DLG2–DLG4 Expression in Lower-Grade Glioma is Associated with Improved Survival and an Excitatory Synaptic Transmission and Plasticity Gene Signature

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Simple Summary

Lower-grade gliomas are brain tumors with highly variable outcomes, and better markers are needed to predict how patients will fare. Recent research suggests that these tumors may use mechanisms normally involved in communication between brain cells, but this is not well understood in these cancer types. In this study, we analyzed large patient datasets to examine genes related to synaptic function. We found that higher expression of three genes involved in synaptic membrane organization, *DLG2*, *DLG3*, and *DLG4* was consistently associated with longer patient survival. These genes were also linked to a broader pattern of gene expression suggestive of neural transmission and plasticity. Our findings suggest that some lower-grade gliomas may adopt characteristics of normal brain cells that are associated with less aggressive behavior. This work may help guide future research on prognostic markers and improve understanding of brain tumor biology.

Abstract

Background/Objectives: Increasing evidence indicates that gliomas co-opt mechanisms of excitatory synaptic transmission and plasticity to support tumor progression, yet these processes remain poorly characterized in lower-grade gliomas (LGGs). Here, we investigated whether genes associated with excitatory synaptic function are linked to patient prognosis in LGG. **Methods**: A curated panel of 36 synaptic genes was analyzed in LGG using RNA-sequencing and clinical data from The Cancer Genome Atlas (TCGA) and Chinese Glioma Genome Atlas (CGGA) datasets. Correlations among gene expression levels were analyzed using the Evergene platform. **Results**: Among the genes investigated, *DLG2*, *DLG3*, and *DLG4*, which encode the postsynaptic scaffolding proteins PSD-93, SAP-102, and PSD-95, respectively, showed strong associations with patient overall survival (OS). Higher expression of each gene was consistently associated with longer OS across both datasets. Expression of *DLG2–DLG4* was higher in oligodendroglioma and IDH-mutant, 1p/19q co-deleted tumors, and lower in astrocytoma and IDH-wild-type tumors. Furthermore, expression of all three genes positively correlated with a broad gene signature related to excitatory synaptic transmission and synaptic plasticity, including multiple components of glutamatergic signaling and postsynaptic organization. **Conclusions**: These findings suggest that elevated expression of *DLG2–DLG4* is associated with

a transcriptional program resembling differentiated neuronal-like features and favorable clinical outcome in LGG.

Keywords: *DLG2*; *DLG3*; *DLG4*; MAGUK; synaptic plasticity; low-grade glioma

1. Introduction

Increasing evidence supports the view that tumor progression hijacks fundamental cellular mechanisms underlying neural transmission and synaptic plasticity. In particular, malignant gliomas exploit signaling pathways normally involved in excitatory synaptic communication, establishing functional interactions with surrounding neural circuits. In glioblastoma (GBM), the most aggressive primary brain tumor in adults, tumorigenesis, growth, and invasion are influenced by the formation of functional neuron–glioma synapses mediated by glutamate receptor-initiated signaling. Pioneering studies demonstrated that glioma cells receive direct synaptic input from neurons through α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors, leading to membrane depolarization and activity-dependent tumor proliferation. These neuron-to-glioma synapses enable electrical and synaptic integration of tumor cells into neural circuits, thereby coupling neuronal activity to tumor growth dynamics [1, 2].

In addition to simple synaptic integration, glioma cells appear to recruit mechanisms of activity-dependent plasticity. Glioma synapses recruit canonical pathways of adaptive synaptic potentiation, supporting tumor progression through activity-driven processes [3]. These observations support a model in which glioma cells adopt and repurpose core components of excitatory synaptic transmission and plasticity to sustain tumor growth. This conceptual framework raises the possibility that genes classically associated with postsynaptic organization and excitatory synapse function may define biologically and clinically relevant programs in glioma. Most studies showing parallels between gliomas and normal synapses focus on GBM, whereas synaptic mechanisms in lower grade glioma (LGG) types remain poorly understood.

In the present study, we selected a panel of genes involved in excitatory synaptic transmission based on the available literature. We then analyzed whether these genes are associated with patient prognosis, assessed by survival, using datasets comprising GBM and LGG. Among the genes analyzed, *DLG2*, *DLG3*, and *DLG4* were among the ones showing particularly strong associations with survival specifically in LGG tumors, which were then chosen as the focus of our analyses. Interestingly, higher expression of each of these three *DLG* genes was associated with better, rather than worse, survival. *DLG2*, *DLG3*, and *DLG4* encode PSD-93 (SAP-93), SAP-102, and PSD-95, respectively, which are members of the membrane-associated guanylate kinase (MAGUK) family of synaptic scaffolding proteins. These proteins help organize the postsynaptic density through interactions with other scaffolding proteins and regulate the clustering of glutamate receptors [4]. PSD-93 is a key postsynaptic protein that links *N*-methyl-D-aspartate (NMDA) receptor activity to downstream signaling complexes and can form heterodimers with PSD-95, enhancing synaptic plasticity [5, 6]. PSD-95 also plays a central role in AMPA receptor trafficking during the induction of long-term potentiation (LTP) of excitatory synaptic transmission [7–9]. During early neurodevelopment, initial synapse formation is strongly influenced by SAP-102, whose role is later largely replaced by PSD-95 [4]. Following the initial screening, we then examined whether expression of *DLG2*–*DLG4* positively correlates with that of the other genes in our panel which are associated with synaptic transmission and plasticity.

2. Materials and Methods

2.1. Gene Panel for Excitatory Synaptic Transmission and Plasticity

Based on a curation of the available literature, we assembled a panel of 36 genes with key roles in excitatory synaptic transmission and synaptic plasticity (Supplementary Table S1).

2.2. Datasets and Gene Expression Analyses

RNA-sequencing and corresponding clinical data for patients with LGG were obtained from two independent cohorts, The Cancer Genome Atlas (TCGA-LGG, https://www.cancer.gov/tcga) [10] and the Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn) [11]. For the TCGA cohort, gene-level expected counts were retrieved from the TOIL hub using the UCSCXenaTools package, and clinical annotations were obtained from the TCGA Pan-Cancer Atlas dataset via cBioPortalData. For the CGGA cohort, both RNA-sequencing and corresponding clinical data from two datasets (CGGA-325 and CGGA-693) were downloaded and merged by retaining genes shared across both datasets and merged by retaining common genes. Samples with missing survival data or incomplete clinical annotations were excluded.

