Screening and Identification of Key Biomarkers in Low-Grade Gliomas: Evidence from Bioinformatic Analysis

Dong He

Shandong Provincial Hospital

Zhen Qin

Shandong University of Traditional Chinese Medicine Affiliated Hospital

Taihong Gao

Shandong Provincial Hospital

Fan Yang

Shandong Provincial Hospital

Hua Guo

Shandong Provincial Hospital

Tao Xin

Shandong Provincial Hospital

Rui Zhang

Shandong Provincial Hospital

Jun Sun

Shandong Provincial Hospital

Qian Liu

Shandong University Cheeloo College of Medicine

Pang Qi (✉ pangqi@sdu.edu.cn)

Shandong Provincial Hospital

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Abstract

Background: Low-grade glioma (LGG) is one of the most common brain tumors in the world. Surgical resection is often regarded as the first choice for treatment. Patients with LGGs have a longer survival time than other types of gliomas, but risks of recurrence exist which often transforms the initial LGGs to malignant glioblastomas (GBM). For standardized therapy, adjustment in the treatment options based on early assessment of the recurrent risk of LGGs may improve the outcome of the patients. Considering that the molecular mechanisms beneath the recurrence of LGGs, as well as the detailed differences between the primary and recurrent LGGs still remain unclear, we aim to identify the critical genes that are specifically involved in the recurrence of LGGs.

Method: The Cancer Genome Atlas (TCGA) dataset of the primary and recurrent LGGs (TCGA-LGG) was therefore downloaded for the subsequent investigation. By performing differential expression and functional enrichment analyses followed by a module examination based on STRING and Cytoscape.

Results: A total of 413 DEGs were identified, consisting of 187 downregulated genes and 226 upregulated genes. We further integrated the enriched functional pathways of these DEGs, which led to the identification of five hub genes— *GFAP*, *AQP4*, *KCNJ10*, *SLC1A2* and *SLC1A3*. The subsequent survival analysis indeed showed that these five genes may be closely involved in the recurrence and progression of LGGs.

Conclusion: The identification of DEGs and hub genes in LGGs help us better understand the development, progression, and recurrence mechanisms of LGGs. Our research evidently suggested that ion/water homeostasis and neurotransmitters, two major factors in tumor microenvironment, may play important roles in the tumorigenesis, especially recurrence of LGGs, and it may provide potential targets for diagnosis and treatment of LGGs.

Introduction

Glioma is derived from the glial cell type in the brain or spinal cord, which accounts for about 30% of all brain tumors and 80% of all malignant brain tumors in the USA(1). Since neuropathological evaluation and diagnosis of brain tumor specimens are usually based on WHO classification of central nervous system tumors(2), gliomas are further classified into grade I to IV depending on their histopathological and genetic characteristics. Among them, gliomas of WHO grade II and below are identified as low grade gliomas (LGGs). Despite of relatively good clinical prognosis, recurrence of LGGs still occurs. As a matter of fact, treating initially LGG that relapses and undergoes malignant transformation to glioblastoma (GBM)(3, 4) has been one of the greatest challenges in neuro-oncology. Some do believe that LGGs should be further divided into two prognostic categories—high-risk and low-risk, which makes their treatment strategies after surgery rather complicated.

Accumulating evidences have demonstrated that abnormal expression and mutations of genes are involved in the development and progression of LGGs. TP53 mutations, for instance, frequently

