GNG5 is a Novel Oncogene Associated with Poor Prognosis in Glioma Patients

Wang Zhang

First Affiliated Hospital of Harbin Medical University

Binchao Liu

First Affiliated Hospital of Harbin Medical University

Miaomiao Jiang

First Affiliated Hospital of Harbin Medical University

Shi Yan

First Affiliated Hospital of Harbin Medical University

Xian Han

First Affiliated Hospital of Harbin Medical University

Hong Shen

First Affiliated Hospital of Harbin Medical University

Meng Na

First Affiliated Hospital of Harbin Medical University

Zhenfeng Jiang

First Affiliated Hospital of Harbin Medical University

Zhiguo Lin (✉ linzhiguo@hrbmu.edu.cn)

First Affiliated Hospital of Harbin Medical University <https://orcid.org/0000-0002-2920-4272>

Research article

Keywords: Glioma, oncogene, biomarker, GNG5

Posted Date: July 16th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-42547/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Although many biomarkers have been reported for detecting glioma, the prognosis for the disease remains poor, and therefore, new biomarkers need to be identified. *GNG5*, which is part of the G-protein family, has been associated with different malignant tumors, though the role of *GNG5* in glioma has not been studied. Therefore, we aimed to identify the relationship between *GNG5* expression and glioma prognosis and to identify a new biomarker for the diagnosis and treatment of gliomas.

Methods: We used datasets from databases including TCGA and GEO, and results from GEPIA, RT-qPCR, and HPA to determine the expression of *GNG5* in glioma. Based on datasets obtained from the CGGA database, we identified the correlation between *GNG5* expression and multiple molecular and clinical features as well as clinical prognosis using a variety of analytical methods. Co-expression analysis and GSEA were performed to detect *GNG5*-related genes in gliomas and possible signaling pathways involved. ESTIMATE, ssGSEA, and TIMER were used to detect the relationship between *GNG5* and the immune microenvironment.

Results: A total of 1826 glioma related datasets were used in our study, including sequencing data, microarray data, and RT-qPCR data. We found that *GNG5* is highly expressed in gliomas, and its expression level is positively correlated with pathological grade, histological type, age, and tumor recurrence and negatively correlated with isocitrate dehydrogenase mutation, 1p/19 co-deletion, and chemotherapy. Moreover, *GNG5* as an independent risk factor was negatively correlated with the overall survival time. GSEA analysis revealed the potential signaling pathways involved in *GNG5* function in gliomas, such as ECM-receptor interaction and the toll-like receptor signaling pathway. The ssGSEA, ESTIMATE, and TIMER based analysis indicated a correlation between *GNG5* expression and various immune cells in glioma, such as B cell, macrophage, and dendritic cells.

Conclusions: Based on the large data platform and the use of different databases to corroborate results obtained using various datasets, our study reveals for the first time that *GNG5*, as an oncogene, is overexpressed in gliomas and can lead to poor prognosis of patients. Thus, *GNG5* is a potential novel biomarker for the clinical diagnosis and treatment of gliomas.

Background

Glioma is the most common malignant tumor of the central nervous system (CNS), showing high recurrence and a high mortality rate [1]. Although extensive research has been performed on the etiology, diagnosis, and treatment of gliomas, the prognosis is still unsatisfactory despite active comprehensive treatment being adopted for glioma patients, including maximum surgical resection, postoperative radiotherapy, and chemotherapy [2]. To improve the diagnostic accuracy of glioma and predict the prognosis of glioma patients more accurately, The World Health Organization (WHO) in 2016 defined the subclassification of glioma using molecular parameters including the co-deletion of chromosomal 1p and 19q(chr1p/19q), and the mutational status of isocitrate dehydrogenase (IDH) [1]. However, as

malignant tumors are highly heterogeneous, the use of these widely used biological markers is not sufficient for the precise diagnosis and treatment of glioma. Therefore, there is an urgent need for novel molecular markers with high specificity and sensitivity to improve the molecular diagnosis and treatment of glioma.

G-proteins are a family of proteins that participate in multiple cellular functions such as cell division, differentiation, and metastasis during embryonic development through direct interaction with G protein-coupled receptors [3, 4]. The formation of gliomas is related to embryonic development; for example, the epithelial-mesenchymal transition (EMT) is a process vital for embryonic development, and it has been shown to regulate the progression and invasion of glioma [5, 6]. Multiple studies have confirmed the significance of G-protein family members in the pathological progress of cancer. For example, *GNG7* is an epigenetic silencing gene involved in the malignant progression of renal clear cell carcinoma and esophageal cancer [7, 8]; *GNG4* function shows a high degree of correlation with liver cancer and colorectal cancer and is used in their diagnosis and prognosis [9, 10]; and *GNG11* promotes the adhesion, migration, and invasion of gastric cancer cells [11]. Thus, the G-protein family is associated with tumor progression and may be used as potential biomarkers for the diagnosis and treatment of tumors.

We focused on the *GNG5* protein of the G-protein family, and few reports describe the role of *GNG5* in tumors. *GNG5*, as a gamma subunit of G-protein, localized to the human chromosome 1p22, and is composed of 3 exons and 2 introns, and regulates the occurrence and development of many diseases [12]. Additionally, it acts as a regulator of islet function regulating the secretion of insulin, and is also used as a marker of melanosis coli to regulate cellular apoptosis [13, 14]. *GNG5* has been shown to play an important role in various cancers such as endometrial carcinoma [15] where it is highly expressed, and in invasive ductal carcinoma of the breast [16] where it regulates the secretion of E-cadherin through the Wnt signaling pathway. However, the role of *GNG5* in contributing to the pathological mechanism in glioma has not been reported.

