Comprehensive Analysis of PLOD Family Members in Low-grade Gliomas Using Bioinformatics Methods

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Research article

Keywords: PLOD family, LGG, bioinformatic analysis, biomarker, prognosis

Posted Date: July 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-40797/v1

Version of Record: A version of this preprint was published at PLOS ONE on January 27th, 2021. See the published version at https://doi.org/10.1371/journal.pone.0246097.

Abstract

Low-grade gliomas (LGGs) is a primary invasive brain tumor that grows slowly but is incurable and eventually develops into high malignant glioma. Novel biomarkers for the tumorigenesis and lifetime of LGG are critically demanded to be investigated. In this study, the expression levels of procollagen-lysine, 2-oxoglutarate 5-dioxygenases (PLODs) were analyzed by ONCOMINE, HPA and GEPIA. The GEPIA online platform was applied to evaluate the interrelation between PLODs and survival index in LGG. Furthermore, functions of PLODs and co-expression genes were inspected by the DAVID. Moreover, we used TIMER, cBioportal, GeneMINIA and NetworkAnalyst analysis to reveal the mechanism of PLODs in LGG. We found that expression levels of each PLOD family members were up-regulated in patients with LGG. Higher expression of PLODs was closely related to shorter disease-free survival (DFS) and overall survival (OS). The findings showed that LGG cases with or without alterations were significantly correlated with the OS and DFS. The mechanism of PLODs in LGG may be involved in response to hypoxia, oxidoreductase activity, Lysine degradation and immune cell infiltration.

In general, this research has investigated the values of PLODs in LGG, which could serve as biomarkers for diagnosis, prognosis and potential therapeutic targets of LGG patients.

Introduction

LGGs are common tumors in the central nervous system, which can progress into high-grade glioma, leading to undesirable prognosis[1, 2]. Advances in genome sequencing have elucidated the genetic and novel biomarkers of high-grade glioma, which provided newly categorization and some promising treatments[3, 4]. However, the molecular mechanisms and targeted gene markers for LGGs are poorly understood, so more promising and therapeutic biomarkers are urgently needed.

PLOD family is composed of three members, PLOD 1/2/3, which is a group of enzymes engaged in stabilizing collagen through cross-linking and hydroxylation of lysine[5, 6]. PLOD family members have binding sites to catalyzing the lysine hydroxylation [7, 8]. Molecular biology mechanism of PLOD family involving a wide range of biological process, such as modulating cancer cell migration, tumorigenesis and development[9]. Many studies show that over expression of PLODs can promote tumor invasion and higher recurrence, suggesting that targeting PLOD family members is potential strategy for cancer treatment[10]. However, the effect of this promising gene family in LGGs is still lacking research.

In the current study, we studied the expression levels and prognosis of PLODs in LGGs based on online databases, platforms, and various data sets. The purpose of this study is to provide insights into the molecular mechanism of LGG and uncover potential new biomarkers for the disease.

Methods

ONCOMINE analysis

According to the **ONCOMINE** (https://www.oncomine.org/) dataset [11], we tested the transcription levels of PLODs in different tumors. Besides, we compared the expression of PLODs between the subtypes in LGG and normal tissues, 'PLOD1, PLOD2 and PLOD3' was selected as the keywords in search, and 'Anaplastic Astrocytoma vs. Normal Analysis' was chosen as Analysis Type. The threshold was set up as *P*-value 0.05, fold change 2, and top 10% gene rank.

HPA analysis

HPA dataset (https://www.proteinatlas.org/) is an open access to enable researchers to freely access data for exploration of the human protein in different tissues[12]. The HPA database was also used to validate the immunohistochemistry of the PLOD s in patients with LGG.

GEPIA analysis

The GEPIA database (http://gepia.cancer-pku.cn/) is an online dataset for comparing the gene expression profile in cancer and paired normal

tissues[13]. We have assessed the prognosis of high and low expression PLODs in LGG, including OS and DFS. The number of high-risk cases, hazard ratios (HRs), 95% confidence intervals (CIs), and *P* values are shown accordingly.

cBioPortal analysis

The cBio Cancer Genomics Portal (http://cbioportal.org) was applied to investigated the genetic mutations of PLODs in LGG [14]. Mutation and a summary of the gene types in LGG was inquiry. According to cBioPortal's online instructions, DFS and OS were analyzed for with or without PLODs mutation in LGG.

GeneMINIA and NetworkAnalyst analysis

GeneMANIA (http://www.genemania.org)[15] was used to analyze the relationship between the PLOD family members and co-expression genes. Using the NetworkAnalyst (https://www.network analyst.ca/) [16], we analyzed the correlated 23 genes screening by GeneMINIA database, which integrates pathway, genetic interactions, co-expression genes and physical interactions.

DAVID

To reveal the functions of PLODs and the twenty interactors from GeneMINIA analysis, DAVID database (https://david.ncifcrf.gov/) was used to explore the functions of PLODs in LGG [17].

TIMER analysis

TIMER is an internet platform resources for comprehensive investigation of the relationship between immune cells and multiple cancer types (https://cistrome.shinyapps.io/timer/)[18]. TIMER applies algorithm method to evaluate the abundance of tumor-infiltrating immune cells from gene expression

profiles. In this dataset, we analyzed the correlation of PLODs expression with the abundance of immune infiltrates in LGG.