TCGA data were back-transformed from expected counts to ensure compatibility with count-based statistical modeling using DESeq2. The raw count matrices from the two CGGA datasets were merged after matching common genes. Duplicate gene symbols were resolved by retaining the entry with the highest mean expression across samples. Variance-stabilizing transformation was applied to normalized count data using DESeq2, and z-score scaling was subsequently performed on a gene-wise basis across all samples within each cohort.

Differences in the expression of *DLG2*, *DLG3*, and *DLG4* were evaluated across TCGA histological types [10, 12, 13], namely astrocytoma (TCGA, n = 188; CGGA, n = 239), oligodendroglioma (TCGA, n = 179; CGGA, n = 145), and oligoastrocytoma (TCGA, n = 123; CGGA, n = 23), and the updated classification incorporating molecular features (molecular subtypes) [14], namely LGG with a mutation in the isocitrate dehydrogenase (IDH) gene and co-deletion of chromosomal arms 1p and 19q (LGG-IDH-mut-codel; TCGA, n = 164; CGGA, n = 113), LGG with a mutation in the IDH gene without co-deletion of chromosomal arms 1p and 19q (LGG-IDH-mut-non-codel; TCGA, n = 235, CGGA, n = 142), and LGG wild-type for IDH (LGG-IDH-wt; TCGA, n = 91; CGGA, n = 88). Pairwise comparisons between groups were conducted using the non-parametric Wilcoxon rank-sum test, with p values being adjusted for multiple testing via the Benjamini-Hochberg method.

2.3. Principal Component Analysis

Principal Component Analysis (PCA) was performed using the Evergene platform (https://bshihlab.shinyapps.io/evergene/) [15] to allow the visualization of *DLG2-DLG4* expression across the different histological types and molecular subtypes of LGG in relation to the first principal component (PC1), which captures the largest proportion of variance in the LGG transcriptomic data. Classification of TCGA LGG tumors within the Evergene platform is: histological types, astrocytoma, oligodendroglioma, oligoastrocytoma, or not available (NA); molecular subtypes, pilocytic astrocytoma (PA)–like, mesenchymal-like, glioma CpG island methylator phenotype (G-CIMP)-high, G-CIMP-low, glioma with 1p/19q co-deletion (codel), classic-like, or not available (NA).

2.4. Survival Analysis

Associations between overall survival (OS) of patients with LGG and expression of analyses of the selected genes were assessed using Kaplan–Meier survival curves. Patients were dichotomized into "high" and "low" expression groups based on an optimal cutpoint determined by using the surv_cutpoint function in the survminer package [12]. Survival distributions were compared using the log-rank test, with p-values adjusted for multiple testing across all genes and cohorts using the Benjamini–Hochberg method.

All analyses were performed in the R statistical environment (version 4.5.1). Variance-stabilizing transformation and batch adjustment were conducted using DESeq2 (version 1.48.1). OS analyses were performed using the survival (version 3.8.3), with optimal expression cutpoints determined using maximally selected rank statistics implemented in survminer (version 0.5.2). Kaplan–Meier curves were generated using survminer. Graphical visualizations were produced using ggplot2 (version 4.0.2). Data acquisition was performed using UCSCXenaTools (version 1.7.0), cBioPortalData (version 2.20.0).

2.5. Gene Expression Correlations

Correlations between expression of each of the three selected *DLG* genes and each of the genes in the synaptic gene panel, or between each *DLG* gene and all genes in the panel pooled together to represent a synaptic signature, were analyzed with the Evergene platform using default settings [15]. Pearson correlation coefficient (r) values equal or higher than 0.5 along with p values of less than 0.05 were considered to indicate moderate to high correlation.

3. Results

3.1. DLG2-DLG4 *Expression Across Different LGG Types*

An early analysis of associations of the genes in the synaptic panel with LGG patient survival revealed *DLG2*, *DLG3*, and *DLG4* among the genes with the most significant association. Their expression was then analyzed in LGG tumors classified into histological types or molecular subtypes. Within histological types, expression of *DLG2* in TCGA tumors was lower in astrocytoma compared to oligodendroglioma and oligoastrocytoma. *DLG3* and *DLG4* were more highly expressed in oligodendroglioma and less expressed in astrocytoma (Figure 1). Within the molecular classification, all three *DLG* genes showed the same pattern of higher expression in LGG-IDH-mut-codel and lower expression in LGG-IDH-wt, with intermediate levels in LGG-IDH-mut-non-codel (Figure 2).

