

Comprehensive Analysis Reveals Promising Immune Target and Prognostic Value of PLK3 in Glioma

Ao Qian

The First Affiliated Hospital of Chongqing Medical University <https://orcid.org/0000-0003-2361-5668>

Gang Huo

The First Affiliated Hospital of Chongqing Medical University

Xiaoshu Wang

The First Affiliated Hospital of Chongqing Medical University

Jiamin Mou

The First Affiliated Hospital of Chongqing Medical University

Dong Gao

The First Affiliated Hospital of Chongqing Medical University

Song Chen (✉ chensong198125@126.com)

The First Affiliated Hospital of Chongqing Medical University <https://orcid.org/0000-0003-1143-5686>

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Abstract

Background

PLK3, a gene played an important role in cell cycle progression and stress response, was identified in different carcinomas. However, the PLK3 expression, molecular characteristics, and prognostic value in glioma still remained unknown.

Methods

Total 2265 glioma samples from the CGGA RNA-seq, TCGA RNA-seq, CGGA microarray, GSE16011, as well as their clinical information and genomic profiles were selected in our research. Survival analysis based on Kaplan–Meier and Cox proportional hazard model methods was performed to evaluate the prognostic value.

Results

Expression of PLK3 was compared in different WHO grade, isocitrate dehydrogenase (IDH) status, and molecular subtype of gliomas. We found that increased level of PLK3 was associated with malignancy and invasiveness of glioma, and high expression of PLK3 may represent malignant entities with amplification of driver oncogenes and deletion of suppressor genes. Moreover, PLK3 played a crucial role in inflammatory and immune response, and was involved in suppressive T cell anti-tumor functions. Furthermore, PLK3 was synergistic with other checkpoint members in glioma. Finally, high expression of PLK3 was associated with malignant process and reduced survival in patients with glioma.

Conclusion

PLK3 may serve as an indicator of poor prognosis in glioma and a potential target for immunotherapy of glioma. These results demonstrated that PLK3 may serve as a biomarker and a potential target of glioma.

1. Introduction

Glioma is the most common malignant tumor in central nervous system. Due to its infiltrative growth, routine surgical resection is difficult to remove the tumor completely[1]. Although comprehensive treatments including total section, radiotherapy, and chemotherapy were adopted, postoperative survival is still limited for high recurrence and malignant transformation rate of gliomas, especially for glioblastoma (GBM)[2, 3]. Historically, according to histopathology, gliomas are divided into low grade gliomas (WHO grade I and II) and high grade gliomas (WHO grade III and IV)[4]. Considering the limitation of this classification, WHO put forward a new classification by integrating tumor morphology, IDH status

and 1p19q co-deletion status in 2016. In recent years, with the development of molecular mechanism research, several genes have been used to evaluate clinical features and prognosis of gliomas[5].

Polo-like kinases 3 (PLK3) is classified as immediate-early genes that plays an important role in cell cycle progression and stress response[6, 7]. Recently, a study reported that IDH mutations may affect prognosis of low grade gliomas by influencing PLK3 and related DNA damage repair pathways, moreover, the sensitivity of temozolomide may be affected by PLK3 in low grade gliomas and GBM[1].

In previous reports, the expression of PLK3 was distinctive in different carcinomas. Reduced PLK3 expression was found in colon cancer, lung cancer, liver cancer and kidney cancer[8–11]. On the contrary, PLK3 upregulated in ovarian and breast cancers may indicate poor prognosis and short survival[12, 13]. However, limited studies of PLK3 function were available in gliomas. To explore the status of PLK3 in gliomas, RNA expression data of glioma samples was enrolled from Chinese Glioma Genome Atlas (CGGA) dataset, TCGA network and GSE16011 microarray. As far as we know, this is the first integrative study that explore PLK3 characteristics in both clinical and molecular area in whole grade glioma.

2. Materials And Methods

2.1 Data collection

Four kinds of transcriptome data from patients who were diagnosed with glioma (WHO II-IV) were used. The Cancer Genome Atlas (TCGA) database (RNAseq, n = **670**) (<http://cancergenome.nih.gov/>), The datasets used were The Chinese Glioma Genome Atlas (CGGA) database (RNAseq, n= **1018**, microarray, n = **301**) (<http://www.cgga.org.cn>), the GSE16011 database (n= **276**) downloaded from Gene Expression Omnibus (GEO). The copy number variation (CNV) profile (n= **667**) and somatic mutation data (n= **654**) were obtained from TCGA data portal whose RNA-seq data was corresponded to those of the cases (<http://cancergenome.nih.gov/>).

