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ORIGINAL RESEARCH

PLP2 Could Be a Prognostic Biomarker and Potential Treatment Target in Glioblastoma Multiforme

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Objective: This study aimed to discern the association between PLP2 expression, its biological significance, and the extent of immune infiltration in human GBM.

Methods: Utilizing the GEPIA2 and TCGA databases, we contrasted the expression levels of PLP2 in GBM against normal tissue. We utilized GEPIA2 and LinkedOmics for survival analysis, recognized genes co-expressed with PLP2 via cBioPortal and GEPIA2, and implemented GO and KEGG analyses. The STRING database facilitated the construction of protein-protein interaction networks. We evaluated the relationship of PLP2 with tumor immune infiltrates using ssGSEA and the TIMER 2.0 database. An IHC assay assessed PLP2 and PDL-1 expression in GBM tissue, and the Drugbank database aided in identifying potential PLP2-targeting compounds. Molecular docking was accomplished using Autodock Vina 1.2.2.

Results: PLP2 expression was markedly higher in GBM tissues in comparison to normal tissues. High PLP2 expression correlated with a decrease in overall survival across two databases. Functional analyses highlighted a focus of PLP2 functions within leukocyte. Discrepancies in PLP2 expression were evident in immune infiltration, impacting CD4+ T cells, neutrophils, myeloid dendritic cells, and macrophages. There was a concomitant increase in PLP2 and PD-L1 expression in GBM tissues, revealing a link between the two. Molecular docking with ethosuximide and praziquantel yielded scores of −7.441 and −4.295 kcal/mol, correspondingly.

Conclusion: PLP2's upregulation in GBM may adversely influence the lifespan of GBM patients. The involvement of PLP2 in pathways linked to leukocyte function is suggested. The positive correlation between PLP2 and PD-L1 could provide insights into PLP2's role in glioma modulation. Our research hints at PLP2's potential as a therapeutic target for GBM, with ethosuximide and praziquantel emerging as potential treatment candidates, especially emphasizing the potential of these compounds in GBM treatment targeting PLP2.

Plain Language Summary: Glioblastoma is the most prevalent and aggressive malignant glioma. PLP2, a protein found to be upregulated in several cancers and neurological diseases, has drawn attention for its potential role in glioblastoma treatment. With advances in immunotherapy and improved understanding of tumor-immune system interactions, PD-1/PD-L1 inhibitors are gaining increasing interest for treating glioblastomas.

In glioblastoma patients, overexpression of PLP2 may worsen overall survival rates. PLP2 is believed to be involved in key pathways related to leukocyte, and a connection was found between PLP2 and PD-L1, suggesting that PLP2 might influence glioblastomas through this interaction. The potential use of PD-1/PD-L1 inhibitors in treating glioblastomas has emerged as a result of these findings.

Moreover, the study highlights the potential of drugs such as ethosuximide and praziquantel to target PLP2 in glioblastoma therapy. This research emphasizes the promising role of PLP2 as a therapeutic target for glioblastoma, where praziquantel and ethosuximide could be employed. Overall, the study presents a new therapeutic target for glioblastoma and demonstrates the potential of PD-1/PD-L1 inhibitors, praziquantel, and ethosuximide in this treatment approach.

Keywords: immune infiltrates, PDL1, programmed cell death-ligand 1, glioma, molecular docking, bioinformatics

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Introduction

Gliomas are typically the most prevalent brain tumors, with glioblastoma (GBM) being the most aggressive and common malignant glioma.[1] As such, a comprehensive investigation of GBM pathogenesis and identification of significant biomarkers would greatly aid in the diagnosis and treatment of this disease.

The identification of Proteolipid protein 2 (PLP2), also known as A4/A4LSB, in colon epithelial cells, is a novel finding. Although the precise function of PLP2 in normal physiological conditions remains uncertain, research has uncovered several intriguing characteristics of the protein. Specifically, PLP2 has been shown to possess ion channel properties and the ability to multimerize.[2] Ion channels have been shown to play an important role in intrinsic and acquired immunity,[3] and PLP2, a type of ion channel, has also been found to be relevant to immunity.PLP2 acts as a downstream regulator of CD45RO-CD8+ T cells in endometrial cancer.[4] PLP2-derived peptide Rb4 exerts effective antimelanoma activity in vivo dependent on the immune system.[5] PLP2 also is a critical host factor for Kaposi's sarcoma- associated herpesvirus immune evasion.[6] Additionally, it is an integral membrane protein that localizes to the endoplasmic reticulum (ER). Notably, PLP2-deficient mice exhibit heightened ER stress, leading to apoptotic cell death in hypoxic conditions.[7] The PLP2 gene has been identified as a potential contributor to normal gastrulation while also being linked to various diseases. In particular, PLP2 has been observed to be upregulated in numerous cancer types such as melanoma,[8] breast cancer,[9] endometrial cancer,[4] and renal cell carcinoma.[10]

Programmed cell death protein 1 (PD1) serves as a crucial immune response checkpoint, regulating the immune system. By binding to its ligands PDL1 and PDL2, PD1 transmits inhibitory signals to T-cells. The expression of PD1 plays a significant role in the development of exhausted effector T-cell phenotype. Furthermore, the presence of PD1 on effector T-cells and PDL1 on cancerous cells allows tumor cells to evade the immune response against them. Consequently, blocking PD1 has emerged as a key immunotherapeutic approach for treating various types of cancer. The role of ion channels in the interaction between PD-1 and PD-L1 has garnered growing attention. Research indicates that blocking the PD-L1/PD-1 interaction leads to the swift activation of specific ion channels in T cells of head and neck cancer patients. Consequently, this encourages us to redirect our focus towards PLP2, an additional ion channel, in order to explore any potential association between PLP2 and PD-1/PD-L1.

The goal of this study is to gain a deeper understanding of the biological role of PLP2 in GBM. To achieve this, we first obtained PLP2 gene expression data from the GEPIA2[11] and LinkedOmics[12] databases and analyzed their prognostic significance in GBM. We then identified the main biological functions and built a network of protein-protein interactions (PPIs) using functional enrichment analysis and Cytoscape software. To determine the correlation between tumor-infiltrating immune cells and PLP2 in GBM, we analyzed the Tumor Immune Estimation Resource (TIMER 2.0) database. Moreover, we assessed the relationship between PLP2 and tumor-infiltrating immune cells using these methods.

We also investigated PLP2 expression in tumor tissues through immunohistochemistry (IHC) and found a correlation with PD-L1 expression. These results significantly contribute to the current literature about PLP2's potential positive impact on GBM. Our findings suggest that PLP2 and tumor immunity may interact through a correlation and mechanism. Furthermore, we explored the pharmacological treatment of GBM and identified praziquantel and ethosuximide as drugs that effectively bind to PLP2 and may potentially treat GBM by affecting PLP2 expression. Figure 1 illustrates the workflow of our study, providing a visual representation of the sequential steps and processes undertaken.

Materials and Methods

Data Acquisition and Survival Analysis

We obtained PLP2 expression in GBM and paired normal tissues from the TCGA database. Subsequently, the effect of PLP2 on survival was assessed using tools such as GEPIA2 and Linkedomics. Log rank test was used to compare differences in survival between these groups. For Kaplan-Meier curves, p-values and hazard ratio (HR) with 95% confidence interval (CI) were generated by Log rank tests and univariate cox proportional hazards regression. p value <0.05 was considered statistically significant. Using Overall Survival as a Survival Indicator. The association between PLP2 expression and survival was quantified using hazard ratios.

