DRAXIN as a Novel Diagnostic Marker to Predict the Poor Prognosis of Glioma Patients

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Abstract

An increasing number of evidences have shown that the carcinogenic effect of DRAXIN plays an important role in the malignant process of tumors, but the mechanism of its involvement in glioma has not yet been revealed. The main aim of this study is to explore the relationship between DRAXIN and the prognosis and pathogenesis of glioma through a large qualities of data analysis. Firstly, thousands of tissue samples with clinical information were collected based on various public databases. Then, a series of bioinformatics analyses were performed to mine data from information of glioma samples extracted from several reputable databases to reveal the key role of DRAXIN in glioma development and progression, with the confirmation of basic experiments. Our results showed high expression of the oncogene DRAXIN in tumor tissue and cells could be used as an independent risk factor for poor prognosis in glioma patients and was strongly associated with clinical risk features. The reverse transcription-quantitative PCR technique was then utilized to validate the DRAXIN expression results we obtained. In addition, co-expression analysis identified respectively top 10 genes that were closely associated with DRAXIN positively or negatively. Finally, four drugs that may have inhibitory effects on DRAXIN were gained by Cmap drug analysis. To sum up, this is the first report of DRAXIN being highly expressed in gliomas and leading to poor prognosis of glioma patients. DRAXIN may not only benefit to explore the pathogenesis of gliomas, but also serve as a novel biological target for the treatment of glioma.

Introduction

Gliomas are malignant tumor of neuroepithelial origin due to the interaction of congenital genetic risk factors and environmental carcinogenic factors, frequently occurs in brain and spinal cord glial cells. The annual incidence rate of glioma is 5.26 per 100,000, or 17,000 new cases diagnosed each year (Omuro and DeAngelis 2013). The diagnostic criteria of glioma based on pathological classification has gradually shifted from microscopic tumor morphology to a combination of morphological and molecular features, including gene mutations, chromosome copies, and gene rearrangements, etc. (Wood et al. 2019). The following standard treatments include surgical resection, chemotherapy, immunotherapy, and a number of innovative treatments known as tumor treatment field or photodynamic therapy (Agostinis et al. 2011; Liu et al. 2019). Although above intervention methods for glioma have reached a comparatively high level, therapeutic effects still do not meet our expectations, probably due to chemoradiotherapy resistance or poor marker specificity. Consequently, there is an urgent need to find better biomarkers that can benefit the accurateness of diagnose and predict the prognosis of glioma.

To date, a number of biomarkers have been identified for the diagnosis and prognosis of glioma. Epidermal growth factor receptor (EGFR) is involved in tumorigenesis and multiple signaling pathways. Disregulated expression of EGFR occurs in approximately 40% of glioblastoma patients, and its mutation, EGFRvIII, reaches to 20% to 30% of glioblastoma (Riemenschneider et al. 2010). Therefore, EGFR has a guiding role in the diagnosis of glioma, and EGFRvIII can be used to distinguish high-grade gliomas and poor prognosis of patients. Another valid biomarker CD133 is a membrane-bound protein
encoded by the gene \textit{PROM1}, which may play a key role in cell differentiation. It is reported that CD133-positive cells derived from human glioma cells are highly susceptible to replicate the original tumor, so increased proportion of CD133 cells indicates a lower survival rate. Thus, the mRNA level of encoding gene PROM1 can be used to differentiate between GBM and low-grade gliomas. There are also several well-known biomarkers such as CD15, A2B5 and Nestin that can benefit to increase the diagnosis and prognosis of glioma (Ludwig and Kornblum 2017). As the fact that many biomarkers have been identified, malignancies are inherited diseases caused by mutations in multiple genes. Therefore, one single gene is not enough to accurately predict and treat malignant tumor. In this study, we are committed to discovering novel predictors for glioma.