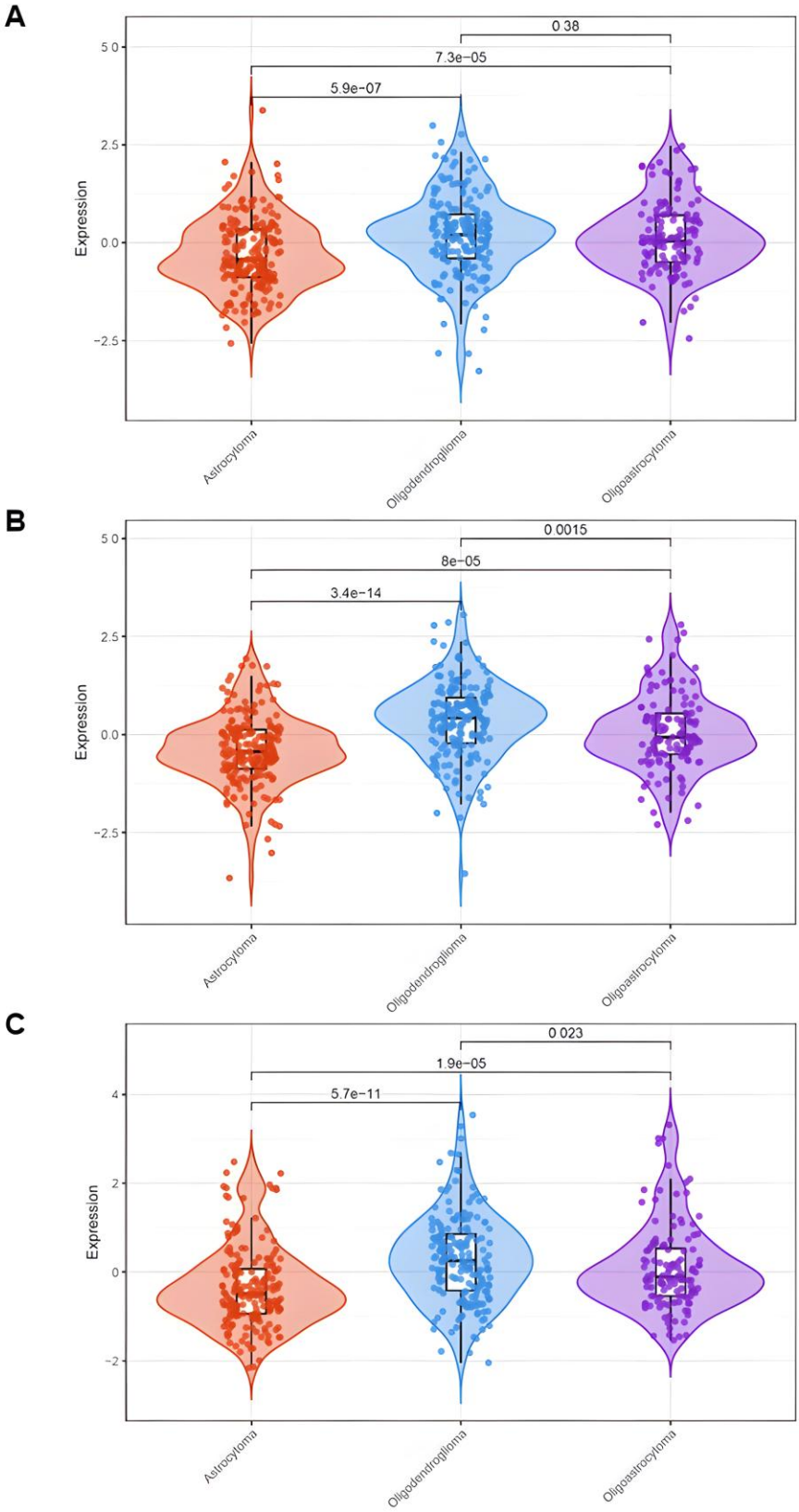


Figure 1. Gene expression levels of (**A**) *DLG2*, (**B**) *DLG3*, and (**C**) *DLG4* in TCGA LGG tumors classified into histological types. Astrocytoma, n = 188; oligodendroglioma, n = 179; oligoastrocytoma, n = 123; p values are indicated in the panels.

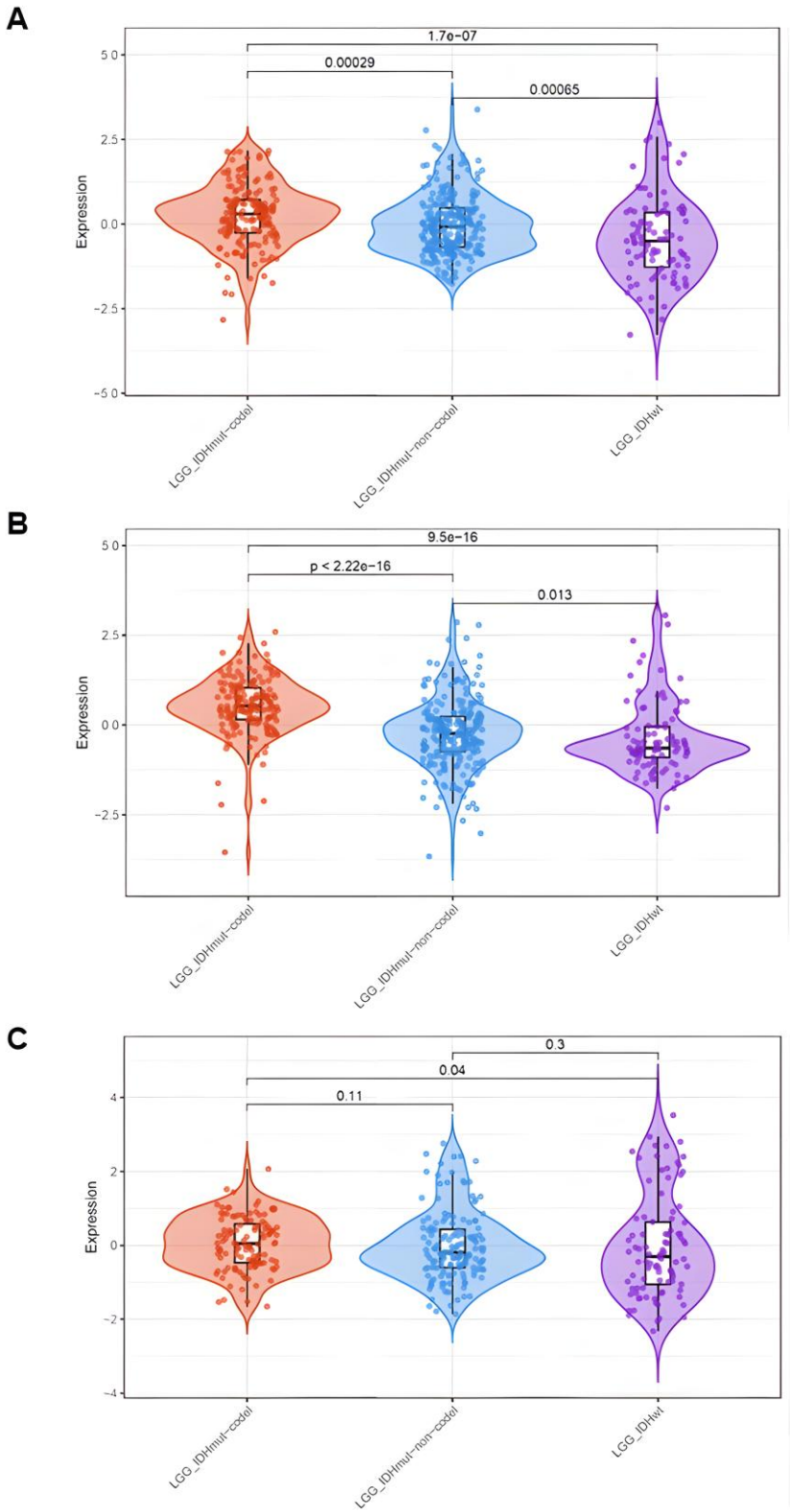
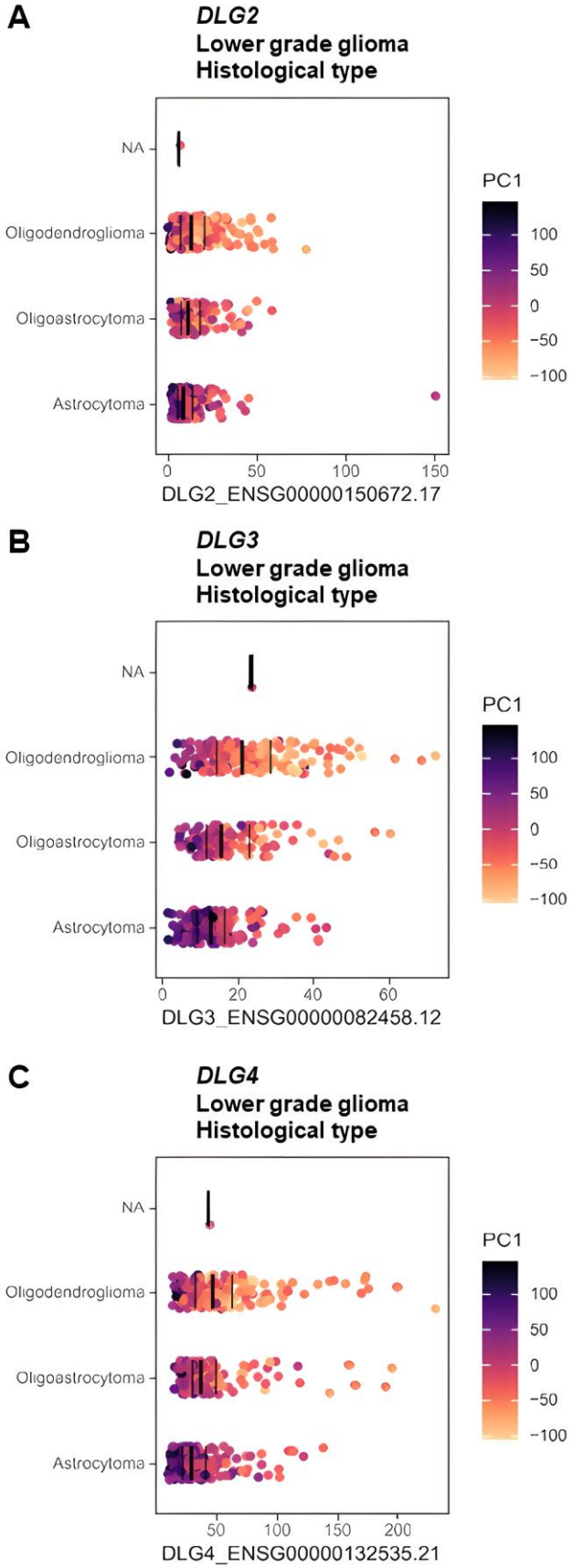


