Cox regression model: (a) Univariate analysis of CGGA RNA-seq database. (b) Multivariate analysis of CGGA RNA-seq database. (c) Univariate analysis of CGGA microarray database. (d) Multivariate analysis of CGGA microarray database. (e) Univariate analysis of TCGA RNA-seq database. (f) Multivariate analysis of TCGA RNA-seq database.

**Figure 4**

Prognostic factors and risk assessment of glioma and the diagnostic value of TROAP. (a) CGGA RNA-seq data set. (b) CGGA microarray data set. (c) TCGA RNA-seq data set.
Figure 5

The relationship between the expression of ESPL1 in glioma and clinicopathological characteristics in the CGGA RNA-seq, CGGA microarray and TCGA RNA-seq data sets: (a) Grade. (b) 1p19q_codeletion status; (c) Age. (d) Chemotherapy status. (e) IDH mutation status. (f) PRS type. (g) Histology.
Figure 6

Gene set enrichment analysis (GSEA) of TROAP in CGGA RNA-seq, CGGA microarray and TCGA RNA-seq database. The results show the common signaling pathway of the three data sets: (a) Cell cycle, (b) Homologous recombination, (c) p53 signaling pathway.

Figure 7

Protein expression in tissues with the immunohistochemistry (IHC). a, d in normal brain tissues. b, e in low grade glioma. C, f in high grade glioma.
Figure 8

Relationship analysis of TROAP and differential expression genes.
Figure 9

The two-dimensional and three-dimensional structures of the four compounds identified by the connectivity map analysis: (a) Bezafibrate. (b) Clobetasol. (c) Scriptaid. (d) Thioguanosine.

**Supplementary Files**

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