

Correlation of Prognostic Biomarker SLC25A1 with Immune Infiltrates in Low-Grade Gliomas

Li Qian

Affiliated Hospital of Nantong University

Shu-min Lu

Shanghai Jiaotong University School of Medicine Xinhua Hospital

Xin He

Affiliated Hospital of Nantong University

Hua Huang

Affiliated Hospital of Nantong University

Jianguo Zhang (✉ jgz_edu@163.com)

Affiliated Hospital of Nantong University <https://orcid.org/0000-0002-6984-8523>

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Abstract

Background: Glioma is the most common primary malignant tumor of the central nervous system (CNS), and low-grade glioma (LGG) is an important pathological type of glioma. Immune infiltration of tumor microenvironment is an independent predictor of survival and prognosis in LGG patients. SLC25A1 is a gene that has not been reported in LGG. We used the TCGA database to study the expression level of SLC25A1 in LGG tissues and its relationship with the prognosis of LGG patients and tumor immune cell infiltration.

Methods: Download gene expression profile data and clinical information of LGG patients from the TCGA database. The expression level of SLC25A1 gene in LGG tissues and normal tissues was compared. The expression level of SLC25A1 gene in LGG tissue samples with different WHO grades and its relationship with the prognosis of patients were analyzed. The correlation between clinical information and SLC25A1 expression was analyzed by Logistic regression. The effect of SLC25A1 on survival of LGG patients was evaluated by survival module. The correlation between SLC25A1 gene expression and tumor immune cell infiltration in LGG and the effect of immune cell infiltration on the prognosis of patients were analyzed by using the relevant modules of GEPIA. The gene groups related to SLC25A1 expression were analyzed by KEGG pathway enrichment analysis. In addition, TCGA dataset was used for gene set enrichment analysis (GSEA).

Result: The expression level of SLC25A1 gene in LGG tissues was higher than that in normal tissues. The expression of SLC25A1 in WHO I LGG tissues was higher than that in WHO II tissues. Multivariate analysis showed that the up-regulated expression of SLC25A1 was an independent prognostic factor for good prognosis in patients with LGG. There was a correlation between the expression level of SLC25A1 and the level of immune cell infiltration of LGG, and the latter affects the prognosis of patients with LGG. In addition, GSEA also found that Neuroactive ligand receptor interaction, cell adhesion molecules and so on were differentially enriched in the phenotypic pathway of high expression of SLC25A1. In the Kyoto Encyclopedia of Genome and Genome (KEGG), Neuroactive ligand-receptor interaction, Morphine addiction et al have been identified as differential enrichment pathways.

Conclusion: SLC25A1 is the prognosis of LGG related to immune cell infiltration, and may become a biomarker of LGG grade diagnosis, immunotherapy and prognosis.

1. Introduction

Glioma is a tumor that originates from neuroectoderm cells or precursor cells, including astrocytoma, oligodendroglioma and ependymoma, which account for 75% of primary malignant tumors in the adult brain[1]. The fatality rate and disability rate are both high[2]. According to the World Health Organization (WHO) central nervous system tumor classification standard, grades I-II are low-grade gliomas (LGGs) and grades III-IV are high-grade gliomas (HGGs). LGG has a relatively good prognosis, but it may also progress to high-grade glioma with higher malignancy and aggressiveness. In recent years, advances in molecular genetics and biomarkers have brought new hope and direction for the precise diagnosis, individualized treatment and prognostic judgment of LGG [3, 4]. Finding reliable and effective biomarkers is of great significance to the basic research and clinical treatment of LGG.

The mitochondrial citrate transporter SLC25A1, also known as CIC or CTP, belongs to a family of proteins embedded in the mitochondrial membrane. It promotes the outflow of tricarboxylic acid citric acid to the cytoplasm in exchange for dicarboxylic acid cytosolic malic acid[5–7]. Our previous work has shown that CIC is highly expressed in several tumor types, and its gene or chemical suppression has anti-tumor activity [8]. But there is no report about LGG and SLC25A1.

In this study, the LGG-related sequencing data and clinical information in the American Cancer Genome Atlas (TCGA) database were used for bioinformatics analysis, the R (v.3.6.3) software and Tumor Immune Estimation Resource (GEPIA) tools were used to analyze the expression differences of SLC25A1 in different tumor tissues and normal tissues, as well as the correlation between SLC25A1 and LGG of different WHO grades and its influence on the prognosis of patients.

In order to understand tumor immune cell infiltration, this study further used the Tumor Immune Estimation Resource (TIMER) database to analyze the correlation between SLC25A1 and tumor infiltrating immune cells in LGG and the impact of tumor infiltrating cells on the prognosis of patients. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed on the gene groups related to the expression of SLC25A1 in LGG to determine the potential pathway of SLC25A1 in the occurrence, development and immune cell infiltration of LGG.

2. Results

2.1 General situation of tissue samples

A total of 529 LGG tissue samples downloaded from TCGA database were included in this study. All of them contained gene expression profile sequencing data and clinical information, including age, sex, grade, overall survival, staging, distant metastasis, survival status and other information (Table 1). Among them, there were 224 cases of WHO grade I LGG (48%) and 243cases of WHO grade II LGG (52%). The median age of the patients was 40.5 years old (32,53).

Table 1
LGG patient characteristics based on TCGA

Characteristic	levels	Overall
n		528
WHO grade, n (%)	G2	224 (48%)
	G3	243 (52%)
IDH status, n (%)	WT	97 (18.5%)
	Mut	428 (81.5%)
1p/19q codeletion, n (%)	codeletion	171 (32.4%)
	non- codeletion	357 (67.6%)
Primary therapy outcome, n (%)	PD	110 (24%)
	SD	146 (31.9%)
	PR	64 (14%)
	CR	138 (30.1%)
Gender, n (%)	Female	239 (45.3%)
	Male	289 (54.7%)
Race, n (%)	Asian	8 (1.5%)
	Black or African American	22 (4.3%)
	White	487 (94.2%)
Age, n (%)	<=40	264 (50%)
	> 40	264 (50%)
Histological type, n (%)	Astrocytoma	195 (36.9%)
	Oligoastrocytoma	134 (25.4%)
	Oligodendroglioma	199 (37.7%)
Laterality, n (%)	Left	256 (48.9%)
	Midline	6 (1.1%)
	Right	261 (49.9%)
OS event, n (%)	Alive	392 (74.2%)
	Dead	136 (25.8%)
DSS event, n (%)	Alive	397 (76.3%)

PD (progressive disease), SD (stable disease), PR (partial response), CR (complete response)

Characteristic	levels	Overall
	Dead	123 (23.7%)
PFI event, n (%)	Alive	318 (60.2%)
	Dead	210 (39.8%)
Age, median (IQR)		40.5 (32, 53)
PD (progressive disease), SD (stable disease), PR (partial response), CR (complete response)		

2.2 Expression level of SLC25A1 gene in tumor and normal tissues

The results of Oncomine database analysis showed that SLC25A1 gene was highly expressed in brain and central nervous system tumor data sets ($P < 0.001$, multiple of difference > 1.5). SLC25A1 was highly expressed in Bladder Urothelial carcinoma, Colon adenocarcinoma, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma, Esophageal carcinoma, Glioblastoma multiforme, Head and Neck squamous cell carcinoma and other data sets ($P < 0.001$, Fig. 1).

In order to further evaluate the expression of SLC25A1 gene in LGG tissues, we analyzed the transcriptional level of SLC25A1 based on TCGA database. We found that the expression of SLC25A1 gene in LGG tissues was higher than that in normal tissues ($p < 0.001$, Fig. 2). In addition, SLC25A1 mRNA was also expressed differently in different age, sex, WHO grade, primary therapy outcome, IDH and 1p19q status groups. The results showed that the expression level of SLC25A1 in IDH Mut group was higher than that in WT group ($P < 0.001$), and the expression level of SLC25A1 in 1p19q non-codeletion group was lower than that in codeletion group ($P = 0.031$). In the primary therapy outcome group, SLC25A1 expression was higher in the PR group than in the PD group ($P < 0.001$), and higher in the CR group than in the PD group ($P = 0.002$). The average level in PR group was higher than that in SD group ($P = 0.028$). The expression of SLC25A1 in Male group was higher than that in Female group ($P = 0.024$). Most patients with high SLC25A1 expression were less than or equal to 40 years old ($P = 0.003$). The expression level of SLC25A1 gene in WHO grade III LGG tissue was higher than that in WHO grade II LGG tissues, and the median difference between the two groups was 0.152(-0.265 - -0.036), with statistical significance ($P = 0.01$, Fig. 3).

2.3 Correlation between clinical characteristics and SLC25A1 gene expression in patients with LGG

In this study, chi-square test was used to analyze the correlation between clinical characteristics and SLC25A1 mRNA expression in patients with LGG (Table 2). According to the expression level of SLC25A1 mRNA, they were divided into high expression group and low expression group. The results showed that the high expression of SLC25A1 mRNA was related to IDH status ($P < 0.001$), Primary therapy outcome (< 0.001), Age ($P = 0.019$), Histological type ($P = 0.015$) and survival status ($P < 0.001$), respectively.

Table 2
Relationship between SLC25A1 mRNA expression and clinical characteristics in LGG.