Results

Transcriptional levels of PLODs in LGG

Diverse transcriptional levels of PLODs have been investigated in twenty human cancers and adjacent normal tissues in the **Oncomine**. As illustrated in Fig. 1A, we have compared the transcriptional levels of PLODs in cancers with those in the normal tissues. In contrast to normal specimens, PLODs mRNA levels of all members were over expression in Brain and CNS Cancers. PLOD1 overexpression was illustrated in 2 datasets[19], followed by PLOD2 in 10 datasets[19–25], and PLOD3 in 2 datasets. All the datasets were summarized in Table 1. Using the **Oncomine** database, we compared the mRNA expression of PLODs in the subtypes of LGG with normal brain tissues. The Fig. 1B showed that the expression levels of PLODs were all observed significantly higher in anaplastic astrocytoma as compared with the normal tissues. We also used the GEPIA database to compare the expression of PLODs between LGG and normal brain tissues. Contrast to normal brain tissues, the expression level of PLODs in LGG was significantly up-regulated **(**Fig. 1C and 1D**)**.

Table 1
The upregulated mRNA expressions of PLOD family genes in GC from ONCOMINE database*

PLOD family genes	Fold change	*P*-Value	Dataset	Group comparison
PLOD1				
	3.742	1.33E-10	TCGA Brain	Brain Glioblastoma vs. Normal
PLOD2	2.082	2.96E-5	Bredel Brain 2	Glioblastoma vs. Normal
PLOD3	3.543	1.71E-25	Sun Brain	Glioblastoma vs. Normal
	2.356	0.004	Sun Brain	Diffuse Astrocytoma vs. Normal
	3.494	3.40E-11	Bredel Brain 2	Glioblastoma vs. Normal
	2.688			Astrocytoma vs. Normal
	3.204	1.45E-4	Rickman Brain	Glioblastoma vs. Normal
	6.368	6.26E-7	Shai Brain	Brain Glioblastoma vs. Normal
	3.326	2.75E-12	TCGA Brain	Glioblastoma vs. Normal
	2.430	0.014	Liang Brain	Anaplastic Oligoastrocytoma vs. Normal
	2.716	0.002	French Brain	Glioblastoma vs. Normal
	2.873	0.004	Lee Brain	Brain Glioblastoma vs. Normal
	2.204	1.03E-10	TCGA Brain	Glioblastoma vs. Normal
		0.003	TCGA Brain	

*Only datasets that meet the criteria *P* value < 0.05 and fold change > 2 are listed.

In summary, the results showed that the transcriptional levels of PLODs were up-regulated in multiple online datasets.

Protein expression levels of PLODs in the human protein atlas

In order to further investigate the expression of PLODs at the protein level, we further verified their expression levels using the Human Protein Atlas (**HPA**) database. As shown in Fig. 2, the protein expressions of PLOD1 and PLOD2 in LGG were higher than corresponding normal tissues, while PLOD3 level was not significantly different.

On the whole, the above results illustrate that PLODs protein expression were also up-regulate in LGG tissues than that in normal tissues.

Prognostic values of PLODs in patients with LGG

To explore the prognostic value of PLODs in patients with LGG, **GEPIA** analysis was performed according to the mRNA expression of individual PLODs family members. The findings showed that the mRNA expression levels of PLODs were closely associated with shorter OS and DFS in patients with LGG **(**Fig. 3**)**. The present results suggested that patients with LGG with a high mRNA expression level of PLOD1/2/3 were predicted lower OS and DFS.

In brief, PLOD family members may be biomarkers for poor prognosis in patients in LGG.

Genetic alteration analysis of PLODs in LGG

In another, we used the **cBioPortal** online tool to analyze the genetic variation of PLOD family members in LGG patients. The results indicated that three categories are shown based on filtering. (Fig. 4A). The ratios of genetic alterations in PLOD family members for LGG different from 3–12% for each member (PLOD1 3%; PLOD2 5%; PLOD3 15%) (Fig. 4B). Furthermore, we analyzed genetic alteration in PLODs and their associations with the OS and DFS of LGG patients. As was shown in Fig. 4C-D, the results indicated that LGG cases with or without alterations were significantly related with the OS and DFS.

Construction of interactive genes network and TF-miRNA coregulation network of PLODs family members in LGG

Moreover, **GeneMANIA** is used to build networks of PLODs family members and their interactive genes. The online tool identified twenty genes closely related to PLODs (FIGURE 5A). After that, we built up the PLODs family members related TF-miRNA regulatory network, FIGURE 5B shows the regulation network composed of miRNAs and target genes, including 23 seeds, 155 edges and 67 nodes **(Supplement 1)**.

In short, these interactive genes and miRNAs may be potential targets for LGG formation and deserve further study.

GO enrichment and KEGG analysis of PLOD1/2/3

In order to further explore the biological functions that these interactive genes of PLODs in LGG, we used DAVID to construct GO and the KEGG pathway. The results of biological process (BP) showed that these genes are primarily involved in response to hypoxia, proline hydroxylation to 4-hydroxy-L-proline, mRNA transport, RNA splicing, collagen fibril organization and mRNA processing (Fig. 6A). For GO cellular component (CC) analysis, the significantly enriched terms were intracellular ribonucleoprotein complex, endoplasmic reticulum, membrane, spliceosomal complex and viral nucleocapsid (Fig. 6B). Significantly enriched molecular function (MF) terms included L-ascorbic acid binding, oxidoreductase activity, procollagen-lysine 5-dioxygenase activity, nucleotide binding and procollagen-proline 4-dioxygenase activity (Fig. 6C). KEGG pathway analysis showed enrichment in Lysine degradation, other types of O-glycan biosynthesis, Arginine and proline metabolism, renal cell carcinoma (Fig. 6D).

To sum up, the results illustrated that PLODs were mainly engaged in tumor-related regulatory mechanisms, such as response to hypoxia, oxidoreductase activity and Lysine degradation.