Figure 2. Gene expression levels of (**A**) *DLG2*, (**B**) *DLG3*, and (**C**) *DLG4* in TCGA LGG tumors classified into molecular subtypes. LGG-IDH-mut-codel, n = 164; LGG-IDH-mut-non-codel, n = 235; LGG-IDH-wt, n = 91; p values are indicated in the panels.

Analysis of the CGGA dataset largely validated the overall pattern of lower *DLG2*-*DLG4* expression in astrocytoma compared to the other histological groups, and *DLG3* was more highly expressed in oligoastrocytoma than in oligodendroglioma (Supplementary Figure S1). In CGGA tumors classified according to molecular subtype, *DLG3* and *DLG4* were more expressed in LGG-IDH-mut-codel, but there were no statistically significant differences among tumor types in *DLG2* expression, although there was a trend towards higher expression in LGG-IDH-mut-codel compared to LGG-IDH-mut-non-codel (Supplementary Figure S2). The relationship between *DLG2*–*DLG4* expression and PC1 in TCGA tumors is illustrated by PCA, showing an overall pattern of greater alignment with PC1 in tumors with lower *DLG* expression levels (Figure 3).


A
DLG2
Lower grade glioma
Histological type
NA
Oligodendroglioma
Oligoastrocytoma
Astrocytoma
0
50
100
150
DLG2_ENSG00000150672.17
PC1
100
50
0
−50
−100
B
DLG3
Lower grade glioma
Histological type
NA
Oligodendroglioma
Oligoastrocytoma
Astrocytoma
0
20
40
60
DLG3_ENSG00000082458.12
PC1
100
50
0
−50
−100
C
DLG4
Lower grade glioma
Histological type
NA
Oligodendroglioma
Oligoastrocytoma
Astrocytoma
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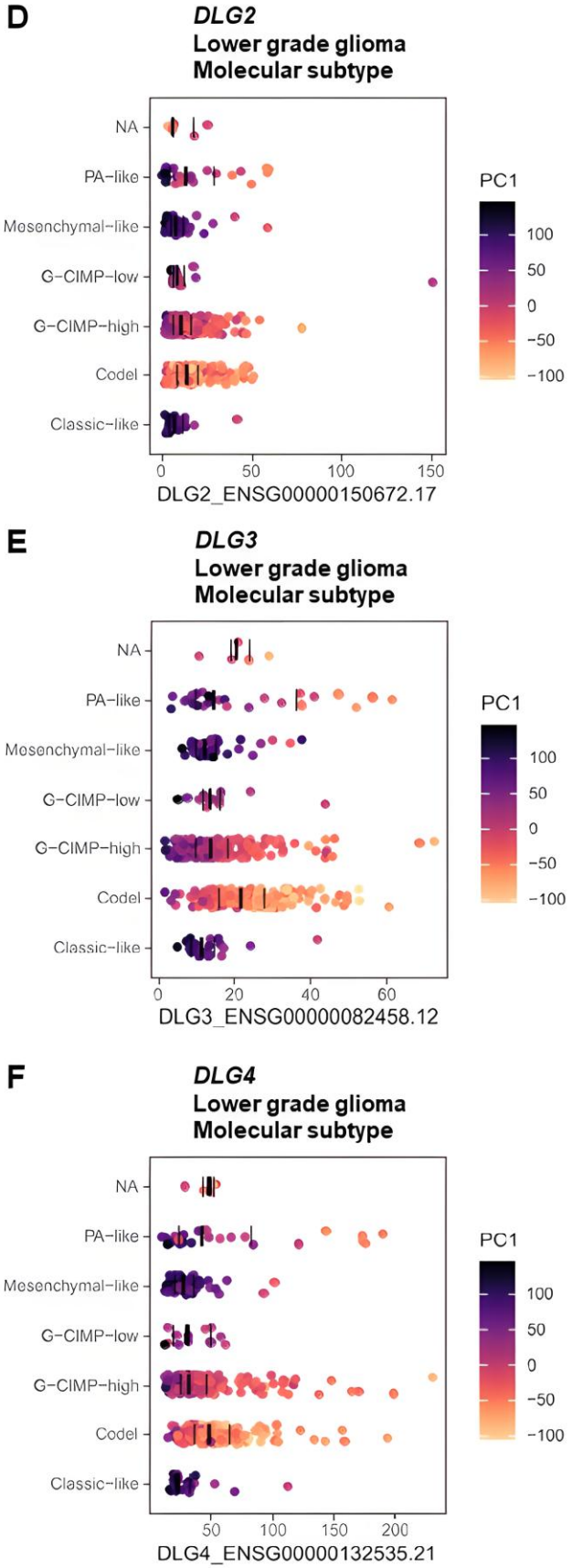
100
150
200
DLG4_ENSG00000132535.21
PC1
100
50
0
−50
−100