Dorsal rejection axon guidance protein (\textit{Draxin}), encoded by the \textit{DRAXIN} gene, is required for the development of spinal cord and forebrain connectivity, as a chemical driver-guiding protein for conjoined axons (Zhang et al. 2010; Sato et al. 2018). It can inhibit or repel the growth of dorsal spinal cord neurons. In recent years, the role of \textit{DRAXIN} in tumors has gradually become apparent, as reported in lung cancer (Sato et al. 2018). However, the expression of \textit{DRAXIN} in glioma has not been reported, so the present study is the first of its kind to clarify the relationship between \textit{DRAXIN} and characteristics of glioma.

Firstly, we demonstrated that \textit{DRAXIN} expression is much higher in glioma tissues and cell lines compared to normal samples. KM and ROC curves exhibited \textit{DRAXIN} was correlated with shorter OS, and univariate and multivariate regression analysis claimed \textit{DRAXIN} was an independent prognostic factors for glioma. We performed pearson correlation coefficient to prove the positive relationship of \textit{DRAXIN} and malignant clinical features. Furthermore, GSEA revealed potential signaling pathways that \textit{DRAXIN} might participate in. At last, individually ten most-related genes and four candidate drugs for glioma were identified. It is believed that this study will provide \textit{DRAXIN} as an effective biological target for the prognosis of glioma and may provide new research directions for the drug treatment of glioma.

**Materials And Methods**

**2.1. Data collection**

GEPIA (Gene Expression Profiling Interactive Analysis) (http://gepia.cancer-pku.cn/) is a database established by Peking University that contains a large of number RNA sequencing data from human tumor tissues and mutually matched normal tissues, creating new opportunities for data mining and a deeper understanding of gene function (Tang et al. 2017). GEO (Gene Expression Omnibus) (https://www.ncbi.nlm.nih.gov/geo/) is an international public repository containing high-throughput microarray and next-generation sequence functional genomic datasets, containing data from multiple pathological and normal samples (Barrett et al. 2013). We screened and obtained the key dataset GSE50161, which contains genetic experimental microarray data from tumor tissues (n=34) and normal control tissues (n=13). The IVY-GAP (Ivy-Glioblastoma Atlas Project) (http://glioblastoma.alleninstitute.org/static/home) database is dedicated to GBM-related studies. Here
we obtained the characteristic annotation of DRAXIN in glioma tissues by ISH/FISH/HE for its expression level in gliomas.

CGGA (Chinese Glioma Genome Atlas) (http://www.cgga.org.cn/) is a database of gene sequencing and clinical characteristics of a large number of glioma patient samples established by Beijing Tiantan Hospital, Capital Medical University (Yan et al. 2012). Samples with incomplete information were censored. The detailed clinical information of the remaining 748 samples was recorded in Table S1. Survival analysis, univariate Cox analysis, multivariate Cox analysis, co-expression analysis, and ROC curve analysis were performed on this basis.

2.2. GSEA analysis of DRAXIN

GSEA (Gene Set Enrichment Analysis) can be used for data analysis of whole genome expression profiles. It is used to compare genes with predefined gene sets (Subramanian et al. 2007). GSEA analysis was performed on mRNA sequencing data obtained from the CGGA database. First, batch calibration and normalization were performed by SVA and LIMMA packages. Then the RNA sequencing data were divided into high expression group (H group) and low expression group (L group) according to the expression of DRAXIN. GSEA (version 4.0.3) jar software was used for enrichment analysis. The number of enrichments was set to 1000 times, and the KEGG cell signaling pathway was selected as the gene database. Normal P<0.05 and FDR<0.25 were regarded as significantly enriched.

2.3. Connectivity map analysis

Connectivity map (Cmap, https://portals.broadinstitute.org/cmap/) is an online tool for drug analysis to predict potential therapeutic drugs for certain diseases according to genome sequencing results (Lamb 2007). On the basis of the co-expression analysis results, Cmap drug analysis can be performed to obtain a number of drugs according to up- or down-regulate gene expression profiles. After screening these drugs (p-value < 0.05, enrichment < -0.8), four drugs were identified that could directly or indirectly inhibit DRAXIN expression. Then we obtained specific drug information in PubChem (https://pubchem.ncbi.nlm.nih.gov/) database, including CID, 2D and 3D structures and chemical formulas of drugs.