The expression of PLOD family members is correlated with immune infiltration levels in LGG

To explore the immune microenvironment, the relationship of the levels of immune infiltration and the expression of PLODs in LGG was analyzed by TIMER database. The results showed that all the PLODs family members were associated with negative tumor purity. The expression level of PLOD1/2 was significantly positively correlated with the infiltration levels of B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages and dendritic cells. Similarly, PLOD3 mRNA expression was positively correlated with infiltrating levels of CD4 + T-cells, neutrophils, macrophages and dendritic cells, except CD8 + T-cells and B-cells (Fig. 7A-C).

Taken together, these results further confirm the key role of PLOD family members in regulating immune activity in the LGG microenvironment.

Disccussion

Low-grade gliomas are infiltrating primary central nervous tumor that most commonly occurs in young patients [26] and cannot be cured by the traditional treatment, such as surgery, radiology or a combined approach[27]. Therefore, new biomarkers are urgently to be discovered, which can promote early diagnose and predict the prognosis of LGG patients [28]. Recent research shows that the functions of PLODs have involved in the tumorigenesis and the prognosis of a lot of cancers, yet functions of PLODs in LGGs are still unclear[29–31]. In this manuscript, we analyzed the expression level and prognostic value of PLOD family members in LGGs, with the purpose of proposing new diagnostic and therapeutic strategy for LGG patients.

PLOD1 has been reported that its aberrant expression level was significantly correlated with various human cancers, including prostate cancer, gastric cancer, colorectal cancer and bladder cancer[32–35]. Moreover, Rolf Warta,etc have presented that the expression PLOD1 can be predicted prognosis of IDH^{mut} glioma patients, which involved in oxygen metabolism[36]. However, the prognostic significance of PLOD1 in LGG patients has not been reported. In our research, it was also found that the expression level of PLOD1 in LGG tissues was up-regulate than normal brain tissues and was significantly related with poorer prognosis in LGG patients.

PLOD2 has been reported up-regulated in many tumors, which affect collagen remodeling through affecting HIF-1α, TGF-β and microRNA-26a/b[37–39]. In breast cancer tumorigenesis, high expression of PLOD2 was positively associated with poorer prognosis[40]. In the central nervous system, some researchers have reported that hypoxia-induced PLOD2 can promote tumorigenesis via PI3K/Akt signaling in glioma[41]. In addition, Li X et al[42], indicated that knockdown of PLOD2 in glioblastoma (GBM) can play antitumor effect under hypoxia conditions. Up to now, the potential effects of PLOD2 on

LGG tumorigenesis remain limited. In this manuscript, PLOD2 was validated a prognostic indicator in patients with LGG, and PLOD2 of significantly overexpressed in LGG tissues.

PLOD3 was also founded in many human solid tumors, containing gastric cancer, lung cancer and hepatocellular cancer[43–45]. In brain tumor, PLOD3 was founded play considerable roles in the proliferation and metastasis of glioblastoma[46].

This research has systematically provided evidence that PLODs may be prognostic factors in the outcome of LGG patients. The results also used multiple bioinformatic platform to validate the over expression levels of PLODs in LGG. The limitation of this research was lack of laboratory experiment procedures and the exact mechanism should be further investigated in further studies. The research showed that PLODs were up-regulated in LGG and can be set as survival risks for this cancer type.

Previous studies revealed that the mechanisms of PLODs in cancer development mainly involved in regulating the collagen metabolism, hypoxia, extracellular matrix construction, and immune microenviroment[10, 33]. In our research, the mutation of PLODs in LGG was founded and closely related to the prognosis of LGG patients. In this study, GO and KEGG pathway enrichment analysis was performed to understand the biological functions of PLODs in LGG, mainly involved in hypoxia, oxidoreductase activity and Lysine degradation. The results illustrated the mechanisms by which PLODs are regulating tumorigenesis. In the recent clinical studies, some immune therapy for LGG has broad prospects, such as CAR-T cell therapy[47]. We interestingly founded that the expression of PLODs was closely related with tumor purity and immune cell infiltration, which may be insight biomarkers for immune therapy for LGG.

Conclusion

This is the first study to our knowledge to investigate the relationship between the expression of all PLOD family genes in LGD and patient outcomes. Taken together, this study strongly suggests that PLOD family members may be potential therapeutic targets and serve as prognostic markers for LGG patients' survival.

Declarations

AUTHOR CONTRIBUTION

Yonghui, Zhao designed this study and contributed substantially to the design of the search strategy, and Yonghui, Zhao performed the analysis and interpreted the data, Yonghui, Zhao wrote the manuscript. Xiang Zhang and Junchao, Y critically reviewed the manuscript. Yonghui, Zhao and Xiang Zhang participated in the data extraction and critically revised it. Yonghui, Zhao andJunchao, Y proofread the final version. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

The authors received no funding.

CONFLICTS of INTEREST

The authors declare no competing financial interest.