D
DLG2
Lower grade glioma
Molecular subtype
NA
PA-like
Mesenchymal-like
G-CIMP-low
G-CIMP-high
Codel
Classic-like
0
50
100
150
DLG2_ENSG00000150672.17
PC1
100
50
0
-50
-100
E
DLG3
Lower grade glioma
Molecular subtype
NA
PA-like
Mesenchymal-like
G-CIMP-low
G-CIMP-high
Codel
Classic-like
0
20
40
60
DLG3_ENSG00000082458.12
PC1
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-50
-100
F
DLG4
Lower grade glioma
Molecular subtype
NA
PA-like
Mesenchymal-like
G-CIMP-low
G-CIMP-high
Codel
Classic-like
50
100
150
200
DLG4_ENSG00000132535.21
PC1
100
50
0
-50
-100

Figure 3. Expression levels of *DLG2*, *DLG3*, and *DLG4* in relation to PC1 in TCGA LGG tumors. (**A**) *DLG2*, histological type; (**B**) *DLG3*, histological type; (**C**) *DLG4*, histological type; (**D**) *DLG2*, molecular subtype; (**E**) *DLG3*, molecular subtype; (**F**) *DLG4*, molecular subtype. For this analysis, molecular subtypes were classified as PA-like, mesenchymal-like, G-CIMP-high, G-CIMP-low, codel, classic-like, or NA [14, 15].

3.2. High DLG2-DLG4 *Expression is Associated with Longer OS in Patients with LGG*

High expression of *DLG2*, *DLG3*, and *DLG4* was significantly associated with longer OS in patients with LGG in both the TCGA (Figure 4) and CGGA (Figure 5) datasets.