Characteristic	levels	Low expression of SLC25A1	High expression of SLC25A1	p
n		264	264	
WHO grade, n (%)	G2	111 (23.8%)	113 (24.2%)	0.196
	G3	136 (29.1%)	107 (22.9%)	
IDH status, n (%)	WT	82 (15.6%)	15 (2.9%)	< 0.001
	Mut	179 (34.1%)	249 (47.4%)	
1p/19q codeletion, n (%)	codeletion	75 (14.2%)	96 (18.2%)	0.063
	non- codeletion	189 (35.8%)	168 (31.8%)	
Primary therapy outcome, n (%)	PD	74 (16.2%)	36 (7.9%)	< 0.001
	SD	79 (17.2%)	67 (14.6%)	
	PR	20 (4.4%)	44 (9.6%)	
	CR	57 (12.4%)	81 (17.7%)	
Gender, n (%)	Female	131 (24.8%)	108 (20.5%)	0.054
	Male	133 (25.2%)	156 (29.5%)	
Race, n (%)	Asian	3 (0.6%)	5 (1%)	0.070
	Black or African American	16 (3.1%)	6 (1.2%)	
	White	240 (46.4%)	247 (47.8%)	
Age, n (%)	<=40	118 (22.3%)	146 (27.7%)	0.019
	> 40	146 (27.7%)	118 (22.3%)	
Histological type, n (%)	Astrocytoma	108 (20.5%)	87 (16.5%)	0.015
	Oligoastrocytoma	53 (10%)	81 (15.3%)	
	Oligodendroglioma	103 (19.5%)	96 (18.2%)	
Laterality, n (%)	Left	118 (22.6%)	138 (26.4%)	0.075
	Midline	5 (1%)	1 (0.2%)	
	Right	139 (26.6%)	122 (23.3%)	

PD (progressive disease), SD (stable disease), PR (partial response), CR (complete response)

Characteristic	levels	Low expression of SLC25A1	High expression of SLC25A1	p
OS event, n (%)	Alive	176 (33.3%)	216 (40.9%)	< 0.001
	Dead	88 (16.7%)	48 (9.1%)	
DSS event, n (%)	Alive	178 (34.2%)	219 (42.1%)	< 0.001
	Dead	82 (15.8%)	41 (7.9%)	
PFI event, n (%)	Alive	136 (25.8%)	182 (34.5%)	< 0.001
	Dead	128 (24.2%)	82 (15.5%)	
Age, median (IQR)		42.5 (33, 57)	38 (31, 49)	0.002
PD (progressive disease), SD (stable disease), PR (partial response), CR (complete response)				

2.4 Diagnostic value of SLC25A1 mRNA expression in LGG

In this study, the diagnostic value of SLC25A1 mRNA expression in LGG was evaluated by ROC curve. The results showed that the area under the curve of SLC25A1 was 0.964, which had good diagnostic value (Fig. 4). Since then, we have constructed a nomogram to predict the 1-, 3- and 5-year survival probability of patients by combining the expression level of SLC25A1 and clinical variables (Fig. 5).

2.5 The high expression of SLC25A1 mRNA is an independent risk factor affecting the overall survival of patients with LGG

The expression of SLC25A1 gene in LGG tissue samples with different WHO grades was calculated according to UALCAN. The results of survival analysis showed that the survival prognosis of the group with high expression of SLC25A1 gene (n = 264) was better than that of the group with low expression (n = 263), overall survival (OS) (HR = 0.54, 95%CI = 0.38–0.77, P = 0.001 [Fig. 6A]) Disease Specific Survival (DSS) (HR = 0.49 [95%CI = 0.34–0.72] P < 0.001 [Fig. 6B]) progression-free interval (PFI) (HR = 0.55 [95%CI = 0.42–0.73] P < 0.001 [Fig. 6C]).

As shown in the Table 3, univariate Cox regression analysis showed that SLC25A1 expression level, WHO grade (P < 0.001), 1p/19q codeletion (P < 0.001), primary therapy outcome (P < 0.001), ERBB2 expression (P < 0.004), TP53 (P < 0.012), IDH status (P < 0.001), Age (P < 0.001) and histological type (P < 0.001) were related to the overall prognosis of the patients.

Table 3

Association between clinicopathologic characteristics and LGG patient OS through univariate analysis with Cox regression survival model.

Characteristics	Total(N)	Univariate analysis	
		Hazard ratio (95% CI)	P value
WHO grade (G3 vs. G2)	466	3.059 (2.046–4.573)	< 0.001
1p/19q codeletion (non- codeletion vs. codeletion)	527	2.493 (1.590–3.910)	< 0.001
Primary therapy outcome (PR&CR vs. PD&SD)	457	0.202 (0.113–0.359)	< 0.001
ERBB2 (High vs. Low)	527	1.676 (1.185–2.370)	0.004
TP53 (High vs. Low)	527	1.558 (1.104–2.197)	0.012
IDH status (Mut vs. WT)	524	0.186 (0.130–0.265)	< 0.001
Gender (Male vs. Female)	527	1.124 (0.800–1.580)	0.499
Age (> 40 vs. <=40)	527	2.889 (2.009–4.155)	< 0.001
Laterality (Midline &Right vs. Left)	522	0.776 (0.548–1.099)	0.154
SLC25A1 (High vs. Low)	527	0.540 (0.379–0.768)	< 0.001
Race (Black or African American &White vs. Asian)	516	3334251.560 (0.000-Inf)	0.993
Histological type (Oligoastrocytoma &Oligodendroglioma vs. Astrocytoma)	527	0.606 (0.430–0.853)	0.004

Multivariate Cox regression analysis showed that WHO grade ($P = 0.005$), 1p/19q codeletion ($P = 0.008$), primary therapy outcome ($P < 0.001$), IDH status ($P = 0.001$), Age ($P < 0.001$) and SLC25A1 expression level ($P = 0.042$) were independent prognostic factors in LGG patients (Fig. 7).

2.6 Relationship between tumor immune cell infiltration and SLC25A1 gene expression and its effect on prognosis of patients

Previous analysis showed that tumor infiltrating lymphocytes were independent predictors of sentinel lymph node status and survival in cancer patients[9]. Therefore, we try to find out whether the expression of SLC25A1 is related to the immune infiltration of LGG. Spearman partial correlation analysis showed that the expression level of SLC25A1 gene was related to 24 kinds of immune cells (Fig. 8). Among them, SLC25A1 was positively correlated with the infiltration level of tumor immune CD8⁺ T cells ($r_s = 0.220$, $P < 0.001$), and negatively correlated with Tcm ($r_s = -0.170$, $P < 0.001$), Thelper ($r_s = -0.140$, $P = 0.001$), TFH ($r_s = -0.220$, $P < 0.001$), Th1 ($r_s = -0.280$, $P < 0.001$), Th2 ($r_s = -0.270$, $P < 0.001$), aDC ($r_s = -0.110$, $P = 0.012$), B cells ($r_s = -0.290$, $P < 0.001$), Cytotoxic ($r_s = -0.160$, $P < 0.001$), Eosinophils ($r_s = -0.320$, $P < 0.001$), iDC ($r_s = -0.097$, $P = 0.025$), Macrophages ($r_s = -0.220$, $P < 0.001$), Mast ($r_s = -0.340$, $P < 0.001$), Neutrophils ($r_s = -0.180$, $P < 0.001$), CD56bright cells ($r_s = -0.140$, $P = 0.001$), CD56dim cells ($r_s = -0.093$, $P = 0.033$), NK cells ($r_s = -0.130$, $P = 0.002$), T cells ($r_s = -0.280$, $P < 0.001$) (Fig. 9).

Through the analysis of the relationship between the infiltration level of different immune cells and the survival and prognosis of patients with LGG, it was found that the infiltration level of B cells, Macrophage, Neutrophil would affect the overall prognosis of patients with LGG (Fig. 10).

2.7 Results of KEGG pathway enrichment analysis of gene groups positively related to SLC25A1 gene expression

As the high expression of SLC25A1 gene is a risk factor for the survival and prognosis of patients with LGG, we used UALCAN correlation analysis to obtain a total of 56494 genes related to SLC25A1 gene expression in LGG tissues, and used DAVID database for KEGG pathway enrichment analysis. The results showed that under the condition of $p_{adj} < 0.05$ and q value < 0.2 , there were 1 BP, 13 CC, 37 MF and 1 KEGG (Table 4 and Fig. 11).

Table 4
The Functions of SLC25A1 and Genes Significantly Associated with SLC25A1 Alterations.