We focused on exploring the expression pattern of *GNG5* in glioma from gene level to protein level, examining the underlying relationship between *GNG5* and the clinical and molecular characteristics of glioma in patients, and revealing the potential biological function of *GNG5* in the pathological progression of glioma. The results obtained could enhance our understanding of the complex molecular mechanisms of glioma and provide a promising novel target for combating the disease.

Methods

Data Collection

A comparison of the transcript levels of *GNG5* in different tumors was performed using Gene Expression Profiling Interactive Analysis (GEPIA, <http://gepia.cancer-pku.cn>). To identify differences in expression for *GNG5* between gliomas and the control group, RNAseq data from 698 gliomas and 5 normal brain tissues were downloaded from The Cancer Genome Atlas (TCGA, <https://portal.gdc.cancer.gov/>), and microarray

data of the GSE131273 dataset from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) database. A Mann-Whitney test was performed to analyze the difference in expression for *GNG5* between glioma and normal brain tissues. RNA sequencing data from 1018 gliomas and their corresponding clinical information was downloaded from the Chinese Glioma Genome Atlas (CGGA, <http://www.cgga.org.cn/help.jsp>) database. The relationship between the expression level of *GNG5* and clinicopathological features was further analyzed using the data obtained from CGGA. The patients were divided into older and younger groups according to the median age of the patients, and high and low expression groups according to the average expression level of *GNG5* in the samples. Additionally, we obtained the overall survival (OS) information for glioma patients from the GSE53733 dataset, including 23 patients who showed long-term survival (> 36 months), 16 patients that showed short-term survival (< 12 months), and 31 patients who displayed intermediate survival. We analyzed the difference in expression of *GNG5* among the three groups of patients with different survival phenotypes. The expression of *GNG5* at the protein level was analyzed using data from the Human Protein Atlas (HPA, <https://www.proteinatlas.org/>) database.

Patients, Tissue Preparation And Rt-qpcr

A total of 40 primary glioma samples and 5 non-glioma samples were selected from the Department of Neurosurgery of the First Affiliated Hospital of Harbin Medical University. The tissues were cut into bean grains and put into liquid nitrogen after removal, as soon as possible. Total RNA was collected using Trizol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and RNA was reverse transcribed using the transcriptor first-strand cDNA synthesis kit (Hoffmann-La Roche, Basel, Switzerland), according to the manufacturer's instructions as described previously [17]. RNA-specific primers (Ruibo Beijing) were used for the *GNG5* reverse transcription (*GNG5*-forward: CGGACTCAACCGCGTAAA, *GNG5*-reverse: GGGTCTGAAGGGATTTGTACTT). RT-PCR was conducted with SYBR Green on a 7500HT Fast Realtime System (Applied Biosystems, Foster City, CA, USA). The glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, forward: TCCAAAAATCAAGTGGGGCGA, reverse: TGATGACCCTTTTGGCTCCC) was used for the normalization of *GNG5*. The relative fold-change in the expression of *GNG5* was determined by using the $-\Delta\Delta CT$ method.

Gene set enrichment analysis (GSEA) and immune correlation analysis

GSEA is a tool developed by research teams at MIT and Harvard University's Broad Institute to analyze genome-wide expression profiling from microarray data [18], and we used it for analyzing *GNG5* related pathways and molecular mechanisms in glioma. We used 1000 gene set permutations for each analysis using the expression level of *GNG5* as a reference phenotype tag. The resulting enriched pathways were analyzed based on nominal (NOM) *P*-values and normalized enrichment scores (NES). We examined the relationship between the expression of *GNG5* in glioma tissues and the immune microenvironment using the single-sample gene set enrichment analysis (ssGSEA) to calculate the enrichment of 29 immune cell geneset signatures in each glioma sample based on data downloaded from the CGGA database [19–21].

Further, a cluster analysis of glioma samples was performed based on the quantitative results obtained from the ssGSEA analysis. The ESTIMATE method (estimate R package) was used to evaluate the immune scores, tumor purity, and the stromal score for each glioma sample [22]. Tumor IMMune Estimation Resource (TIMER, <https://cistrome.shinyapps.io/timer/>) is an ideal public database for studying the infiltration abundance of tumor-infiltrating immune cells. The database pre-evaluated the immune infiltration levels of six immune infiltrating cells (including B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages and dendritic cells) in 10897 cancer samples by complex statistical methods. We examined the relationship between the expression of *GNG5* and the infiltration abundance of the above six immune cells in glioma using TIMER [23].

Statistical analysis

We used R (v3.5.1) for statistical analysis. The Wilcoxon rank-sum test was used to analyze the correlation between the expression level of *GNG5* and clinical data of glioma patients. A Cox's regression model and the Kaplan-Meier method was used to analyze the relationship between the expression level of *GNG5* and the OS of the patients. COX test was used for univariate and multivariate analysis to reveal the risk factors affecting the prognosis of glioma. The Mann-Whitney test was used to analyze the differences in the quantitative reverse transcription PCR (RT-qPCR) results using the Graphpad Prism 8.0 software (GraphPad Software Inc., San Diego, U.S.A.).

Results

Clinical characteristics of the patients involved in the study

We used data from the CGGA database that contains a large number of glioma gene expression profiles and a vast amount of clinical information data to further analyze the relationship between *GNG5* expression and the clinical features of glioma. RNA sequencing data of 749 patients, including 442 males and 307 females, with complete clinical information were screened from 1018 samples based on the CGGA database. The mean age and survival range were 43.26 years and 8–79 years for males, and 3.22 years and 0-11.99 years for females, respectively. In total, we analyzed 502, 222, and 25 cases of primary, recurrent, and secondary gliomas, respectively. An analysis of the pathological results showed that there were 218, 240, and 291 cases with WHO grade II, grade III, and grade IV gliomas, respectively. In addition, 40.28% of the patients (n = 410) showed mutations in IDH, and 33.30% patients (n = 339) were wildtype for IDH. Only 15.23% of patients (n = 155) showed a 1p19q co-deletion, whereas 58.35% (n = 594) did not have this genotype. These results are summarized in Table S1.