2.4. Tissue acquisition and cell culture

Clinical specimens of 17 glioma tissue samples and 7 normal brain tissues were obtained from patients in the clinical department of Henan Provincial People's Hospital, and patients signed a written informed consent. Collected samples were stored in liquid nitrogen environment, and then transferred to -80°C freezer for use. Human glioma cell lines (U251, T98, A172) and human astrocytes (HA) were purchased from Shanghai Shunran HAKATA Cell Bank (http://www.xrshbio.com/). All cells were cultured in an incubator at 37°C and 5% CO2 using DMEM medium (HyClone) containing 10% fetal bovine serum (FBS, Gibco). This study was approved by the Ethics Committee of Henan Provincial People's Hospital.
2.5. RNA extraction and quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis

RT-qPCR was used to detect the expression of **DRAXIN** in glioma tissues and glioma cell lines. Tissue RNA and cellular RNA were extracted with Tri-Reagent and QIA symphony™ 120RNA kit (QIAGEN, Hilden, Germany). Subsequently, the RNA solubility was determined using an ultra-micro spectrophotometer (NanoDrop One spectrophotometer, Thermo Fisher Scientific). The NovoStart SYBR qPCR SuperMix Plus (Novoprotein) kit was used to perform RT-qPCR. GADPH was used as an internal reference. The primer sequences used for **DRAXIN** were 5'-CGACTGGACCGATTATGAAGAC-3'(F) and 5'-CGGCTGGTGATGTTTCGTTAC-3'(R). "2^{-ΔΔCT}" and "2^{-ΔCT}" methods were individually applied to analyze the **DRAXIN** expression of glioma cells and tissues. The unpaired t test was used for the statistical difference between the two groups. When the p value was<0.05, the difference between the two groups was statistically significant.

2.6. Statistical Analysis

The R software (v.4.0.3) was frequently used to analyze the above multiple sets of data and perform statistical analysis. The Wilcox test was used to determine the expression of **DRAXIN** in glioma and non-tumor brain tissues. Cox regression and Kaplan-Meier method were used to examine the relationship between the expression level of **DRAXIN** and the patient’s OS, and to draw survival curves and ROC curve. Univariate Cox analysis and multivariate Cox analysis based on statistical data sheet was performed. Use Wilcox or Kruskal test to detect the relationship between clinically relevant information and **DRAXIN** in patients with glioma expression, p<0.05 is statistically significant.

Results

3.1. Clinical characteristics information

After censoring some invalid information in 1018 samples, the sample data of 748 glioma patients can be obtained from the CGGA database. It includes general clinical information, such as gender, age, pathological type, and WHO classification of glioma, in addition to PRS (primary, recurring, secondary) type, IDH (isocitrate dehydrogenase) mutation and some data on the missing status of the 1p19q codon. Detailed clinical information is shown in Table 1.

3.2. **DRAXIN** is highly expressed in glioma tissues and cell lines relative to normal control

Through the GEPIA database, the **DRAXIN** gene is highly expressed; it was found that it in glioblastoma (Fig 1A). It was verified in GSE50161 that the expression of **DRAXIN** in glioma tissue was much higher than normal brain tissue. At the same time, at the nucleic acid level, the high expression of **DRAXIN** was proved by the IVY-GAP database using ISH images (Fig 2). The results showed that the expression level of tumor cells in the same glioma tissue is higher than that of normal brain adjacent tissue. In order to verify the results of previous studies, we performed RT-qPCR at the tissue and cell levels. The results show that **DRAXIN** is highly expressed in glioma tissues and cells compared with normal brain tissues.
and human brain astrocytes, especially the expression level of A172 cell line is 1569 times of that in normal cells (Figure 1D, E). In summary, the high expression of DRAXIN has been proven on multiple levels.