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Figures

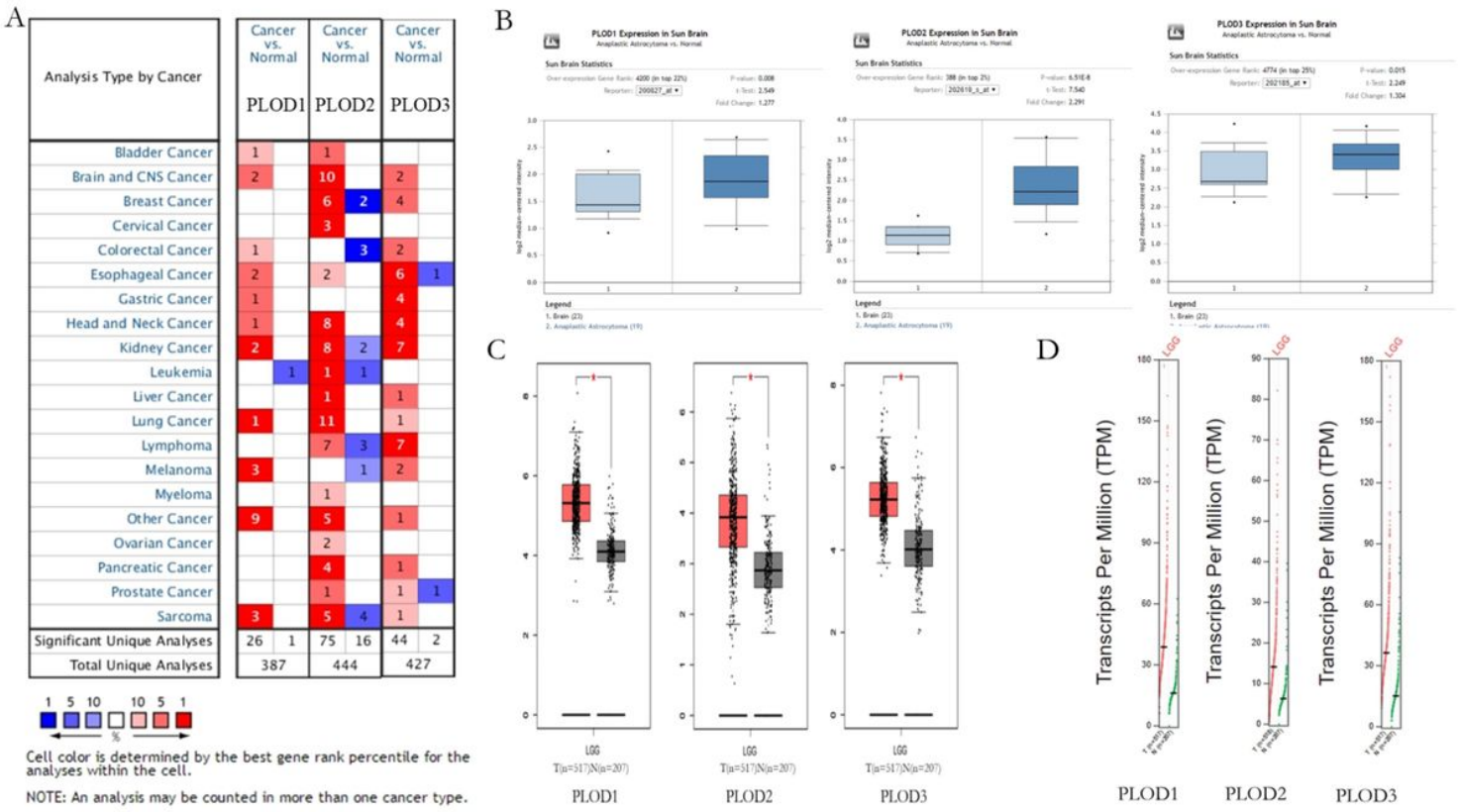

Figure 1

The expression of PLOD family members in different tissues. (A) The transcription levels of PLOD family members in different types of cancers Red, upregulation; blue, downregulation. The number in each cell indicates the datasets that met the set threshold in each cancer type. Cell color was defined as the gene rank percentile for analyses within the cell. (B) Expression levels of PLODs family members compared between subtypes of LGG and normal tissues from the Oncomine. (C) Boxplot results of the expression levels of PLODs family members in LGG analyzed using GEPIA. Red box, tumor samples; green box, normal samples. T, tumor; N, normal. (D) Expression profile of PLODs family members in LGG analyzed using GEPIA. Red trace, tumor samples; green trace, normal samples.