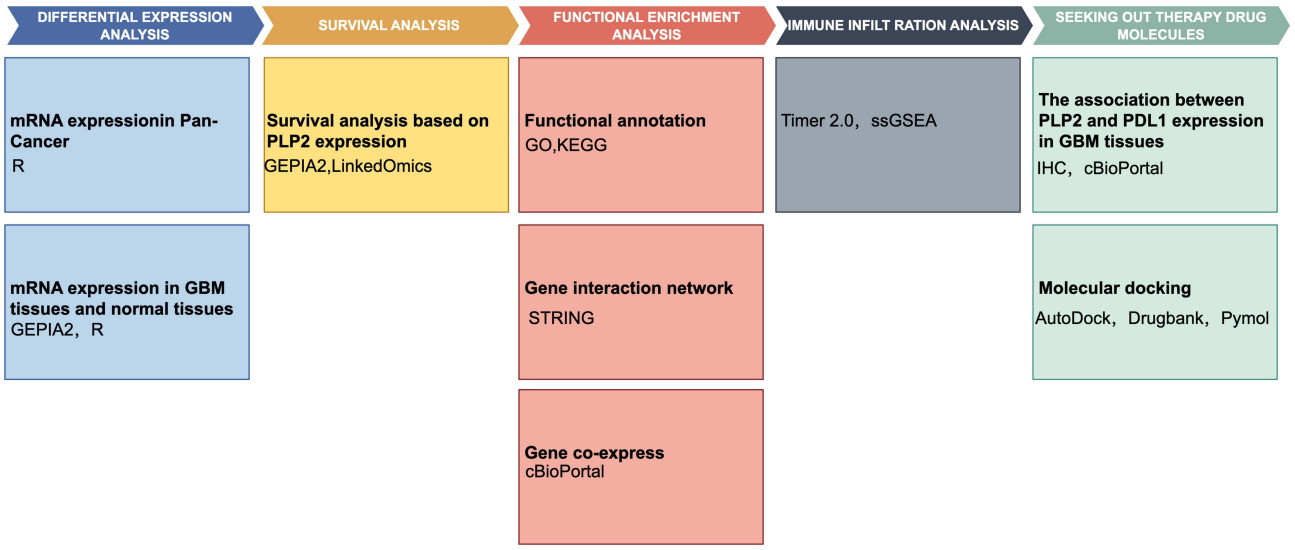

Figure 1 Flowchart.
Abbreviations: GEPIA2, Gene Expression Profiling Interactive Analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; STRING, search tool for recurring instances of neighbouring genes; TIMER, Tumor Immune Estimation Resource; IHC, Immunohistochemistry.

Gene Enrichment Analysis and PPI Network Analysis

To investigate the fundamental roles of genes co-expressed with PLP2, enrichment analyses of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were performed by R (4.2.1). The R packages used are clusterProfiler and ggplot2. p value <0.05 was considered statistically significant. A protein-protein interaction (PPI) network was constructed utilizing the STRING database, with a confidence score of ≥ 0.4. The Cytoscape software 3.7.1 was employed for network visualization. Additionally, correlation analysis between PLP2 and the 7 hub genes was conducted using cBioPortal and the GEPIA2 database.

Immune Infiltration Analysis

The R software (version 4.2.1) was utilized to conduct analyses. The PLP2 expression levels of 349 GBM samples from TCGA were measured and subsequently categorized into two groups based on median levels. To compare PLP2 expression in GBM with immune cell proportions, ssGSEA analyses were employed. Additionally, the TIMER 2.0 database was utilized to investigate the correlation between PLP2 expression and levels of tumor-infiltrating immune cells.

Immunohistochemical (IHC) Assay

From 48 patients with GBM diagnosed at the Affiliated Hospital of Qingdao University, we collected formalin-fixed (10% formalin at room temperature for 24 h) and paraffin-embedded tumor tissues. The inclusion criteria for patients with glioblastoma were defined as follows: i) individuals who underwent surgical resection for glioblastoma; ii) individuals with a confirmed pathological diagnosis of glioblastoma; iii) individuals who had not received any pre-operative therapy; iv) individuals with complete case data available. Conversely, the following exclusion criteria were applied: i) patients with glioblastoma lacking a histopathological diagnosis; ii) patients with incomplete data records; iii) patients presenting with additional severe medical conditions; iv) patients diagnosed with other tumors or central nervous system (CNS) diseases. Informed consent was obtained from all patients before surgery. Deparaffinized sections (4 μm thickness) underwent antigen retrieval by incubating them in Tris/EDTA buffer (pH 9.0). We blocked endogenous peroxidase and non-specific epitopes using the UltraSensitive IHC kit.

Slides were first incubated at 4°C overnight with primary antibodies (PLP2 Antibody 1:200; PD-L1 Antibody 1:200), then at 37°C for 30 minutes with secondary antibodies (Mouse and Rabbit General Antibodies) conjugated to biotin, and with streptavidin-peroxidase for 10 minutes at room temperature, all according to the UltraSensitive IHC kit protocol.

Slides were immunostained with 3.3′ diaminobenzidine, counterstained with hematoxylin, dehydrated, and mounted. Following this, mounted specimens were scanned using the NanoZoomer-S60 (Hamamatsu Photonics) to generate high-resolution digital slides.

Microscopic observation was performed, photographs were taken, and no staining was defined as negative, <50% of cells stained was defined as half-positive and >50% of cells stained were defined as positive.

Molecular Docking

In the current study, AutodockVina 1.2.2,[13] a software specifically designed for protein-ligand docking, was employed to investigate the binding affinities and interaction modes between drug candidates and their respective targets. The molecular structures of Praziquantel and Ethosuximide were retrieved from the PubChem Compounds database,[14] while the 3D coordinates of PLP2 were obtained from the AlphaFold Protein Structures Database.[15,16]

Before performing the docking analysis, all protein and molecular files were converted into PDBQT format, with water molecules and polar hydrogen atoms excluded. To allow unrestricted molecular mobility, the grid box was positioned to encompass the domains of each protein. In this simulation, the grid box dimensions were 110 Å × 120 Å × 120 Å, with a grid point spacing of 0.05 nm. Semi-flexible docking was used, and the number of dockings was 30. To verify the correctness of the docking process, three protein-ligand complexes with known docking results were randomly selected from the PDBbind database, and the above method was repeated, and the results obtained were the same as the known results.

The docking analyses were carried out using Autodock Vina 1.2.2 (http://autodock.scripps.edu/), which provided valuable insights into the binding affinities and modes of interaction between the drug candidates and their targets, suggesting their potential use in the treatment of GBM based on their interaction with PLP2.

Statistical Analysis

All statistical analyses were conducted using SPSS 22.0 and R 4.2.1 software. The results are presented as the mean ± standard deviation (SD). The distribution of variables was assessed using Shapiro–Wilk tests. If the data followed a normal distribution, Student's *t*-test or one-way ANOVA was employed for data analysis. Conversely, when the data did not exhibit a normal distribution, Mann–Whitney tests were used. Survival curves were estimated using the Kaplan-Meier method, and the Log rank test was utilized for comparison. Correlation analyses were conducted using either Pearson Correlation Coefficient or Spearman rank-order correlation. All tests were two-sided, and statistical significance was considered with P-values < 0.05. The level of statistical significance is denoted as follows: ns (not significant), *P < 0.05, **P < 0.01, ***P <0.001.

Results

PLP2 Expression in Cancerous via Online Databases

We conducted an investigation to explore the possible roles of PLP2 by examining its expression patterns in 33 different types of human cancers. Our results showed that the PLP2 gene was significantly upregulated in 21 cancer types, including Adrenocortical carcinoma (ACC), breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Cholangiocarcinoma (CHOL), Colon adenocarcinoma (COAD), Esophageal carcinoma (ESCA), Glioblastoma multiforme (GBM), Head and Neck squamous cell carcinoma (HNSC), Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Acute Myeloid Leukemia (LAML), Brain Lower Grade Glioma (LGG), Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma (LUAD), and Lung squamous cell carcinoma (LUSC) (Figure 2A). However, we observed no significant difference in PLP2 expression levels in Bladder Urothelial Carcinoma (BLCA) or Thymoma (THYM). In contrast, our analysis revealed that the PLP2 gene was significantly downregulated in Lymphoid Neoplasm Diffuse Large B-cell Lymphoma (DLBC), Pheochromocytoma and Paraganglioma (PCPG), Prostate adenocarcinoma (PRAD), Skin Cutaneous Melanoma (SKCM), Testicular Germ Cell Tumors (TGCT), Uterine Corpus Endometrial Carcinoma (UCEC), and Uterine Carcinosarcoma (UCS).