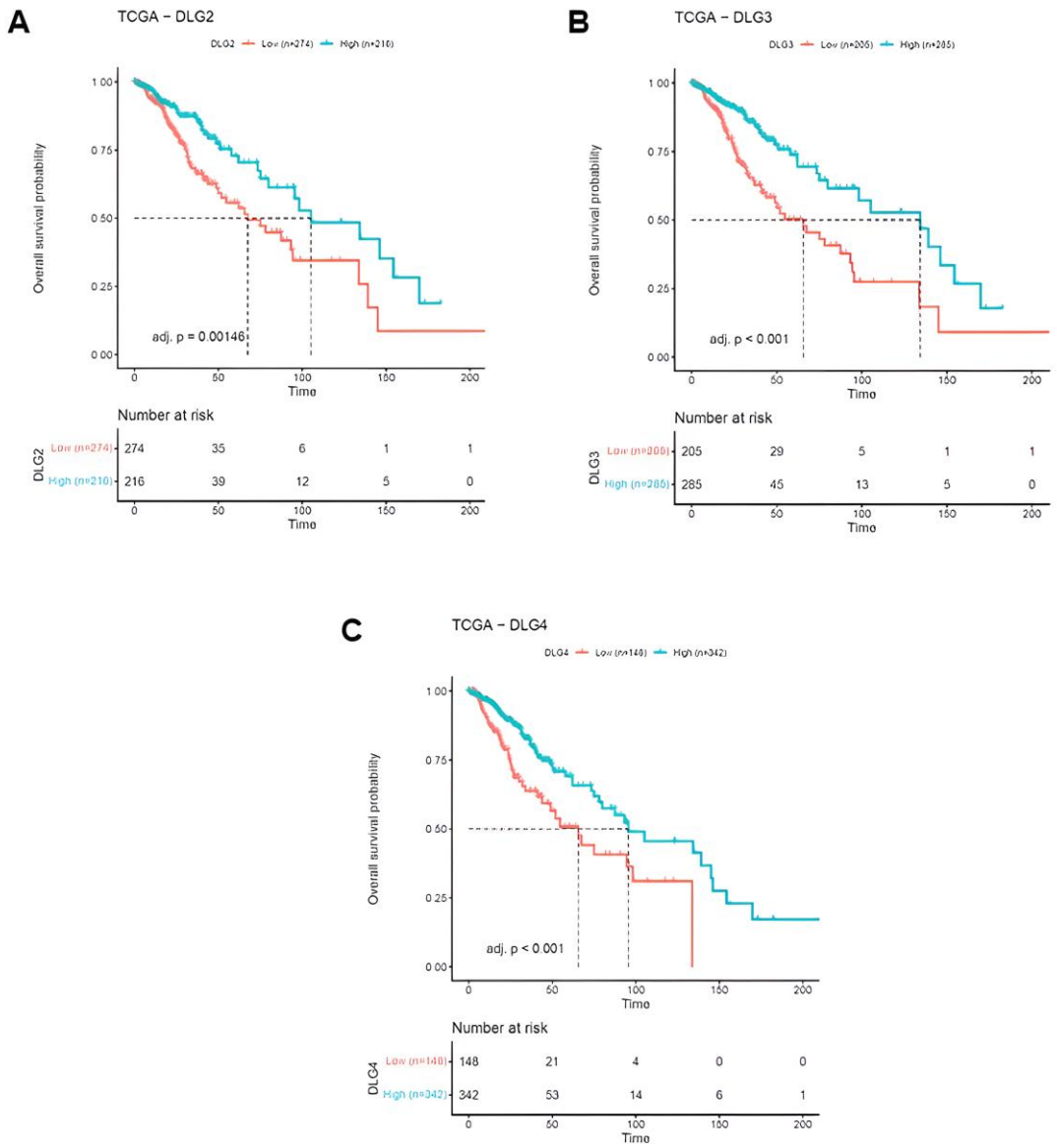

Figure 4. Kaplan-Meier analysis of OS in patients bearing TCGA LGG tumors with higher or lower expression of (**A**) *DLG2*, (**B**) *DLG3*, and (**C**) *DLG4*. The number of samples and adjusted *p* values are indicated in the panels.

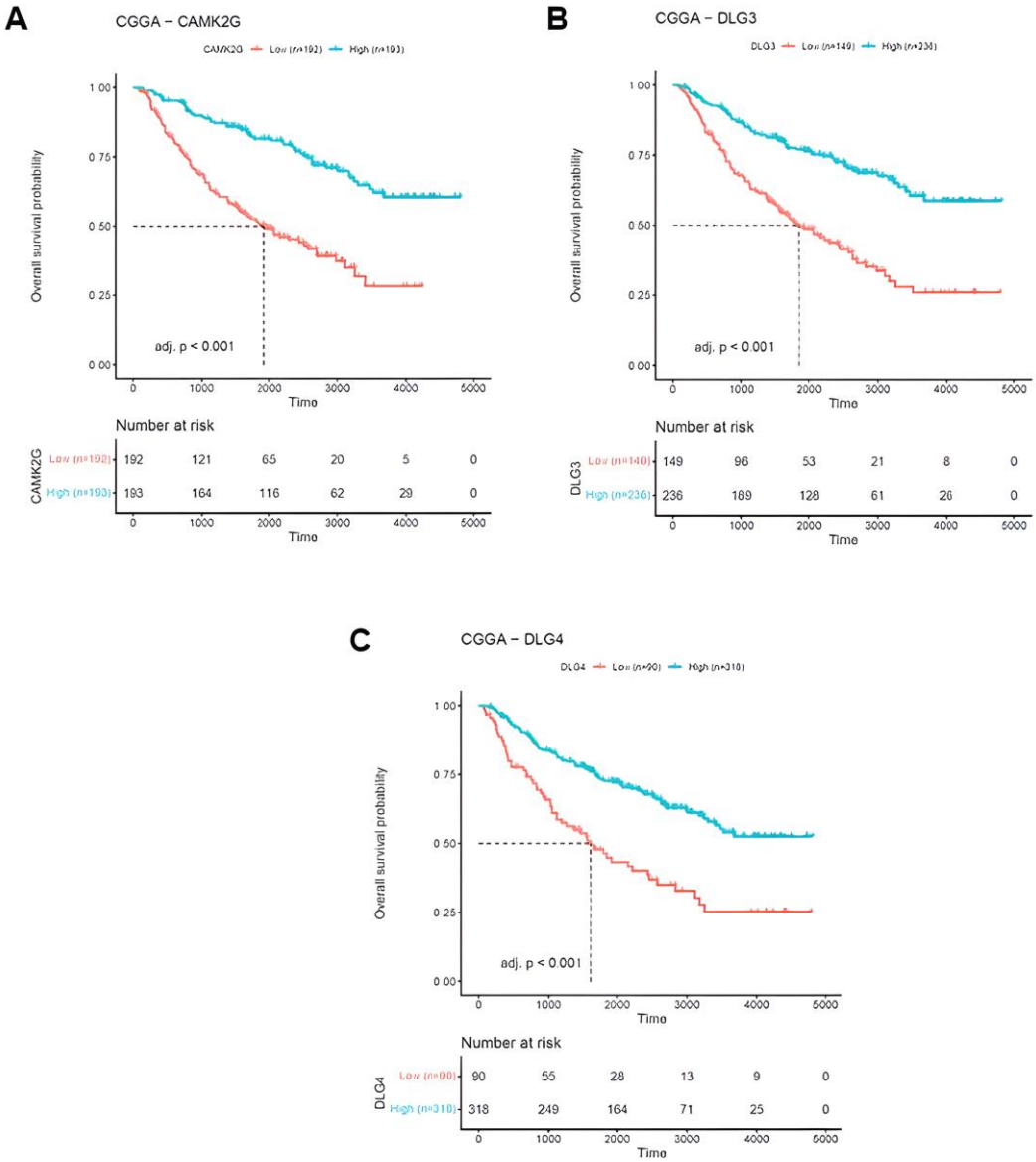

Figure 5. Kaplan-Meier analysis of OS in patients bearing CGGA LGG tumors with higher or lower expression of (**A**) *DLG2*, (**B**) *DLG3*, and (**C**) *DLG4*. The number of samples and adjusted *p* values are indicated in the panels.

3.3. Expression of DLG2-DLG4 *is Positively Correlated with Expression of Genes Associated with Excitatory Synaptic Transmission and Synaptic Plasticity*

Among the genes included in our synaptic panel, moderate to strong positive correlations were found between 21 non-*DLG* genes and *DLG2* and between 24 non-*DLG* genes and either *DLG3* or *DLG4*. Genes showing correlations with all three *DLG* genes were *CAMK2A*, *CAMK2B*, *DLGAP1*, *GRIN1*, *GRIN2B*, *HOMER1*, *NEFL*, *NEFM*, *PPP3CA*, *PRKCG*, *RBFOX3*, *SHANK1*, *SHANK2*, *SHANK3*, *SLC17A6*, *SLC17A7*, *SYNGAP1*, and *TBR1*. There were also positive correlations in expression among the *DLG* genes themselves (Supplementary Figure S3, Table 1). Moreover, each individual *DLG* gene showed a positive correlation with the overall synaptic gene signature derived from the full gene set (Figure 6).