2.2 Bioinformatic analysis

GISTIC 2.0 was performed to analyze the copy number alternation which associated with expression of PLK3. Meanwhile, if alternation peak was observed, GISTIC analysis was used to obtained the thresholded copy number at alternation peak. Spearman correlation analysis to acquire genes related to PLK3, then GO analysis in R project was performed to detected biological functions of related genes to draw a Heatmap, and T cell specific genesets was obtained from AmiGO 2 Web portal. The inflammatory related metagenes and the calculation of metagene expression value were described in previous study[14, 15].

2.3 Statistical analysis

Differences in variables between groups were evaluated by Student t-test, one-way ANOVA, or Pearson's Chi-squared test. The prognostic value of PLK3 was estimated by Kaplan–Meier analysis and Cox proportional hazard model analysis using R project. Other statistical computations and figures drawing

were performed with several packages (ggplot2, pheatmap, pROC and corrgram) in R project, version 4.1.0 (<http://www.r-project.org>). For all statistical methods, $p < 0.05$ was considered as significant difference.

3. Results

3.1 Expression of PLK3 associated with malignancy of molecular and clinical characteristics in gliomas.

With the analysis of RNA-sequence and microarray data of glioma, expression of PLK3 increased with grade of gliomas, especially highest in WHO grade IV gliomas (Figure 1A). Furthermore, in comparison with other subtypes, like astrocytoma, oligodendroglioma and oligoastrocytoma, as well as their anaplastic variations, GBM was related to higher PLK3 expression (Figure 1B). As an essential indicator of prognosis, gliomas with IDH wild-status had a considerably higher expression of PLK3 compared with gliomas with IDH mutation-status (Figure 1C). Thus, the level of PLK3 was positively associated with malignance and aggressiveness of gliomas.

Molecular classification provides a new method to predict outcomes of different patients with gliomas. Classical, mesenchymal, neural and proneural were known as molecular subtypes of glioma with the definition of TCGA network. As shown in Figure 1D, significant difference was found in the distribution of PLK3 expression among these four subtypes. Moreover, compared with other subtypes, the highest expression of PLK3 was detected in mesenchymal subtype, which showed worst prognosis for patients[16]. Given the intimate relationship with malignance of glioma, PLK3 may play an important role in the progression of glioma. Meanwhile, PLK3 may be considered as a biomarker for the mesenchymal subtype.

3.2 Expression of PLK3 associated with distinct patterns of genomic alterations.

As the primary malignant tumor in central nervous system, genetic mutation is pervasive in molecular mechanism. Therefore, the TCGA database was analyzed to explore the characteristics of somatic mutations and copy number alterations in gliomas. Parallel analyses were performed in 2 groups, 3 groups, or 4 groups that classified with ascending order of PLK3 expression. Low and high levels group of PLK3 were formed to compare the frequency of mutations. More somatic mutations were revealed in cases with high PLK3 expression (1st vs. 2nd half, 6938 vs. 31404 mutations; 1st vs 3rd tertile, 4104 vs. 7446 mutations; 1st vs. 4th, 3018 vs. 5833 mutations). In low PLK3 expression group, mutations in IDH1, CIC, and FUBP1 was significantly detected (Figure 2A; Supplementary Figure 1). On the other hand, mutations in TTN, EGFR, ATRX and PTEN was enriched in high PLK3 expression group (Figure 2A).

Then somatic copy number alternations were investigated between cases with low and high PLK3 expression. More segment count of CNAs was found in cases with high PLK3 expression (1st vs. 2nd

half, 10149 vs. 15455 CNAs; 1st vs 3rd tertile, 5627 vs. 11223 CNAs; 1st vs. 4th, 4254 vs. 8224 CNAs). As shown in Figure 2B, with increased level of PLK3, ascending amplification of Chr7 and deletion of Chr10, as well as declining rate of 1p/19q codeletion were detected. In high PLK3 expression group, focal amplification peaks were found in EGFR (7p11.2), PDGFRA (4q12), CDK4 (12q14.1), CCND2 (12p13.32), which were characterized by driver oncogenes. On the contrary, a focal deletion peak was observed in 9p21.3 and 1p32.3 (CDKN2A and CDKN2C) (Figure 2C; Supplementary Figure 1). Meanwhile, in high PLK3 expression group, amplification peaks were observed in 7p11.2, while there were deleted genomic regions in 9p21.3, 9p21.2 and 9p21.1 (Supplementary Dataset S1)