3.3. Relationship between the expression of DRAXIN and the clinical features and prognosis of patients with glioma

To further explore whether the high expression of DRAXIN has a certain clinical significance, such as a certain impact on the prognosis, OS curve was drawn and it was found that the survival time of the high expression group was lower than the low expression group in these queues, indicating a poor prognosis (Fig 3A). In addition, combined with the conditions such as WHO classification, IDH mutation, and 1p19q co-deletion status, OS diagrams were further obtained (Fig 3B-D). Moreover, ROC curves were drawn with above categories, we discovered that expression of DRAXIN, accompanied with 1p19q deletion status, and IDH mutation with 1p19q co-deletion status is statistical significance (AUC>0.7). In the end, univariate and multivariate logistic regression analysis exhibited three independent risk factors (HR>1, p<0.001) for glioma, the expression of DRAXIN, PRS grade and high-grade glioma leading to poor prognosis (Fig 3I, J). IDH mutation type and 1p19q co-deletion status were present as protective factors (HR<1, p<0.001). Based on the above data, high expression of DRAXIN could be used to indicate a poor prognosis for patients, and it had a certain clinical diagnostic value.

3.4. The relationship between the expression of DRAXIN and clinical features in patients with glioma

Not only of overall survival, the relationship between various clinical characteristics and expression of DRAXIN was also estimated via correlation analysis. The data of glioma was screened for the complete information including gender, age, pathological type, and WHO grade of glioma, PRS (primary, recurring, secondary) type, IDH (isocitrate dehydrogenase) mutation and the status of the 1p19q codon. After Wilcox and Kruskal tests, it was concluded that the increased expression of DRAXIN was positively correlated with higher WHO classification and recurrent and secondary glioma (p<0.001; Fig 4A-B). In the primary glioma, the wild-type expression of DRAXIN was higher than that of the mutant (p<0.001, Fig 4C). Compared with the 1p19q co-deletion status, the gene expression level of DRAXIN was lower than that with non-co-deletion state (p<0.001, Fig 4D). In addition, histocytology showed that the expression level of DRAXIN in the GBM group and the relapsed GBM group was significantly increased. In summary, expression of DRAXIN was significantly related to the malignant features of glioma.

3.5. GSEA reveals DRAXIN-related cell signaling pathways

To further study the specific mechanism of DRAXIN in glioma, GSEA was used to explain the cancer-related cell signaling pathways that DRAXIN involved in. Several items were obtained by screening with p<0.05, FDR q-value<0.25, which turned out that focal adhesion, cell cycle, DNA replication, pyrimidine metabolism, Toll-like receptor signaling pathway, VEGF signaling pathway, the DRAXIN high expression group showed significant differential enrichment (Fig 5).
3.6. Co-expression analysis and drug analysis

Furthermore, to explain the role of gene itself in glioma, individual top ten most related genes were obtained after screening the p value and correlation coefficient value of co-expressed genes (Fig 6). Through co-expression analysis, it was found that ten genes were positively correlated with DRAXIN, including CDCA8, KIF2C, DLGAP5, KIFA4, CCNB1, MELK, KIF23, GTSE1, ASPM and GAS2L3, and other ten genes, AKR1C3, RASL10A, SLC25A18, CYP17A1-AS1, ETNPPL, FBXW4, LDHD, SLC22A6, SLC25A48 and MRVI1, were negatively correlated. Finally, Cmap drug analysis using these 20 genes found that there were 4 drugs, as camptothecin, doxorubicin, etoposide and lomustine, that might have therapeutic effects. Drug-related information such as chemical formula and PubChem CID were obtained on PubChem and shown in Fig 7.

Discussion

DRAXIN is commonly recognized as an axon guidance cue for thalamocortical development (Shinmyo et al. 2015). It can regulate hippocampal neurogenesis through the inhibition of neuroblasts apoptosis (Tawarayama et al. 2018). When this idea is extended to tumor field, DRAXIN is reported to be a key factor in the progress of lung adenosquamous carcinoma via the regulation of cell proliferation and apoptosis (Sato et al. 2018). Nevertheless, the molecular mechanism of DRAXIN in glioma has not been reported. The aim of this study was to reveal the function of DRAXIN in glioma from multiple perspectives and its impact on patient treatment and prognosis.