Table 1. Correlations between expression of *DLG2–DLG4* and genes involved in excitatory synaptic transmission and synaptic plasticity.

GENE	*DLG2*	*DLG3*	*DLG4*
ARC	NC	NC	NC
CAMK2A	+	+	+
CAMK2B	+	+	+
DLG2	1	+	+
DLG3	+	1	+
DLG4	+	+	1
DLGAP1	+	+	+
EGR1	NC	NC	NC
FOS	NC	NC	NC
FOSB	NC	NC	NC
GRIA1	NC	NC	NC
GRIA2	NC	+	+
GRIA3	NC	+	NC
GRIA4	NC	NC	NC
GRIN1	+	+	+
GRIN2A	+	NC	+
GRIN2B	+	+	+
HOMER1	+	+	+
HOMER2	NC	NC	NC
MAP2	NC	+	+
NEFL	+	+	+
NEFM	+	+	+
NPAS4	NC	NC	NC
PIK3R5	NC	+	+
PPP3CA	+	+	+
PRKCG	+	+	+
RASGRF1	NC	NC	NC
RBFOX3	+	+	+
SATB2	NC	NC	NC
SHANK1	+	+	+
SHANK2	+	+	+
SHANK3	+	+	+
SLC17A6	+	+	+
SLC17A7	+	+	+
SYNGAP1	+	+	+
TBR1	+	+	+

Positive correlations (+) were defined as Pearson's $r \geq 0.5$ with a statistically significant p value; NC, no correlation defined by $r < 0.5$.

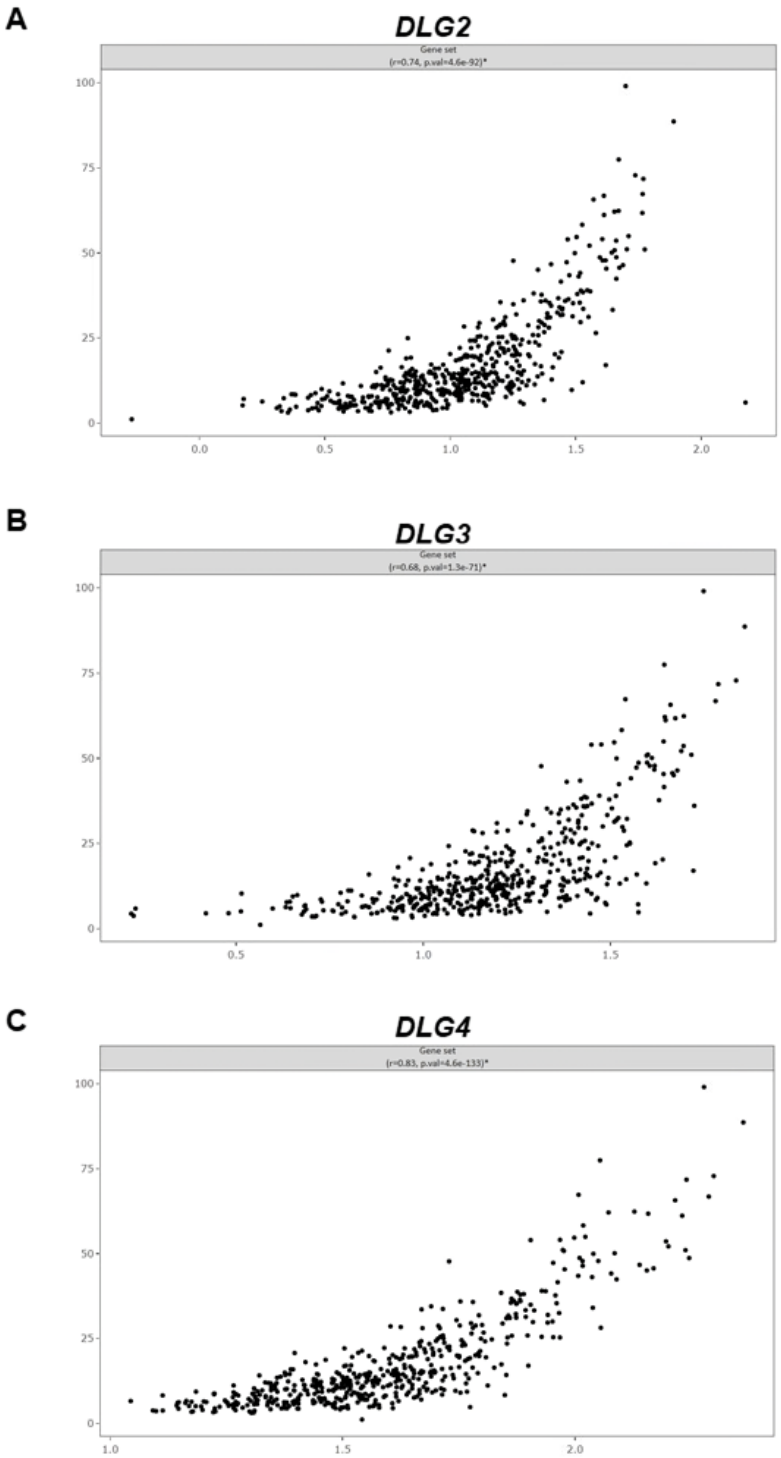

Figure 6. Expression of (**A**) *DLG2*, (**B**) *DLG3*, and (**C**) *DLG4* correlates with a postsynaptic excitatory transmission and synaptic plasticity gene signature, represented by a composite gene set score calculated from all genes in the panel, in TCGA LGG tumors. The horizontal

axis represents *DLG* gene expression and the vertical axis represents the gene set score. Pearson's *r* and *p* values are shown in the panels.

4. Discussion

LGGs, especially IDH-mutant astrocytomas, can eventually progress to aggressive, malignant tumors with GBM-like features [10, 16]. Increasing attention has been given to neural transmission and plasticity pathways co-opted by GBM tumors to stimulate brain growth [1-3]. In contrast, synaptic components in LGG remain to be characterized. We provide early evidence, based on mRNA transcription as a measure of gene expression, suggesting that excitatory neurotransmission and post-synaptic plasticity features may be present in LGG tumors and associate with patient prognosis.

