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Prognostic marker PTPN6 in glioma and its relationship with immune infiltration

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Abstract

Background & Objective: Glioma is a type of malignant tumor that develops in the neurological system, and identification of glioma biomarkers is critical. PTPN6 is a protein tyrosine phosphatase. The role of PTPN6 in glioma is unclear. This is the first study to investigate the expression of PTPN6 in glioma patients and its prognostic value, potential biological functions, and impact on the immune system. *Methods:* Gene expression and clinicopathological analysis, enrichment analysis, and immune infiltration analysis were based on The Cancer Genome Atlas (TCGA) data with additional bioinformatics analysis. Statistical analysis was performed using TIMER and ssGSEA to analyze the immune response to PTPN6 expression in glioma. In addition, CGGA, K-M survival analysis, and data from HPA were used to validate the results. *Results:* PTPN6 played a vital role as an independent prognostic factor in glioma patients. PTPN6 expression correlated with age, WHO grade, IDH status and 1p/19q codeletion. GSEA found that PTPN6 is closely related to cell adhesion, immunological. PTPN6 expression was positively correlated with infiltration of B cells, CD8+ T cells, CD4+ T cells, macrophages, dendritic cells, and neutrophils and was co-expressed with immune-related genes and immune checkpoints.
Conclusion: The expression of PTPN6 is increased in gl666ioma, and the high expression of PTPN6 is associated with poor prognosis. PTPN6 may affect tumor development by regulating tumor-infiltrating cells in the tumor microenvironment (TME). PTPN6 may be a potential target for immunotherapy.

Keywords: PTPN6, glioma, prognosis, tumor microenvironment, tumor immune cell infiltration

INTRODUCTION

Glioma is a type of malignant tumor that develops in the neurological system. Gliomas are classified into four classes (I, II, III, and IV) by the World Health Organization (WHO), with glioblastoma multiforme (glioblastoma, WHO grade IV) being the most frequent and aggressive strong malignant variety. Gliomas are the most prevalent and lethal malignant central nervous system tumors, with current treatments such as surgical resection, chemotherapy, and radiation therapy having minimal efficacy.[1] Immunotherapy, as represented by PD-1/PD-L1 inhibitors[2], provides a novel alternative for the treatment of glioma in light of recent findings indicating the crucial role of immunity in carcinogenesis and progression. However, due to the suppressive immunological milieu of gliomas and the intricacy of systemic immunosuppression, currently existing immunotherapies are inadequate for gliomas.[3,4] Because the pathophysiological mechanisms behind the formation of glioma are not fully understood, progress in glioma therapy has been limited. Identifying new therapeutic targets and developing glioblastoma treatment techniques will be aided by understanding the molecular mechanism of glioma development.

PTPN6 is a protein tyrosine phosphatase that is not a receptor and is hypothesized to be a master regulator of inflammation.[5] PTPN6 suppresses caspase-8 and Ripk3/Mlkl-dependent

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inflammation, according to studies.[6] Studies indicate that PTPN6 is a key player in regulating systemic inflammation.[7] By inhibiting syphilis kinase activity, PTPN6 prevents IL-1-induced neutrophilic skin disease.[8] Furthermore, inhibiting PTPN6 activated the STAT pathway, which worsened alumina nanoparticle-induced COPD in mice.[9] By dephosphorylating oncogenic kinases, it also works as a tumor suppressor. PTPN6 has been linked to the prognosis and progression of cancers such as liver cancer, renal cell carcinoma, and gastric cancer in previous studies.[10-12] Furthermore, PTPN6 improves chemotherapy efficacy and can be used in cancer immunotherapy when paired with blocking antibodies. However, the link between PTPN6 and glioma prognosis is unknown. Therefore, exploring the molecular mechanism of PTPN6 development in glioma will help identify new therapeutic targets and develop therapeutic strategies for glioma. PTPN6 is mostly found in hematopoietic and epithelial cells, and it is hypothesized to be implicated in pro-inflammatory chemicals in cancer.[13] Numerous signaling pathways can be negatively affected by PTPN6, according to studies.[14,15] PTPN6 regulates the cell cycle and cell proliferation in a variety of malignancies.[16,17] The PTPN6 gene, as well as its mRNA and protein expression, are downregulated in natural killer T-cell lymphomas and 95% of different other malignant lymphomas, according to Plutsky *et al*.[18] and loss of PTPN6 expression may cause tumors to become malignant. and a rise in intrusiveness. In advanced cases of chronic myeloid leukemia, breast cancer, and liver cancer, PTPN6 gene expression was down-regulated.[19] As a result, PTPN6 is frequently considered to be a tumor suppressor gene associated with the onset and progression of cancer.[20] Hepatocellular carcinoma growth has been demonstrated to be slowed by PTPN6, which has also been proven to suppress the activation of JAK/STAT, NF-B, and AKT signaling pathways. Furthermore, it has been demonstrated that PTPN6 increases apoptosis and reduces prostate cancer cell growth.[21] PTPN6, despite being regarded as a tumor suppressor gene, has been found to exhibit up- and down-regulation in a number of malignancies. These findings imply that the role of PTPN6 varies on the type of tumor and its biological environment. It may both promote and inhibit tumorigenic processes, including growth factor receptor downstream signaling and proliferation.

Sooman's research shows that PTPN6 expression may be a factor leading to poor survival in patients with de novo gliomas. In glioma - derived cells, its expression is regulated epigenetically and impacts the response to chemotherapy.[22]

In the present study, we identified the prognostic value of PTPN6 expression in glioma based on the TCGA database. In addition, we explored the potential molecular mechanisms of PTPN6 in glioma and the relationship between PTPN6 and immune cell infiltration in the microenvironment of glioma.