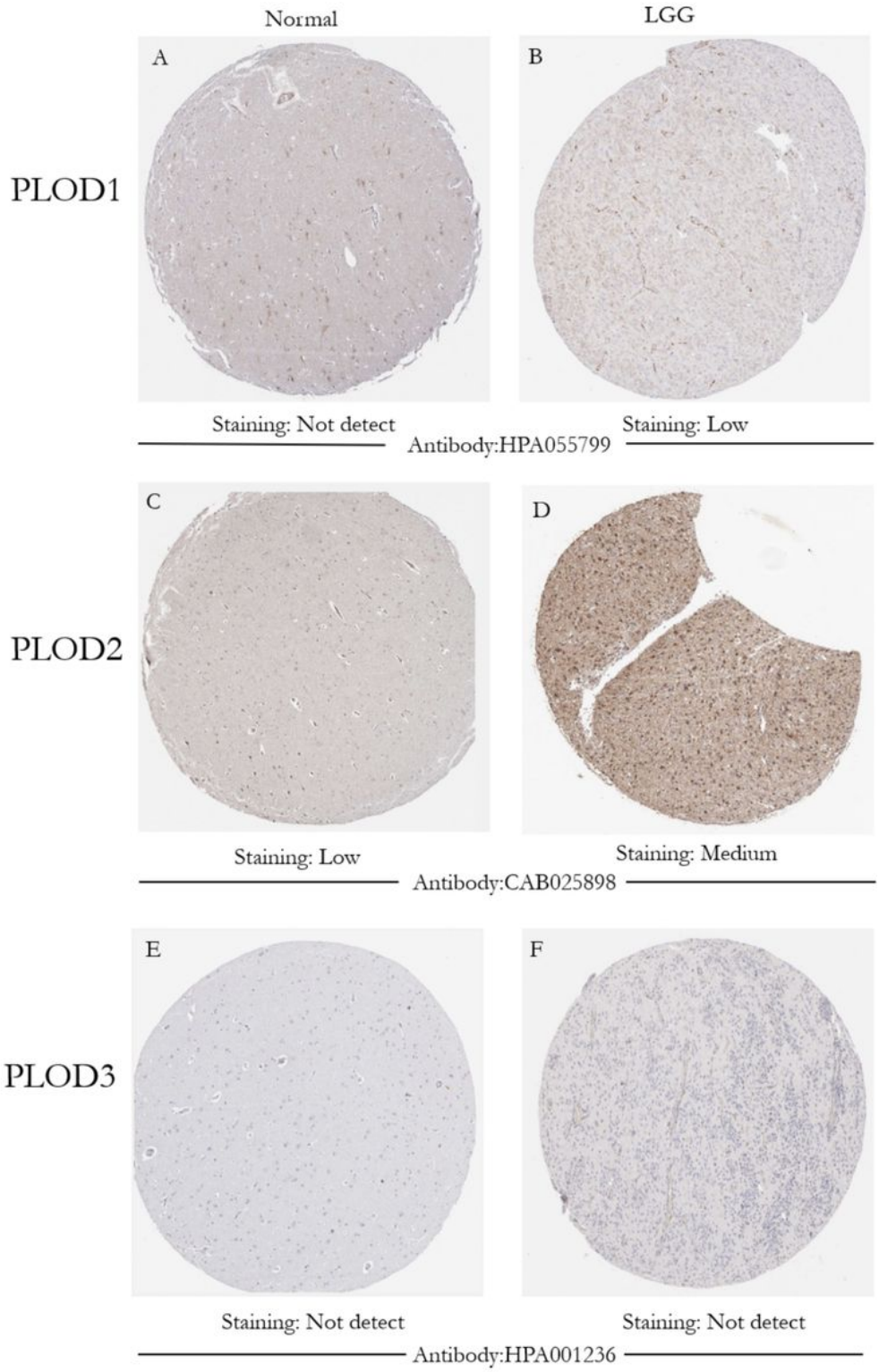

Figure 2

The protein expression of PLOD family members in patients with LGG. (A-B) Representative immunohistochemistry images of PLOD1 in LGG and non-cancerous brain tissues derived from the HPA database. (C-D) Representative immunohistochemistry images of PLOD2 in LGG and non-cancerous brain tissues derived from the HPA database. (E-F) Representative immunohistochemistry images of

PLOD3 in LGG and non-cancerous brain tissues derived from the HPA database. HPA, Human Protein Atlas