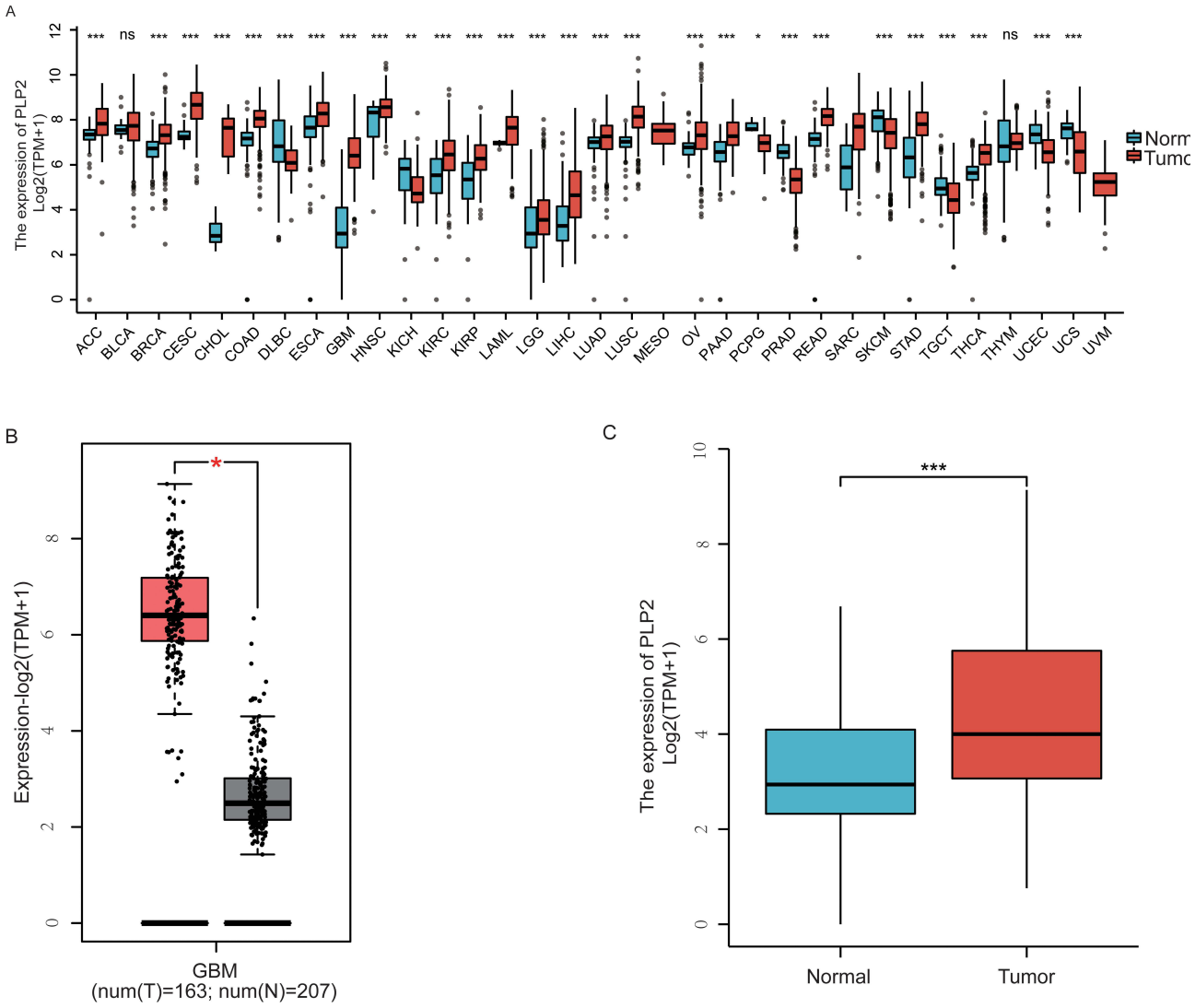

Figure 2 PLP2 expression in cancerous. (**A**) PLP2 expression in 33 types of human cancer. (**B**) Expression of PLP2 between GBM and paired normal brain tissues based on the GEPIA2 database. *P < 0.05, **P < 0.01, ***P <. 001, ns (no significance) P>0.05. (**C**) PLP2 expression was upregulated in GBM using TCGA dataset analysis.

To further validate our findings, we analyzed the expression levels of PLP2 in GBM using the GEPIA2 database, which consisted of 163 GBM samples and 207 normal tissue samples. Our results showed that PLP2 mRNA expression was significantly more abundant in GBM than in normal tissue (Figure 2B). Additionally, data from The Cancer Genome Atlas (TCGA) revealed that PLP2 was overexpressed in GBM (Figure 2C).

In summary, our results suggest that PLP2 may play a role in the development and progression of several human cancers. These findings warrant further investigation into the underlying mechanisms and potential therapeutic implications.

PLP2 Survival Analysis via Online Database

To examine the function of PLP2 in GBM, we conducted an investigation into the relationship between its expression and patient survival prognosis. Initially, we analyzed survival data from the GEPIA2 database (Figure 3A). Subsequently, we evaluated the prognostic significance of PLP2 in GBM patients through the TGCA database (Figure 3B). The results showed that the overall survival of the patients was shorter in the high PLP2 expression group than in the low PLP2 expression group, and the difference was statistically significant. (p=0.046, HR=1.4)

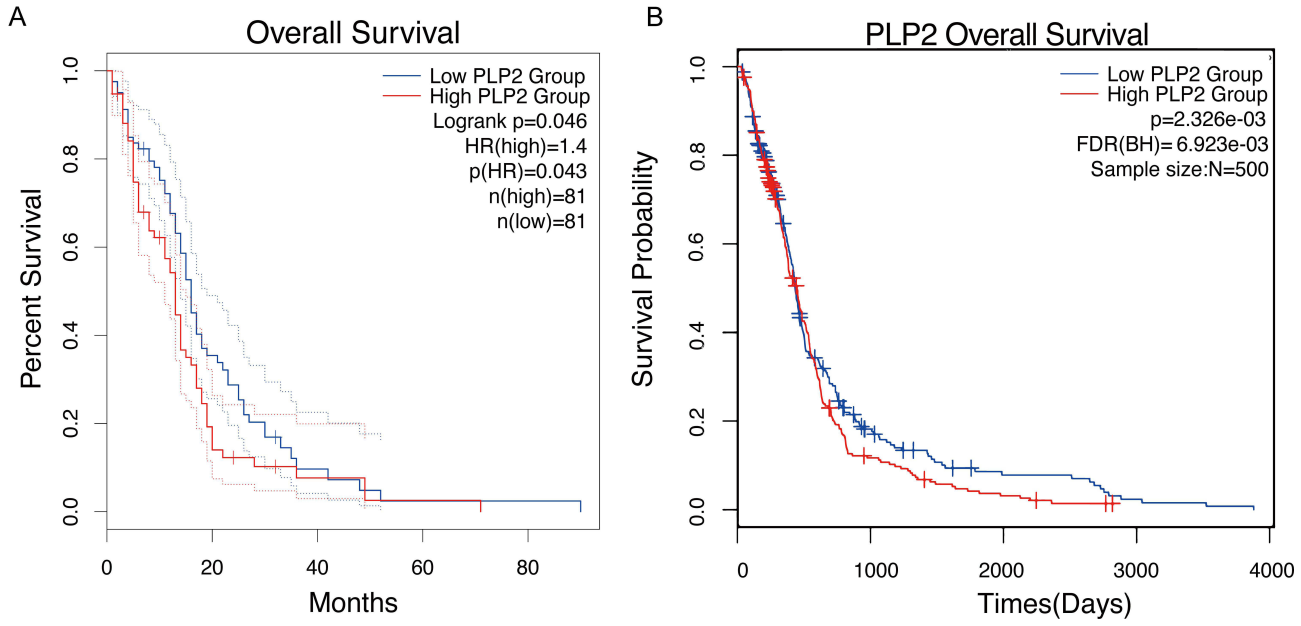

Figure 3 Survival analysis of PLP2 in GBM. (**A**) OS curve of PLP2 in GBM patients in GEPIA2 dataset. (**B**) OS curve of PLP2 in GBM patients in TCGA.