ONTOLOGY	ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
BP	GO:0007218	neuropeptide signaling pathway	7/67	104/18670	8.97e-08	8.63e-05	7.67e-05
BP	GO:2001257	regulation of cation channel activity	5/67	178/18670	4.46e-04	0.090	0.080
BP	GO:0048562	embryonic organ morphogenesis	6/67	288/18670	5.80e-04	0.090	0.080
BP	GO:0021761	limbic system development	4/67	109/18670	6.35e-04	0.090	0.080
BP	GO:0034776	response to histamine	2/67	11/18670	6.83e-04	0.090	0.080
CC	GO:0034702	ion channel complex	9/73	301/19717	1.65e-06	1.51e-04	1.23e-04
CC	GO:1902495	transmembrane transporter complex	9/73	324/19717	3.01e-06	1.51e-04	1.23e-04
CC	GO:1990351	transporter complex	9/73	332/19717	3.68e-06	1.51e-04	1.23e-04
CC	GO:0034703	cation channel complex	7/73	220/19717	1.71e-05	5.25e-04	4.27e-04
CC	GO:0008076	voltage-gated potassium channel complex	4/73	87/19717	3.05e-04	0.008	0.006
MF	GO:0022839	ion gated channel activity	10/68	334/17697	5.52e-07	5.42e-05	3.63e-05
MF	GO:0022836	gated channel activity	10/68	343/17697	7.04e-07	5.42e-05	3.63e-05
MF	GO:0005216	ion channel activity	10/68	416/17697	3.98e-06	1.97e-04	1.32e-04
MF	GO:0022838	substrate-specific channel activity	10/68	428/17697	5.12e-06	1.97e-04	1.32e-04
MF	GO:0015267	channel activity	10/68	456/17697	8.93e-06	2.33e-04	1.56e-04
KEGG	hsa04080	Neuroactive ligand-receptor interaction	8/25	341/8076	5.36e-06	2.95e-04	2.59e-04

ONTOLOGY	ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
KEGG	hsa05032	Morphine addiction	3/25	91/8076	0.003	0.073	0.064

2.8 GSEA identifies signaling pathways associated with SLC25A1

GSEA is used to identify signaling pathways that are activated in LGG. The results showed that Neuroactive ligand receptor interaction, cell adhesion molecules cams, core matrisome, CD8⁺ T cell downstream pathway, acetylcholine neurotransmitter release cycle, barrestin pathway were differentially enriched in the negatively correlated with SLC25A1 mRNA expression phenotype (Fig. 12).

3. Discussion

Glioma is the most common primary malignant tumor of the central nervous system (CNS). It originates from glial cells in the brain and has a high degree of malignancy. The World Health Organization (WHO) (2016) classifies gliomas into grades I-IV, of which grade I and II are low-grade gliomas (LGG) [10], accounting for 40%-50% of CNS tumors under 18 years of age. LGG is characterized by slow growth and even growth stagnation [11]. Most of the patients with LGG are treated with comprehensive therapy, including surgery, radiotherapy and chemotherapy. However, the existing treatment methods can only improve the clinical symptoms of patients, cannot be cured, often occur drug resistance and tumor recurrence [12], and more than half of LGG can develop into high-level LGG that is difficult to treat [13].

In recent years, some new treatments, such as immunotherapy, have been adopted, but the prognosis of patients is still not ideal, which may be related to the lack of effective diagnosis and treatment targets. The SLC25A1 we studied in this article may provide a potential molecular target for the diagnosis, treatment and prognosis of LGG. The discovery of the relationship between SLC25A1 and tumor immune cell infiltration may contribute to the development of LGG immunotherapy.

The mitochondrial citrate transporter, SLC25A1 belongs to the family of ion transporters embedded in the mitochondrial membrane, and its defects have been indirectly related to a variety of human diseases[5, 6, 14]. The human SLC25A1 gene is located on the chromosome 22q11.21 [15, 16]. The expression of SLC25A1 is induced by insulin and inflammation, and its activity is lost in type II diabetes. SLC25A1 can also be expressed in a variety of cancer tissues, including ovarian cancer, colon cancer and so on[17]. Previous studies have also shown an increase in SLC25A1 activity in liver tumors [18–21]. In addition, mutations in members of the tricarboxylate transporter family in fruit fly, INDY, which can prolong the lifespan, thus indicating that the citrate transporter pathway also controls longevity[22].

The potential prognostic effects of SLC25A1 in LGG have not been reported. Compared with normal cells, most tumor cells show metabolic changes, which is closely related to the development, progression and invasiveness of cancer[23]. These metabolic changes are largely due to the different utilization of citric acid from normal cells. In fact, it has been suggested that in cancer cells, citric acid is mainly exported from the mitochondria through CIC, and then cleaved in the cytoplasm by citrate lyase (CLY) to support lipid, acetyl-CoA

and macromolecular biosynthesis[24–26]. This shift in citric acid metabolism from mitochondria to cytoplasm is thought to explain the acquisition of lipogenic phenotypes, as well as the high percentage of anaerobic glycolysis (Warburg effect), which represents the characteristics of many cancer cells[27]. Therefore, we studied the potential role of SLC25A1 in LGG and analyzed the expression of SLC25A1 in a large number of human glioma tumor specimens for the first time. Under the background of clinical and RNA sequence data, 529 patients with LGG were retrospectively analyzed and histologically confirmed. Through the study, we observed that the expression of SLC25A1 in normal LGG tissues was different from that in tumor tissues, and the expression of SLC25A1 in LGG tissues was higher than that in normal tissues. In addition, the expression level of SLC25A1 mRNA was related to tumor grade. The expression level in LGG tissues of WHO III was higher than that in LGG tissues of WHO II. The results of survival analysis showed that the expression level of SLC25A1 gene was related to the prognosis of LGG, and the prognosis of patients with high expression might be better. Multivariate Cox regression analysis showed that the level of SLC25A1 gene expression was an independent risk factor for the prognosis of patients with LGG. At the same time, the results of ROC curve suggest that SLC25A1 has a good diagnostic value.

The prognosis of LGG is affected by age, location, degree of nerve injury, resection extent, gene phenotype and so on. The prognosis and progression of LGG in children and adults are different, and adult LGG is more likely to develop into malignant LGG [28]. Because of the heterogeneity of LGG, the tumor usually shows different molecular characteristics, so the prognosis is different [28]. In recent years, the research on prognostic biomarkers of LGG has been gradually deepened and enriched. According to the status of IDH mutation and 1p/19q deletion, WHO (2016) can be divided into three categories: IDH wild type, IDH mutation and 1p/19q intact, IDH mutation and 1p/19q co-deletion. Among them, the prognosis of IDH wild type is the worst[29], while the expression of IDH mutation in LGG patients indicates a good prognosis [30]. In an article published by Professor Jiang et al. [31], 77% of WHO III gliomas had IDH1 mutations, with a mutation rate of 55% in WHO III and only 6% in WHO IV. And Professor Yan et al. [32] believe that IDH1 mutation is positively related to the prognosis of tumor treatment. Previous experiments have once again confirmed the significance of IDH1 mutation, that is, detection of IDH1 mutation can be used as an independent index to evaluate the prognosis of glioma patients. IDH1 mutation is more common in low-grade glioma patients, and the progression-free survival time of this type of patients is also relatively long[33]. An article published by Professor Brat et al. [34] pointed out that loss of heterozygosity of 1p/19q is common in oligodendrogliomas, and it is often accompanied by IDH mutation. The view of this study is that the molecular pathological typing based on IDH1 gene mutation combined with 1p/19q co-deletion can more accurately judge the recovery and survival of patients than traditional histopathological typing. The results of this study showed that the expression level of SLC25A1 in IDH mutation group and chromosome 1p19q co-deletion group was significantly higher than that in wild type group ($P < 0.001$) and non-deletion group ($P < 0.001$). The progress in the study of molecular markers will also promote the development of new technologies for brain tumors and further reduce the pain of patients while accurate diagnosis and treatment. The immunotherapy of LGG needs to be further studied.

Some studies have shown that tumor infiltrating immune cells are independent predictors of sentinel lymph node status and prognosis of tumor patients [9]. The genomic variation of tumor cells may produce tumor antigens, which are systematically recognized as non-self-components, thus triggering cellular immune response[35]. In recent years, it is considered that T cells are related to the positive prognosis of patients [36]. The role of B cells in solid tumors show that the expression of CD20 is related to the prolongation of overall

survival in patients with breast and ovarian cancer [37]. Another previous study showed that there was a high correlation between T and B cell gene expression in all tumor types that had been evaluated [38, 39]. The results of a previous study showed that there was a high correlation between T cell and B cell gene expression in all assessed tumor types. The expression of immune signals has a strong correlation between different types of immune cells, showing a diversified, but quite predictable and consistent tumor immune infiltration, and B cells support anti-tumor immune response[40, 41].

LGG has significant heterogeneity in genetics and immune level, and finding a suitable target is still a key factor affecting LGG immunotherapy [42]. In this study, we analyzed the correlation between SLC25A1 gene expression and LGG immune cell infiltration and the effect of immune cell infiltration on the prognosis of patients. Our results showed that different immune markers and immune infiltration levels were related to the expression of SLC25A1 in LGG. Among them, SLC25A1 was positively correlated with tumor immune T cells and pDC infiltration, and negatively correlated with Tcm, Thelper, TFH, Th1, Th2, aDC, B cells, Cytotoxic, Eosinophils, iDC, Macrophages, Mast, Neutrophils, CD56bright cells, CD56dim cells, NK cells, T cells. In addition, the infiltration levels of B cells, Macrophage and Neutrophil all affect the overall prognosis of LGG patients. Therefore, we think that SLC25A1 may have a potential effect on tumor immunity. In addition, it can also be used as a promising biomarker of cancer.