The rigor of the analysis for aberrant expression of DRAXIN in glioma was improved by validation using GEPIA, CGGA database, IVY-GAP database and basic experimental RT-qPCR. High expression of oncogenes is often closely associated with poor prognosis and malignant features of tumors. Our study also demonstrated the consistent results by plotting survival curves, univariate and multivariate Cox analysis, as well as correlation coefficient analysis. Besides the correlation with shorter OS, DRAXIN expression was found positively correlated with glioma WHO classification, 1p19q non-co-deiciency, GBM and recurrent GBM. Interestingly, these factors are reported to act as independent risk factors for glioma. (Ducray et al. 2008; Louis et al. 2016). For example, in 1p/19q non-codel gliomas, the expression of 1p19q is highly associated with the poor prognosis and malignancy of the tumor (Chai et al. 2019). Interestingly, after we attempted to group the overall samples according to two factors, 1p19q and IDH, subsequent survival analysis and ROC curves showed that abnormally high DRAXIN expression were still significantly associated with poor prognosis. This result strongly suggest that DRAXIN is a novel prognostic factor for glioma beyond 1p19q co-deletion and IDH mutant.

To further discover the potential mechanism, GSEA analysis were performed to reveal the possible signaling pathways of DRAXIN might participate in leading to the carcinogenesis of glioma. Enrichment results showed cell cycle, DNA replication, pyrimidine metabolism, VEGF signaling pathway, focal adhesion and Toll-like receptor signaling pathway, which had influential functions in the development of glioma. First of all, the cell cycle signaling pathway exists throughout the cell division cycle and is
activated in almost all tumors (Liu et al. 2019). Aberrant anabolism of DNA and proteins contributes to the hyperproliferation of glioma and DNA replication is an excellent target to increase the sensitivity of glioma to radiotherapy (Lim et al. 2020). And neovascularization is a potent process for the development of malignancy, which owes to the VEGF signaling pathway. As its contribution to the growth and invasion of tumors (Zhao and Adjei 2015), VEGF/VEGFR pathway is brought to inhibition treatment, leading to rapid and long-lasting anti-angiogenesis and anti-tumor responses (Carmeliet and Jain 2011). And because VEGF is also a key factor in regulating glioma angiogenesis, the results of anti-VEGF therapy showed improved progression-free survival of relapsed GBM (Robles Irizarry et al. 2012). Enrichment in focal adhesion and Toll-like receptor signaling pathway also remind us that \textit{DRAXIN} may partake in migration and tumor immunity of glioma. From the above conclusions, it can be inferred that the mechanism of \textit{DRAXIN} in the poor prognosis of glioma depend on synergy of multiple pathways.

As itself, genes with similar functions or variations are somewhat linked and will have a tendency to co-express, which can be used to screen genes with similar sequences. Hence, by co-expression analysis of the data in the CGGA-seq database, the ten most positively and negatively co-expressed genes of \textit{DRAXIN} were shown separately, among which, \textit{CDCA8} gene is the one with the strongest positive association to \textit{DRAXIN}. According to previous studies, \textit{KIF23}, a member of the kinesin family positively associated with \textit{CDCA8}, is involved in the regulation of cytoplasmic division, and its high expression is associated with poor prognosis of gliomas (Gao et al. 2020). The idea mentioned above may give us an inspiration to \textit{DRAXIN} standing in glioma. On the other hand, \textit{ETNPPL}, a gene inversely associated with \textit{DRAXIN}, is also negatively associated with the grade of glioma as its expression is not detected in glioblastomas. Overexpression of \textit{ETNPPL} protein can exhibit tumor suppressive effect and inhibit the proliferation of glioma stem cells (Leventoux et al. 2020). Other co-expressed genes are also involved in the progression of glioma, such as \textit{KIF2C} (Bie et al. 2012), \textit{DLGAP5} (Zhou et al. 2021) and \textit{CCNB1} (Yang et al. 2020). Above biomarkers show a coordinated association with glioma progression, indicating their co-expressed gene \textit{DRAXIN} is inevitably functional in the development of glioma.