3.3 PLK3 related malignant biological processes in glioma.

In previous studies, PLK3 played an essential role in biological functions in both physiological and pathological states. However, the impact of PLK3 in gliomas remains largely elusive. Hence, 2455 genes from CGGA dataset and 1218 genes from TCGA dataset that strongly correlated with PLK3 by Pearson correlation analysis (Pearson $R > 0.5$) were selected for GO analysis to analyze their biological functions. In CGGA dataset, genes with PLK3 correlation, were mostly related to inflammation response and immune response (Figure 3A). Meanwhile, the similar result was observed in TCGA dataset (Figure 3B). In both CGGA cohort and TCGA cohort, the normal biological processes, such as chemical synaptic transmission and others, were more common in functions of negatively correlated genes (Supplementary Figure 2).

3.4 PLK3 was related to immune response

PLK3 may be involved in immune response. So immune responses related genes from AmiGO 2 Web portal were sought to explore the correlation between PLK3 expression and immune response[17]. 872 genes from CGGA dataset and 990 genes from TCGA dataset, all of which were most related to PLK3 (Pearson $|R| > 0.4$), were enrolled to draw the heatmaps. In CGGA dataset, 864 genes were positively associated with expression of PLK3, while 8 genes were negatively related (Fig. 3C). 855 genes were positively associated with PLK3 expression, while 135 genes were negatively related in TCGA dataset (Fig. 3D). Accordingly, in gliomas, most relevant immune responses were positively associated with PLK3 expression.

3.5 PLK3 was correlated with other checkpoint members.

With the development of immunotherapy, immune checkpoints may become potential therapeutic targets in various tumors. PD-1 and its receptor (PD-L1) are crucial immune checkpoints in series tumor, for binding of PD-L1 and PD-1 restrains anti-tumor function of T cell and enhances Tregs activation that enables tumor to achieve immune evasion[18–20]. Further, PD-L1 also interacts with CD80 on surface of activated CD8+T cell to suppress its anti-tumor activity [2]. High expression of PD-1 and PD-L1 is associated with poor prognosis of patients with glioma[21, 22]. Given the relationships between PLK3 and PD-1/PD-L1, as well as CD80 remained unknown, Pearson correlation analysis was performed with PLK3, PD-L1, PD-1 and CD80 expression, both in the CGGA dataset and TCGA dataset. We found PLK3 showed high correlation with PD-1, PD-L1 and CD80 in whole grade gliomas, low-grade gliomas and glioblastoma of CGGA dataset (Figure 4A). Moreover, similar results of the correlations were observed in

TCGA database (Figure 4B). We believe that PLK3 may play an important role in the regulation of PD-1/PD-L1 pathway.

To further explore the relationship between PLK3 and immune checkpoints, several genes of immune checkpoints were enrolled to explore the relationship between PLK3 and gliomas, including VISTA, TIM3, PSGL1, LAG3, IDO1, CTLA4, BTLA, B7H4, and B7H3. In CGGA and TCGA dataset, PLK3 correlated tightly with TIM3 and B7H3 in both whole grade gliomas, low-grade gliomas and GBM (Figure 4C and D).

3.6 PLK3 was associated with T cell immunity in glioma

Although PLK3 plays an important role in various tumors, the relationship between PLK3 and T cell immunity remains poor understanding. Thus, GSVA analysis was performed to explore the connection between PLK3 and T cell immunity in glioma. In both CGGA and TCGA databases, the connection between PLK3 and T cell immunity was found in the form of positive regulation of T cell tolerance induction, positive regulation of T cell mediated immune response to tumor cell, positive regulation of T cell cytokine production, positive regulation of regulatory T cell differentiation, positive regulation of T cell proliferation, and positive regulation of T cell receptor signaling pathway. Meanwhile, we also found that PLK3 was positively associated with negative regulation of alpha-beta T cell activation. (Figure 5A and B). These results indicated that PLK3 played function of T-cell immunity inhibition in glioma.

3.7 PLK3 was connected with inflammation activities in gliomas.

As close association between PLK3 and inflammation as well as immune response in gliomas described before, seven metagenes that representing different inflammation and immune response were selected to analyze the role of PLK3 in inflammatory activities. In CGGA databases, the expression of PLK3 was positively associated with HCK, interferon, LCK, MHC-1, MHC-2 and STAT-1 that were closely associated with activating of macrophages, signaling transduction of T cells and antigen-presenting. Negative correlation with IgG that represented activity of B lymphocytes was detected (Figure 5C and D).