In this study, KEGG pathway enrichment analysis was carried out on the genes with significant correlation with SLC25A1 gene expression in LGG tissues, in order to obtain the pathways that mainly produce biological functions. The results showed that there were gene enrichment in Neuroactive ligand-receptor interaction, Morphine addiction and neuropeptide signaling pathway pathways, which suggested that most of the genes positively related to SLC25A1 gene expression might regulate the immune cell infiltration of LGG and affect the prognosis of patients through these pathways.

Finally, based on GSEA, we further studied the function of SLC25A1 and the possible mechanism of SLC25A1 affecting the progress and metastasis of LGG. GSEA has wide applicability and is one of the most used methods in path enrichment analysis. Compared with traditional pathway enrichment analysis such as Gene Ontology (GO) and Kyoto Encyclopedia of Gene and Genome (KEGG), GSEA can detect changes in the expression of gene sets rather than individual genes, and can detect subtle enrichment signals, which makes the results more reliable and flexible [43]. With the development of GSEA, we found that Neuroactive ligand receptor interaction, cell adhesion molecules cams, core matrisome, CD8 tcr downstream pathway, acetylcholine neurotransmitter release cycle, barrestin pathway were differentially enriched in the negatively correlated with SLC25A1 mRNA expression phenotype

Although our current study improves our understanding of the role of SLC25A1 in LGG, some limitations remain. First, the sample size of cancer patients in the TCGA database was significantly higher than that of the control group. Second, the lack of specific details of patient medication and/or surgical treatment in public databases can also affect the evaluation of patient outcomes. Third, the protein level of SLC25A1 in KGG and its direct role in LGG progression and metastasis need to be further verified in vitro. Fourth, due to the limitations of GSEA, there are still few studies on SLC25A1, which may miss other important signaling pathways regulated by SLC25A1. Finally, this study is a retrospective study, and prospective studies should be conducted in the future to make up for the limitations of retrospective studies. Although this study has certain

limitations, it does provide clues for studying the function of SLC25A1 in LGG, and provides targets and potential prognostic markers for the treatment of LGG.

4. Conclusion

SLC25A1 expression is a potential molecular marker for evaluating the prognosis of LGG patients. Signal pathways such as Neuroactive ligand-receptor interaction, neuropeptide signaling pathway regulated by SLC25A1 in LGG tissues may affect tumor immune cell infiltration, which in turn affects tumor occurrence, development and patient prognosis. As an independent prognostic factor of LGG patients, SLC25A1 has the potential to become a molecular target for the treatment of LGG.

5. Materials And Methods

5.1. Data collection and preprocessing

From the TCGA database (<https://portal.gdc.cancer.gov>), we screened and downloaded the clinical information and tissue gene expression profile data of 529 LGG patients, all of which were tumor tissues and no normal tissues. Due to the lack of normal tissue control, the gene expression profile data of 5 normal brain tissues of patients with glioblastoma multiforme were screened and downloaded from the TCGA database for control analysis. Check the integrity of the downloaded data and delete cases that completely lack clinical information or lack key data such as survival status or survival time. Convert the gene code to the standard gene name of the American Gene Nomenclature Committee (HUGO Gene Nomenclature Committee, HGNC). The gene expression and clinical characteristics of TCGA. The relevant data provided by TCGA is public and open, and does not require the approval of the local ethics committee.

5.2 Analysis of the expression level of SLC25A1 gene in tumor tissues and normal tissues

The Oncomine database (<https://www.oncomine.org/resource/login.html>) was used to identify the expression level of SLC25A1 gene in various types of tumor tissues. Screening conditions: $P < 0.05$, multiple of difference > 1.5 , the top 10% of genes are ranked, and the data type is mRNA. To compare and analyze the expression level of SLC25A1 gene in some tumor tissues and normal tissues in the TCGA database.

5.3 Expression analysis of SLC25A1 gene in LGG tissues of different WHO grades and its relationship with patient prognosis

Use the cancer data online analysis and mining website UALCAN (<http://ualcan.path.uab.edu/index.html>) to analyze the LGG data in the TCGA database. Calculate the expression of SLC25A1 gene in LGG tissue samples of different WHO grades. GEPIA (<http://gepia.cancer-pku.cn/index.html>) was used to compare the expression of SLC25A1 gene in normal brain tissues and LGG tissues. According to the median of SLC25A1 gene expression, the cases were divided into high expression group and low expression group, and survival curves were drawn.

5.4 Analysis of clinical characteristics and SLC25A1 gene expression on prognosis of LGG patients

The classification of case data was converted to assign values, and the clinical characteristics and SLC25A1 gene expression level of LGG patients were analyzed by univariate and multivariate Cox regression analysis with R (v.3.6.3) software to analyze the impact on the prognosis of patients.

5.5 Analysis of the relationship between tumor immune cell infiltration and SLC25A1 gene expression and its effect on the prognosis of patients

The markers of 24 immune cells were extracted from the study of Bindea and his colleagues[44]. The infiltration of 24 kinds of immune cells in tumor was analyzed by GSEA method, and the correlation between SLC25A1 and these 24 kinds of immune cells was analyzed by Spearman correlation. The absolute values of binding strength of immune infiltrating cells to SLC25A1 were as follows: 0.00–0.05, very weak; 0.06 – 0.10, weak; 0.11–0.15, moderate; > 0.15, strong. In statistical analysis, $p < 0.05$ was considered to be statistically significant.

5.6 Enrichment analysis of GSEA

GSEA is a method that can be used for analysis and calculation to determine whether a priori defined set of genes has consistent and statistically significant differences between the two biological states[43]. In this study, we used R (v.3.6.3) to analyze the correlation between SLC25A1 mRNA expression and all genes, and then used the cluster Profiler software package in R (v.3.6.3) for GSEA analysis. $|ES| > 1$ ($P < 0.05$) and $FDR < 0.25$ are significantly enriched[43, 45].

5.7 Enrichment Analysis of KEGG Pathway of genes positively correlated with SLC25A1 Gene expression

The gene groups which were positively correlated with SLC25A1 gene expression in LGG tissues were obtained by correlation analysis by UALCAN. The KEGG pathway enrichment analysis was carried out by using DAVID database (<https://david.ncifcrf.gov/home.jsp>). The pathways with the top 10 enrichment ratios and $P < 0.01$ were selected, and the bubble chart was drawn by R (v.3.6.3) software.

5.8 Statistical analysis by R (v.3.6.3)

SPSS26.0 and R (v.3.6.3) software were used for analysis. Measurement data are expressed as median (lower quartile, upper quartile), using nonparametric Mann-Whitney U test; counting data are expressed as examples and percentages. The survival curve was drawn by Kaplan-Meier method, and the survival rate was compared by log-rank test. Univariate and multivariate Cox regression were used to analyze the clinical characteristics and the effect of SLC25A1 gene expression on the prognosis of LGG patients. Spearman partial correlation analysis was used to analyze the relationship between SLC25A1 gene expression and tumor immune infiltrating cells. The test level (α) was 0.05.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors agreed to submit the manuscript to BioMedical Engineering OnLine

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Qian Li and Lu Shu-min conceived the project and wrote the manuscript. Qian Li and He Xin participated in data analysis. Huang Hua and Zhang Jian-guo participated in discussion. Zhang Jianguo-guo reviewed the manuscript.

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Figures

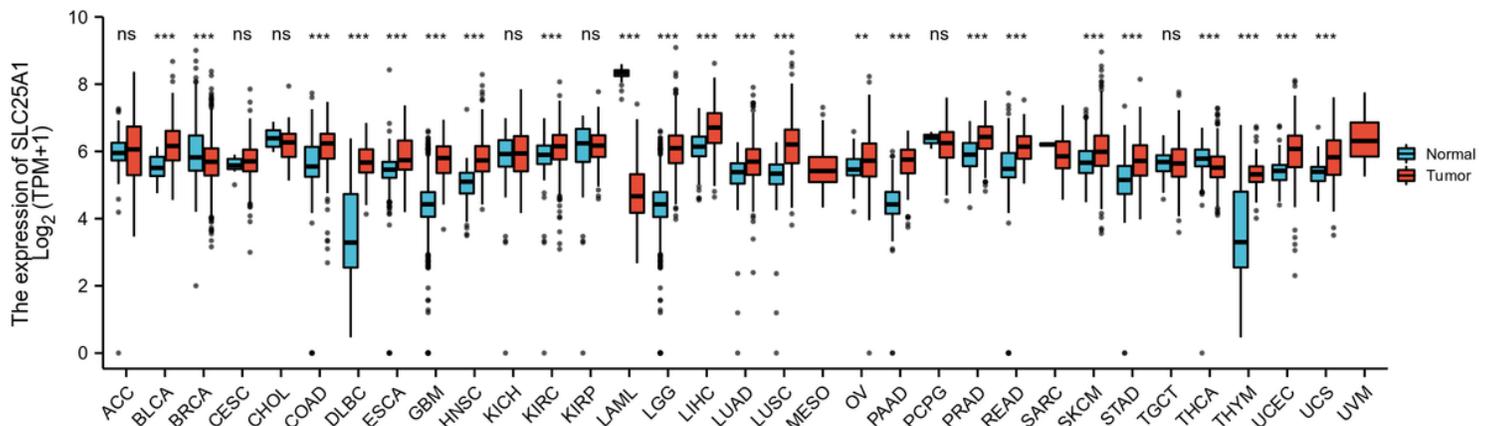


Figure 1

Expression analysis of SLC25A1 in different types of human cancers.