recognized in most low-grade astrocytomas, are rare in oligodendrogliomas (5–7). Mutations in IDH1 or IDH2 are currently well understood, and both are considered to be the earliest genetic events in glioma development, while mutations in IDH1 or IDH2 are associated with malignant transformation of low-grade gliomas. The risk of low-grade glioma patients with IDH1 or IDH2 mutations is significantly different from that of wild-type patients(8–10). A (1;19)(q10;p10) translocation, on the other hand, mediates the combined deletion of chromosome 1p/19q and is associated with superior overall survival and progression-free survival in LGG patients(11). However, due to the lack of detailed knowledge of the molecular differences between the primary and recurrent LGGs, no treatment regimens or bona fide biomarkers that significantly translate into a survival benefit to LGG patients have been identified. It was once thought that a larger dose of radiation therapy for patients with LGGs and the use of temozolomide(TMZ) may bring benefits, but studies have been proven that treatment with temozolomide(TMZ) in some patients with LGGs, rather unexpectedly, increased tumor aggressiveness and relapse(12). Therefore, the molecular mechanism involved in the recurrence of LGGs may be more complicated, and it is difficult to prevent recurrence by simply adjusting standardized treatment, clarifying the molecular mechanisms involved in the development, especially in the recurrence of LGGs would provide a theoretical basis for developing more effective treatment strategies or identifying new therapeutic targets.

In recent decades, with the development of microarray technology and bioinformatics analysis, the expression and function of differential expressed genes(DEGs) have been better understood after screening based on genetic alterations at the genomic level. Identification and functional analysis of DEGs in LGGs can help us better understand the development and progression of LGGs. In the present study, the TCGA dataset of the primary and recurrent LGGs (TCGA-LGG) was downloaded. By performing the differential expression and functional enrichment analyses followed by a module examination based on STRING and Cytoscape, a total of 413 DEGs were identified. We further integrated the enriched functional pathways of these DEGs, which led to the identification of five hub genes. The subsequent survival analysis indeed showed that these five genes may be closely involved in the recurrence and progression of LGGs, which may provide more effective targets for the diagnosis and treatment of LGGs.

Materials And Methods

Microarray data

TCGA (The Cancer Genome Atlas, <https://cancergenome.nih.gov/>) is a public cancer functional genomics data repository of high throughout gene expression data. This gene expression dataset was downloaded from the TCGA GDC data portal(<https://portal.gdc.cancer.gov/>). The TCGA-LGG dataset included 530 samples in total, among which 498 were derived from the patients who only had primary tumors and 32 were derived from the 14 patients who recurred. Among the latter 32 samples, 14 were primary tumor tissues and the rest 18 were recurrent tissues. Recurrent patients' metadata were shown in **Table S1**.

Identification of DEGs

The DEGs between the primary and recurrent samples were screened using R software such as GDCRNAtools package (13), DT package, EdgeR package(14) and Limma package(15). Limma-voom with quality weights and duplicate correlation in RNA-seq data were used. The adjusted P-values (adj. P) and Benjamini and Hochberg false discovery rate were applied to provide a balance between discovery of statistically significant genes and limitations of false-positives. logFC (fold change) > 1.5 and adj. P-value < 0.01 were considered statistically significant.

GO and KEGG enrichment analyses of DEGs

GO(Gene Ontology) is a bioinformatics tool for annotating genes and analyzing the biological processes of DEGs. KEGG(Kyoto Encyclopedia of Genes and Genomes) is a database resource that enriches molecular data and integrates molecular data generated from high-throughput experimental techniques. GO and KEGG enrichment analyses can help us understand the biological functions and cellular signaling pathways of DEGs. GO and KEGG enrichment analyses were performed using R software (org. Hs. eg. db package, clusterProfiler package(16) and ggplot2 package)

PPI network construction and module analysis

The STRING(Search Tool for the Retrieval of Interacting Genes,[http:// string-db.org](http://string-db.org))(17) online platform is a genetic and protein interaction relationship retrieval tool that helps users easily access unique, wide-ranging experiments and predictive interaction information. Analysis of functional interactions between proteins may provide insight into the mechanisms of LGG recrudescence. In the present study, prediction of PPI networks of DEGs through STRING database, and the interaction with a combined score > 0.4 was considered statistically significant. The predicted PPI network used Cytoscape for data visualization. Cytoscape is an open source bioinformatics software platform for visualizing molecular interaction networks. MCODE(Molecular Complex Detection) is a plugin of Cytoscape for clustering a given network based on topology to discover densely connected regions. MCODE was used to find the most significant module in PPI network after it was constructed. The screening criteria were as follows: MCODE scores > 5, node score cut-off = 0.2, Max depth = 100, degree cut-off = 2 and k-score = 2