DLG2, *DLG3*, and *DLG4* encode the MAGUK scaffold proteins PSD-93, SAP-102, and PSD-95. These are core proteins of the PSD at excitatory synapses, where they serve as organizational hubs that assemble and stabilize signaling complexes. All three proteins share a modular structure (PDZ, SH3, and GK domains) that allows them to bind multiple partners simultaneously. Through these interactions, they anchor and cluster NMDA and AMPA receptors at the synaptic membrane and link them to intracellular signaling pathways. They also connect receptors to other scaffold proteins such as Shank1-3, helping maintain synapse structure and efficiency. In excitatory synaptic transmission, MAGUK proteins ensure proper receptor localization and density at the postsynaptic membrane, which directly influences synaptic strength and responsiveness to glutamate release. In synaptic plasticity, especially LTP, these proteins play a central role by regulating activity-dependent changes in receptor trafficking and signaling. PSD-95 (*DLG4*) is particularly important for stabilizing AMPA receptors at synapses and promoting their insertion during LTP, whereas PSD-93 (*DLG2*) participates in organizing NMDA receptor–associated signaling complexes and can modulate synaptic strength, partly through interactions with PSD-95. SAP-102 (*DLG3*) is more prominent during development, supporting early synapse formation and plasticity by regulating NMDA receptor trafficking [4-9, 17, 18]. The role of MAGUK proteins in synaptic function leads to implications in memory formation and neurodegenerative diseases [19, 20].

We found consistent associations of all three *DLG* genes with better prognosis, as assessed by longer OS in patients with LGG, which is somewhat counterintuitive given the proposed contributing role of synaptic mechanisms in glioma progression indicated by studies in GBM. If there is any causal relationship between high *DLG2*-*DLG4* expression and survival, which cannot be concluded based in our current results, it is possible that *DLG* genes and MAGUK proteins, together with their associated network of synaptic components, confer a more differentiated, neuron-like transcription profile to LGG tumors, thus reducing their aggressiveness and likelihood of progressing towards GBM. In neuroblastoma, it has been shown that low *DLG2* expression induces forced cell cycle progression and predicts poor patient survival [21].

Our study has several limitations. First, for some of the analyses we used the glioma histological classification, which is now outdated. The definitions for LGG entities were updated in the World Health Organization (WHO) 2021 classification to integrate

molecular signatures with histological features [13, 22]. The histological classification of gliomas in the TCGA LGG dataset is based on WHO 2007/2016 criteria and therefore does not fully reflect the integrated molecular classification introduced in the WHO 2021 guidelines. This limitation is inherent to TCGA and other datasets, which were generated prior to the adoption of the 2021 classification. However, retaining the original histological annotations ensures reproducibility and consistency with prior TCGA-based studies, and these annotations remain relevant as they represent well-characterized morphological entities that form the basis of much of the existing glioma literature. The WHO 2021 classification recognizes that legacy datasets generated before its implementation may not be fully compatible with the updated integrated framework and explicitly supports the continued use of prior WHO histological classifications in such contexts [13]. In addition, we have also included analyses using updated classifications that incorporate molecular features.

A second limitation is that associations between gene expression and patient prognosis, or correlations with expression of other genes, do not imply mechanistic roles and causative effects. These associations should be interpreted primarily as a basis for generating mechanistic hypotheses and identifying candidate prognostic factors. Also, it is well established that higher gene expression at the mRNA level does not necessarily represent increased protein content. Some studies specifically addressing this issue in different model systems have reported broad agreement between mRNA and protein levels, whereas others have found a weak positive correlation [23-25]. Further research is required to examine the expression and possible role of AMPAR subunit proteins in LGG.

5. Conclusions

High expression of *DLG-DLG4* shows a consistent association with better OS in patients with LGG, suggesting the possibility of a prognostic role. In addition, *DLG2-DLG4* gene expression is correlated with a signature of components of excitatory neural transmission and synaptic plasticity, raising the possibility that LGG tumors may replicate cellular mechanisms involved in normal neuronal function.

Supplementary Materials: The following supporting information can be downloaded: **Supplementary Table S1:** Panel of genes chosen as representative of an excitatory synapse and synaptic plasticity signature; **Supplementary Figure S1:** Gene expression levels of (**A**) *DLG2*, (**B**) *DLG3*, and (**C**) *DLG4* in CGGA LGG tumors classified into histological types; **Supplementary Figure S2:** Gene expression levels of (**A**) *DLG2*, (**B**) *DLG3*, and (**C**) *DLG4* in TCGA LGG tumors classified into molecular subtypes; **Supplementary Figure S3:** Correlations found between expression of (**A**) *DLG2*, (**B**) *DLG3*, and (**C**) *DLG4* and individual genes from the synaptic dataset in TCGA LGG tumors; **Supplementary references**.

Author Contributions: Conceptualization, F.G., R.R. and G.I.R.; methodology, F.G., H.R.D.P., R.R. and G.R.I; formal analysis, F.G., H.R.D.P. and R.R.; investigation, F.G., H.R.D.P., R.R. and G.R.I.; resources, F.G., O.M., R.R. and G.R.I.; data curation, F.G., H.R.D.P. and R.R.; writing—original draft preparation, H.R.D.P. and R.R.; writing—review and editing, F.G., H.R.D.P., O.M., R.R. and G.R.I.; supervision, R.R. and G.R.I.; project administration, R.R. and G.R.I.; funding acquisition, F.G., O.M., R.R. and G.R.I. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CGGA	Chinese Glioma Genome Atlas
Codel	Co-deletion
GBM	Glioblastoma
G-CIMP	Glioma CpG island methylator phenotype
LGG	Lower-grade glioma
LGG-IDH-mut-codel	Lower-grade glioma with a mutation in the isocitrate dehydrogenase (IDH) gene and co-deletion of chromosomal arms 1p and 19q
LGG-IDH-mut-non-codel	Lower-grade glioma with a mutation in the IDH gene without co-deletion of chromosomal arms 1p and 19q
LGG-IDH-wt	Lower-grade glioma wild-type for IDH
LTP	Long-term potentiation
MAGUK	Membrane-associated guanylate kinase
NA	Not available
NC	No correlation
NMDA	*N*-methyl-D-aspartate
OS	Overall survival

PA	Pilocytic astrocytoma
PC1	First principal component
PCA	Principal Component Analysis
TCGA	The Cancer Genome Atlas
WHO	World Health Organization

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