***GNG5* is highly expressed in gliomas**

We analyzed *GNG5* expression in different tumors and control groups using GEPIA. A total of 15,203 patient samples were included, including 9,663 samples from 33 tumors and 5,540 corresponding control samples. *GNG5* expression was elevated in tumor tissues including glioblastoma (GBM), lower-grade

glioma (LGG), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), skin cutaneous melanoma (SKCM), and uterine carcinosarcoma (UCS), and showed significantly lower expression in acute myeloid leukemia (LAML) relative to the control group (Fig. 1A). These findings suggest that *GNG5* is highly expressed in a variety of tumors. To further analyze the expression of *GNG5* in gliomas, we analyzed the expression level of *GNG5* in glioma and normal brain tissues based on transcriptome data in TCGA database and chip data in GSE131273 dataset, respectively. And the results showed that *GNG5* expression was significantly higher in gliomas relative to normal brain tissue (Fig. 1B, C, $P < 0.001$), which was consistent with the results of RT-qPCR (Fig. 1D, $P < 0.05$). Furthermore, immunohistochemical data from HPA revealed that *GNG5* expression in glioma was higher than that in non-glioma tissues (Fig. 1E-F).

Relationship between *GNG5* and the underlying molecular and clinical characteristics in glioma patients

We analyzed the relationship between *GNG5* expression level in the 749 samples from the CGGA database and the various tumor subtypes, their pathological classification, molecular classification, tumor treatment, and the age of the patients. The results indicated that the *GNG5* expression level in gliomas was significantly correlated with the pathological grade and the age of the patients (Fig. 2A, C, $P < 0.001$). The average level of *GNG5* expression in tissue from patients with recurrent gliomas was higher than in patients with primary and secondary tumors (Fig. 2B, $P < 0.001$). Similar results were found based on a correlation analysis between *GNG5* expression level in gliomas and different pathological subtypes, such as the expression levels of *GNG5* in recurrent astrocytoma, recurrent anaplastic astrocytoma and recurrent anaplastic oligodendroglioma were higher than that in corresponding primary pathological subtypes, as shown in Fig. 2G.

Reports from research conducted previously shows that patients with mutations in the *IDH* gene or a 1p/19q co-deletion had longer survival and better prognosis [24]. Interestingly, we found that the expression level of *GNG5* in patients with mutations in the *IDH* gene was lower than in patients with wildtype *IDH* (Fig. 2D). Similarly, patients with a 1p/19q co-deletion had significantly lower expression of *GNG5* than in patients without the co-deletion (Fig. 2E). Together, these results suggest that *GNG5* expression may correlate with the prognosis of glioma patients. When a grouping of patients based on whether they received chemotherapy or not was performed, we found that tissue from patients who received chemotherapy showed an upregulation of *GNG5* expression relative to those who did not receive chemotherapy (Fig. 2F). However, radiotherapy did not affect *GNG5* expression (data was not shown). One explanation for this observation may be that the surgical resection of the tumor and subsequent sequencing data analysis are from samples collected from patients before they underwent chemotherapy. Taken together, these results indicate that a correlation exists between *GNG5* expression and the molecular and clinical characteristics of glioma.

High expression of *GNG5* is a predictor of poor prognosis

The average follow-up time for the 749 patients whose data for survival analysis is included in this study was 3.22 years. The results from the survival analysis showed that high expression of *GNG5* is

significantly correlated with reduced survival of glioma patients (Fig. 3A, $P < 0.001$); these results were verified by Kaplan-Meier method based on TCGA database and the *GNG5* expression analysis based on GSE53733 dataset (Figure S1A, B). Moreover, a time-dependent receiver operated characteristic (ROC) analysis showed that the values for area under the ROC curve (AUC) were 0.714, 0.792, and 0.821 for one, three, and five-year OS respectively (Fig. 3B). Therefore, our results indicate that *GNG5* may serve as a biomarker for glioma patients, especially for the five-year OS group.

The results of a univariate analysis suggest that the high expression level of *GNG5* in glioma patients is associated with reduced OS, primary recurrence or secondary (PRS) type, histological type, pathological grade, age, chemotherapy status, presence of mutations in the *IDH* gene, and a 1p/19q co-deletion (Fig. 4A). Additionally, results from the multivariate analysis indicate that the expression of *GNG5*, PRS type, pathological grade, age, chemotherapy status, presence of mutations in the *IDH* gene, and 1p/19q co-deletion were independently correlated with OS (Fig. 4B). The above data indicate *GNG5* may serve as a prognostic factor and increased *GNG5* expression is associated with poor OS.

Co-expression analysis of *GNG5*

To further explore the function of *GNG5*, the limma package (Bioconductor) was used in the R statistical software to analyze the coexpression genes with *GNG5*. We found 4517 genes associated with *GNG5* (correlation coefficient (Cor) > 0.5 , $P < 0.001$), of which 29 genes were negatively correlated with *GNG5* and a total of 4488 showed a positive correlation. A heatmap of the top 20 genes associated with *GNG5* is shown in Fig. 5A and the five genes showing significant positive or negative correlation with *GNG5* is shown in Fig. 5B. The 5 genes that showed positive correlation with *GNG5* expression were *RPF1* (Cor = 0.895, $P = 0.000$), *AK2* (Cor = 0.893, $P = 0.000$), *TMSB4X* (Cor = 0.875, $P = 4.249 \times 10^{-322}$), *POP4* (Cor = 0.874, $P = 4.247 \times 10^{-320}$) and *RER1* (Cor = 0.866, $P = 1.400 \times 10^{-307}$) (Fig. 5C-E and S2A, B), while the top 5 genes that were negatively correlated with *GNG5* expression were *RIMS1* (Cor = -0.665, $P = 4.201 \times 10^{-131}$), *CDYL2* (Cor = -0.61, $P = 6.459 \times 10^{-105}$), *RN7SL4P* (Cor = -0.605, $P = 1.370 \times 10^{-102}$), *ATRNL1* (Cor = -0.595, $P = 1.953 \times 10^{-98}$) and *TUB* (Cor = -0.589, $P = 3.049 \times 10^{-96}$), (Fig. 5F-H and S2C, D;). Thus, *GNG5* expression is correlated with various genes in gliomas.