To achieve the ultimate goal of clinical application, we uploaded the co-expression profiles to Cmap drug analysis and obtained four drugs most likely to restrict the specific effect of \textit{DRAXIN}. Among them, camptothecin is the most recognized. It is a topoisomerase inhibitor that shows anticancer activity by interfering with DNA dissociation (Pommier 2004), which exactly regulate cell cycle and DNA replication pathway that \textit{DRAXIN} participate in (Kim et al. 2009). Lomustine, also known as CCNU (chloroethylcyclohexylNitrosourea), cross-links DNA and exerts therapeutic effects by affecting cell cycle signaling pathways. As a lipid-soluble drug, lomustine has a good blood-brain barrier permeability and becomes a good candidate for the treatment of endogenous brain tumors (Weller and Le Rhun 2020). These drugs, including doxorubicin and etoposide, not only have anti-glioma effects but also regulate the specific pathway consistent with GSEA results, which further confirms scientific consistency of the present study. Furthermore, our analysis can extend the application of developed drugs and acquire better effectiveness of glioma treatment.
In summarize, we have studied a large amount of high-throughput sequencing data based on several databases, which reveals the association between \textit{DRAXIN} and the prognosis of glioma for the first time. However, there are some shortcomings in this study worth noting. Foremost, due to different medical environments and data processing methods, clinical information is collected based on different standards, resulting in unsatisfactory consistency of the data profiles. However, we collected data from several public databases with a large amount of glioma sample, to the best elimination of data bias. Secondly, the statistical results may be affected by the lack of normal samples in the database, which has a large gap with the tumor sample size. To compensate for these shortcomings, we used RT-qPCR to verify the differences in \textit{DRAXIN} expression at the tissue level and cellular level. And numerous analysis methods also make a guarantee for accurate results.

**Conclusions**

Collectively, for the first time, \textit{DRAXIN} was found to be highly expressed in gliomas as an oncogene leading to shorter survival and poor prognosis of patients. In addition, \textit{DRAXIN} may be involved in the cell cycle and VEGF pathway to affect the progression of gliomas. Also, we found that camptothecin and doxorubicin may play an anti-glioma role by inhibiting \textit{DRAXIN} related pathways. This study provides a molecular basis for subsequent studies on \textit{DRAXIN} as a potential biomarker for glioma prognosis and treatment.

**Abbreviations**

RT-qPCR, reverse transcription polymerase chain reaction; GEO, Gene Expression Omnibus; GEPIA, Gene Expression Profiling Interactive Analysis; \textit{Draxin}, Dorsal Inhibitory Axon Guidance Protein; CGGA, Chinese Glioma Genome Atlas; Cmap, Connectivity map; GSEA, Gene Set Enrichment Analysis; ROC, receiver operating characteristic; OS, overall survival; IDH, isocitrate dehydrogenase; GBM, glioblastoma; VEGF, vascular endothelial growth factor; CCNU, chloroethylcyclohexylnitrosourea.

**Declarations**

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**Credit authorship contribution statement**

\textbf{Zhendong Liu:} Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing - original draft. \textbf{Runze Liu:} Data curation, Visualization, Writing - original draft. \textbf{Xingbo Cheng:} Writing - review & editing. \textbf{Hongbo Wang:} Writing - review & editing. \textbf{Yongjie Zhu:} Writing - review & editing. \textbf{Yaoye Zhao:} Writing - review & editing. \textbf{Zhibin Han:} Conceptualization, Investigation, Supervision, Writing - review & editing. \textbf{Mengjun Zhang:} Conceptualization, Investigation, Supervision, Writing - review & editing. \textbf{Binfeng Liu:} Writing - review & editing. \textbf{Xiaoyu Lian:}
Conceptualization, Investigation, Supervision, Writing - review & editing. **Yanzheng Gao:**
Conceptualization, Investigation, Supervision, Project administration, Writing - review & editing.