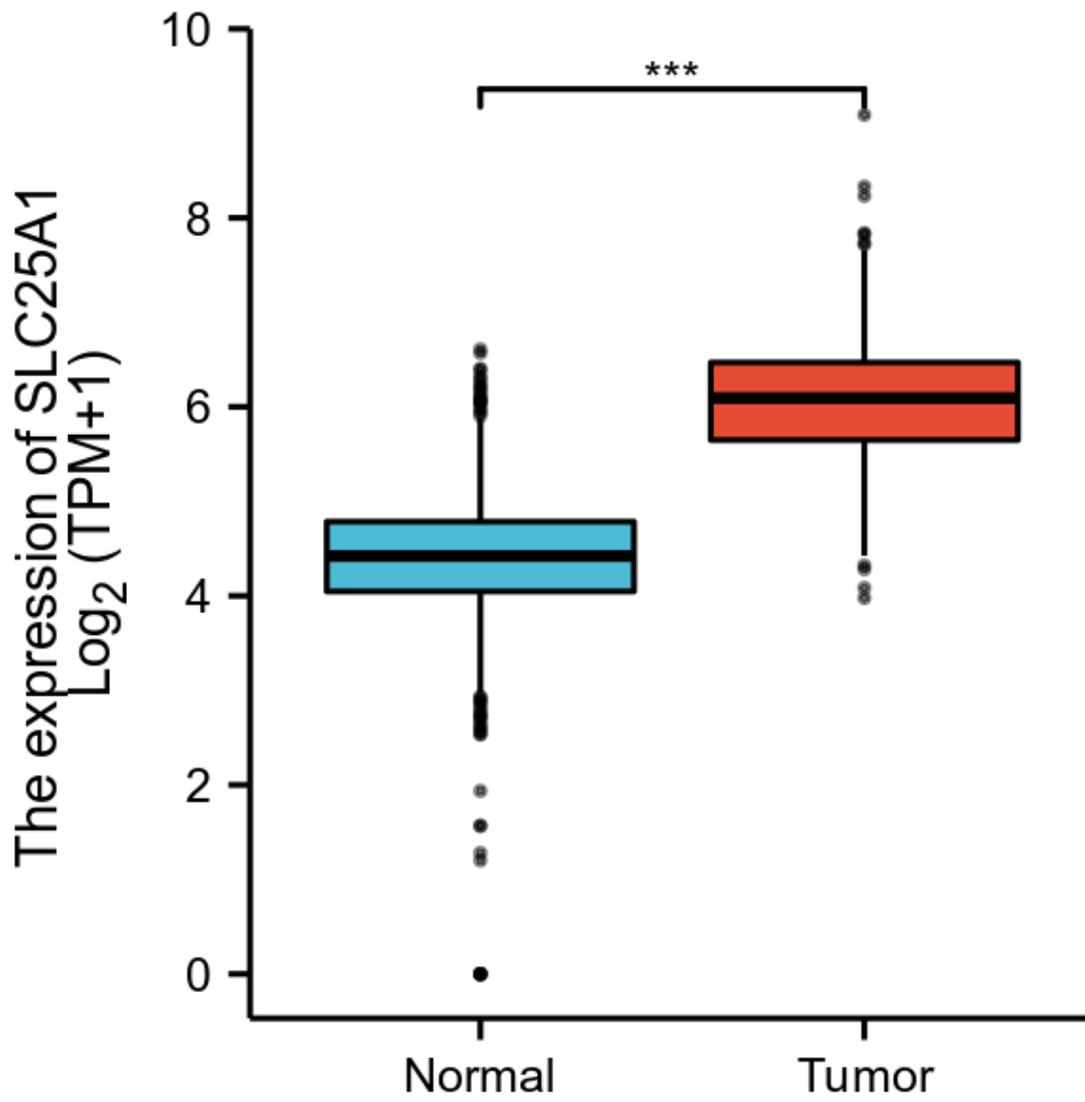


Figure 2

SLC25A1 is elevated in LGG. SLC25A1 showed prominently high expression in HCC samples than in normal samples via Wilcoxon rank sum test.

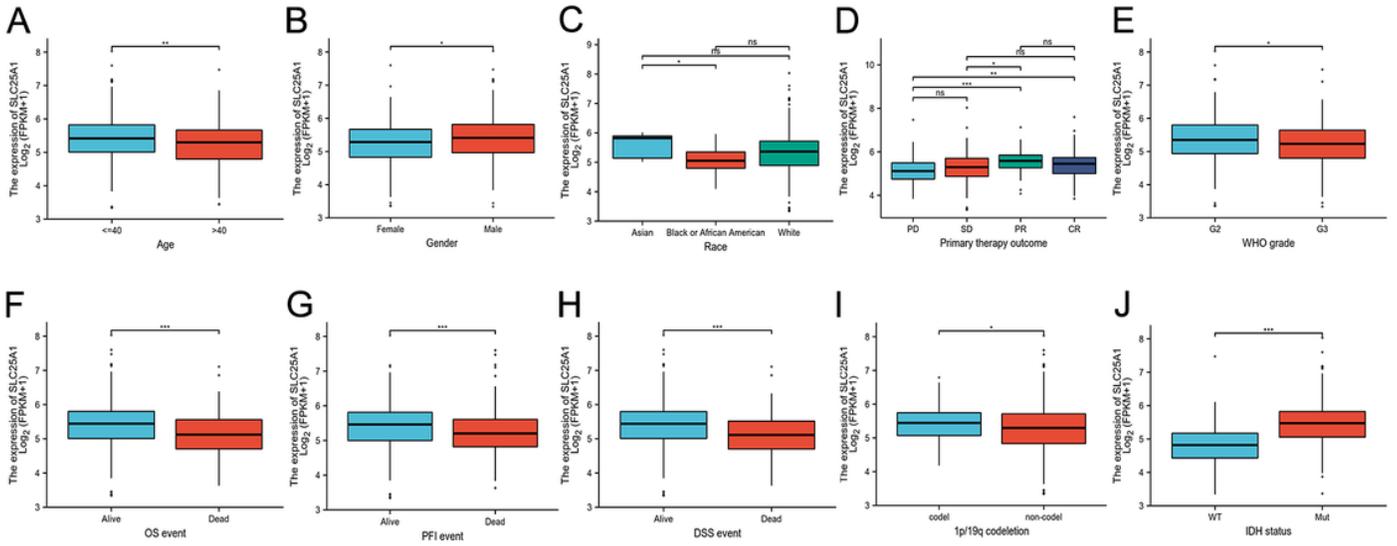


Figure 3

Association between SLC25A1 expression and clinicopathologic characteristics. Fig. 2 (A–J), elevated SLC25A1 was significantly correlated with (A) age, (B) gender, (C) race, (D) primary therapy outcome, (E) WHO grade, (F) OS event (G) PFI event, (H) DSS event, (I) 1p/19q codeletion, (J) IDH status.