GNG5 related signaling pathways based on GSEA

GSEA was used to identify *GNG5* related signaling pathways involved in gliomas. A $P < 0.05$ and a false discovery rate (FDR) < 0.25 represented a significant enrichment in the results (in the enrichment of MSigDB Collection). Six pathways, including those involving the ECM-receptor interaction, focal adhesion, cell adhesion molecules, toll-like receptor signaling pathway, nod-like receptor signaling pathway, and the rig-like receptor signaling pathway showed significantly differential enrichment in samples from patients showing the *GNG5* high expression phenotype based on NES, NOM P -values, and FDR values (Fig. 6; Table 1), indicating a potential role for *GNG5* in the development of glioma.

Table 1
Signaling pathways enriched in high expression of *GNG5*.

Gene set name	NES	NOM <i>P</i> -value	FDR q-value
KEGG_ECM_RECEPTOR_INTERACTION	1.85	0.008	0.1
KEGG_FOCAL_ADHESION	1.81	0.004	0.076
KEGG_CELL_ADHESION_MOLECULES_CAMS	1.77	0.026	0.096
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	1.76	0.002	0.092
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	1.75	0.016	0.093
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	1.74	0.008	0.093
<i>GNG5</i> is related to the immune microenvironment			
<p>Next, we explored whether there is a correlation between <i>GNG5</i> expression and the tumor immune microenvironment. We analyzed 29 immune-system related gene sets characterizing different types and functions of immune cells (Table S2). Based on data from the CGGA database, ssGSEA was used to quantify and hierarchically cluster immune cells in tumor samples into three groups. According to the clustering heat map of immune cell gene sets in the three groups, a high immunity group (high-immune), medium immunity group (mid-immune), and low immune activity group (low-immune) were defined (Fig. 7A). Moreover, the high-immune group had a significantly higher immune score than that of the low-immune group, though the tumor purity showed the opposite characteristics (Fig. 7B, C). Interestingly, we found that the expression of <i>GNG5</i> was significantly increased in the high-immune group, and decreased in the low-immune group ($P < 0.001$, Fig. 7D). Further, we used TIMER to analyze the relationship between <i>GNG5</i> expression and infiltration abundance of six immune cells (B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells) after purity correction in glioma [23, 25]. We found that <i>GNG5</i> expression was positively correlated with the infiltrating abundance of CD8 + T cell (Cor = 0.154, $P = 7.29 \times 10^{-4}$), B Cell (Cor = 0.453, $P = 1.40 \times 10^{-25}$), macrophages (Cor = 0.544, $P = 9.10 \times 10^{-38}$), CD4 + T cells (Cor = 0.549, $P = 6.68 \times 10^{-39}$), neutrophils (Cor = 0.554, $P = 1.25 \times 10^{-39}$), dendritic cell (Cor = 0.582, $P = 2.06 \times 10^{-44}$) in LGG. B Cell (Cor = 0.112, $P = 0.022$), macrophages (Cor = 0.179, $P = 0.000$), and dendritic cells (Cor = 0.241, $P = 5.88 \times 10^{-7}$) were corrected with <i>GNG5</i> expression in GBM (Fig. 7E). These results suggest that <i>GNG5</i> may be a potential factor influencing the immune microenvironment in glioma.</p>			

Discussion

G-proteins consisting of α , β and γ subunits are also known as GTP binding proteins, which mediate the transduction of a variety of hormones and neurotransmitters across the membrane and subsequently trigger a series of physiological and biochemical reactions in cells [26, 27]. G-proteins regulate basic life processes such as cell metabolism, secretion, growth, proliferation, differentiation, distortion, pathological changes, and cell death [4]. *GNG5*, a subunit of G-protein, has been reported to promote the proliferation and migration of tumor cells [28]. However, to date, few studies have reported the role and mechanism of action for *GNG5* in tumors.

To explore the expression patterns of *GNG5* in various tumors, we searched for *GNG5* in the GEPIA database. We found that *GNG5* was significantly highly expressed in a variety of cancers, including gliomas. (Fig. 1A). To further understand the role of *GNG5* in tumors, we focused on its role in glioma. We obtained *GNG5* expression data from TCGA and GEO databases and clinical samples. Our results showed that *GNG5* expression was increased in glioma based on data from sequencing, microarrays, and PCR data (Fig. 1B-D). Previous studies have suggested that *GNG5* shows elevated expression in endometrial cancer and in infiltrating ductal carcinoma of the breast [15, 16]. Furthermore, *POP4*, a *GNG5* coexpressing promoter gene, is highly expressed in prostate cancer cells, while *RIMS1*, a *GNG5* coexpressing suppressor gene, is lowly expressed in craniopharyngioma [29, 30]. Further, we searched the HPA database for *GNG5* protein expression, and the results indicate that a high level of *GNG5* is observed in glioma (Fig. 1E-F). Interestingly, we found that *GNG5* expression levels are closely related to the clinical features associated with glioma prognosis, especially with the pathological grade and primary recurrence status of glioma (Fig. 2). Some studies have reported that glioma patients with a mutation in the *IDH* gene and a 1p/19q co-deletion have a better prognosis [31, 32]. However, we found that the average expression level of *GNG5* was lower in samples from patients with mutations in the *IDH* gene and those with a 1p/19q co-deletion. Therefore, we hypothesized that *GNG5* may be an oncogene in glioma.