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Figures
Comparison of DRAVIN expression levels in glioma and normal tissues at different levels. (A) Based on the GEPIA database, differences in the expression levels of DRAVIN in various tumors. DRAVIN is highly expressed in GBM (n=163) compared to normal tissues (n=207). The different colors of the tumor name represent different meanings. Red means that the expression of DRAVIN in tumor tissue is higher than that of normal tissue, Black indicates that the expression of DRAVIN in tumors is not statistically
significant compared to the expression of normal tissues; (B) Based on the GEPIA database, the expression level of DRAXIN gene in GBM (n=163) and corresponding normal tissues (n=207) expression level box plot; (C) The expression level of DRAXIN gene in glioma (n=34) and corresponding normal tissues (n=13) based on GSE50161; (D) Based on RT-qPCR, the expression level of DRAXIN in glioma and corresponding normal tissues; (E) The expression level of DRAXIN gene in the glioma cell lines U251, T98, A172 and the corresponding normal control cell line (HA) based on RT-qPCR (p < 0.05).

Figure 2

The expression difference of DRAXIN in different tumor characteristic areas of the same Glioblastoma tissue is based on the IVY-GAP database. (A) In situ hybridization; (B) HE staining technique; (C) Fluorescence in situ hybridization; (D) tumor feature annotation. Green represents tumor tissue. Blue represents advanced tissue. Purple represents the infiltration zone. Black represents necrosis. Orange represents Hyperplastic blood vessels.
Based on the CGGA database, the high expression of DRAXIN gene can be used as an independent risk factor to cause poor prognosis, and can be used as a prognostic assessment method; (A-D) Kaplan-Meier survival curve based on CGGA database shows that patients with four different molecular subtypes with high expression of DRAXIN have a lower survival rate; (E-H) ROC curve shows that the expression of DRAXIN has a higher prognosis for the five-year survival rate of glioma patients assessment value (AUC>0.7); (I) univariate cox analysis of the prognosis of glioma patients; (J) multivariate cox analysis of the prognosis and/or posterior of glioma patients.
The expression of DRAXIN in glioma is related to a variety of clinical features. (A) WHO classification; (B) PRS classification; (C) IDH mutation status; (D) 1p19q co-deletion status; (E) pathological tissue typing.

Figure 4
Figure 5

GSEA enrichment analysis results of DRAXIN. (A) Focal adhesion, (B) Cell cycle, (C) DNA replication, (D) Pyrimidine metabolism, (E) Toll-like receptor signaling pathway, (F) VEGF signaling pathway.
Figure 6

The co-expression analysis results of DRAXIN gene and 20 related genes in glioma. (A) Twenty genes are positively correlated and negatively correlated with the expression of DRAXIN. (B) 20 DRAXINN related genes and their correlation coefficients.

| Gene           | Correlation coefficient | P-value     |
|---------------|-------------------------|-------------|
| CDC2A8        | 0.681                   | 9.15E-140   |
| KIF2C         | 0.68                    | 3.18E-139   |
| DLGAP5        | 0.675                   | 2.59E-136   |
| KIF4A         | 0.67                    | 1.06E-133   |
| CCNB1         | 0.665                   | 3.64E-131   |
| MELK          | 0.665                   | 6.66E-131   |
| KIF23         | 0.664                   | 2.33E-130   |
| GTSE1         | 0.659                   | 1.02E-127   |
| ASPM          | 0.657                   | 7.69E-127   |
| GAS2L3        | 0.656                   | 4.68E-126   |
| AKR1C3        | -0.432                  | 1.94E-47    |
| RASL10A       | -0.438                  | 6.66E-49    |
| SLC25A18      | -0.442                  | 6.13E-50    |
| CYP17A1-AS1   | -0.444                  | 2.03E-50    |
| ETNPPL        | -0.457                  | 8.71E-54    |
| FBXW4         | -0.46                   | 2.01E-54    |
| LDHD          | -0.462                  | 5.66E-55    |
| SLC22A6       | -0.475                  | 1.71E-58    |
| SLC25A48      | -0.489                  | 2.39E-62    |
| MRVI1         | -0.493                  | 2.18E-63    |
Figure 7

Based on the Cmap database and PubChem database, it is possible to achieve targeted therapy through drugs that affect the expression of DRAXIN. It includes drug name, PubChem CID, chemical structure formula and two-dimensional structure diagram and three-dimensional structure diagram. (A) Camptothecin; (B) Etoposide; (C) Adriamycin; (D) Lomustine.
Supplementary Files

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- TableS1.docx