METHODS

Data collection

The experimental cohort primarily used the The Cancer Genome Atlas (TCGA) official website (http://tcgadata.nci.nih.gov/tcga/) to gather gene expression profiles and clinical information of glioma patients.[23]

We downloaded cancer-related RNA sequences, clinicopathological, and survival data of gliomas on the TCGA. GTEx (http://commonfund.nih.gov/GTEx/) contains publicly available gene expression data from the RNA sequencing of 54 normal tissue sites from about 1,000 individuals (GTEx Consortium, 2013). Normal samples from the GTEx database and tumor samples from TCGA were applied to compare the differential expressions of PTPN6 between cancer and normal tissue. Our study excluded samples with missing or insufficient data on age, WHO stage, OS time, IDH, and local invasion. We retained RNA-Seq and clinical data for further study. Our research complies with publication guidelines provided by the TCGA.

Validation cohort data were mostly gathered from the Chinese Glioma Genome Atlas (CGGA) website (http://www.cgga.org.cn/).

We created survival curves to confirm the variations in PTPN6 expression in various clinicopathological characteristics of gliomas.

Gene Set Enrichment Analysis (GSEA)

GSEA is a statistical test that determines if a group of genes displays statistically significant changes between two biological states. GSEA was initially used to produce an ordered gene list based on the association between all genes and PTPN6 expression in order to uncover the underlying mechanism by which PTPN6 expression impacts the prognosis of glioma patients. GSEA was used to explain the observed substantial survival differences between the high and low PTPN6 groups. The expression level of

PTPN6 was utilized as a phenotypic marker, and genome permutations were conducted 1,000 times each study. Significantly enriched gene sets were classified as those with normalized (NOM) $p < 0.05$and false discovery rates (FDRs) $p < 0.05$.

Correlation analysis of PTPN6 expression and immune cell infiltration in glioma

PTPN6 has been found to alter cancer prognosis by modulating immunological processes and changing the immune milieu of tumors as an immune-related gene. To study the association between PTPN6 expression and immune cell infiltration, we used the Tumor Immunity Estimation Resource (TIMER), a notable resource for thorough characterization of tumor-infiltrating immune cells.[24]

Users may estimate the makeup of six tumor-infiltrating immune cell subsets using the TIMER method (B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils, and dendritic cells). Immune cell infiltration levels in glioma patients were gathered from the TIMER site, and R was used to construct correlations between PTPN6 expression and six tumor-infiltrating immune cells.

Immunopenetration analysis

A work by Bindea[25] led to the use of marker genes for 24 distinct immune cell types. The ssGSEA approach was used to examine the infiltration of 24 immune cell types in malignancies. The degree of association between PTPN6 and the aforementioned 24 immune cells, as well as the analysis of immune cell infiltration between high and low expression groups of PTPN6, were investigated using the Spearman correlation approach.

Immune checkpoint analysis

SIGLEC15, TIGIT, CTLA4, CD274, HAVCR2, LAG3, PDCD1LG2, and PDCD1LG2 were chosen as immune checkpoint-related transcripts, and their expression data was collected. To determine the expression of immunological checkpoints and if PTPN6 is co-expressed with these checkpoints. The TIDE algorithm was used to predict probable immune checkpoint blockade responses.

Immunohistochemical (IHC) staining

Immunohistochemical (IHC) staining Immunohistochemical pictures of PTPN6 protein expression analyses were done in normal and glioma tissue from HPA (http://www.proteinatlas.org/) to assess the difference in PTPN6 expression at the protein level. IHC was performed using the antibody HPA001466.

Statistical analysis

R was used to conduct all statistical analyses (v3.6.3). For unpaired samples, the Wilcoxon rank sum test was performed. The researchers employed receiver operating characteristic (ROC) curves to see whether PTPN6 expression may be used as a diagnostic sign. The connection between clinicopathological characteristics and PTPN6 expression was investigated using the Kruskal-Wallis test, Wilcoxon signed-rank test, and logistic regression. The connection between PTPN6 expression and clinicopathological characteristics was investigated using the Chi-square test or Fisher's exact test. The prognostic significance of PTPN6 expression was assessed using Cox risk regression analysis or the Kaplan-Meier technique. Variables having a $P<0.05$ in univariate Cox's risk regression analysis were included in multivariate Cox's risk regression analysis. The P value for statistical significance was fixed at 0.05. A thorough analysis of each clinical category was not feasible due to inadequate clinical information in the TCGA database and the fact that not every sample documented clinical baseline information such as age, WHO stage, treatment result, and so on. As a result, the total number of samples and the number of samples in various clinical categories varies in the table in the Results section.

RESULTS

Survival outcomes and variable analysis

We analyzed any expression changes in PTPN6 mRNA levels in normal and tumor tissues in pan-cancer using RNA-seq data from the TCGA database and GTEx. Except for malignancies for which no standard tissue data were available, we discovered that 26 of 33 tumours exhibited substantially altered PTPN6 expression, including Bladder Urothelial Carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Cholangiocarcinoma (CHOL), Colon adenocarcinoma (COAD), Esophageal carcinoma (ESCA), Glioblastoma, Head and Neck squamous cell carcinoma (HNSC), Kidney Chromophobe (KICH), Kidney renal clear cell carcinoma (KIRC), Kidney renal papillary cell carcinoma (KIRP), Kidney renal papillary cell carcinoma (LAML),

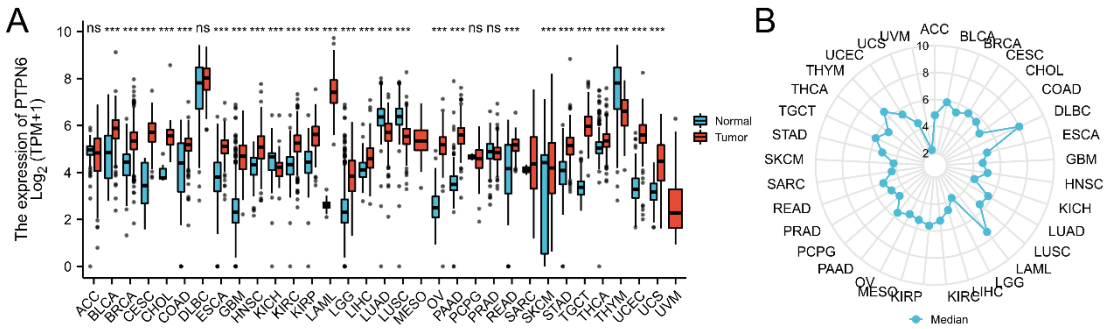

Figure 1. The expression level of PTPN6 in normal tissues and pan-cancer tissues (A) Radar map of PTPN6 expression in pan-cancer tissues (B).