To clarify whether *GNG5* is an oncogene and may lead to a poor prognosis for glioma, we obtained clinical data from thousands of glioma samples from TCGA, CGGA, and GEO databases. The results from our analysis show that increased expression of *GNG5* could indeed cause poor prognosis of glioma patients (as shown in Fig. 3A, S1). Moreover, as shown in Fig. 3B, *GNG5* has a high diagnostic value and can be used as a biomarker for predicting the prognosis of glioma. Additionally, genes coexpressed with *GNG5* such as *AK2* are highly expressed in lung cancer and can lead to poor prognosis in patients [33]; besides, *RER1* as a coexpressed gene with *GNG5*, could promote the progression of pancreatic cancer and reduce the survival rate of patients [34]. Although, the high expression of *GNG5* in glioma could reduce the OS rate of patients, whether the expression of *GNG5* is necessarily correlated with such a prognosis for glioma patients was further examined. Results from univariate and multivariate analyses showed that increased expression of *GNG5* is an independent risk factor for glioma patients. However, the mechanism explaining how *GNG5* leads to a poor prognosis for glioma patients is yet to be elucidated.

We next undertook a GSEA to understand the functioning of *GNG5* in glioma. We found that high expression of *GNG5* promotes the activation of a series of cancer-related signaling pathways (Fig. 6). Similar interactions have been described previously; for example, the ECM-receptor interaction is involved in the proliferation and invasion of cancer cells [35, 36], and focal adhesion is involved in the metastasis and invasion of cancer cells [37]. Additionally, the toll-like receptor promotes the immune evasion of glioma by down-regulating the MHC II molecules in microglia [38], and cell adhesion molecules and the nod-like receptor play a significant role in the proliferation, invasion, and metastasis of glioma cells [39–42]. A particular gene may play a role in a variety of signaling pathways in the pathological process of diseases. Used as a tool to reveal the molecular mechanisms underlying diseases, GSEA is widely used and has high reliability. Compared with traditional methods of analysis, GSEA has the advantage of using

large sample sizes and can avoid the biases inherent in experimental results caused by artificial threshold setting [18]. Previous studies have suggested the reliability of GSEA as a predictive tool. For example, Liu Z et al. reported that overexpression of *HOXA2* can activate the JAK-STAT and focal adhesion signaling pathways in glioma [43], and Wang et al. elucidated the autophagy-related signaling pathway in glioblastoma through the GSEA method [44]. Therefore, we believe that the signaling pathways enriched with *GNG5* in glioma as revealed in our study could provide the basis for further investigations into the specific mechanisms underlying *GNG5* functioning in glioma.

Recently, immunotherapy has been shown to be a new treatment strategy for glioma, and genes related to glioma immunity have been reported frequently [45, 46]. However, the association of *GNG5* with glioma immunity has not been reported. Our results suggest that *GNG5* may participate in the malignant progression of glioma through ECM-receptor interaction, toll-like receptor pathway, and the nod-like receptor signaling pathways, which are reported to be related to the immune response process [47, 48]. Therefore, we hypothesized that *GNG5* may be involved in the immune response in glioma. To verify this, we analyzed 29 immune-associated gene sets in glioma samples using ssGSEA and ESTIMATE. The results showed that the expression of *GNG5* in glioma was correlated with immune activity (Fig. 7D). Previous studies have reported a correlation between programmed death-1/ligand-1 (PD-1/PD-L1) and immune activity in tumors in triple-negative breast cancer [20]; Moreover, a large number of studies have shown that ssGSEA and ESTIMATE are highly reliable in the evaluation of tumor immune stratification and immune invasion analysis [22, 49–52]. Furthermore, we analyzed the correlation between the expression of *GNG5* and infiltration level of tumor-infiltrating immune cells using TIMER. The results suggest that *GNG5* is correlated with the infiltration abundance of various immune cells in glioma, especially macrophage, dendritic cells and neutrophils in low-grade glioma (Fig. 7E). Though there are currently no reports describing the relationship between the G-protein family and cancer immunity, correlations between single genes and cancer immunity have been observed. For example, CD70, which is highly expressed in glioma, can promote macrophage infiltration into a tumor [45], and JAK1 was shown to be positively correlated with the infiltration of various immune cells in breast cancer, such as CD8 + T cells and dendritic cells, etc. [53]. Additionally, the expression of *LAYN* was found to be positively correlated with the infiltration of CD4 + T and CD8 + T cells, macrophages, neutrophils, and dendritic cells in colon and gastric adenocarcinomas [54]. These results indicate that *GNG5* is a highly expressed gene in glioma, is associated with immune activity, and future research could help establish the immune response related to *GNG5* function in glioma.

Although we used bioinformatics analysis and large datasets to uncover the role of *GNG5* in glioma, our study has a few limitations. First, as extensive clinical sample information in the public databases are not always available, it is difficult to comprehensively evaluate the correlation between *GNG5* and the clinical features of glioma patients. However, it is difficult to avoid the problem of the lack of clinical sample information in public databases, as this data is collated from different research centers. Second, although multicenter research in public databases can make up for the deficiencies inherent in single-center research, there are still some shortcomings, especially the inconsistency of intervention measures

and the lack of clinical information. Thus, prospective studies implemented in the future will need to minimize bias in experimental results due to retrospective analysis.