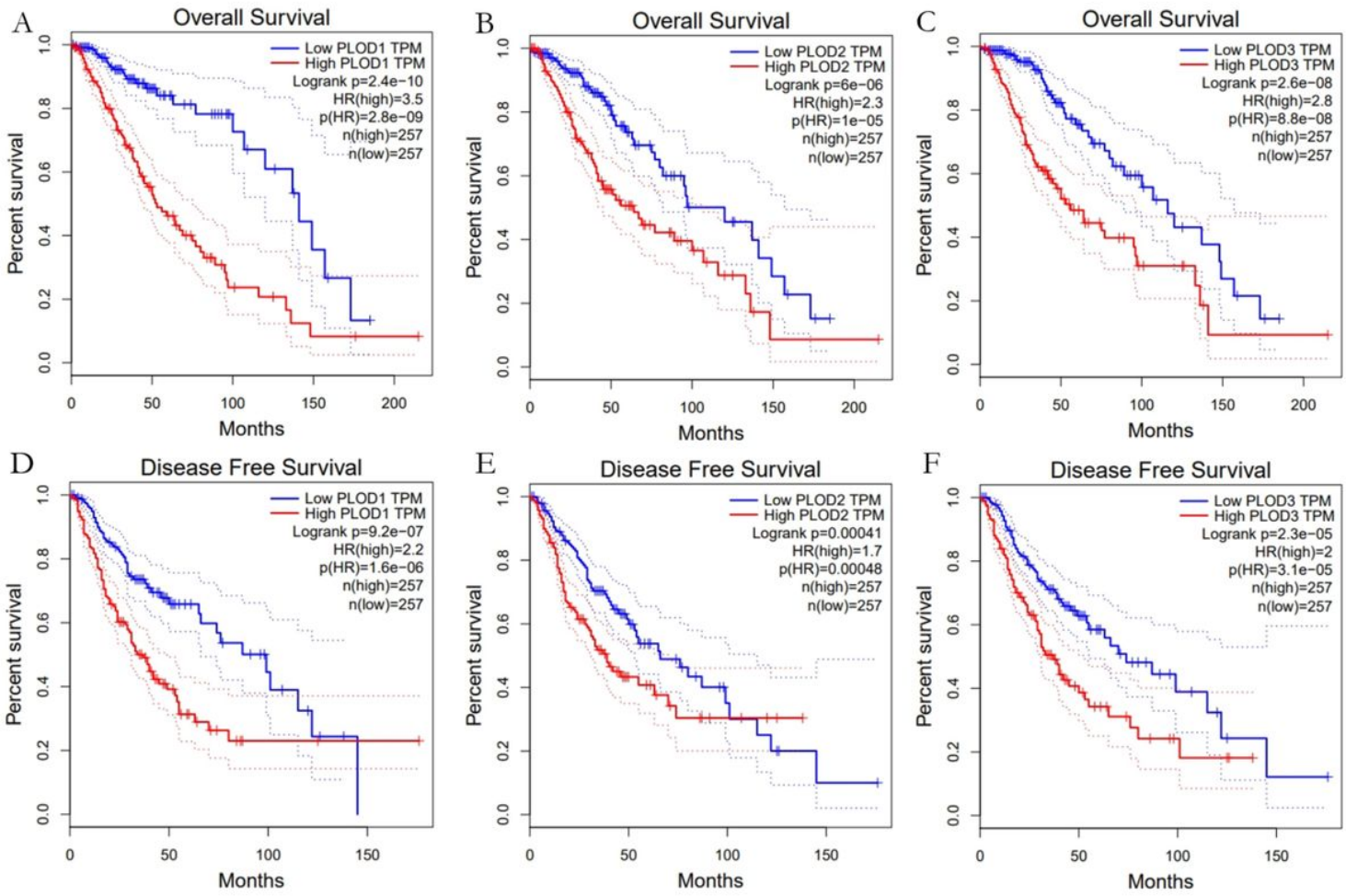

Figure 3

The survival analysis of PLOD family members in LGG.(A-C) Overexpression level of PLOD1,PLOD2 and PLOD3 were associated with shorter OS in LGG. (D-F) Overexpression level of PLOD1,PLOD2 and PLOD3 were associated with shorter DFS in LGG.

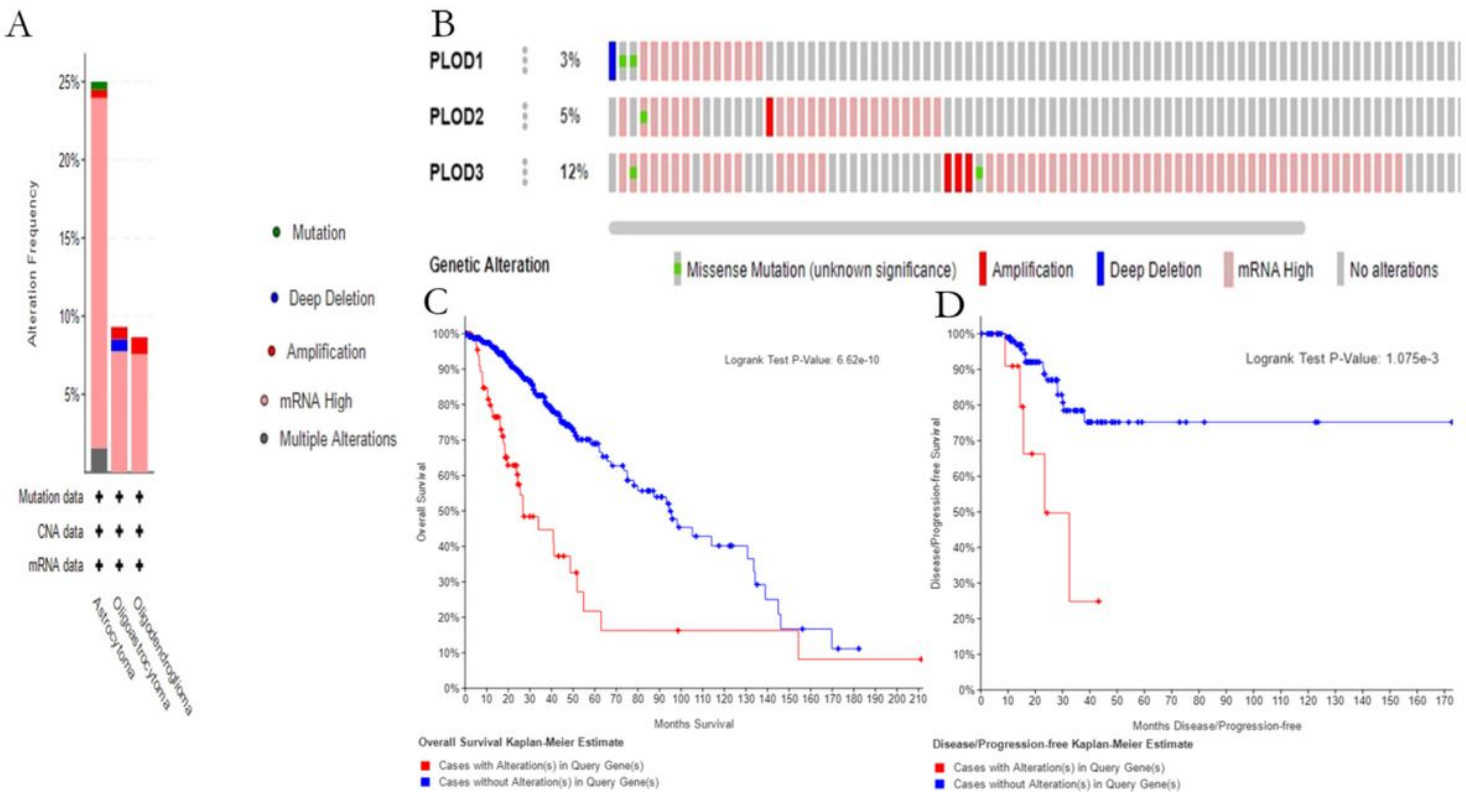

Figure 4

Alteration frequency and prognosis of the PLOD family genes in patients with LGG. (A) The summary of the cancer types in the cBioPortal was used to calculate the percentages of LGG cases with of the PLOD family genes. (B) mRNA expression alterations (RNA Seq V2 RSEM) of of the PLOD family genes in LGG patients. (C) OS and DFS/PFS of LGG patients with altered (red) and unaltered (blue) mRNA expression of the PLOD family genes.