Functional Enrichment Analysis of PLP2

The analysis of genes based on Gene Ontology (GO) includes the study of biological process, cell composition, and molecular function (as shown in Figure 4I). Based on a GO biological process analysis, PLP2 gene expression is primarily enriched in leukocyte migration, regulation of leukocyte migration, mononuclear cell migration, macrophage migration, and T cell extravasation. In terms of cell composition, differentially expressed genes (DEGs) play a role in ion channel complex, phagocytic vesicle, and chloride channel complex. Molecular functions of these genes show their main involvement with ion channel activity, carbohydrate binding, and gated channel activity. Based on these results, DEGs play a significant role in ion channel and Immunity.

According to KEGG analysis, these genes are enriched in pathways associated with proteoglycans in cancer, focal adhesion, TNF signaling pathway, complement and coagulation cascades, and NF-kappa B signaling pathway (as shown in Figure 4J).

Identification and Validation of PPI Network and the Hub Genes

Figure 4A illustrates that the PPI network comprises 41 nodes, which was constructed using Cytoscape 3.7.1 and the STRING database. To investigate the associations between PLP2 expression and the hub genes that are most closely linked to PPI, we utilized the cBioPortal and GEPIA2 database. Our analysis revealed a positive correlation between PLP2 expression and IRF7 (rho=0.224, p=0.004), IRF3 (rho=0.279, p<0.001), BCL2L1 (rho=0.221, p=0.004), and PPP1R15A (rho=0.448, p<0.001). Conversely, a negative correlation was observed between PLP2 expression and CCT3 (rho=−0.212, p=0.006), UVRAG (rho=−0.269, p<0.001) and SH2D3C (rho=−0.268, p<0.001) as depicted in Figure 4B–H.

Correlation Between PLP2 and Immune Cells Infiltration

As a component of our investigation, we conducted an assessment of the immune cell infiltration status in glioblastoma multiforme (GBM) with the aim of elucidating the potential interrelationship between PLP2 expression and this condition. Drawing upon data from The Cancer Genome Atlas (TCGA) and a sample of 349 GBM cases, Figure 5A illustrates the observed differences in immune infiltration between the high and low PLP2 expression groups. Our analysis of the single-sample gene set enrichment analysis (ssGSEA) data revealed a significant correlation between PLP2 expression and various immune cell types, including NK cells, Neutrophils, Macrophages, Th1 cells, Tgd, TFH, iDC, DC, B cells, aDC, Treg cells. (Figure 5A).

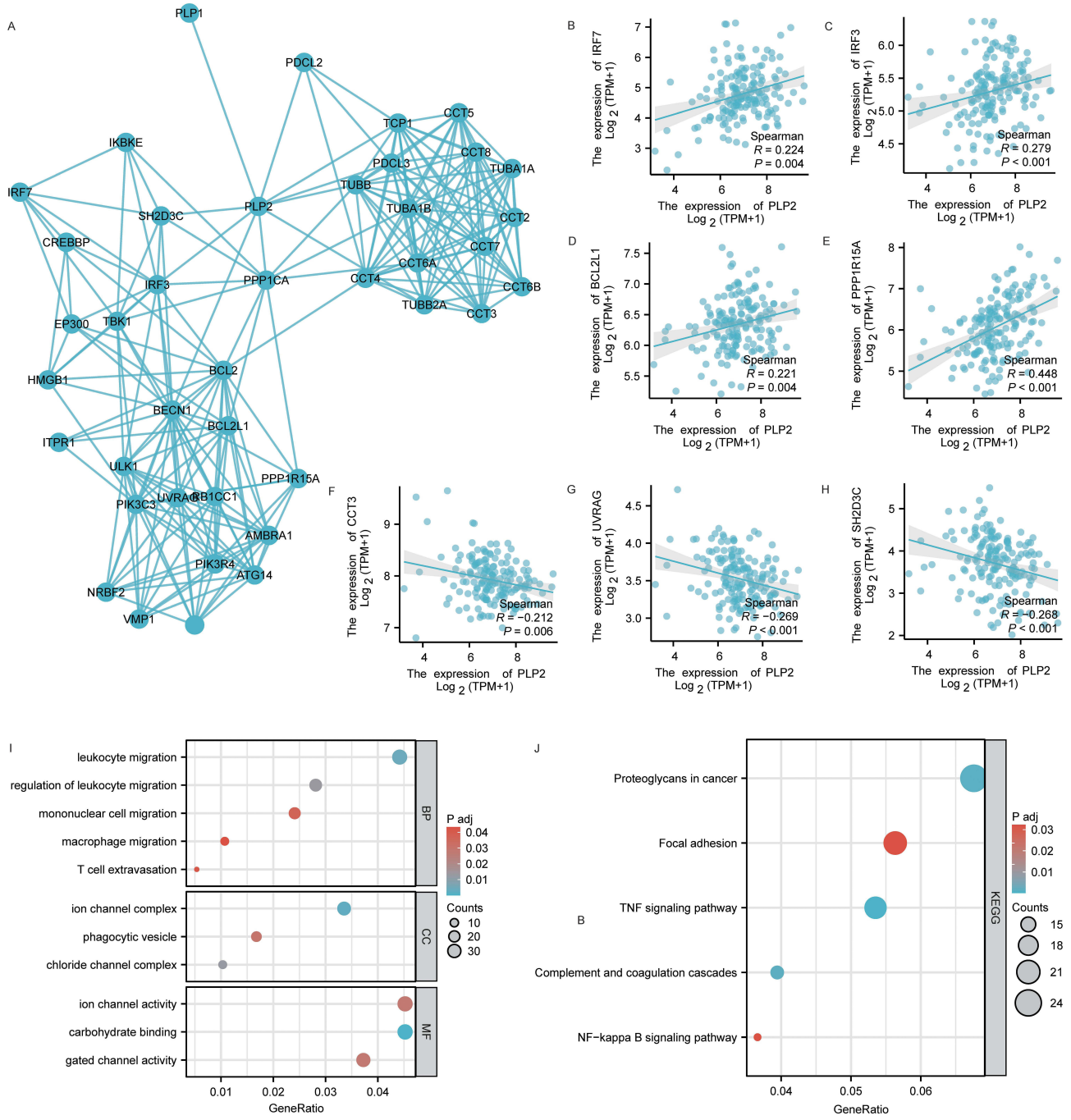

Figure 4 Functional enrichment analysis, PPI Network and the Hub Genes (**A**) The PPI network of PLP2 co-expression genes. (**B–H**) The expression of PLP2 was significantly correlated with the top hub genes. (**I**) GO enrichment analysis. (**J**) KEGG enrichment analysis.

PLP2 Expression with Immune Infiltration Status

The expression of PLP2 was found to have a significant positive correlation with the infiltration of immune cells in glioblastoma (GBM) tissue samples. Specifically, PLP2 expression was positively correlated with the infiltration of CD4+ T cells (rho=0.27, p=0.001), neutrophils (rho=0.31, p=9.51e-05), myeloid dendritic cells (rho=0.48, p=4.08e-10), and macrophages (rho=0.2, p=0.016), as shown in Figure 5B–G.