Furthermore, the same result was also verified in TCGA database. Accordingly, in gliomas, the function of PLK3 in inflammation and immune response were crucial.

3.8 PLK3 indicated poor prognosis in gliomas.

To figure out the prognostic value of different PLK3 level, Kaplan-Meier analysis and Cox proportional hazard model analysis were performed in CGGA and TCGA database. As shown in Figure 6, in CGGA database with high PLK3 expression, significantly shorter survival was not only found in whole gliomas, but also detected in gliomas of WHO Grade II, WHO Grade III and WHO Grade IV. As well, these results were verified in TCGA cohort. In TCGA databases, univariate analysis revealed that PLK3, age at diagnosis, radiotherapy, chemotherapy and IDH mutation status were significantly associated with overall survival. Further, the same results except radiotherapy were observed in CGGA database. Importantly, significant correlation was still detected between PLK3 and overall survival with multivariate analysis

performed (Table 1). These results demonstrated that high expression of PLK3 obviously decreased the prognostic value of patients with glioma.

Table 1
Univariate and multivariate cox analyses in gliomas in CGGA and TCGA datasets

Characteristic	CGGA database (n=631)			TCGA database (n=629)		
	P	HR	95%CI	P	HR	95CI
Univariate						
PLK3*	<0.001	1.871	1.689–2.072	<0.001	1.223	1.189–1.258
Age†	<0.001	2.213	1.764–2.776	<0.001	4.825	3.502–6.648
Gender (male vs. female.)	0.762	1.034	0.832–1.285	0.196	1.185	0.916–1.531
Radio_status (No vs Yes)	0.334	1.175	0.847–1.632	<0.001	2.864	1.951–4.203
Chemo_status (No vs Yes)	<0.001	1.656	1.290–2.125	<0.001	2.623	1.814–3.792
IDH_status (mutation vs wild)	<0.001	0.214	0.170–0.271	<0.001	0.099	0.075–0.132
Multivariate						
PLK3*	<0.001	1.374	1.220–1.548	0.046	1.053	1.001–1.107
Age†	<0.001	1.565	1.219–2.009	0.064	1.647	0.971–2.794
Gender (male vs. female.)	0.924	1.011	0.805–1.270	0.201	1.245	0.890–1.740
Radio_status (No vs Yes)	0.611	0.914	0.648–1.290	0.612	0.874	0.519–1.471
Chemo_status (No vs Yes)	0.224	1.177	0.905–1.532	0.616	0.885	0.549–1.427
IDH_status (mutation vs wild)	<0.001	0.331	0.251–0.436	<0.001	0.092	0.053–0.160
* PLK3: increased expression.						
† Age: increased years.						

4. Discussion

As the most common primary tumor in brain parenchyma, the treatment of gliomas has always been a sticky challenge. Although advance was made in conventional treatment regimens, outcome and prognosis were still inferior, due to their characteristic of propensity to infiltrate into surrounding parenchyma[2, 3]. Immunotherapy has achieved certain benefit in cancer treatment. The combination of immune checkpoint inhibitors has extended survival of some patients with melanomas or non-small-cell lung cancer[23]. However, the efficacy of immune checkpoint inhibitors was limited and unpredictable in

gliomas, particularly in glioblastomas[24]. Recently, Pang et al suggested that stress response and double strand break repair in which PLK3 involved might play important roles in modulation of glioma therapeutic response[1]. However, the status of PLK3 in gliomas still remains unclear. Determining the molecular characteristics of PLK3 may be a new target for glioma therapy.