Lower Grade Glioma (LGG), Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma (LUAD), Lung adenocarcinoma (LUSC), Ovarian serous cystadenocarcinoma (OV), Pancreatic adenocarcinoma (PAAD), Rectum adenocarcinoma (READ), Skin Cutaneous Melanoma (SKCM), Stomach adenocarcinoma (STAD), Testicular Germ Cell Tumors (TGCT), Thyroid carcinoma (THCA), Thyroid carcinoma (THYM). PTPN6 expression was elevated in 22 tumors and reduced in the remaining four cancers as compared to control tissues. (Figure 1A) We looked at how PTPN6 was expressed in each tumor (Figure 1B). The expression of PTPN6 in glioma and normal tissue demonstrated a difference in expression levels between the two tissues, with tumor tissue expression being strongly elevated (P<0.001, Figure 2A). The ROC curve was also used to examine the diagnostic value of PTPN6. The area under the curve (AUC) of PTPN was 0.897 (Figure 2B), suggesting that the protein may be used as a diagnostic biomarker.

Patient characteristics

As shown in Table 1, baseline data was collected from TCGA in April 2022 and included 698 primary tumors with clinical and gene expression data. The patients were divided into two groups by age, with 552 patients (79.4%) under 60 years old and 143 patients (21.6%) over 60 years old. Gender: Female: 297 (42.7%) and 398 (57.2%) male. Non-Asian patients: 669 patients (98.1%) Asian patients: 13 patients (1.9%) WHO grades were divided into G2 grades in 223 cases (35.2%) and G3&G4 grades in 411 cases (64.8%). IDH status was divided into WT: 246 cases (35.9%) and Mut: 439 (64.1%). 1p/19q codeletion is divided into codel: 170 (24.7%) non-codel: 518 (75.3%). PTPN6 was divided into high expression 348 (50.1%) and low expression 347 (49.9%). We further applied univariate and multivariate Cox regression models to investigate the independent prognosis of the features. Univariate analysis showed that age (HR 4.668, p < 0.001), who grade (HR 5.642, p < 0.001), IDH status (HR 0.117, p < 0.001) 1p/19q codeletion (HR 4.428, p < 0.001)

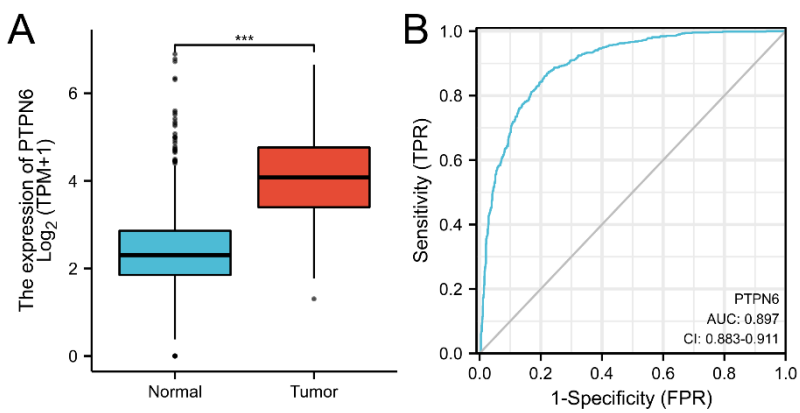

Figure 2. Expression of PTPN6 in glioma and normal tissues (A). ROC curve of PTPN6 for glioma (B).

Table 1: Baseline data on PTPN6 expression in glioma patients, univariate and multivariate Cox proportional hazards analysis

Characteristics	Total (N)	Univariate analysis Hazard ratio (95% CI)	P	Multivariate analysis Hazard ratio (95% CI)	P
Age	695				
<=60	552	Reference			
>60	143	4.668 (3.598-6.056)	**<0.001**	1.974 (1.467-2.657)	**<0.001**
Gender	695				
Female	297	Reference			
Male	398	1.262 (0.988-1.610)	0.062	1.257 (0.953-1.659)	0.106
Race	682				
White&Black or African American	669	Reference			
Asian	13	0.837 (0.268-2.617)	0.760		
WHO grade	634				
G2	223	Reference			
G3&G4	411	5.642 (3.926-8.109)	**<0.001**	2.216 (1.478-3.324)	**<0.001**
IDH status	685				
WT	246	Reference			
Mut	439	0.117 (0.090-0.152)	**<0.001**	0.211 (0.146-0.305)	**<0.001**
1p/19q codeletion	688				
codel	170	Reference			
non-codel	518	4.428 (2.885-6.799)	**<0.001**	1.329 (0.786-2.247)	0.289
PTPN6	695				
Low	347	Reference			
High	348	2.398 (1.869-3.078)	**<0.001**	1.398 (1.048-1.865)	**0.023**

and PTPN6 (HR 2.398, $p < 0.001$) had prognostic value. In multivariate stepwise cox regression analysis of glioma, age (HR 1.974, $p < 0.001$), who grade (HR 2.216, $p < 0.001$), IDH status (HR 0.211, $p < 0.001$) and PTPN6 (HR 1.398, $p = 0.023$) (Table 1). These results suggest that PTPN6 expression is an independent prognostic factor in gliomas.