Selection and analysis of hub genes

The genes in the most significant module screened in MCODE were considered to be hub genes. The hub genes' biological processes analysed by BiNGO(Biological Networks Gene Oncology Tool, a plug-in of Cytoscape, version 3.0.3). The hub genes network and its co-expressed genes were analyzed using the cBioPortal online platform(<http://www.cbioportal.org>)(18, 19) Hierarchical clustering of hub genes was constructed using UCSC Xena Browser (<http://genome-cancer.ucsc.edu>). The overall survival and disease-free survival analyses of hub genes were performed using Kaplan-Meier curve and the expression profiles of hub genes were analyzed and displayed using GEPIA (Gene Expression Profiling Interactive Analysis, <http://gepia.cancer-pku.cn/>)(20).

Results

Identification of the DEGs in the LGGs.

After standardization of the TCGA-LGG dataset, We first performed differential gene expression screening in the tumor samples derived from the 14 patients with recurrent LGGs. The results showed that there was no eligible difference of gene expression in the 14 primary tumor samples compared with the 18 recurrent tumor samples (Fig. 1A and 1B). This indicated that the key to tumor recurrence depends on the patient's own genetic characteristics, not the genetic difference between the primary or recurrent tumors. Therefore, the primary tumor tissues of these 14 patients with recurrent LGGs should be deleted from the primary group. In this case, the actual DEGs screening was performed between the primary samples from the 498 patients who only had primary tumors compared with the 32 samples from the 14 patients with recurrent LGGs. The result showed that a total of 413 DEGs were identified, consisting of 187 downregulated genes and 226 upregulated genes. (**Table S2**, Fig. 1C and 1D)

GO and KEGG enrichment analyses of the DEGs.

In this study, R software was used to analyze the biological classification, function and pathway enrichment of DEGs. The result of GO analysis showed that changes in the biological processes (BP) of DEGs were significantly enriched in the chromosome segregation, nuclear division, nuclear chromosome segregation and mitotic nuclear division (Fig. 2A). Changes in molecular function (MF) of the DEGs were mainly enriched in the chromosomal region, proteinaceous extracellular matrix, spindle and condensed chromosome (Fig. 2A). Changes in cell component (CC) of the DEGs were mainly enriched in the monovalent inorganic cation transmembrane transporter activity, microtubule binding, growth factor binding and motor activity (Fig. 2A). KEGG pathway analysis, on the other hand, revealed that changes of the DEGs were mainly enriched in the cell cycle, protein digestion and absorption, ECM-receptor interaction and Fanconi anemia pathway (Fig. 2B). In a corollary, the recurrence of the LGGs was closely related to higher activities of tumor division and growth.

PPI network construction and module analysis.

Subsequently, the PPI network of the DEGs was constructed (**FigureS1**) and the most significant module was obtained using Cytoscape(MCODE app) (Fig. 3A and 3B). *KCNJ10*, *SLC1A3*, *SLC1A2*, *GFAP* and *AQP4* were identified. After a module analysis based on Cytoscape(BiNGO app) and cBioportal, the functions of the genes involved in this significant module were analyzed. These most influential functions included cellular response to IFN- γ , gamma aminobutric metabolic process, multicellular organisimal water homeostasis and amino acid transport (Fig. 4B).

Hub gene selection and analysis.

We further integrated the enriched functional pathways of the above DEGs. A total of 5 genes (*KCNJ10*, *SLC1A3*, *SLC1A2*, *GFAP* and *AQP4*) were identified as the hub genes which were associated with the recurrence of the LGGs. The names, abbreviations and functions of these five genes were presented in **Table I**. Additionally network of the hub genes and the genes co-expressed with them were examined

using the cBioPortal online platform. The result showed that there were some differences in the expression profiles of the five hub genes. The expression of *KCNJ10* was relatively independent, which was correlated with *GABBR2* to some extent. There was no direct regulatory relationship, however, between the other four hub genes (Fig. 4A). Hierarchical clustering of hub genes was constructed using UCSC Xena Browser. *SLC1A3* and *SLC1A2* these two genes were downregulated relatively significant in recurrent patients (Fig. 4C).