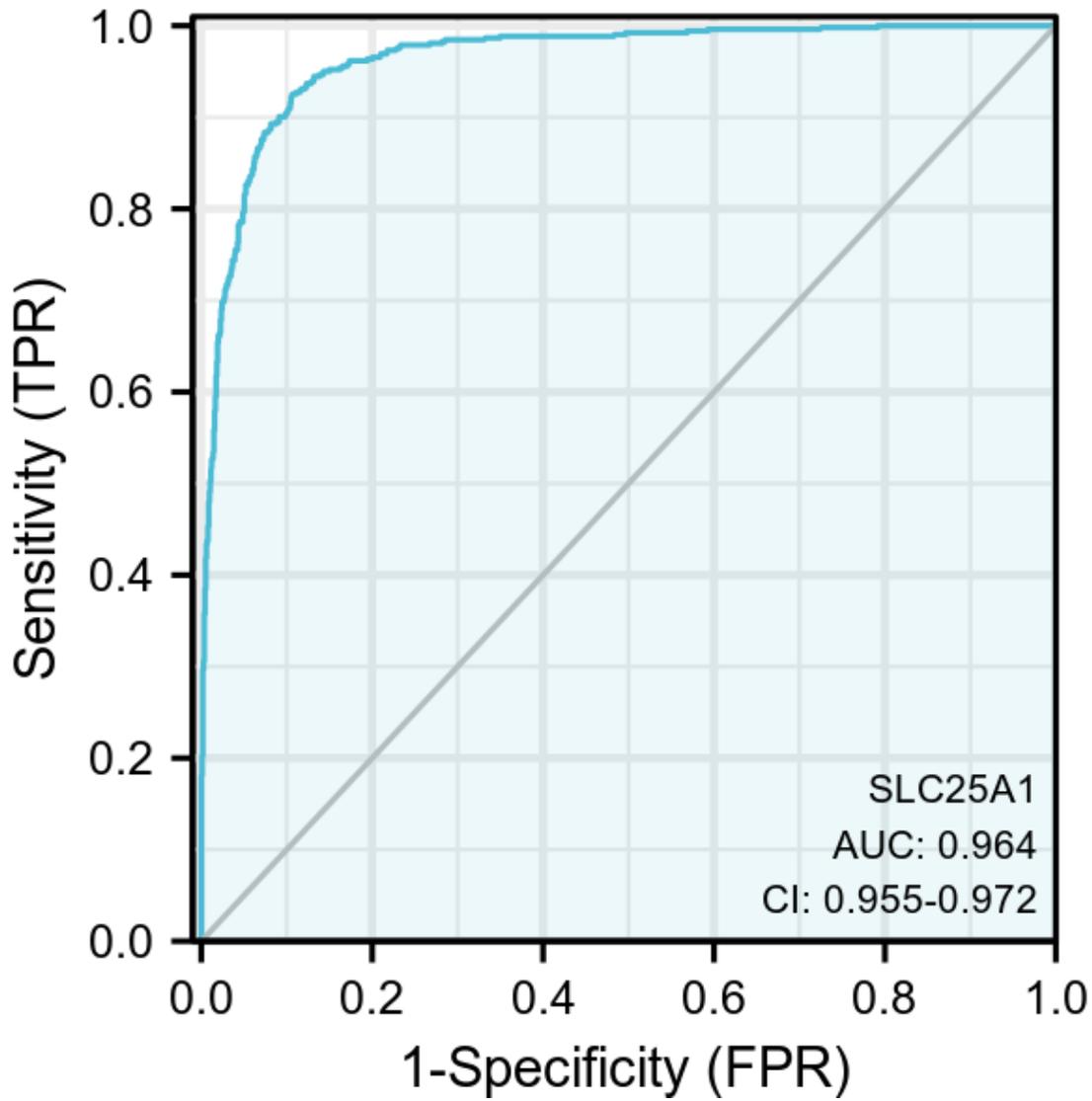


Figure 4

Diagnostic value of SLC25A1 expression in LGG. ROC curve for SLC25A1 in normal tissue and tumor tissue.

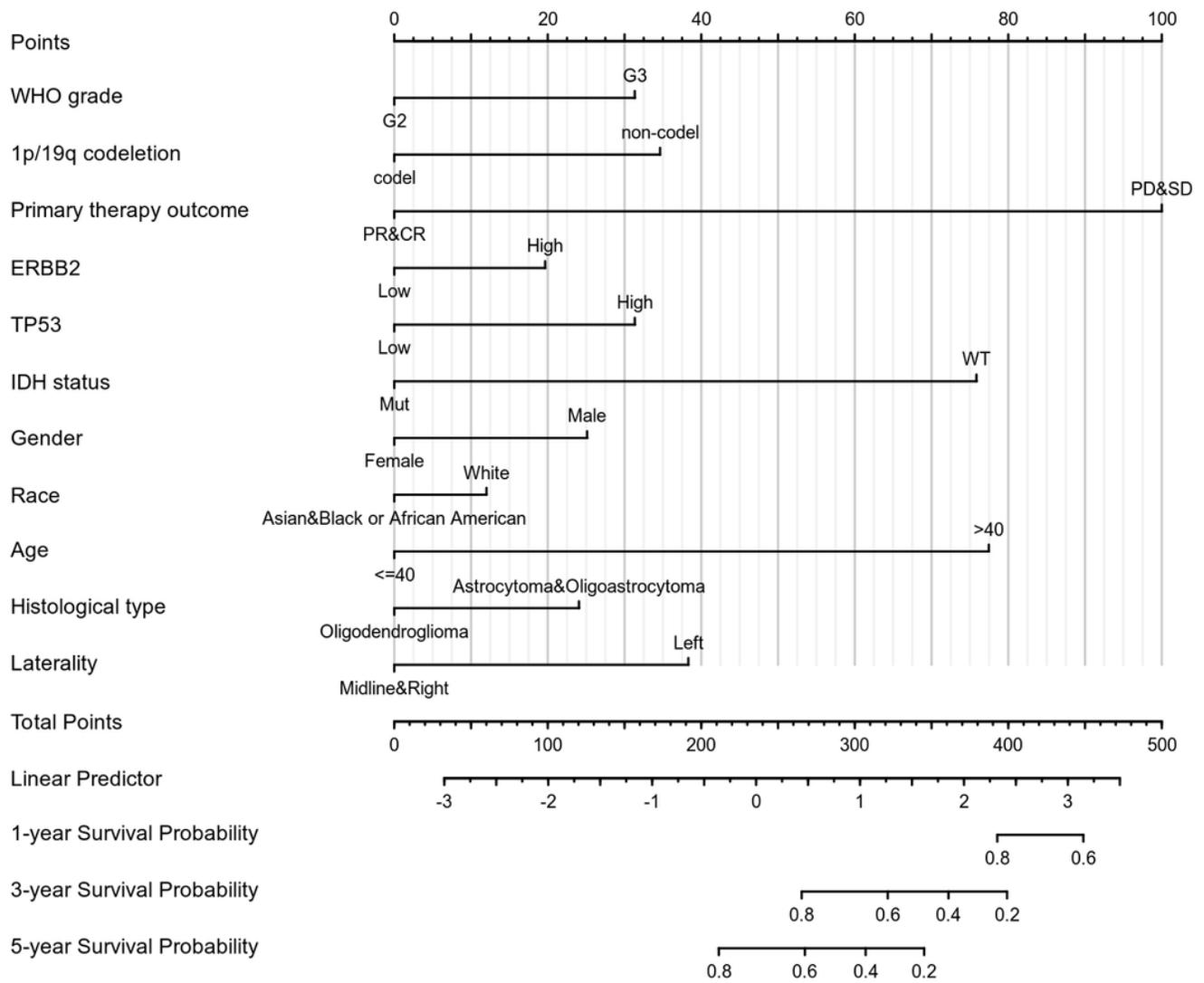


Figure 5

Nomogram for predicting probability of patients with 1-, 3- and 5-year overall survival. For risk estimation, determine the status of each clinical factor and the expression value of SLC25A1, and draw a straight line on the point axis to see the number of points generated by a single factor. Repeat the above steps until the scores of all factors are determined. Sum these points and locate the summation points on the total point axis. Then draw the straight line down to the danger axis to get the survival probability related to 1, 3 and 5 years.

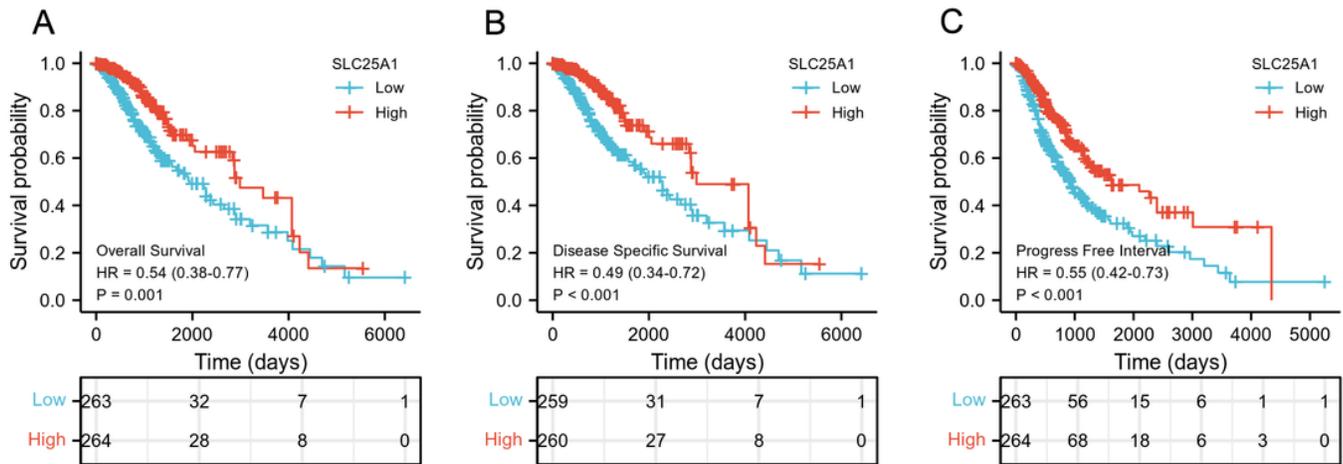


Figure 6

Survival outcomes based on Kaplan-Meier analysis. Kaplan-Meier survival analysis showed that increased SLC25A1 was prominently associated with better (A) over survival, (B) disease-specific survival, (C) progression-free interval.

Characteristics	Total(N)	HR(95% CI) Multivariate analysis	P value Multivariate analysis
WHO grade (G3 vs. G2)	466	2.001 (1.227-3.262)	0.005
1p/19q codeletion (non-codel vs. codel)	527	2.245 (1.238-4.068)	0.008
Primary therapy outcome (PR&CR vs. PD&SD)	457	0.265 (0.140-0.501)	<0.001
ERBB2 (High vs. Low)	527	1.503 (0.964-2.344)	0.072
TP53 (High vs. Low)	527	1.280 (0.814-2.014)	0.285
IDH status (Mut vs. WT)	524	0.447 (0.275-0.728)	0.001
Age (>40 vs. <=40)	527	2.990 (1.873-4.774)	<0.001
SLC25A1 (High vs. Low)	527	0.628 (0.401-0.984)	0.042
Histological type (Oligoastrocytoma&Oligodendroglioma vs. Astrocytoma)	527	1.058 (0.661-1.695)	0.814

Figure 7

Association between clinicopathologic characteristics and survival outcome of LGG patient through multivariate Cox regression analysis. SLC25A1 can independently predict overall survival.