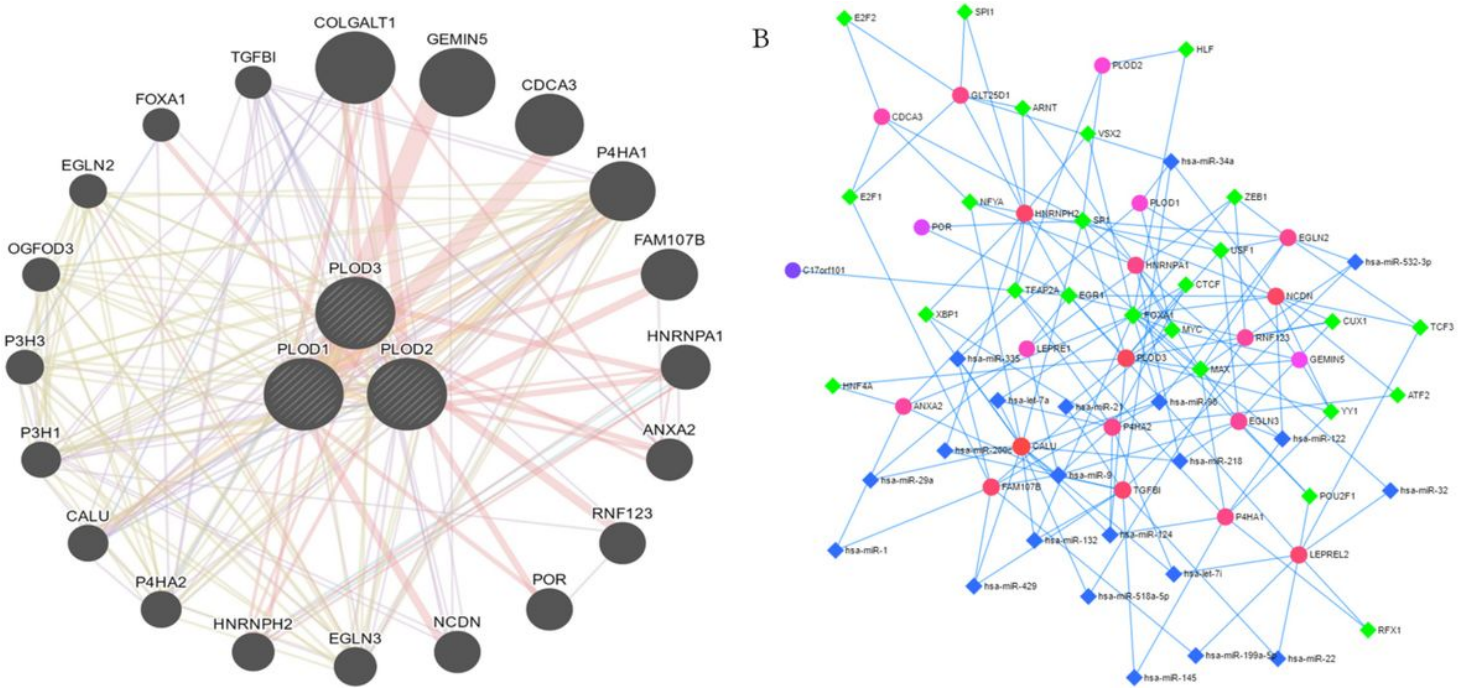

Figure 5

Gene-gene network and miRNA/TF prediction of PLOD family members in LGG. (A) Gene-gene interaction network among PLOD family members. Each node represents a gene. The node size represents the strength of interactions, and the line color represents the types of interactions. (B) Transcription factor-miRNA (TF-miRNA) coregulatory network of significantly PLODs correlated genes.