High expression of PTPN6 is an independent risk factor for overall survival

As demonstrated in Figure 3A, increased PTPN6 expression was linked to a poor overall prognosis in gliomas ($p<0.001$). As demonstrated in Figures 3A-3I, high PTPN6 expression was highly linked with poor prognosis in glioma patients over 60 years old ($p<0.001$), younger than 60 years old ($p<0.001$), who graded G3&G4 ($p< 0.001$), 1p/19q codeletion non-codel ($p<0.001$), IDH status: wt ($p<0.001$), IDH status: mut ($p = 0.044$). Patients' 1-, 3-, and 5-year survival were predicted using nomograms created by integrating PTPN6 expression levels with clinical factors (Figure 4A). The calibration curve demonstrated a reasonable predictive value (Figure 4B)

Logistic regression analysis of the correlation between PTPN6 expression and clinicopathological factors

Correlation between PTPN6 expression and clinicopathological and prognostic features. Age (>60 vs. =60, $p = 0.004$), WHO grade (G3&G4 vs. G2, $p<0.001$), IDH status (Mut vs. WT, $p<0.001$), and 1p/19q codeletion (non-codel vs. Codel, $p<0.001$) were all factors in PTPN6 expression in gliomas. These findings imply that glioma patients with high levels of PTPN6 expression were more likely than those with low levels of PTPN6 expression to develop high-grade IDH-mut and 1p/19q co-deletion tumors (Table 2 Figure 5).

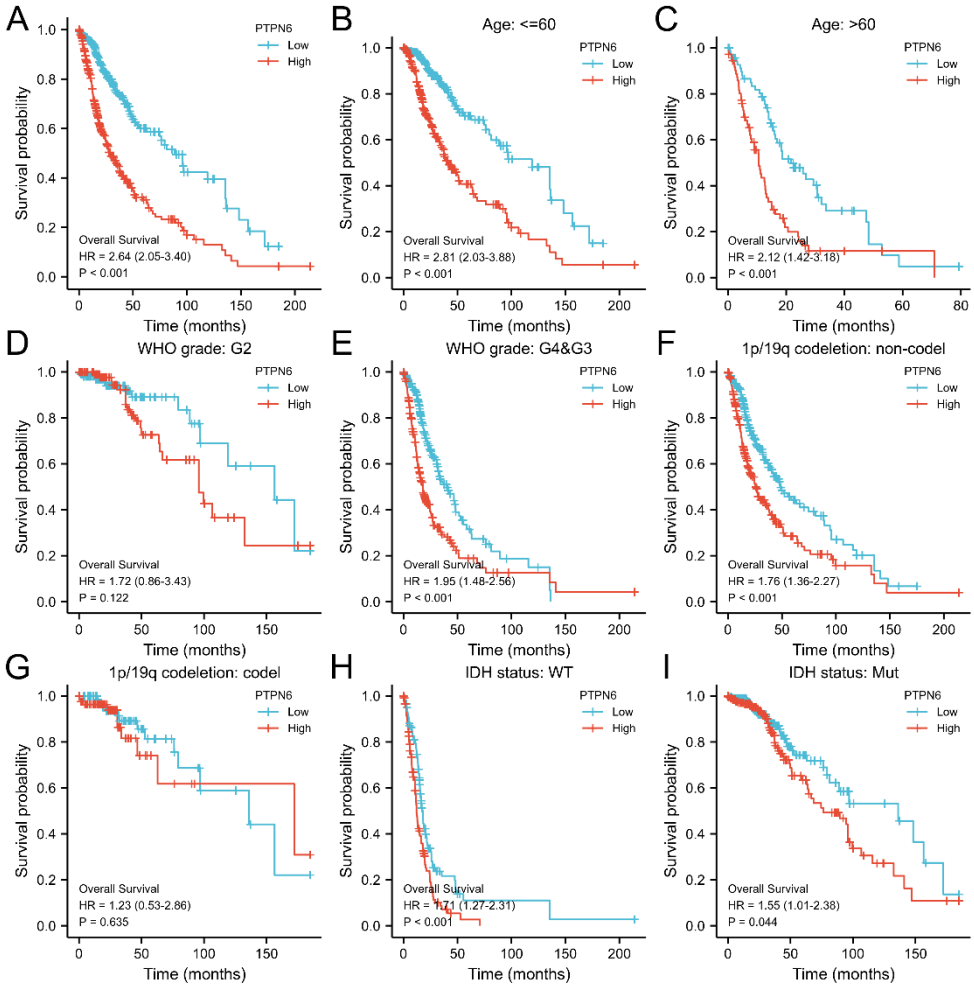

Figure 3. Kaplan-Meier estimate of overall survival of patients by all(A), ≤60(B),>60(C) , G2 (D) G4&G3(E), 1p/19q codeletion:non-condel(F) 1p/19q codeletion:codel (G), IDH status: WT(H), IDH status: Mut (I)

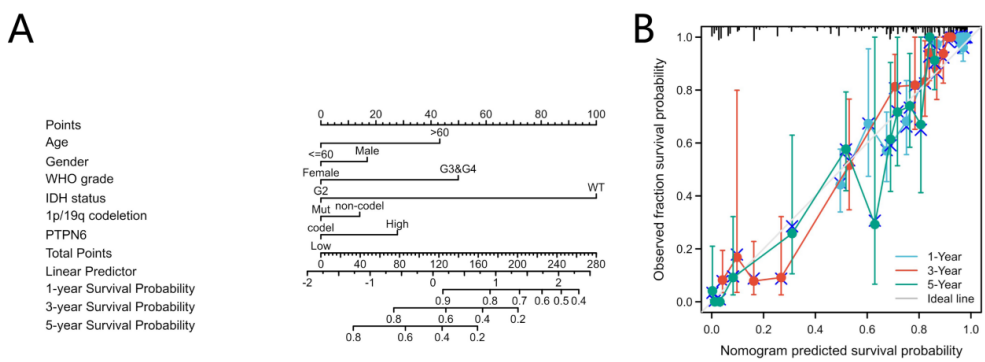

Figure 4. Multivariate analysis nomogram of clinical features based on PTPN6 expression (A) Calibration plot showing the predictive performance of the model constructed using multivariate Cox regression analysis (B)