To analyze the expressions of these five hub genes in gliomas, a box plot was made using GEPIA tools. This time not only LGGs, but also glioblastomas (GBMs, high-grade gliomas) were selected to better reflect the expressional trends of these genes. The results showed that the expressions of *AQP4* and *GFAP* in LGGs and GBMs were significantly higher than that in normal brain tissues. The expressions of *SLC1A2*, in contrast, were opposite. The expression of *KCNJ10* in LGGs was notably higher in comparison to normal brain tissues, however, there was no significant difference in the expressions between GBMs and normal brain tissues. The expression of *SLC1A3*, on the other hand, seemed to be modestly higher in all grades of tumors, but there was no statistical difference (Fig. 5).

To elucidate whether these hub genes were correlated with the prognosis of the LGG patients, the survival analysis was performed using Kaplan-Meier curve tool on the GEPIA online platform (Fig. 6). The results showed that the overall survival and disease-free survival rates of the LGG patients differed, which were associated with different patterns of *KCNJ10*, *SLC1A3*, *SLC1A2*, *GFAP* and *AQP4* expression. Among them, the LGG patients with low level of *AQP4* or *GFAP* TPM (Transcripts Per Kilobase of exon model per Million mapped reads) exhibited a better overall survival outcome ($P = 0.0033$ and $P = 0.035$). Additionally, the LGG patients with high level of *KCNJ10* TPM had a better overall survival outcome ($P = 0.0076$). There was no significant difference between the expression of *SLC1A3/SLC1A2* and the overall survival of the patients. Furthermore, the TPM changes of all five hub genes in correlation to the disease-free survival were not statistically significant as well. In a word, out the five critical genes involved in LGG recurrence, *AQP4*, *GFAP* and *KCNJ10* may play more important roles in the development and progression of the LGGs.

Discussion

Low-grade glioma (LGG) is a general term for groups of heterogeneous tumors which include ependymoma, astrocytoma, oligodendroglioma, brainstem glioma and optic nerve glioma. Since the neuropathological evaluation and diagnosis of brain tumors are based on the WHO Classification of Tumors of the Central Nervous System (2, 21), LGGs (WHO grade II or below) are usually well-differentiated and tend to exhibit benign tendencies and portend a better prognosis for the patients. Patients who were diagnosed with LGG were 17 times more likely to die than matched patients in the general population. The age-standardized 10-year relative survival rate is approximately 47% (22). After comprehensive treatment, the median survival time of patients with low-grade glioma ranges from 8–10 years. A study of low-grade oligodendroglioma showed that patients have a median survival of 11.6 years (23). However, their pathological grade increased accompanied by rate of recurrence over time, and

therefore were suggested to be classified as malignant. For this reason, it is hard to predict the outcome of the LGGs just by pathological typing. There are indeed some controversies about the difference between the high risk and low risk of LGGs and the choice of treatment options(7). At the same time, more radical treatment options may have limited benefits in preventing the recurrence of LGGs, and studies have confirmed that treatment with temozolomide (TMZ) in some patients with LGGs, rather unexpectedly, increased tumor aggressiveness and relapse(12).

Since the molecular mechanisms of LGGs remain poorly investigated, potential biomarkers for diagnosis and treatment of LGGs with high specificity and high efficiency are urgently needed. Nowadays, microarray technology has been proved to be a useful approach that enables us to explore the genetic alterations in LGGs, which may ultimately help to identify new biomarkers to differentiate the high-risk patients and low-risk patients. Our study here specifically aimed to focus on the critical genes that are involved in the recurrence of LGGs, one of the key factors affecting prognosis of the patients.