Conclusion

Our study shows that *GNG5* is highly expressed in gliomas, and this expression is correlated with various molecular and clinical features of glioma patients. High expression levels of *GNG5* predict a poor prognosis in glioma patients. Additionally, *GNG5* may participate in the pathological progress of glioma through the signaling pathways related to cancer, such as ECM-receptor interaction, the toll-like receptor signaling pathway, and the nod-like receptor signaling pathway. These results indicate that *GNG5* is a novel oncogene in glioma and could provide a potential biomarker for the diagnosis and treatment of gliomas.

Abbreviations

AUC

area under the receiver operated characteristic curve; CGGA:Chinese Glioma Genome Atlas; CNS:central nervous system; Cor:correlation coefficient; EMT:epithelial-mesenchymal transition; FDR:false discovery rate; GAPDH:glyceraldehyde-3-phosphate dehydrogenase; GBM:glioblastoma; GEO:Gene Expression Omnibus; GEPIA:Gene Expression Profiling Interactive Analysis; HPA:Human Protein Atlas; IDH:isocitrate dehydrogenase; LAML:acute myeloid leukemia; LGG:lower-grade glioma; NES:normalized enrichment scores; NOM:nominal; OS:overall survival; OV:ovarian serous cystadenocarcinoma; PAAD:pancreatic adenocarcinoma; PD-1/PD-L1:programmed death-1/ligand-1; PRS:primary recurrence or secondary; ROC:receiver operated characteristic; SKCM:skin cutaneous melanoma; ssGSEA:single-sample gene set enrichment analysis; TCGA:The Cancer Genome Atlas; TIMER:Tumor IMMune Estimation Resource; UCS:and uterine carcinosarcoma; WHO:World Health Organization.

Declarations

Ethics approval and consent to participate

All protocols of this study were approved by the Ethics Committee of the First Clinical College of Harbin Medical University. All clinical samples involved in this study have been informed consent of the patients. The public data involved in this study were all downloaded from public databases, therefore there was no new informed consent required.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the CGGA database (<http://www.cgga.org.cn/>), TCGA database (<https://portal.gdc.cancer.gov/>), and datasets from GEO (<https://www.ncbi.nlm.nih.gov/geo/>). The data of RT-qPCR during the current study are available from the corresponding author on reasonable request.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81772678 and 81601130), Natural Science Foundation of Heilongjiang Province (No. LH2019H082), Postdoctoral Research Foundation of China (No. 2018M631951) and Heilongjiang Provincial Postdoctoral Science Foundation (No.LBH-Z17144).

Authors' Contributions

Hong Shen, Zhenfeng Jiang, and Zhiguo Lin conceived and designed the experiment. Wang Zhang, Binchao Liu, Miaomiao Jiang, Shi Yan, and Xian Han analyzed and checked the data, Wang Zhang, and Meng Na wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgments

We would like to thank Dr. Zhendong Liu for the help in data analysis and topic discussion, and also we would like to thank Editage (www.editage.com) for English language editing.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol.* 2016;131:803–20.
2. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352:987–96.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144:646–74.
4. Syrovatkina V, Alegre KO, Dey R, Huang XY. Regulation, Signaling, and Physiological Functions of G-Proteins. *J Mol Biol.* 2016;428:3850–68.
5. Meng L, Wang X, Liao W, Liu J, Liao Y, He Q. BAF53a is a potential prognostic biomarker and promotes invasion and epithelial-mesenchymal transition of glioma cells. *Oncol Rep.* 2017;38:3327–34.

6. Lu Y, Xiao L, Liu Y, Wang H, Li H, Zhou Q, Pan J, Lei B, Huang A, Qi S. MIR517C inhibits autophagy and the epithelial-to-mesenchymal (-like) transition phenotype in human glioblastoma through KPNA2-dependent disruption of TP53 nuclear translocation. *Autophagy*. 2015;11:2213–32.
7. Ohta M, Mimori K, Fukuyoshi Y, Kita Y, Motoyama K, Yamashita K, Ishii H, Inoue H, Mori M. Clinical significance of the reduced expression of G protein gamma 7 (GNG7) in oesophageal cancer. *Br J Cancer*. 2008;98:410–7.
8. Xu S, Zhang H, Liu T, Chen Y, He D, Li L. G Protein γ subunit 7 loss contributes to progression of clear cell renal cell carcinoma. *J Cell Physiol*. 2019;234:20002–12.
9. Ding J, Li Y, Fan H, Xu W, Gao R, Bai S, Zhu Z, Yang W, Gong Y, Yang J, Zhou J. Knockdown of PSMC3IP suppresses the proliferation and xenografted tumorigenesis of hepatocellular carcinoma cell. *J Cell Biochem*. 2019;120:5449–58.
10. Chen L, Lu D, Sun K, Xu Y, Hu P, Li X, Xu F. Identification of biomarkers associated with diagnosis and prognosis of colorectal cancer patients based on integrated bioinformatics analysis. *Gene*. 2019;692:119–25.
11. Miwa T, Kanda M, Tanaka H, Tanaka C, Kobayashi D, Umeda S, Iwata N, Hayashi M, Yamada S, Fujii T, Fujiwara M, Kodera Y. FBXO50 Enhances the Malignant Behavior of Gastric Cancer Cells. *Ann Surg Oncol*. 2017;24:3771–9.
12. Ahmad W, Li S, Chen H, Tuck-Muller CM, Pittler SJ, Aronson NN Jr. Lysosomal chitobiase (CTB) and the G-protein gamma 5 subunit (GNG5) genes co-localize to human chromosome 1p22. *Cytogenet Cell Genet*. 1995;71:44–6.
13. Taneera J, Fadista J, Ahlqvist E, Atac D, Ottosson-Laakso E, Wollheim CB, Groop L. Identification of novel genes for glucose metabolism based upon expression pattern in human islets and effect on insulin secretion and glycemia. *Hum Mol Genet*. 2015;24:1945–55.
14. Hua X, Chen J, Wu L. Identification of candidate biomarkers associated with apoptosis in melanosis coli: GNG5, LPAR3, MAPK8, and PSMC6. *Biosci Rep*. 2019;39.
15. Orchel J, Witek L, Kimsa M, Strzalka-Mrozik B, Kimsa M, Olejek A, Mazurek U. Expression patterns of kinin-dependent genes in endometrial cancer. *Int J Gynecol Cancer*. 2012;22:937–44.
16. Alsaleem M, Toss MS, Joseph C, Aleskandarany M, Kurozumi S, Alshankyty I, Ogden A, Rida P, Ellis IO, Aneja R, et al. The molecular mechanisms underlying reduced E-cadherin expression in invasive ductal carcinoma of the breast: high throughput analysis of large cohorts. *Mod Pathol*. 2019;32:967–76.
17. Tang C, Gu Y, Wang H, Wu H, Wang Y, Meng Y, Han Z, Gu Y, Ma W, Jiang Z, et al. Targeting of microRNA-21-5p protects against seizure damage in a kainic acid-induced status epilepticus model via PTEN-mTOR. *Epilepsy Res*. 2018;144:34–42.
18. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2005;102:15545–50.

19. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C, et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature*. 2009;462:108–12.
20. He Y, Jiang Z, Chen C, Wang X. Classification of triple-negative breast cancers based on Immunogenomic profiling. *J Exp Clin Cancer Res*. 2018;37:327.
21. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformatics*. 2013;14:7.
22. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, Treviño V, Shen H, Laird PW, Levine DA, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun*. 2013;4:2612.
23. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B, Liu XS. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res*. 2017;77:e108–8e110.
24. Mu L, Long Y, Yang C, Jin L, Tao H, Ge H, Chang YE, Karachi A, Kubilis PS, De Leon G, et al. The IDH1 Mutation-Induced Oncometabolite, 2-Hydroxyglutarate, May Affect DNA Methylation and Expression of PD-L1 in Gliomas. *Front Mol Neurosci*. 2018;11:82.
25. Li B, Severson E, Pignon JC, Zhao H, Li T, Novak J, Jiang P, Shen H, Aster JC, Rodig S, Signoretti S, Liu JS, Liu XS. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biol*. 2016;17:174.
26. Ilyaskina OS, Lemoine H, Bünemann M. Lifetime of muscarinic receptor-G-protein complexes determines coupling efficiency and G-protein subtype selectivity. *Proc Natl Acad Sci U S A*. 2018;115:5016–21.
27. Duc NM, Kim HR, Chung KY. Structural mechanism of G protein activation by G protein-coupled receptor. *Eur J Pharmacol*. 2015;763:214–22.
28. Hansen CA, Schroering AG, Carey DJ, Robishaw JD. Localization of a heterotrimeric G protein gamma subunit to focal adhesions and associated stress fibers. *J Cell Biol*. 1994;126:811–9.
29. Romanuik TL, Ueda T, Le N, Haile S, Yong TM, Thomson T, Vessella RL, Sadar MD. Novel biomarkers for prostate cancer including noncoding transcripts. *Am J Pathol*. 2009;175:2264–76.
30. Yang J, Hou Z, Wang C, Wang H, Zhang H. Gene expression profiles reveal key genes for early diagnosis and treatment of adamantinomatous craniopharyngioma. *Cancer Gene Ther*. 2018;25:227–39.
31. Cancer Genome Atlas Research Network. Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, Cooper LA, Rheinbay E, Miller CR, Vitucci M, et al. Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med*. 2015;372:2481–98.
32. Dimitrov L, Hong CS, Yang C, Zhuang Z, Heiss JD. New developments in the pathogenesis and therapeutic targeting of the IDH1 mutation in glioma. *Int J Med Sci*. 2015;12:201–13.
33. Liu H, Pu Y, Amina Q, Wang Q, Zhang M, Song J, Guo J, Mardan M. Prognostic and therapeutic potential of Adenylate kinase 2 in lung adenocarcinoma. *Sci Rep*. 2019;9:17757.