Table 2: Logistic analysis of the association between PTPN6 expression and clinical Characteristics

Characteristics	Total(N)	Odds Ratio(OR)	P value
Age (>60 vs. <=60)	696	1.738 (1.198-2.539)	0.004
Gender (Female vs. Male)	696	0.703 (0.519-0.949)	0.022
WHO grade (G3&G4 vs. G2)	635	2.763 (1.975-3.887)	<0.001
IDH status (Mut vs. WT)	686	0.263 (0.188-0.366)	<0.001
1p/19q codeletion (non-codel vs. codel)	689	13.251 (8.156-22.689)	<0.001
Race (No Asian vs. Asian)	683	2.291 (0.738-8.521)	0.171

KEGG pathway analysis to explore potential biological functions of PTPN6

GSEA revealed that samples with high PTPN6 levels had substantially different enrichment of the KEGG pathway (FDR<0.050, p-value<0.050). Based on their normalized enrichment ratings, we chose the most enriched signaling pathways (NES). Cytokine receptor, systemic lupus erythematosus, hematopoietic cell lineage, allograft rejection, and graft-versus-host disease were the five pathways most strongly related with PTPN6 expression, according to KEGG pathway analysis. Gap junctions, cardiac contraction, long-term potentiation, amyotrophic lateral sclerosis, and terpenoid biosynthesis were the five pathways with the largest negative connection (Figure 6A,6B). PTPN6 expression is linked to pathways that are important for cell interaction and immunological responses.

The relationship between PTPN6 expression and tumor-infiltrating immune cells

Independent tumor-infiltrating lymphocytes are important predictors of survival14. As a result, we employed TIMER to look into the possibility of a link between PTPN6 expression in LGG and glioblastoma and immune infiltration levels. In glioblastoma, PTPN6 expression was substantially linked with B cells (p=2.02e-03) and CD4+ T cells (p=4.27e-13),. Macrophages (p = 3.11e-03), neutrophils (p = 2.98e-11), and dendritic cells (p= 3.15e-30) all had a positive connection. B cells (p=1.49e-61), CD8+ T cells (p=2.28e-02), CD4+ T cells (p=9.83e-151), giant Phagocytes (p= 3.17e-94), neutrophils (p=2.04e-124), and dendritic cells (p= 9.98e-148) were all positively

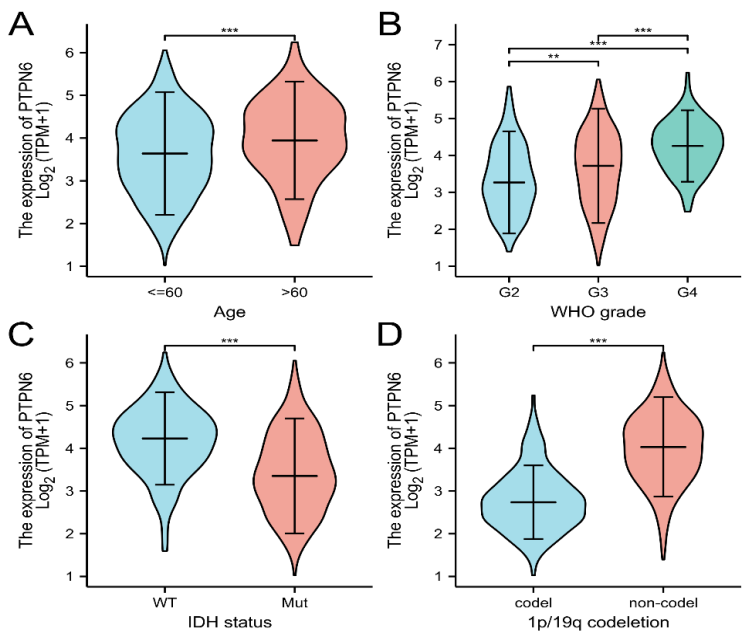

Figure 5. Expression of PTPN6correlated significantly with Age(A) WHO grade (B) IDH status (C) and 1p/19q codeletion(D).

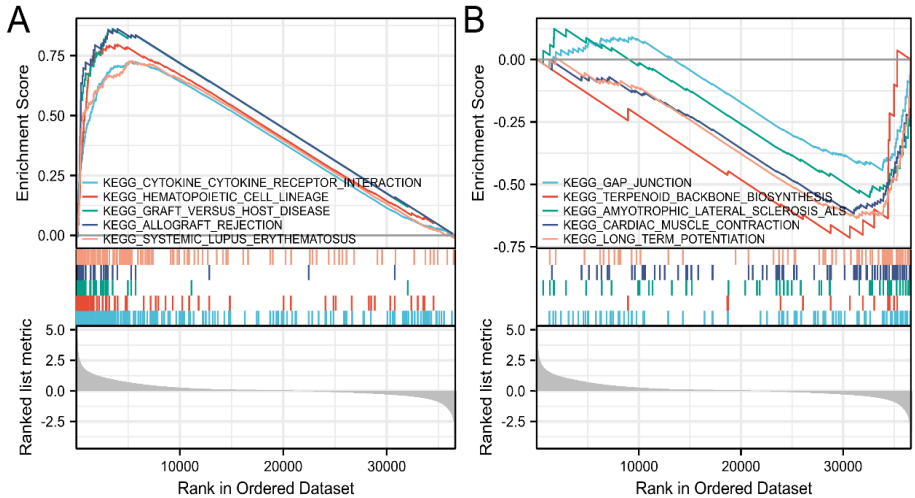

Figure 6. KEGG pathway showed five positively correlated groups (A) and five negatively correlated groups (B).

connected with PTPN6 in LGG. (Figure.7A) These findings imply that PTPN6 is involved in glioma immune infiltration.