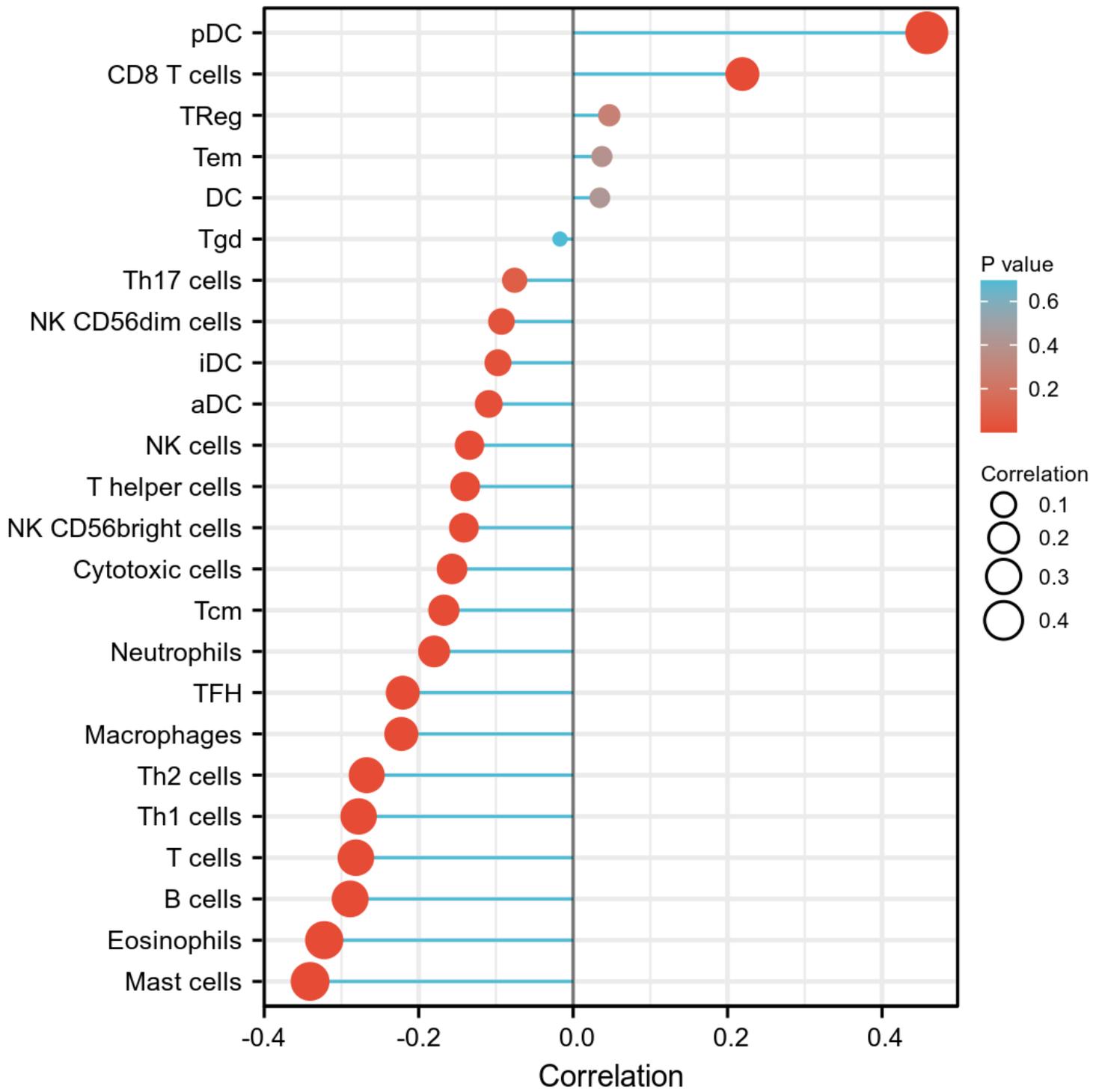


Figure 8

SLC25A1 expression level has significant correlations with immune infiltration level in LGG.

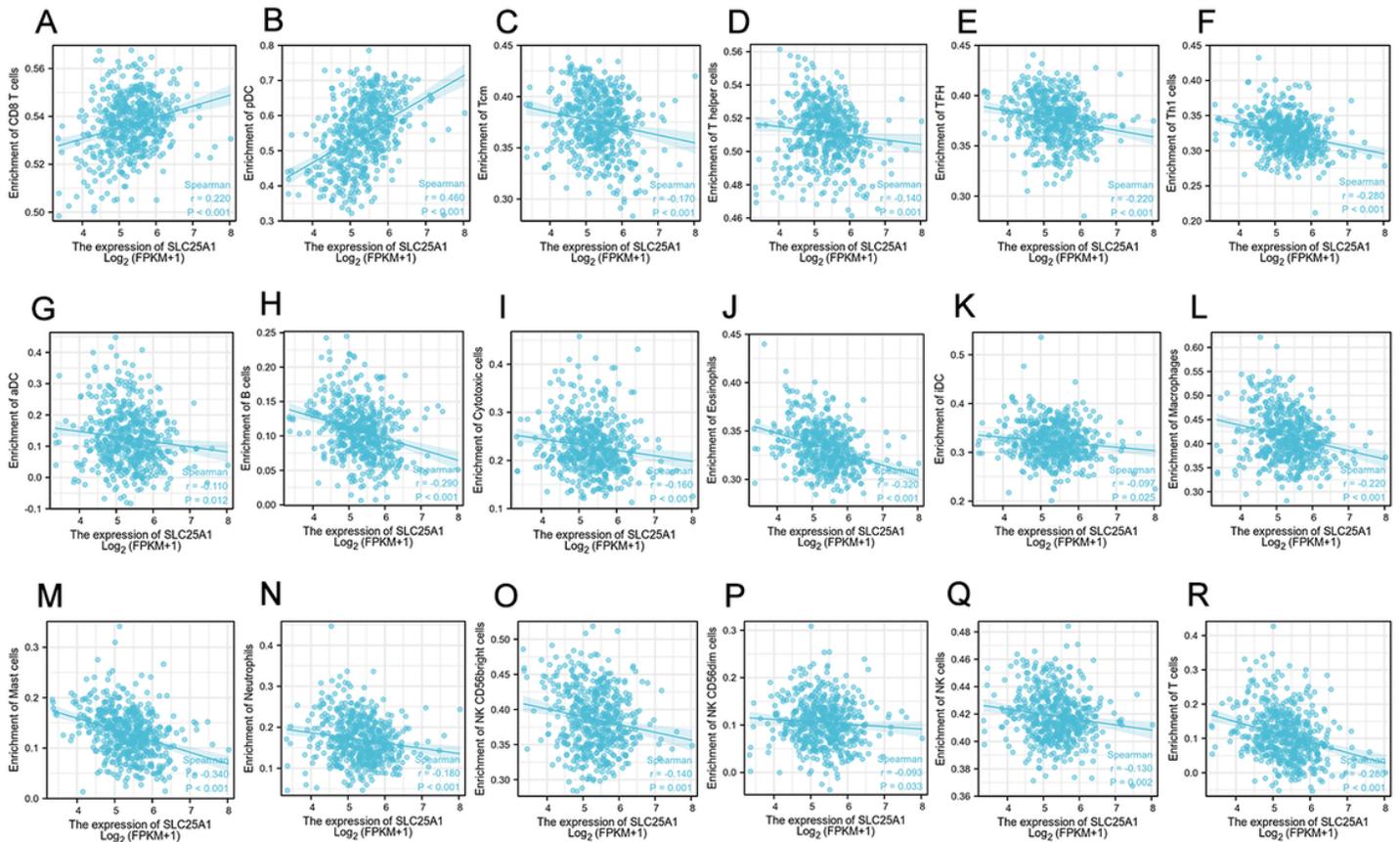


Figure 9

SLC25A1 expression level is correlated with immune infiltration level in LGG. (A-B) positive correlation exists between the SLC25A1 expression level and infiltrating levels of CD8+ T cell, pDC. (C-R) negative correlation between the SLC25A1 expression level and infiltrating levels of Tcm, Thelper, TFH, Th1, Th2, aDC, B cells, Cytotoxic, Eosinophils, iDC, Macrophages, Mast, Neutrophils, CD56bright cells, CD56dim cells, NK cells, T cells.