34. Chen S, Zhang J, Chen J, Wang Y, Zhou S, Huang L, Bai Y, Peng C, Shen B, Chen H, et al. RER1 enhances carcinogenesis and stemness of pancreatic cancer under hypoxic environment. *J Exp Clin Cancer Res.* 2019;38:15.
35. Zhang HJ, Tao J, Sheng L, Hu X, Rong RM, Xu M, Zhu TY. Twist2 promotes kidney cancer cell proliferation and invasion by regulating ITGA6 and CD44 expression in the ECM-receptor interaction pathway. *Onco Targets Ther.* 2016;9:1801–12.
36. Yang J, Wang L, Xu Z, Wu L, Liu B, Wang J, Tian D, Xiong X, Chen Q. Integrated Analysis to Evaluate the Prognostic Value of Signature mRNAs in Glioblastoma Multiforme. *Front Genet.* 2020;11:253.
37. Maziveyi M, Dong S, Baranwal S, Alahari SK. Nischarin regulates focal adhesion and Invadopodia formation in breast cancer cells. *Mol Cancer.* 2018;17:21.
38. Qian J, Luo F, Yang J, Liu J, Liu R, Wang L, Wang C, Deng Y, Lu Z, Wang Y, et al. TLR2 Promotes Glioma Immune Evasion by Downregulating MHC Class II Molecules in Microglia. *Cancer Immunol Res.* 2018;6:1220–33.
39. Liu N, Yang C, Bai W, Wang Z, Wang X, Johnson M, Wang W, Zhang P, Yang H, Liu H, et al. CADM2 inhibits human glioma proliferation, migration and invasion. *Oncol Rep.* 2019;41:2273–80.
40. Jiang Q, Xie Q, Hu C, Yang Z, Huang P, Shen H, Schachner M, Zhao W. Glioma malignancy is linked to interdependent and inverse AMOG and L1 adhesion molecule expression. *BMC Cancer.* 2019;19:911.
41. Xue L, Lu B, Gao B, Shi Y, Xu J, Yang R, Xu B, Ding P. NLRP3 Promotes Glioma Cell Proliferation and Invasion via the Interleukin-1 β /NF- κ B p65 Signals. *Oncol Res.* 2019;27:557–64.
42. Saxena S, Jha S. Role of NOD- like Receptors in Glioma Angiogenesis: Insights into future therapeutic interventions. *Cytokine Growth Factor Rev.* 2017;34:15–26.
43. Liu Z, Shen F, Wang H, Li A, Wang J, Du L, Liu B, Zhang B, Lian X, Pang B, et al. Abnormally high expression of HOXA2 as an independent factor for poor prognosis in glioma patients. *Cell Cycle.* 2020;19:1632–40.
44. Wang Z, Gao L, Guo X, Feng C, Lian W, Deng K, Xing B. Development and validation of a nomogram with an autophagy-related gene signature for predicting survival in patients with glioblastoma. *Aging.* 2019;11:12246–69.
45. Ge H, Mu L, Jin L, Yang C, Chang YE, Long Y, DeLeon G, Deleyrolle L, Mitchell DA, Kubilis PS, et al. Tumor associated CD70 expression is involved in promoting tumor migration and macrophage infiltration in GBM. *Int J Cancer.* 2017;141:1434–44.
46. Jin L, Ge H, Long Y, Yang C, Chang YE, Mu L, Sayour EJ, De Leon G, Wang QJ, Yang JC, et al. CD70, a novel target of CAR T-cell therapy for gliomas. *Neuro Oncol.* 2018;20:55–65.
47. Satoh T, Akira S. Toll-Like Receptor Signaling and Its Inducible Proteins. *Microbiol Spectr.* 2016;4.
48. Wang JQ, Liu YR, Xia Q, Chen RN, Liang J, Xia QR, Li J. Emerging Roles for NLRC5 in Immune Diseases. *Front Pharmacol.* 2019;10:1352.
49. Li J, Liu C, Chen Y, Gao C, Wang M, Ma X, Zhang W, Zhuang J, Yao Y, Sun C. Tumor Characterization in Breast Cancer Identifies Immune-Relevant Gene Signatures Associated With Prognosis. *Front*

Genet. 2019;10:1119.

50. Xue Y, Tong L, LiuAnwei Liu F, Liu A, Zeng S, Xiong Q, Yang Z, He X, Sun Y, Xu C. Tumor–infiltrating M2 macrophages driven by specific genomic alterations are associated with prognosis in bladder cancer. *Oncol Rep.* 2019;42:581–94.
51. Yue C, Ma H, Zhou Y. Identification of prognostic gene signature associated with microenvironment of lung adenocarcinoma. *PeerJ.* 2019;7:e8128.
52. Jia D, Li S, Li D, Xue H, Yang D, Liu Y. Mining TCGA database for genes of prognostic value in glioblastoma microenvironment. *Aging.* 2018;10:592–605.
53. Chen B, Lai J, Dai D, Chen R, Li X, Liao N. JAK1 as a prognostic marker and its correlation with immune infiltrates in breast cancer. *Aging.* 2019;11:11124–35.
54. Pan JH, Zhou H, Cooper L, Huang JL, Zhu SB, Zhao XX, Ding H, Pan YL, Rong L. LAYN Is a Prognostic Biomarker and Correlated With Immune Infiltrates in Gastric and Colon Cancers. *Front Immunol.* 2019;10:6.

Figures

Figure 1

Expression level of GNG5 in gliomas. (A): Expression of GNG5 in different types of tumors in GEPIA, red, and green represent significant differences. Red represents a high expression of GNG5 in tumors, and the green represents a low expression of GNG5 in tumors; (B-D): expression level of GNG5 in gliomas and normal tissues based on TCGA (B), GSE131273 (C) and the result of RT-qPCR (D); (E): Immunohistochemical results of GNG5 in glioma and normal tissues based on human protein atlas. *** $P < 0.01$, * $P < 0.05$.

Figure 2

Expression characteristics of GNG5 in gliomas based on CGGA.

Figure 3

Correlation between GNG5 and prognosis of glioma patients. (A) The Kaplan-Meier survival curve reveals the high expression of GNG5 leads to a poor prognosis in gliomas. (B) The ROC curve shows the good diagnosis value of GNG5 in gliomas.

Figure 4

Univariate and multivariate analyses of prognostic in patients with glioma. (A): Univariate regression analysis; (B): Multivariate analysis.

Figure 5

Co-expression analysis of GNG5. (A): Heatmap of the top 20 genes associated with GNG5; (B): Circos diagram shows the five most significant genes of positive and negative correlating with GNG5; (C-H): The correlation between GNG5 and RPF1 (C), AK2 (D), TMSB4X (E), RIMS1 (F), CDYL2 (G), RN7SL4P (H).

Figure 6

GSEA enrichment analysis results. (A): the ECM-receptor interaction; (B): the focal adhesion; (C): the cell adhesion molecules; (D): the toll-like receptor signaling pathway; (E): the nod-like receptor signaling pathway; (F): the rig-like receptor signaling pathway;

Figure 7

Correlation analysis of GNG5 and immune microenvironment. (A): Cluster analysis and immune activity quantification based on ssGSEA and ESTIMATE; (B): The difference of immune score between groups in glioma; (C): Relationship between tumor purity and immunity in glioma; (D): Relationship between GNG5 immunity; (E): Correlation between GNG5 and immune cells in gliomas based on TIMER. ***P < 0.001.

Supplementary Files

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

- Additionalfile2TableS2.xlsx
- Additionalfile1TableS1.docx