IDH is a one of the most essential catalytic enzyme in tricarboxylic acid cycle that catalyzes the oxidative decarboxylation of isocitrate to 2-oxoglutarate[1]. In some earlier reports, IDH mutation status was regarded as a significant predictor of prognosis in gliomas, for the association with better prognosis and longer survival compared with IDH wild-type[1, 25]. Moreover, mesenchymal subtype of glioma was relevant to a high frequency of NF1 abnormalities, which presented immunosuppressive and aggression[2, 16]. Crucially, compared with other subtypes, patients with mesenchymal subtype of glioma had the worst prognosis, no matter in primary or recurrent tumor[16]. In this study, we found that increased expression of PLK3 was associated more malignant gliomas in terms of higher WHO grade, IDH wild-type status and mesenchymal subtype in glioma samples. Combining these results, high level of PLK3 showed a correlation with malignant entities. According to analysis of somatic mutations in different PLK3 expression level, we found that mutations of TTN, EGFR, PTEN and NF1, all of which were associated with high expression level, indicated poor prognosis[16, 26–28]. On the contrary, mutations of IDH1, CIC and FUBP1 that were significantly high in low expression level of PLK3 were correlated with longer overall survival[29, 30]. In the low expression of PLK3, high rate of 1p/19q co-deletion was observed. Since FUBP1 and CIC located on 1p and 19q, respectively[30], this might be the reason for high level of mutations in these two genes in the low PLK3 expression group. In contrast, high level of chr7 amplification with chr10 deletion, characteristic molecular in IDH wild- type GBM, was observed in high expression of PLK3[31]. For somatic copy number alternations, cases of high level PLK3 not only demonstrated significant amplification peaks of PIK3C2B, PDGFRA, EGFR and CDK4, which were defined as oncogenic drivers[32, 33], but also showed deletion peaks of tumor suppressor genes, such as CDKN2A and CDKN2C[34]. Thus, we believe that PLK3 expression may play an important role in malignant biological process. Clarifying the mechanism of PLK3 in gliomas may become a crucial step to cure this disease.

The immune checkpoint inhibition, which obtained success in treating recurrences from a variety cancer types, is an attractive therapeutic method[25]. As a vital immune checkpoint, few reports about the correlation between PLK3 and other immune checkpoint were available. PD-1, PD-L1 and CD80 are negative regulators of anti-tumor immune that suppress anti-tumor function of T cell. Recently, a meta-analysis suggested that high expression of PD-L1 indicated poor overall survival in patients with glioma[19]. Liu et al reported that high level of PD-1 was associated with malignancy and invasiveness of glioma[22]. In this study, we firstly revealed the tight correlations between PLK3 and PD-1/PD-L1, as well as CD80. Furthermore, we analyzed the relationship among PLK3 and several genes of immune checkpoints. In both CGGA dataset and TCGA dataset, a suppressive role PLK3 played in the anti-tumor immune response was observed, and the poor prognosis for patients with high expression PLK3 in gliomas, that both of B7H3 and TIM3 were found positively correlated with high WHO grade and aggressiveness of gliomas[32, 35, 36], was also detected. As desirable correlations for PLK3 with some

immune checkpoints were obtained, immune treatment combined with PLK3 inhibitor may be a novel approach for glioma therapy. Studies, elucidating the immunoregulatory role of PLK3 in microenvironment of glioma, and demonstrating that anti-PLK3 can prevent growth and aggressiveness of gliomas, are essential in future.

The relationship between PLK3 and T cell immunity has not studied before. In our analysis of immune function of PLK3 in glioma, positive association between PLK3 and T cell immunity was found in regulatory T cell differentiation and T cell tolerance induction, both of which implied anergy of T cell and aggressiveness of glioma[37]. Furthermore, negative regulation of alpha-beta T cell activation was also detected. For T-cell receptor (TCR) $\alpha\beta$ was expressed in about 95% of T cell, and TCR $\alpha\beta$ + T cells were thought to play a prototypical role in adaptive immunity[38], negative regulation of TCR $\alpha\beta$ + T cells may restrain T cell immunity in glioma. Since we found that PLK3 has close connection with PD-1, PD-L1 and CD80, it is possible that suppression of T cell immunity by PLK3 may attribute to the regulation of PD-1, PD-L1, or CD80 through PLK3. Nevertheless, these findings were correlative, further studies that elucidate the relationship between PLK3 and T cell functions are essential.

As for prognosis, the present study unveiled that high expression of PLK3 indicated shorter overall survival in patients with different WHO grade of gliomas and GBM. Therefore, the immunotherapy restraining activity of PLK3 may improve prognosis of patients with glioma.

Conclusion

Through the analysis of transcriptomic and genomic profiling data, we found that the expression of PLK3 was high in more malignant gliomas. Furthermore, PLK3 was associated with inhibition of anti-tumor immunity of T cell, and played an important role in inflammatory and immune response in glioma. As well, obvious association between PLK3 and other immune checkpoints was found. Crucially, high PLK3 was revealed to be related with poor clinical prognosis. These results demonstrated that PLK3 may serve as a biomarker and a potential target of glioma.