Next, the relationship between PTPN6 expression and 24 different immune cell types was assessed in gliomas. PTPN6 is positively correlated with macrophages, neutrophils, aDC, eosinophils, iDC, cytotoxic cells, T cells, Th17 cells, NK CD56dim cells, Th1 cells, NK cells, T helper cells, B cells, Th2 cells, DC and Tem, mast cells, CD8 T cells are closely related and negatively related to Tgd, Tcm, TReg, pDC, NK CD56bright cells. Further studies showed that PTPN6 expression levels were significantly different in infiltrating immune cells, including macrophages, neutrophils, aDCs, eosinophils, iDCs, cytotoxic cells, T cells, Th17 cells, NK cells CD56dim cells, Th1 cells, NK cells, T helper cells, B cells, Th2 cells, DC, Tem, CD8 T cells Tgd, TReg, pDC, NK CD56bright cells were affected by PTPN6 expression. PTPN6 expression and NK CD56bright cells, pDCs, Tgd, TReg, macrophages, neutrophils, aDCs, eosinophils, iDCs, cytotoxic cells, T cells, Th17 cells, NK CD56dim cells, Th1 cells, NK cells, T helper cells were closely and positively correlated, and B cells, Th2 cells, DC, Tem, and CD8 T cells were closely and negatively correlated. We also assessed possible correlations between 24 immune cells. The resulting heatmap showed strong to moderate correlations in the ratios of different tumor-infiltrating immune cell subsets. (Figure 7B-D).

Table 3: Correlation of PTPN6 with immune checkpoints in glioma

Genes	Cor	P
SIGLEC15	0.347	<0.001
PDCD1LG2	0.749	<0.001
PDCD1	0.680	<0.001
LAG3	0.396	<0.001
HAVCR2	0.923	<0.001
CD274	0.619	<0.001
CTLA4	0.509	<0.001
TIGIT	0.226	<0.001

The expression of immune checkpoints, including SIGLEC15, TIGIT, CTLA4, CD274, HAVCR2, LAG3, PDCD1, and PDCD1LG2, was further investigated in gliomas. In tumors, immune checkpoints were positively associated with PTPN6. Furthermore, the results showed that PTPN6 was positively co-expressed with these immune checkpoints (Fig. 7E Table 3), gliomas with low PTPN6 expression had lower TIDE scores, better immune checkpoint blockade (ICB) treatment, and better survival after ICB treatment high (Figure 7F).

Data verification

Using the CGGA website (http://www.cgga.org.cn/), we found that the expression of PTPN6 increased with the increase of who grade. Kaplan-Meier survival analysis showed that high PTPN6 expression was associated with poor overall prognosis in glioma. Correlation ($p<0.001$), as shown in Figure 8D. Subgroup analysis by different who grades showed that high PTPN6 expression was closely associated with poor