Next, we investigated the association between PLP2 and PDL1 expression in GBM tissues by cBioPorta (Figure 6A) l. Using immunohistochemistry (IHC), we detected the expression of both PLP2 and PDL1 in GBM tissues from our

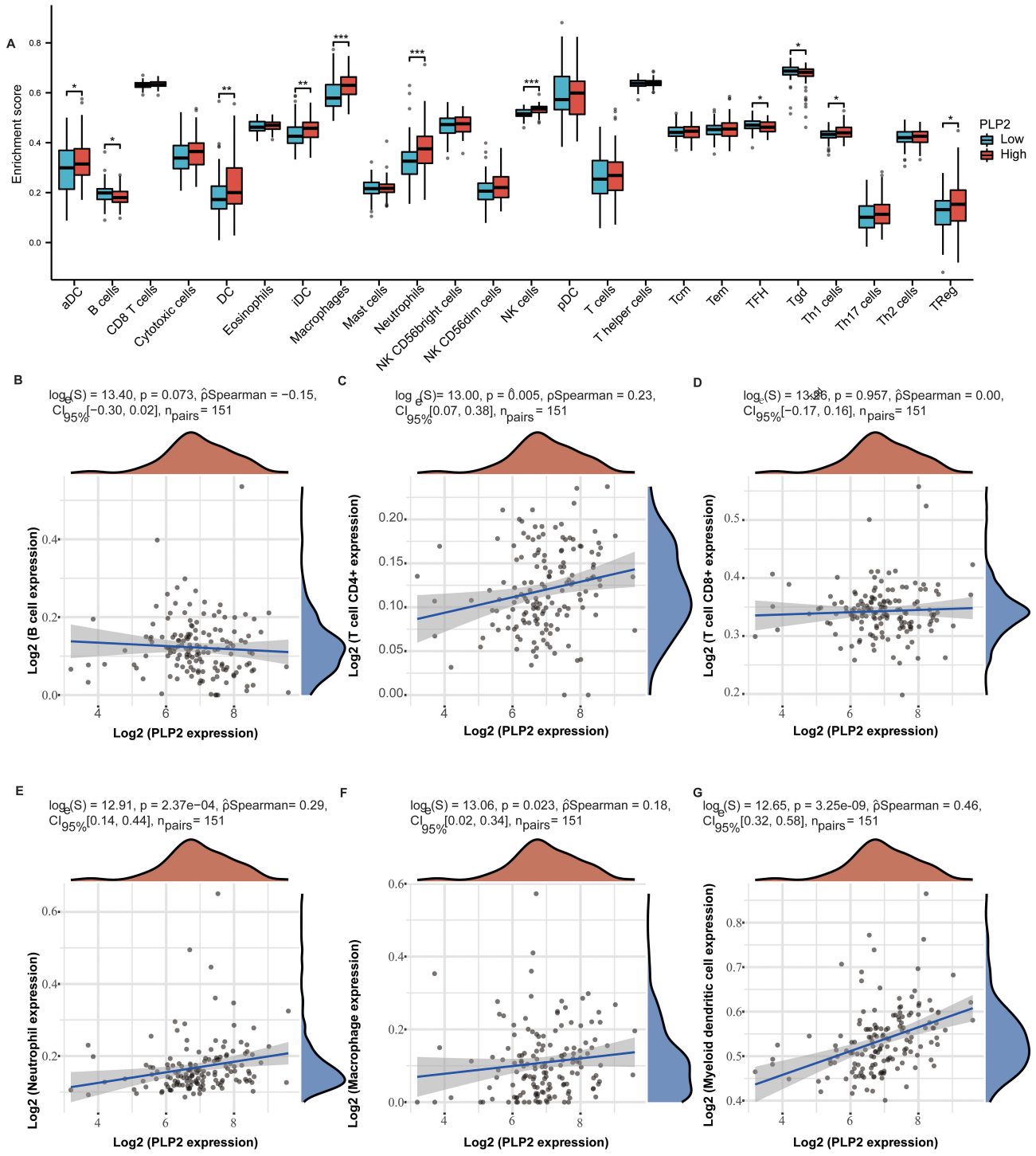

Figure 5 The proportion of immune cells was associated with the expression of PLP2 in TCGA dataset. ns: no significance, *P < 0.05, **P < 0.01, ***P < 0.001.(**A**) Analysis of the proportion of immune cells from ssGSEA. (**B**–**G**) The correlation between tumor immune infiltrated cell levels and PLP2 expression in GBM.

hospital. (Figure 6B–G) We did a total of 54 pathology slides, 40 of which showed PLP2 positive or half-positive, and of those 40, 35 showed PD L1 positive or half-positive. The results showed a considerable increase in the expression of both PLP2 and PDL1 in GBM tissues (p<0.05), indicating a positive correlation between their expression levels.

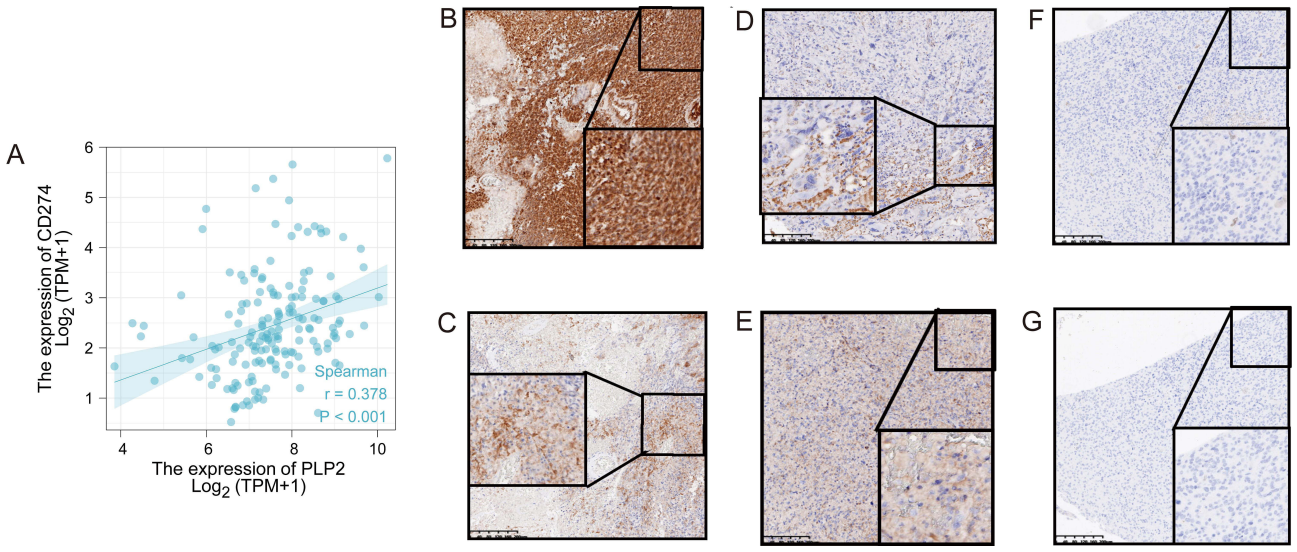

Figure 6 Correlation between PLP2 and PDL1 expression. (**A**) The expression of PLP2 was significantly correlated with the PDL1. (**B** and **C**) PLP2 and PDL1 immunohistochemical positive. (**D** and **E**) PLP2 and PDL1 immunohistochemical half positive. (**F** and **G**) PLP2 and PDL1 immunohistochemical negative.

Molecular Docking

In order to assess the binding affinity of the potential pharmaceutical agents to their respective targets, a molecular docking analysis was conducted. The Autodock Vina v.1.2.2 software was utilized to obtain the binding poses and interactions between the drug candidates and PLP2. The findings indicated that the drug candidates formed visible hydrogen bonds, engaged in hydrophobic interactions, and underwent π-stacking (perpendicular) with their protein targets. Moreover, the drugs demonstrated proficient occupancy of the hydrophobic pockets of every target. For PLP2, Praziquantel and Ethosuximide are the two drug candidates whose binding energies for the top ten docking results are Praziquantel: −6.1 kcal/mol,-6.11 kcal/mol, −6.222 kcal/mol, −6.228 kcal/mol, −6.238 kcal/mol, −6.529 kcal/mol, −6.605 kcal/mol, −6.763 kcal/mol, −7.441 kcal/mol and Ethosuximide:-3.69 kcal/mol, −3.99 kcal/mol, −3.588 kcal/mol, −3.677 kcal/mol, −3.758 kcal/mol, −3.918 kcal/mol, −3.3731 kcal/mol, −4.142 kcal/mol, −4.295 kcal/mol, suggesting that their binding is very stable.(Figure 7A–F)