Before running the entire TCGA-LGG dataset of all 530 LGG samples, we first analyzed the differential gene expressions between the primary and recurrent tumor tissues from the 14 patients with recurrent LGGs. However, no positive result was obtained that could meet the screening criteria. This evidently suggested that the key to tumor recurrence depends on the patient's own genome, not the genetic difference between the primary or recurrent tumor. In another word, the high-risk patients with LGGs may be different from low-risk patients since the onset of the disease.

After reorganizing the specimen distribution, we re-analyzed the TCGA-LGG dataset between the LGG samples derived from the patients who only had primary tumors and the patients who recurred. A total of 413 DEGs were identified, which included 187 downregulated genes and 226 upregulated genes. We further integrated the enriched functional pathways of these DEGs by GO and KEGG enrichment analyses, which led to the identification of five hub genes—*GFAP*, *AQP4*, *KCNJ10*, *SLC1A2* and *SLC1A3*.

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein that is mainly expressed by astrocytes(24). It is thought to help maintain the mechanical strength(25), as well as the shape of the cell. It has also been reported to play a role in mitosis by adjusting the filament network. GFAP is commonly used as a marker for gliomas as well, with the primary purpose of distinguishing them from the CNS tumors of other pathological resources. The GEPIA database has demonstrated a high expression of GFAP in GBMs (grade IV). Our results, combined with the subsequent survival analysis, on the other hand, suggested a critical role of GFAP in LGGs, indicating that patients with high level of GFAP in LGGs are at higher risk and associated with worse prognosis.

KCNJ10 encodes a protein called ATP-sensitive inward rectifier potassium channel 10 (Kir4.1), which forms a heterodimer with another potassium channel protein, and potassium can flow into cells rather than outside the cell. It is responsible for the potassium buffering of brain glial cells. It was also reported that *KCNJ10* was downregulated in higher-grade gliomas. Furthermore barium, a Kir4.1 blocker increased the outgrowth of filopodia in GBM cell lines, suggesting its critical role in the invasive ability of

gliomas(26). Our results, on the other hand, confirmed the tumor suppressive role of *KCNJ10* in LGGs and disclosed that decreased expression of *KCNJ10* in LGGs indicated a worse prognosis of patients.

Aquaporin-4, also known as AQP4, is a water channel protein encoded by the *AQP4* gene in humans. Studies have reported that the synergy of *AQP4* and *KCNJ10*-encoded protein Kir4.1 altogether maintain the stability of water and ion states in the central nervous system(27, 28), which may affect neuronal excitability and cellular homeostasis, such as epileptic symptoms and cerebral edema in the patients with gliomas. However, our study suggested that deregulation of AQP4 and Kir4.1 was different in low- and high-grade gliomas. Considering that TMZ might have therapeutic potentials to control the proliferation and invasion of malignant gliomas by inhibiting *AQP4* expression(29), the significance of TMZ treatment in LGGs may have certain limitations.

SLC1A2(Solute Carrier Family 1 Member 2) encodes a member of a family of solute transporter proteins, which is also known as Excitatory amino acid transporter 2 (EAAT2) and Glutamate transporter 1 (GLT-1). An important paralog of this gene is *SLC1A3*. The expressions of *SLC1A2* and *SLC1A3* are closely related to neuronal excitability. These two membrane-bound proteins are the principal transporter that clears the excitatory neurotransmitter glutamate from the extracellular space at synapses in the central nervous system. Disregulation of *SLC1A3/SLC1A2* is thought to be associated with several neurological disorders. Although we have identified *SLC1A3/SLC1A2* as the hub genes involved in the recurrence of LGGs, there was no significant difference between their expression and the overall survival of the patients. However, if excitatory neurotransmitters are not effectively cleared, they may affect neuroelectric activity, which is related to water/ion homeostasis and may confirm the reasons for *KCNJ10* and AQP-4 as hub genes. Therefore the exact mechanisms of *SLC1A3/SLC1A2* in the LGGs and whether they could still be considered as proper targets to treat LGGs requires further investigations.