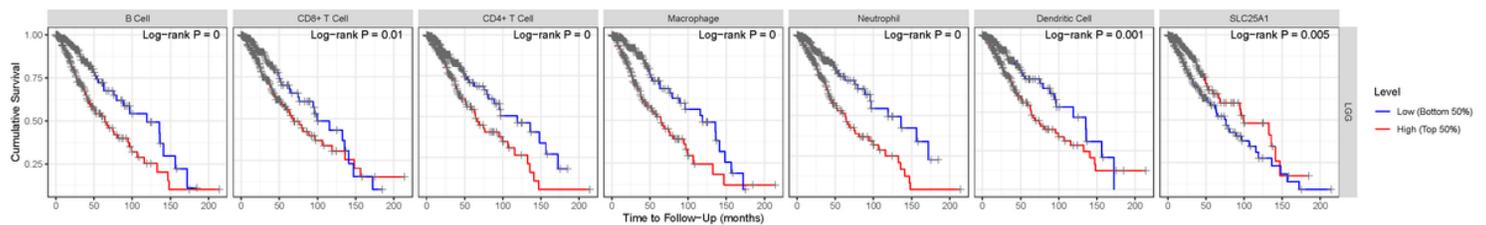


Figure 10

Cumulative survival is related to B cell, CD8+ T cells, CD4+ T cells, Macrophage, Neutrophils and DCs in LGG. (The B cell, T cells, Macrophages, Neutrophils, and DCs are factors related to the cumulative survival rate of LGG over time).

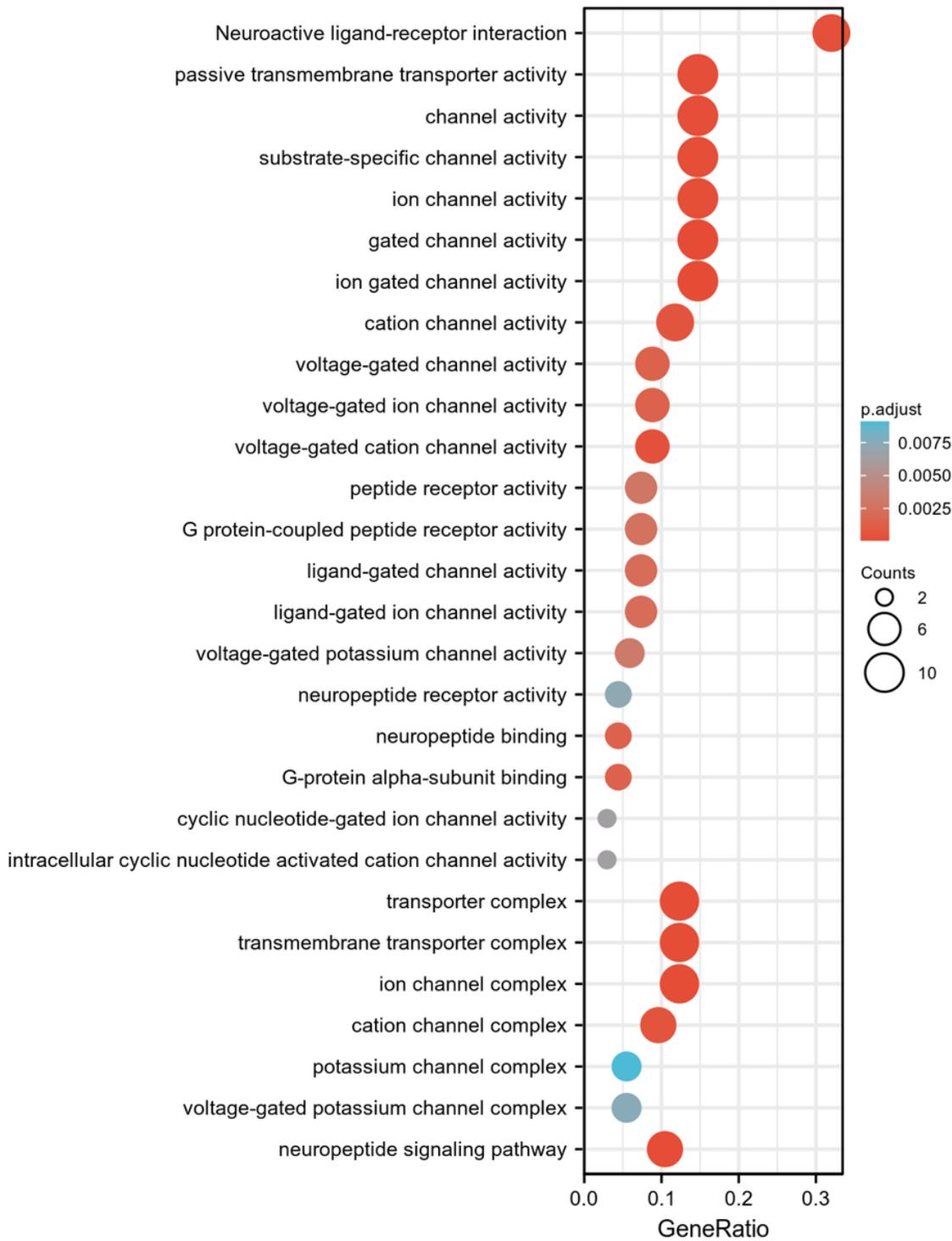


Figure 11

The Functions of SLC25A1 and Genes Significantly Associated with SLC25A1 Alterations. The functions of SLC25A1 and genes significantly associated with SLC25A1 alterations were predicted by the analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) by DAVID tools (<https://david.ncifcrf.gov/summary.jsp>).

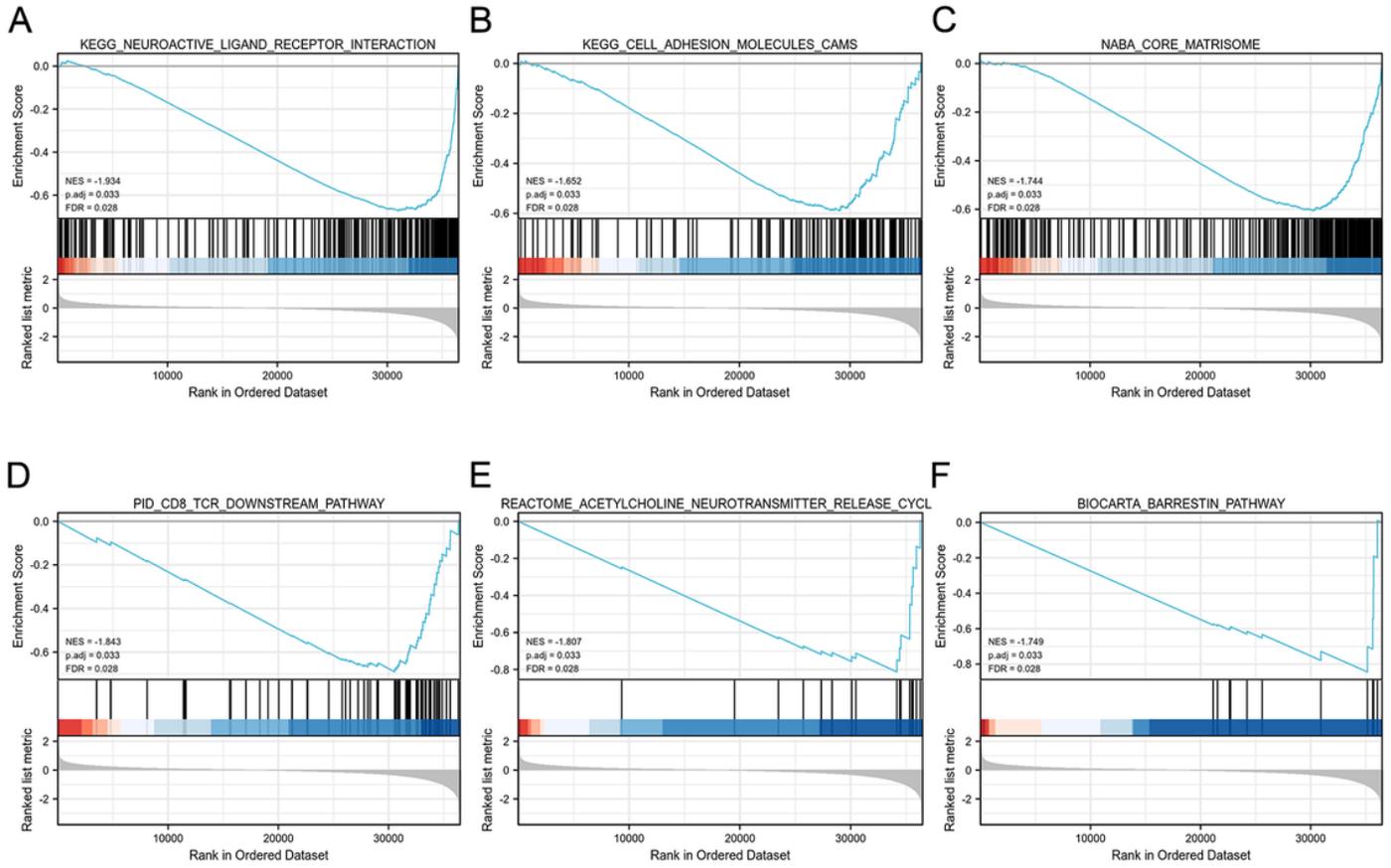


Figure 12

Enrichment plots by GSEA. (A) Neuroactive ligand receptor interaction, (B) cell adhesion molecules cams, (C) core matrisome, (D) CD8 tcr downstream pathway, (E) acetylcholine neurotransmitter release cycle, (F) barrestin pathway.