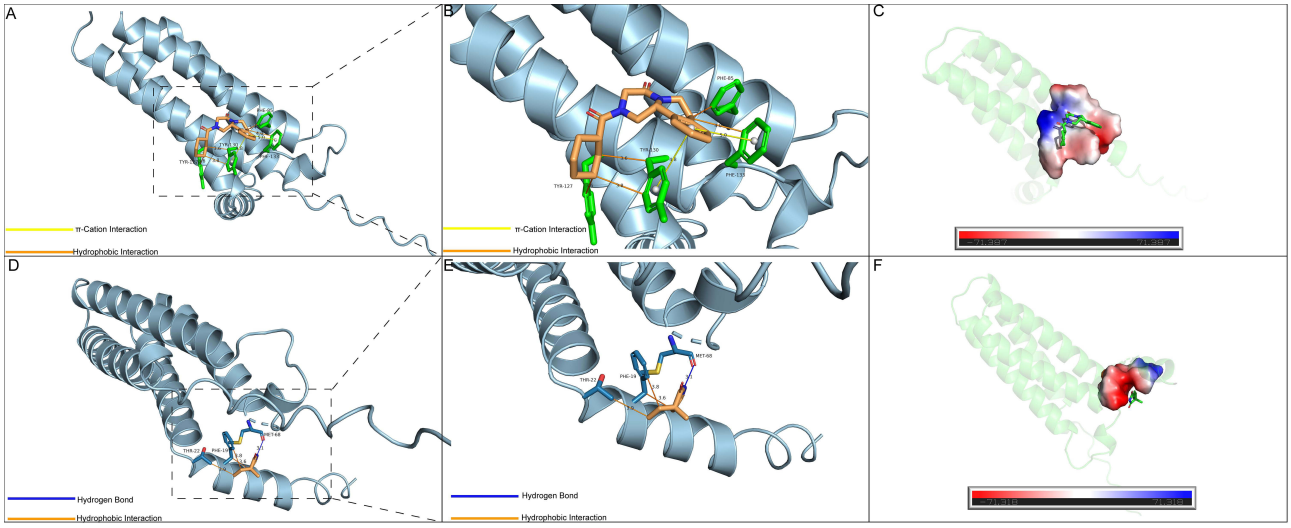

Figure 7 Drugs' molecular docking mode of binding to their targets. (**A** and **B**) Binding mode of Praziquantel to PLP2. (**C**) PyMOL software provided a three-dimensional view of the binding pockets. (**D** and **E**) Binding mode of Ethosuximide to PLP2. (**F**) PyMOL software provided a three-dimensional view of the binding pockets.

Discussion

Glioblastoma, or glioblastoma multiforme (GBM), is a prevalent and highly malignant brain tumor in adults. Despite recent therapeutic advancements, favorable outcomes remain infrequent, and the prognosis and quality of life for GBM patients are typically unfavorable. Despite significant efforts, there has been limited progress in prolonging the survival of GBM patients.

PLP is a hydrophobic integral membrane protein that constitutes about 50% of the protein content of myelin in the adult central nervous system. The PLP gene encodes a 276-amino acid polypeptide consisting of five strongly hydrophobic membrane-spanning domains that interact with myelin lipid bilayers and contribute to the stability of myelin cells.[17] This protein is exclusively found in the mammalian CNS and primarily involved in membrane contact.[18] Moreover, it has been commonly assumed that PLP plays a crucial role as a structural component of the intraperiod line, due to the presence of its extracellular loops in this region.[19]

PLP2 is a type of proteolipid located in the endoplasmic reticulum of colonic epithelium, which exhibits ion channel characteristics and undergoes multimerization. This protein has been demonstrated to facilitate cell growth, proliferation, and migration in various cancers, such as colorectal cancer,[20] clear cell renal cell carcinoma,[10] Multiple myeloma,[21] Ovarian carcinoma,[22] esophageal squamous cell carcinoma,[23] Estrogen-dependent cancers[4] We examined data from multiple databases to study PLP2 expression and related gene functions in GBM. We found that GBM tissues had higher PLP2 expression than normal tissues, which was associated with worse survival.

We constructed PPI network and carried out subsequent analysis based on the PPI network Using GEPIA2 and cBioPorta, we identified 7 real hub genes that correlated with PLP2 expression: IRF7 ($p=0.004$), IRF3 ($p<0.001$), BCL2L1 ($p=0.004$), PPP1R15A ($p<0.001$), CCT3 ($p=0.006$), UVRAG ($p<0.001$), SH2D3C ($p<0.001$). Among the 7 hub genes, IRF3, IRF7, BCL2L1 and PPP1R15A are positive correlation hub genes. CCT3, UVRAG and SH2D3C are negative correlation hub genes.

IRFs, or Interferon Regulatory Factors, represent a class of transcription factors that modulate the expression of genes containing interferon (IFN) motifs.[24] The IRF family has a variety of functions including, but not limited to, apoptosis, oncogenesis, host defense, and viral latency.[25,26] Both IRF3 and IRF7 play critical roles in the production of IFN. These two genes have structural and sequence similarities and may have derived from one ancestral gene.[27]

IRF3 is retained in the cytoplasm, and when stimulated by upstream signals, such as viral infections and double-stranded RNA (ds RNA), IRF3 is activated by phosphorylation and translocated into the nucleus, where it binds with the coactivator CBP/p30 and induces the expression of IFNa/p and IFN stimulatory genes, thereby exerting its anti-infective and immunological effects.[28–30] IRF3 plays a role in tumors by regulating different pathways. For example, in gastric cancer, IRF3 mainly regulates the Hippo and TLR4/I RF3 signaling pathways.[31–33] In melanoma, I RF 3 mainly regulates the expression of IFN-γ.[34,35] In breast cancer, IRF3 regulates STING, TLR3 and TLR4-mediated signaling pathways.[36–41] In ovarian cancer, IRF3 is involved in the regulation of the signaling pathway mediated by STING[42] and TLR4.[38]

In glioblastoma, IRF3 mainly regulates the STING signaling pathway.[43,44] The cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway has emerged as a critical innate immune pathway that, following engagement by DNA, promotes distinct immune effector responses that can impact virtually all aspects of tumorigenesis, from malignant cell transformation to metastasis.[45]

IRF7 also plays an important role in tumour development and metastasis.[46] Although IRF7 inhibits the growth and metastasis of breast cancer[47] and melanoma,[48] it promotes glioblastoma. IRF7 is a major regulator of the inflammatory response in GBM, upregulates the expression of CCL2, CXCL1, and IL-6, and promotes the progression and invasion of GBM and induces GBM cells to acquire tumor stem cell properties.[49–51] Currently, IRF-7 and IL-6 have emerged as independent factors affecting the probability of overall GBM survival.[51]

BCL2L1 is a member of the BCL2 protein family. The BCL2 family proteins are situated on the external membrane of the mitochondria and are recognized as pivotal regulators in the apoptotic response, exerting control over the permeabilization of the mitochondrial membrane. These members of the BCL2 family were initially identified as inhibitors of cell death and regulators of apoptosis.[52] Resisting cell death is a hallmark of cancer as well as an essential feature of acquired drug resistance.[53–55] To maintain their growth and survival, cancer cells often modulate cell death mechanisms by overexpression

of anti-apoptotic BCL2 family members, including BCL2, BCL2L1 and MCL1. Currently, clinical trials are investigating the potential of drugs that act as BCL2 inhibitors as a promising approach to manage malignancies.[56]

Protein phosphatase 1 regulatory subunit 15A (PPP1R15A), also known as growth arrest and DNA damage-inducible protein 34 (GADD34),[57] plays a pivotal role in a fundamental biological process within mammalian cells known as the integrated stress response (ISR).[58] This process, which is evolutionarily conserved, can be linked to both the unfolded-protein response (UPR) and the activation of Heme-regulated eukaryotic translation initiation factor 2α (eIF2α) kinase (HRI).[59,60] Activation of the ISR can be triggered by stimuli from both the endoplasmic reticulum (ER) and the cytosolic lumen,[61] highlighting its critical importance for cellular, tissue, and organismal adaptation to varying environmental conditions and the maintenance of homeostasis.[62] PPP1R15A interacts with the catalytic subunit of protein phosphatase 1 (PP1c) to facilitate the dephosphorylation of eIF2α.[63,64] This phosphorylation and dephosphorylation of eIF2α is a key regulatory mechanism in the ISR.[62,65] When eIF2α is phosphorylated, global protein synthesis is attenuated, leading to the activation of the transcription factor ATF4,[66] which promotes cellular survival and recovery.[67,68] Conversely, eIF2α dephosphorylation enables the cell to resume normal protein synthesis processes. ATF4 can form a complex with the basic region/leucine zipper motif (bZIP) transcription factor, specifically, CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP).[69,70] This ATF4-CHOP complex is critically involved in various aspects of mammalian autophagy, including the induction of autophagy and the activation of autophagy-related genes[69,71] like activating transcription factor 3 (ATF3), PPP1R15A, and Tribble's pseudo kinase 3 (TRIB3).[72] Given the crucial role of the integrated stress response (ISR) in mammalian cells, its impact extends to a wide array of cell types and diseases. Numerous studies have underscored the involvement of the ISR in cognitive and neurodegenerative disorders,[73–75] metabolic disorders,[76,77] and various types of cancers.[78,79] The influence of the ISR on cancer appears to be dual, as induced hypoxia can either trigger apoptosis in tumor cells or bolster the growth of tumor cells adapted to hypoxic conditions.[80,81]