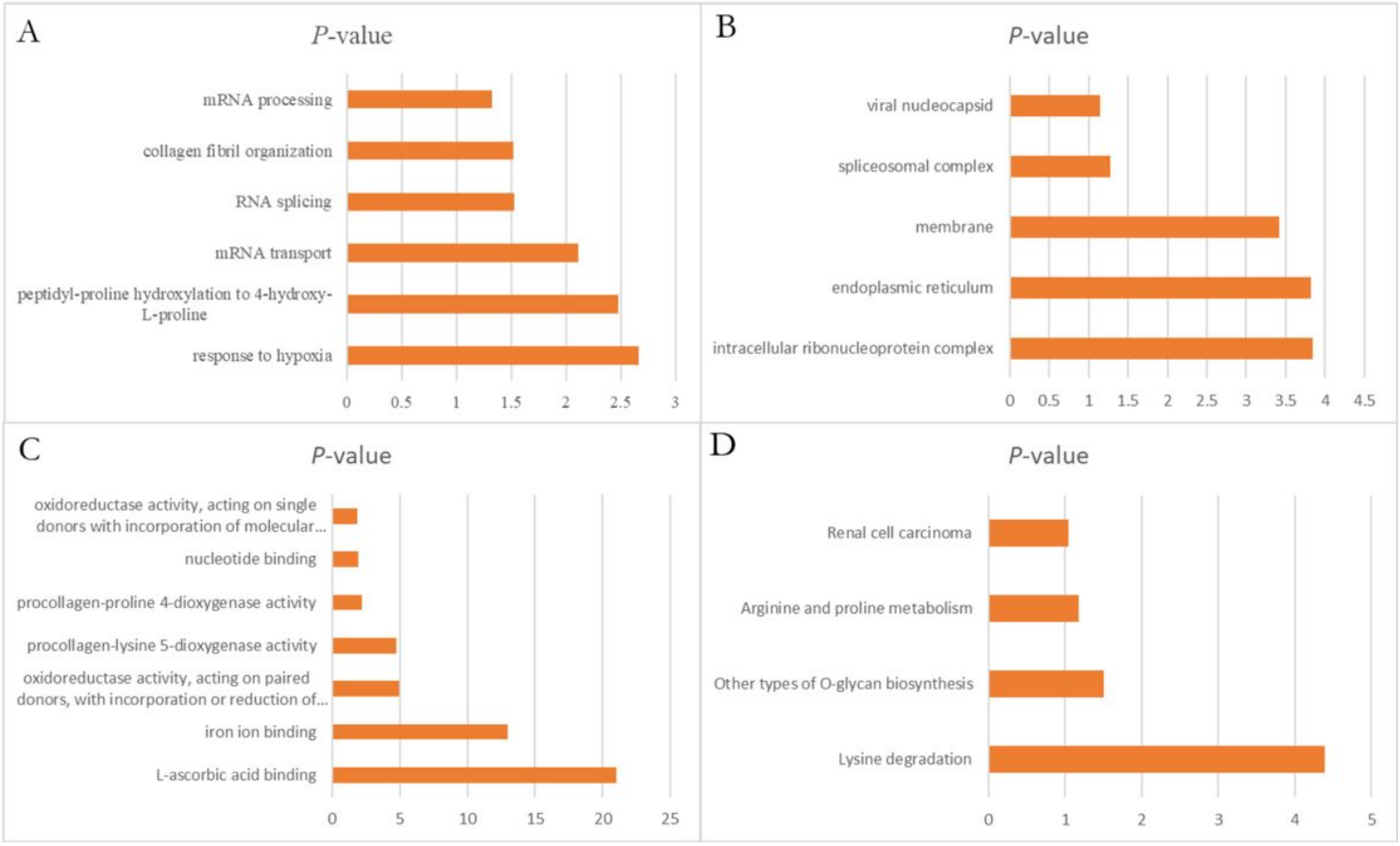

Figure 6

GO annotation and KEGG pathway enrichment analysis of PLOD family members in LGG. The top enriched GO (A) BP, (B) CC and (C) MF terms as well (D) KEGG pathways. GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; BP, biological process; CC, cellular component; MF, molecular function.

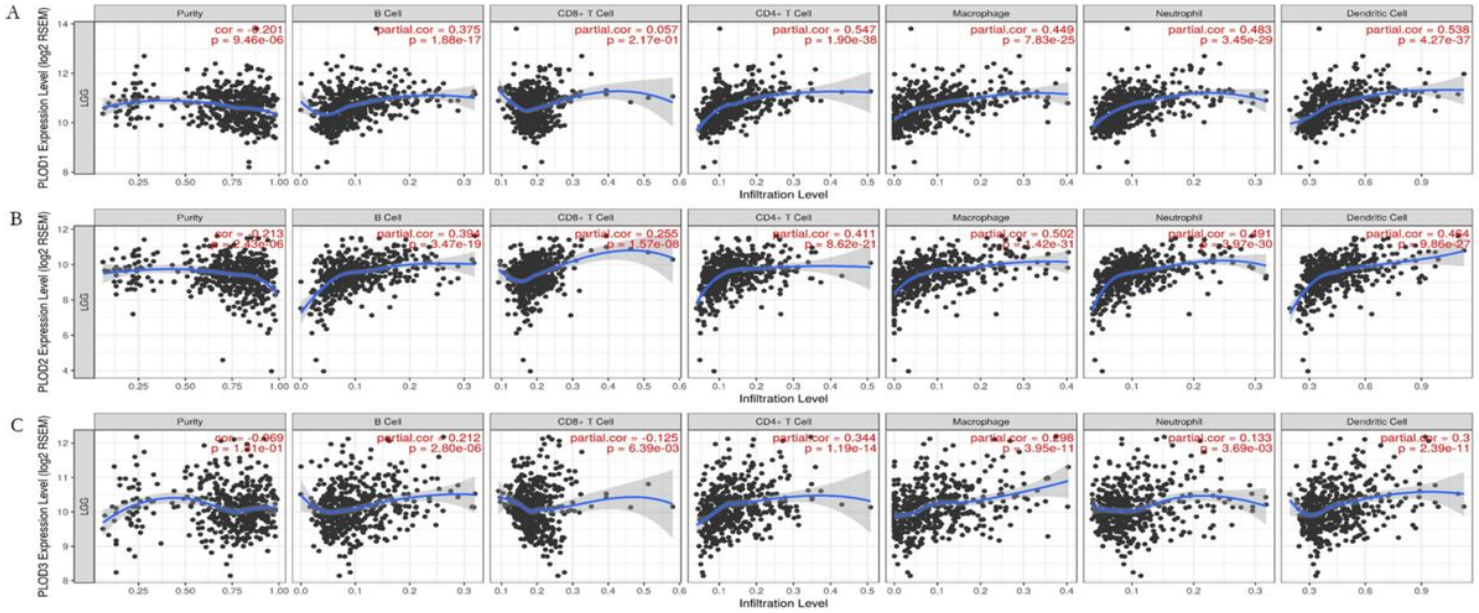

Figure 7

Correlations of PLODs expression with immune infiltration level in LGG. (A) The correlation between each type of TIICs (B-cells, CD4+ T-cells, CD8+ T-cells, neutrophils, macrophages and dendritic cells) and PLOD1. (B) The correlation between each type of TIICs (B-cells, CD4+ T-cells, CD8+ T-cells, neutrophils, macrophages and dendritic cells) and PLOD2. (C) The correlation between each type of TIICs (B-cells, CD4+ T-cells, CD8+ T-cells, neutrophils, macrophages and dendritic cells) and PLOD3.

Supplementary Files

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

- seedproteins.txt
- subnetwork1.sif
- subnetwork1abundance.NA
- origedgelist.csv
- Rhistory.R
- subnetwork1Label.NA
- siggenes.txt
- orignodelist.csv
- subnetwork1name.NA