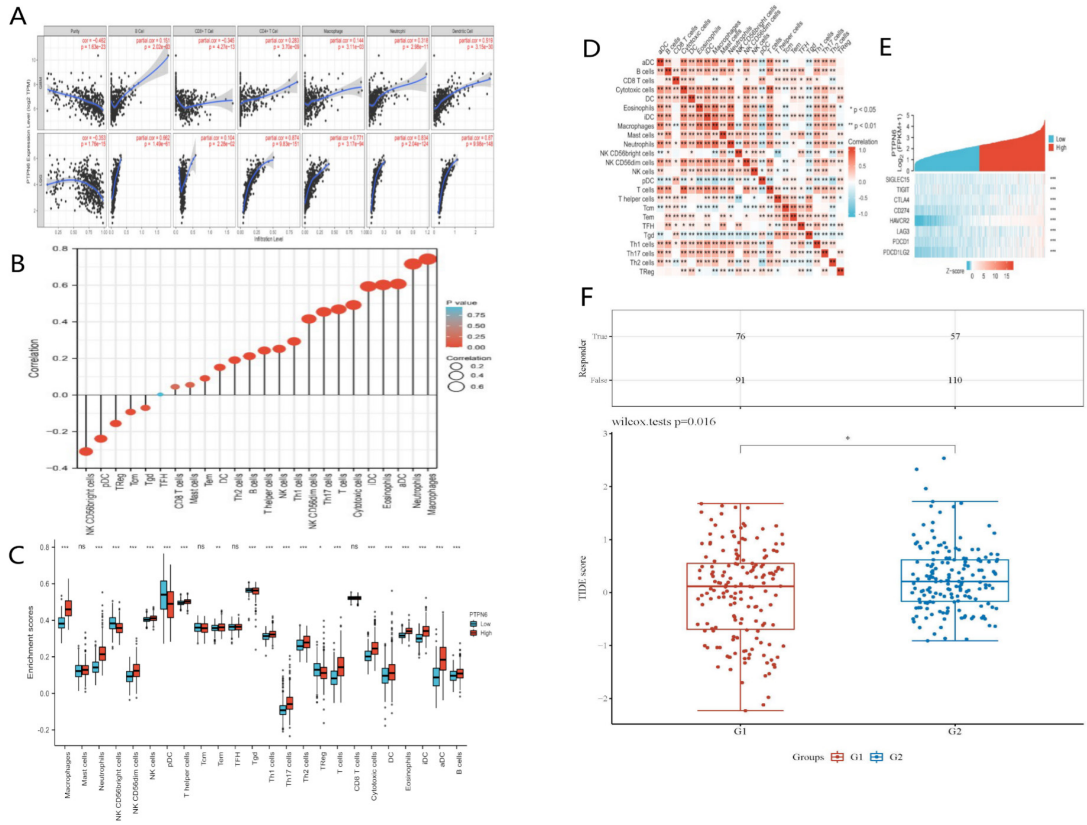

Figure 7. Correlation of PTPN6 expression in the tumor microenvironment with immune cell infiltration and expression of immune checkpoints in gliomas. (A) The relationship between PTPN6 expression and the infiltration level of six types of immune cells. (B) The relationship between PTPN6 expression and 24 types of immune cells (presents a correlation analysis between PTPN6 expression levels and immune infiltration matrix data, utilizing the Spearman correlation analysis method). (C) Change ratio of 24 immune cell subtypes in high and low PTPN6 expression groups in tumor samples. (D) Heat map of 24 immune infiltrating cells in tumor samples. (E) Immune checkpoints with low and high PTPN6 expression different expressions. (F) Differential responses of low (G1) and high (G2) PTPN6 expression to immune checkpoint blockade.

prognosis G2 (p = 0.007), G3 (p = 0.034), G4 (p = 0.02), (Figure. 8E-F)SIGLEC15, TIGIT, CTLA4, CD274, HAVCR2, LAG3 IHC, PDCD1, and PDCD1LG2 were positively correlated with PTPN6 in gliomas (Figure 9A-H). Furthermore, immunohistochemical analysis available from HPA showed that PTPN6 IHC staining was absent in normal cerebral cortex (Figure 10A), Low staining was observed in LGG tumor tissues, while moderate to high PTPN6 staining was observed in glioblastoma. (Figure 10B-E)

DISCUSSION

One of the most prevalent forms of brain cancer in people is glioma, which has a complicated pathophysiology that includes altered gene expression, malfunction, and changes in several signaling networks. Only a few prognostic indications for gliomas can be used in clinical work, despite the fact that research have revealed several indicators. Therefore, there is still a need to identify additional potential and useful glioma prognostic markers.

In this work, we investigated the association between PTPN6 expression, prognosis, clinicopathological characteristics, tumor mutations, and tumor immunity using a publically available dataset of clinically characterized glioma patients. According to our research, elevated PTPN6 expression is a reliable predictor of clinical characteristics related to gliomas and can be exploited as a target for immunotherapy. To properly assess the predictive significance of PTPN6 in gliomas, we separately conducted a survival analysis of gliomas. Allograft rejection,

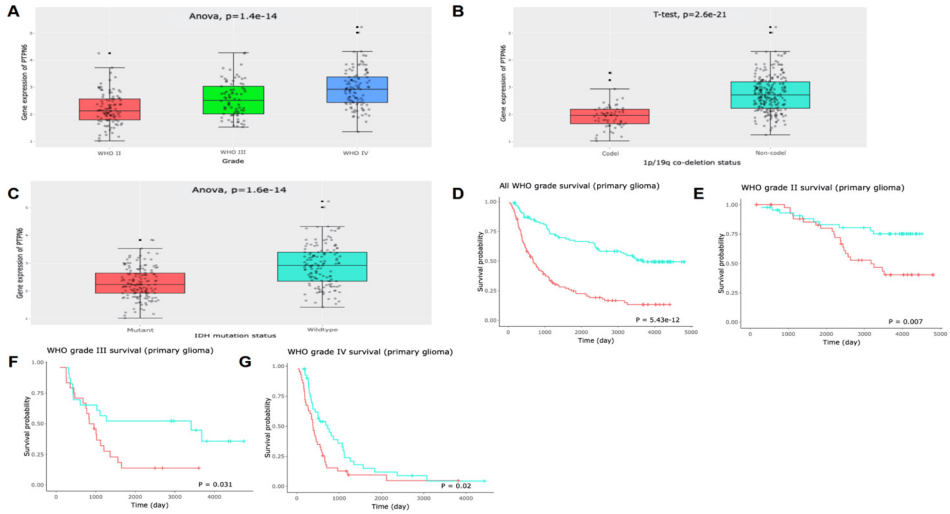

Figure 8. Expression of PTPN6 in each who grade glioma in the CGGA database (A, B, C) Kaplan-Meier estimate of overall survival of patients by who grade in the CGGA database (D-G) (Red Group: Glioma; Blue Group: Normal

graft-versus-host disease, hematopoietic cell lineage, and immune-related responses are predominantly promoted by increased PTPN6. according to the current study's KEGG pathway analysis. The linked effects of gap junction, cardiac contraction, long-term potentiation, amyotrophic lateral sclerosis, and terpenoid biosynthesis were all suppressed by up-regulating PTPN6, in contrast. It is important to note that further validation through wet-lab experiments using molecular and biochemical techniques on glioma cells and patient samples is required to confirm these findings and their clinical relevance.

Tumor occurrence and growth depend heavily on the immunological microenvironment.[26,27] TME profiles can affect tumor prognosis in addition to acting as biomarkers for determining tumor cell immunotherapy responsiveness.[28] Anti-PD-1 therapy, a type of immunotherapy, has demonstrated encouraging outcomes in preclinical glioma investigations in recent years.[29,30] However, the majority of clinical research on immunotherapy fall short of delivering the best possible therapeutic outcomes.[31] The intricate immunosuppressive TME is a major factor in the limited efficacy of immunotherapy in glioma. Additionally, peripheral immunosuppression that is particular to gliomas may affect how well