Moreover, the ISR exerts a profound influence on mammalian immunity. Research has demonstrated its ability to modulate the innate immune response[82,83] and the secretion of specific cytokines such as interleukin-1β (IL1β) and interleukin-6 (IL6).[84,85] These effects are closely tied to the phosphorylation and dephosphorylation dynamics of the eIF2 complex,[86] notably the eIF2α subunit. Thus, PPP1R15A plays a role in tumor and immunity.

As for negative correlation hub genes. They play an important role in normal cellular functions. Chaperonin-containing T-complex protein-1 (CCT) is a chaperonin composed of two stacked identical complexes, each consisting of eight different subunits (CCT1-8).[87] CCT, belonging to the HSP60 family group II, plays a crucial role in various essential biological processes, including the cell cycle and cytoskeleton formation.[88–90] In contrast, CCT3 acts as a molecular chaperone complex that helps protein folding during ATP hydrolysis and mediates WRAP53/TCAB1 folding, thereby regulating telomere maintenance.[91] UVRAG (UV radiation resistance-associated gene) was originally discovered and identified in a UV-sensitive gene screen for xeroderma pigmentosum (XP) cells. It is now found to be associated with autophagy,[92–94] apoptosis,[95] and maintenance of gene stability.[96] What's more, UVRAG was considered as a tumor suppressor.[97,98] The function of SH2D3C has been less well studied, with reports suggesting that it promotes cell migration and invasion through the extracellular mesenchyme[99] and can act as an interface protein for cell signaling pathways regulated by the immune response.[100]

The analysis of hub genes reveals interesting insights into the potential role of PLP2 in tumorigenesis and the tumor microenvironment. Genes that are positively associated with PLP2 appear to be primarily involved in tumour-related processes and pathways, including immune responses, apoptosis, and autophagy. This suggests that PLP2 may play a role in these processes within the context of tumorigenesis.On the other hand, genes that are negatively associated with PLP2 are mostly linked to normal cellular functions. This contrast suggests that PLP2's influence may shift the balance towards tumorigenesis-related processes and away from normal cellular functions.Furthermore, PLP2 might have an impact on the immune system's response to tumors. This is a crucial aspect of cancer biology, as the immune system can either promote or inhibit tumor growth, and understanding how PLP2 influences immune responses in the tumor microenvironment could have important implications for cancer research and therapy.

In summary, the analysis suggests that PLP2 may be associated with tumorigenesis and the modulation of immune responses within the tumor microenvironment, potentially making it a relevant target for further investigation in cancer research.

Based on the results of our present GO enrichment analysis, PLP2 may be involved in leukocyte migration (corrected p-value= 0.00385774321662888), especially mononuclear (corrected p-value=0.0372230201942272) cell and macrophage (corrected p-value=0.0433053710693439). The migration of leukocytes, a natural phenomenon, occurs from the bloodstream to the surrounding tissue by crossing the vascular barrier. This process serves as a defense mechanism against pathogenic invasions and may provide insights into the tumor microenvironment.[101] Moreover, leukocyte migration plays a crucial role in facilitating an effective immune response against tumors.[102] We also found PLP2 participates in ion channel (corrected p-value=0.00242844132322669), especially chloride channel complex (corrected p-value=0.0125402382709211). Multiple electrophysiological studies conducted in the 1990s have documented the existence of a minimum of three distinct Cl− channels that are activated by osmosis in human T lymphocytes.[103] Additionally, the subunit LRRC8A of the Cl− channel has been identified as crucial for the development of T cells.[104] Furthermore, various Cl− channels have been observed to play a role in the functioning of lymphocytes, potentially influencing T cell apoptosis regulation.[3]

In light of the analysis of hub genes associated with PLP2 and the enrichment of Gene Ontology (GO) functions, it becomes evident that PLP2 plays a pivotal role in the realm of immunity. Consequently, our study delved into the intricate relationship between PLP2 and immune cell infiltration. The findings of our investigation reveal a compelling positive correlation between PLP2 expression and various immune markers, as well as the infiltration levels of immune cells within glioblastoma (GBM).

Specifically, employing single-sample Gene Set Enrichment Analysis (ssGSEA), we observed that heightened PLP2 expression was concomitant with increased levels of immune cells, including NK cells, neutrophils, macrophages, Th1 cells, Tgd cells, TFH cells, immature dendritic cells (iDC), mature dendritic cells (DC), B cells, activated dendritic cells (aDC), and regulatory T cells (Treg). Furthermore, analysis conducted through the TIMER algorithm corroborated these findings, indicating that elevated PLP2 expression was associated with heightened levels of macrophages, neutrophils, NK cells, and CD4+ T cells.

Macrophages are key phagocytes in host defence against endogenous or exogenous pathogens in innate immunity. In tumors, the main function of macrophages is not tumor killing, but rather inducing immune dysfunction by promoting angiogenesis at the site of tumorigenesis, metastasis of tumour cells,[105,106] chemoresistance, and interaction with other immune cells in the tumor microenvironment, and by recruiting other immune-suppressive cells, resulting in the escape of tumour cells from the immune system.[107] It has also been shown that the main immune cell type expressing PD-L1 is the macrophage in the tumor microenvironment.[108] For glioblastoma, the degree of macrophage enrichment is closely related to its grade and poor prognosis,[109] and glioma stem cells have been shown to recruit macrophages that accumulate at the tumor site releasing a large number of factors that stimulate tumor growth and invasion and increase the invasion of glioma stem cells that promote tumour vascularity,[110,111] and it has been demonstrated that the number of macrophages in the perivascular area correlates positively with the density of capillaries in the GBM.[112,113]

Neutrophils are the most abundant leukocytes in the circulatory system, originating from hematopoietic stem cells in the bone marrow, accounting for 50% to 70% of all circulating leukocytes in humans, and as a key factor in the tumor microenvironment, they play an important role in tumor progression.[114,115] As a key factor in the tumor microenvironment, it plays an important role in tumor progression, and there is increasing evidence that neutrophils are involved in the inflammatory response to tumorigenesis and proliferation, immunomodulation, pro-angiogenesis, and tumor cell metastasis and invasion.[1]

CD4+ T cells can assist in the activation of other immune cells. Initial CD4+ T cells (TH0) proliferate and differentiate into various effector cell subpopulations: TH (T helper) 1, TH2, TH17 and regulatory T cells (Tregs). The inhibitory function of Treg cells is achieved through a variety of mechanisms; it secretes inhibitory cytokines (TGFD, IL-10, etc),[116,117] releases perforin and granzymes to kill effector cells,[118,119] interferes with the metabolic pathways of effector cells,[120,121] and regulates the inhibitory properties of DC cells.[117]

The activation and maturation of DCs depends on the local microenvironment, and DC maturation can be inhibited by inhibitory factors in the microenvironment, resulting in the formation of a subpopulation of DCs with tolerogenic and immunosuppressive activity, which can then be used to promote immune tolerance. Treg cells, mentioned above, can induce DC immune tolerance, which reduces the contact time between effector T cells and DCs prior to effector T cell activation.[122] And this means that DC cells are the key to Treg cell suppression of anti-tumor immune responses

target.[123] It also blocks CD80 and CD86 expression during DCs maturation.[124] Finally, Treg cells can regulate the production of cytokines in DCs, Treg cells can inhibit the production of IL-6 and promote the production of IL-10.[125] Similarly, in the presence of the tumor microenvironment, DCs can inhibit T cell proliferation.[126] It can also inhibit T cell activation by inducing these inhibitory factors such as nitric oxide, IL-10, or IDO, leading to immune tolerance.[126–128]

Natural killer cells are one of the components of the tumor microenvironment that infiltrate and exert corresponding immune effects, and they are the body's intrinsic immune cells, playing an important role in anti-tumour, anti-viral infection, and immune regulation. It is possible that there are special subpopulations of NK cells that have just not been clearly typed at this time. For example, there is a subpopulation of natural killer cells that express CD57, and CD57+ NK cells have higher cytotoxicity, lower cytokine responsiveness, and lower proliferative capacity; however, these CD57+ cells do not appear to be able to inhibit the growth of malignant tumor cells and may even inhibit immune responses to tumor-associated antigens, potentially competing for resources such as cytokines or nutrients.[129–131] A similar subpopulation of NK cells may exist in glioblastoma, functioning to suppress tumour immunity.