In conclusion, the present study was designed to investigate the DEGs that were involved in the development, progression and recurrence of LGGs. A total of 413 DEGs and 5 hub genes were identified, which can be considered as potential diagnostic biomarkers for LGGs. It is worth noting that although these tumors are classified as LGGs based on pathological typing, the risks of recurrence and prognosis differ greatly. Furthermore, based on the functions of the 5 hub genes, our research evidently suggested that ion/water homeostasis and neurotransmitters, two major factor in tumor microenvironment, may play a more important role in the tumorigenesis, especially recurrence of LGGs. In addition, treatment strategies for LGGs may affect their recurrence possibility, and the use of temozolomide and higher doses of radiation may affect ion water homeostasis and neurotransmitters and affect the tumor microenvironment. These may be the reasons why patients of LGGs taking radical treatment strategies fail to achieve a better prognosis. Magnetic resonance spectroscopy(MRS) can evaluate the preoperative tumor metabolism, especially the level of excitatory neurotransmitters. Preoperative and postoperative excitatory neurotransmitter levels may be used as a prognostic tool. Patients with LGGs may have a higher risk of recurrence if their preoperative excitatory neurotransmitter levels are high. Increased brain tissue excitatory neurotransmitter levels during postoperative follow-up may help us identify tumor recurrence and pseudo-progression. Further research is needed on the choice of treatment strategy and

the application of MRS. These biomarkers may give us a better understanding of the recurrent process of LGGs and provide potential targets for diagnosis and treatment of LGGs.

Conclusion

Unlike other TCGA datasets, the data provided by the LGG dataset includes the recurrence information of clinical patients. In previous studies, few people discovered the DEGs and hub genes related to recurrence based on bioinformatics methods.

The identification of DEGs and hub genes in LGGs help us better understand the development, progression, and recurrence mechanisms of LGGs. Our research evidently suggested that ion/water homeostasis and neurotransmitters, two major factors in tumor microenvironment, may play important roles in the tumorigenesis, especially recurrence of LGGs, and it may provide potential targets for diagnosis and treatment of LGGs.

Declarations

Authors' contributions

Dong He, Zhen Qin, Qi Pang and Qian Liu conceived the concept, Dong He, Zhen Qin, Fan Yang and Qian Liu wrote the manuscript. Dong He, Tao Xin, Rui Zhang and Taihong Gao carried out the dataset analyses. Hua Guo revised the discussion section of the manuscript. All the authors read and approved the final manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. All raw data used in this manuscript are available on reasonable request.

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Ethics approval

This study was reviewed and approved by the Ethical Board at Shandong Provincial Hospital, Cheeloo College of Medicine, Shandong University.

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interests.

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R Software and packages

R software:

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Ggplot2 package:

Hadley, W. (2016). ggplot2: Elegant Graphics for Data Analysis. *Springer-Verlag New York*

DT package:

Xie, Y.(2018). A wrapper of the javascript library 'datatables'. *R package DT version 0.4*

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Tables

Table I The names, abbreviations and functions of these five hub genes

Figures

No.	Gene symbol	Full name	Function
1	AQP4	Aquaporin 4	This gene encodes a member of the aquaporin family of intrinsic membrane proteins that function as water-selective channels in the plasma membranes of many cells. This protein is the predominant aquaporin found in brain and has an important role in brain water homeostasis
2	KCNJ10	Potassium Voltage-Gated Channel Subfamily J Member 10	This gene encodes a member of the inward rectifier-type potassium channel family, characterized by having a greater tendency to allow potassium to flow into, rather than out of, a cell. The encoded protein may form a heterodimer with another potassium channel protein and may be responsible for the potassium buffering action of glial cells in the brain.
3	GFAP	Glial Fibrillary Acidic Protein	This gene encodes one of the major intermediate filament proteins of mature astrocytes. It is used as a marker to distinguish astrocytes from other glial cells during development.
4	SLC1A3	Solute Carrier Family 1 Member 3	<p>This gene encodes a member of a member of a high affinity glutamate transporter family.</p> <p>This gene functions in the termination of excitatory neurotransmission in central nervous system. Mutations are associated with episodic ataxia, Type 6. Alternative splicing results in multiple transcript variants</p>
5	SLC1A2	Solute Carrier Family 1 Member 2	<p>This gene encodes a member of a family of solute transporter proteins.</p> <p>The membrane-bound protein is the principal transporter that clears the excitatory neurotransmitter glutamate from the extracellular space at synapses in the central nervous system. Glutamate clearance is necessary for proper synaptic activation and to prevent neuronal damage from excessive activation of glutamate receptors</p>