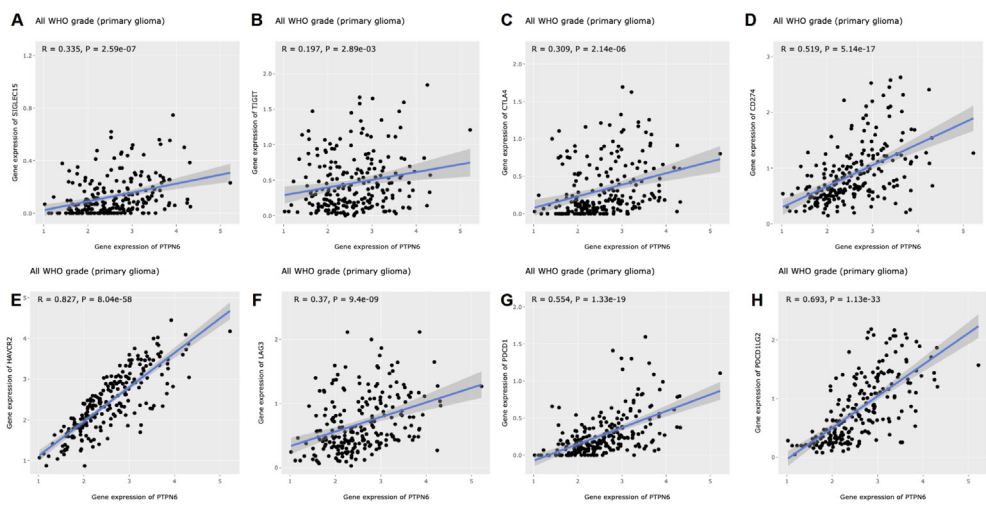

Figure 9. Co-expression of PTPN6 and immune checkpoints in the CGGA database (A-H)

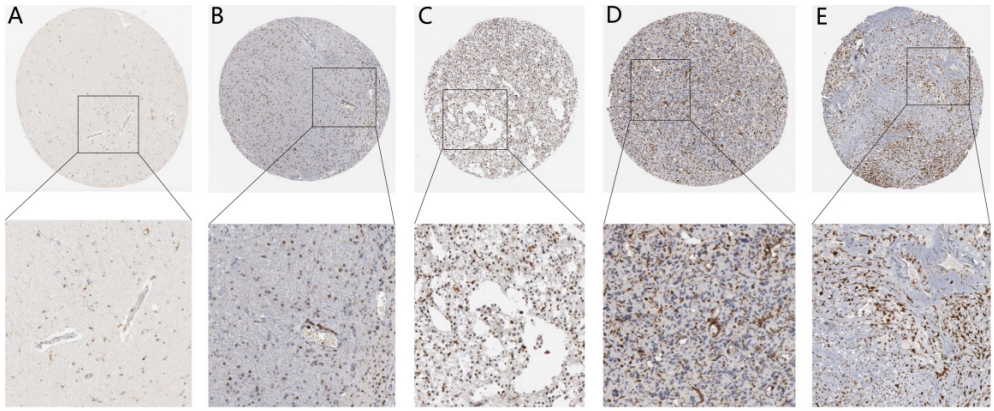

Figure 10. The expression of PTPN6 protein in glioma and normal tissues was observed by HPA immunohistochemistry (A-E). Normal tissue(A): LGG(B-C) glioblastoma (D-E)

immunotherapy works. Our study finds PTPN6 as an important immune-related prognostic marker in the glioma tumor microenvironment by analyzing single-cell databases. We discovered a connection between PTPN6 expression and the level of immune infiltration in gliomas using the TIMER database. These connections imply that PTPN6 is essential for controlling the immunology of glioma tumors. The findings demonstrated a statistically significant variation in PTPN6 expression levels in immune cells. Overall, our findings imply that PTPN6 is essential for controlling the immunological activity of gliomas. Our experimental results, together with those of Zhang *et al*., have collectively demonstrated that PTPN6 has an immunosuppressive role in glioma.[32]

In recent years, tumor immunotherapy has attracted more and more attention and research. Tumor immunotherapy is beneficial to improve overall survival and long-term therapeutic benefits without significant side effects. By modulating and promoting the patient's immune system to kill cancer cells, various immunotherapeutic approaches have emerged as promising anti-tumor modalities, especially those that unleash anti-cancer immunity through immune checkpoint inhibitors.[33]

This study found that PTPN6 was co-expressed with immune checkpoints such as TIGIT, CD274, PDCD1, HAVCR2, PDCD1LG2, SIGLEC15, LAG3 and CTLA4. Patients with high PTPN6 expression in gliomas responded better to immune checkpoint blockade therapy than patients with low PTPN6 expression, resulting in reduced efficacy. Watson and colleagues have found that low expression of PTPN6 can inhibit tumor development by enhancing the ability to adoptively transfer CD8(+) T cells.[34]

Previous studies have shown that PTPN6 is involved in multiple immune cell functions, including neutrophil survival time, B cell activation, and T cell activation and survival time.[35]

In conclusion, therefore, we believe that PTPN6 regulates immune function and immunity plays an important role in glioma initiation and progression. However, our study has several shortcomings. First, the data for this study were drawn from a regularly updated and expanded online platform database; therefore, the results of this study are subject to change. Second, our study lacks information on difficult and in-depth treatment options. Third, no in vivo and in vitro studies were performed to verify the role of PTPN6 in glioma immunity and its molecular mechanism. In future studies, we will pay more attention to the patients' overall baseline information and conduct further tests to confirm the expected results. This is the first study to identify PTPN6 as a novel prognostic marker in glioma and link it to immune function. With a clearer understanding of its functional scope, PTPN6 could serve as a useful diagnostic and therapeutic tool for glioma, and biomarker therapy could represent a viable future treatment option for glioma. The mechanism by which PTPN6 promotes tumor growth and metastasis in glioma deserves further study.

DISCLOSURE

Data availability: The RNA-sequencing expression profiles and corresponding clinical information data used to support the findings of this study have been passed by the TCGA Database and the CGGA Website and the GTEx Website.

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Conflicts of interest: None

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