These results collectively suggest a substantial involvement of PLP2 in modulating the immune microenvironment of GBM, potentially influencing disease progression and therapeutic strategies. As mentioned earlier, PLP2 is associated with immunosuppressive cells, and if PLP2 is used as a therapeutic target, it will reduce the number of immunosuppressive cells in the tumor milieu, thus improving tumor immunosuppression, and thus inhibiting tumor development.

Next, we analyzed PLP2-related pathways using KEGG We found that the proteoglycans in cancer (corrected p-value= 0.00127044286008173), focal adhesion (corrected p-value= 0.0322796416654873), complement and coagulation cascades (corrected p-value= 0.00152255010659654), NF-kappa B signaling pathway (corrected p-value= 0.0322796416654873) and TNF signaling pathway (corrected p-value= 0.0000855856148414451) were involved. Proteoglycans play a pivotal role in cancer and angiogenesis by exerting diverse functions. This can be attributed to their complex structure and their ability to interact with various ligands and receptors that govern tumor growth and the formation of new blood vessels.[132,133] Specifically, they contribute to the creation of a provisional matrix that facilitates tumor growth and influences interactions between cells and the extracellular matrix, as well as signal transduction within tumor cells. Additionally, proteoglycans regulate the phenotype of tumor cells and angiogenesis in the tumor stroma.[134] Kanteti et al have further indicated that focal adhesion kinase plays a crucial role in determining the phenotype of tumor cells, including their survival, proliferation, migration, and invasion abilities.[135] The coagulation and complement systems are separate entities that play distinct pathophysiological roles, serving as innate defense mechanisms against external threats.[136] The cascades of complement and coagulation mutually induce each other, creating a vicious cycle that can hinder immune cells from targeting cancer cells. This process promotes immune evasion, ultimately leading to tumor progression and metastasis.[137,138] The TNF signaling pathway plays a crucial role in various cellular processes including cell growth, proliferation, inflammation, and immunity. Upon activation, this pathway triggers the translocation of NF-κB into the nucleus, thereby facilitating the synthesis and secretion of inflammatory factors such as TNF-α, IL-8, and IL-6.[139] These TNF inflammatory cytokines, which are highly abundant in the tumor microenvironment, exert significant influence on tumor growth, disrupt the equilibrium between cell proliferation and apoptosis, and impair the innate immune response against cancer cells.[140]

Our findings strongly indicate that PLP2 is intricately associated with various immune cells within the tumor microenvironment, including immunosuppressive macrophages and regulatory T cells (Treg cells). Furthermore, functional enrichment analysis of PLP2 has revealed its close linkage with processes such as immune cell migration and proteoglycans in tumors. These associations suggest that PLP2 may exert its influence on the migration of immune cells, potentially affecting key immune pathway components or modifying proteoglycans within tumor polysaccharides. These actions could, in turn, have a significant impact on immune cell infiltration within the glioblastoma tumor microenvironment, ultimately contributing to the promotion of GBM development.

These findings offer valuable insights into the intricate interactions between PLP2 and the immune microenvironment in GBM. They hold substantial clinical implications, where PLP2 could potentially serve as a therapeutic target to counteract the immunosuppressive state of the tumor. Alternatively, it could be utilized in combination with other drugs to enhance the efficacy of existing treatments. Consequently, we would like to present our perspectives on harnessing the therapeutic potential of PLP2 for the treatment of glioblastoma.

Immunotherapy, particularly PD-1/PD-L1 inhibitors, has shown promise in treating GBM as it has in other cancers due to its modulation of the tumor-immune system interaction.[141] To examine the potential of PD-1/PD-L1 inhibitors in GBM, we analyzed bioinformatic data from the TCGA databases. Our study found a positive correlation between PLP2 and PD-L1 expression.

To validate our bioinformatic findings, we used an IHC assay to detect PLP2 and PD-L1 expression in GBM tissue. The results showed that PLP2 expression was positively correlated with PD-L1 expression. This correlation suggests the potential use of PD-1/PD-L1 inhibitors for GBM treatment.

These findings open up new avenues for developing immunotherapeutic strategies against GBM and provide a new perspective on the relationship between PLP2 and PD-L1 in the tumor microenvironment. Further studies are necessary to confirm our findings and explore the underlying mechanisms of PLP2 and PD-L1 interaction in GBM.

Through the utilization of the Drugbank database, chemical compounds targeting PLP2 were identified. Among these compounds, their ability to cross the blood-brain barrier was analysed, and a number of compounds were finally screened for successful docking with PLP2 through a secondary screening of simulated molecular docking, and among the compounds remaining in the final screening, the researchers paid special attention to praziquantel and ethosuximide. Praziquantel, an anti-parasitic agent, has demonstrated efficacy in treating various forms of schistosomiasis. Notably, PZQ has been reported to augment both humoral and cellular immune responses in the host against diseases.[142,143] Wu reported that PZQ exhibits synergistic effects with paclitaxel, resulting in the inhibition of cancer cell growth.[40,144] Whereas in the previous study we demonstrated that PLP2 is closely related to the immune response, we put our focus on PZQ. Based on the ion channel properties of PLP2, we wanted to find a drug that could act on ion channels and could cross the blood-brain barrier, so we chose ethosuximide. Although ethosuximide is commonly prescribed for the treatment of epilepsy, its efficacy in tumor treatment remains unproven. We performed a preliminary screening of compounds that might act on PLP2 and selected compounds of interest for molecular docking to initially verify the feasibility of the interaction. We hope that these two drugs can improve the state of tumour immunosuppression by acting on PLP2 to treat GBM.

Despite our promising findings, our study has several limitations. First, we solely relied on public databases to assess the correlation between PLP2 expression and immune infiltration markers in GBM. Future studies could incorporate functional experiments in cell lines or animal models to better understand the role of PLP2 in GBM.

Nevertheless, our study provides important insights into the potential of PLP2 as a biomarker for predicting the efficacy of immunotherapy in GBM. A better understanding of the biological functions of PLP2 in GBM could facilitate the development of novel immunotherapeutic strategies that could improve patient prognosis.

Conclusion

Our study found that PLP2 is upregulated in GBM, and its overexpression correlates with poor overall survival. We identified potential pathways involving leukocyte that may be impacted by PLP2.

Moreover, we observed a correlation between PLP2 and PD-L1 in GBM, suggesting a potential role for PD-1/PD-L1 inhibitors in treating GBM. Additionally, our study suggests that PLP2 could serve as a promising therapeutic target for GBM through the use of praziquantel and Ethosuximide.

Overall, our findings provide new insights into the pathogenesis and potential treatment strategies for GBM. Further studies are needed to fully elucidate the underlying mechanisms of PLP2 in GBM and assess the clinical utility of targeting PLP2 in GBM therapy.

Ethical Statement

Patients were consented by an informed consent process and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. This study has been approved by Qingdao University Affiliated Hospital Ethics Committee.

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Disclosure

The authors report no conflicts of interest in this work.

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