Figure 1

Data standardization and Volcano Plot TCGA-LGG dataset standardization (A) 14 patients with recurrent tumors and their primary tumor data after standardization (B) showed there is no eligible differential genes ($\log_2(\text{fold change}) > 1.5$ - $\log_{10}(\text{FDR}) > 2$) (C) data standardization of 497 primary tumor samples versus 14 patients' 32 recurrence samples. (D) volcano plot showed the up and down regulated DEGs of these two groups. A total of 413 DEGs were identified, consisting of 187 downregulated genes and 226 upregulated genes.

Figure 2

GO and KEGG pathway enrichment analysis of DEGs (A) biological processes (BP) of DEGs were significantly enriched in chromosome segregation, nuclear division, nuclear chromosome segregation, mitotic nuclear division et.al molecular function (MF) were mainly enriched in chromosomal region, proteinaceous extracellular matrix, spindle and condensed chromosome et.al cell component (CC) of DEGs were mainly enriched in the monovalent inorganic cation transmembrane transporter activity, microtubule binding, growth factor binding and motor activity et. Al (B) KEGG pathway analysis revealed that the changes mainly in cell cycle, protein digestion and absorption, ECM-receptor interaction and Fanconi anemia pathway.

Figure 3

PPI network and most significant module of DEGs (A) DEGs were selected with a fold change >1.5 and P-value <0.01 among mRNA expression profiling set TCGA-LGG The PPI network of DEGs was constructed using STRING online platform and Cytoscape (B) The most significant module was founded by the MCODE app in Cytoscape

Figure 4

Interaction network and biological process analysis of the hub genes. (A) Hub genes and their co-expression genes were analyzed using cBioportal. Nodes with bold black outline represent hub genes. Nodes with thin black outline represent the co-expression genes. (B) The biological process analysis of hub genes was constructed using BiNGO app in Cytoscape. The color depth of nodes refers to the corrected P-value of ontologies. The size of nodes refers to the numbers of genes that are involved in the ontologies. $P < 0.01$ was considered statistically significant. (C) Hierarchical clustering of hub genes was constructed using UCSC Xena Browser. The sample with red bar are recurrent tumors and the sample with blue bar are primary tumors. Upregulation of genes is marked in red; downregulation of genes is marked in blue.

Figure 5

Association between the expression of hub genes and grade of gliomas. AQP4 and GFAP expression increased in both low-grade gliomas and glioblastomas compared with normal tissues and was statistically significant. SLC1A2 expression decreased in both different grade gliomas compared with normal tissues and was statistically significant,too. The expression of KCNJ10 in low grade gliomas was increased and statistically significant compared to normal brain tissue, but was reduced in glioblastoma compared to lower grade gliomas. And the latter is not statistically significant.SLC1A3 expression increased in both groups,but it is not statistically significant.(Normal brain tissue expression data form

GTEx(Genotype-Tissue Expression,<https://commonfund.nih.gov/gtex>) online platform,Boxplot performed using GEPIA online platform)

Figure 6

Overall survival and disease-free survival analyses of hub genes were performed using GEPIA online platform. $P<0.05$ was considered statistically significant

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [FigureS1.jpg](#)
- [TableS1TCGALGGmetadata.xlsx](#)
- [TableS2allDEGs.xlsx](#)