List Of Abbreviations

PLK3: Polo-like kinases 3; PD-1: programmed cell death protein 1; CGGA: Chinese Glioma Genome Atlas; TCGA: The Cancer Genome Atlas;

Declarations

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Not applicable

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Authors' Contributions

SC conceived the study and performed bioinformatics and statistical analysis. AQ, and DG interpreted the data. AQ, XSW and JMM drafted the paper. GH supervised the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All data was searched from public databases, and the ethics approval was not required.

Consent for publication

We assure that the manuscript is original and it has not been published elsewhere yet.

Availability of Data

The datasets presented in this study can be found here: <http://cancergenome.nih.gov/>; <http://www.cgga.org.cn> and <http://cancergenome.nih.gov/>

Competing interests

The authors declared that no competing of interests existing in this study.

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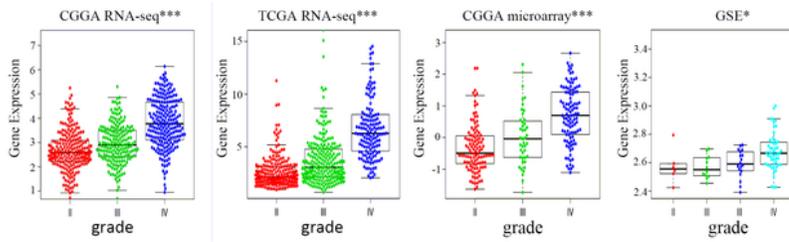
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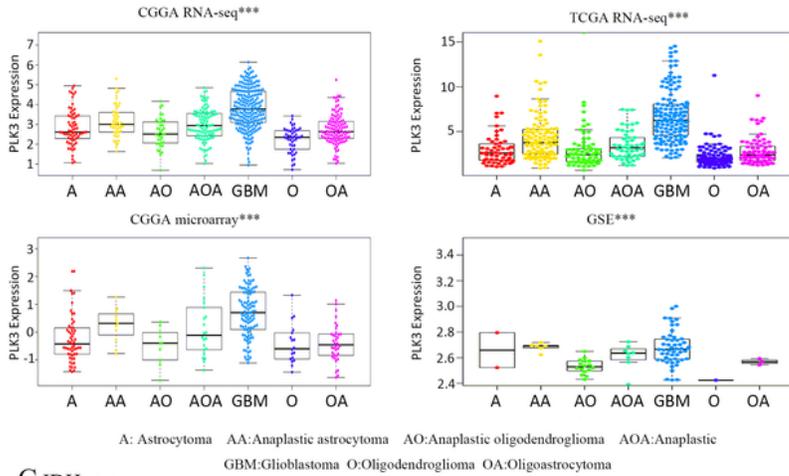
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Figures

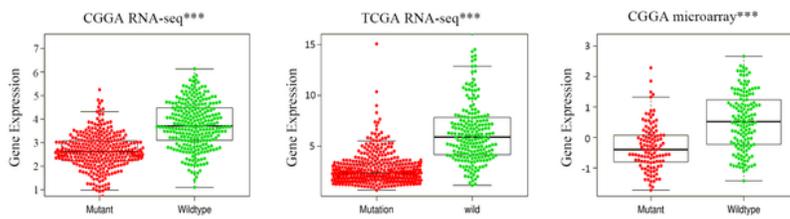
A WHO grade



B histology



C IDH status



D TCGA.molecular.classification

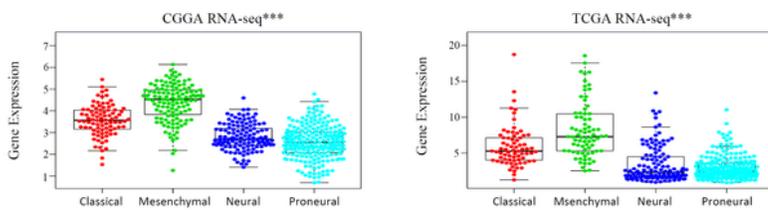


Figure 1

Clinical and molecular glioma parameters in association with PLK3 expression. A. PLK3 expression increased by WHO grade. B. PLK3 was enriched in GBM. C. PLK3 highly expressed in IDH wild-type glioma. D. PLK3 highly expressed in Mesenchymal subtype of glioma. In our cohort, ns, *, *** indicated no significant difference, $P < 0.05$, $P < 0.001$, respectively.

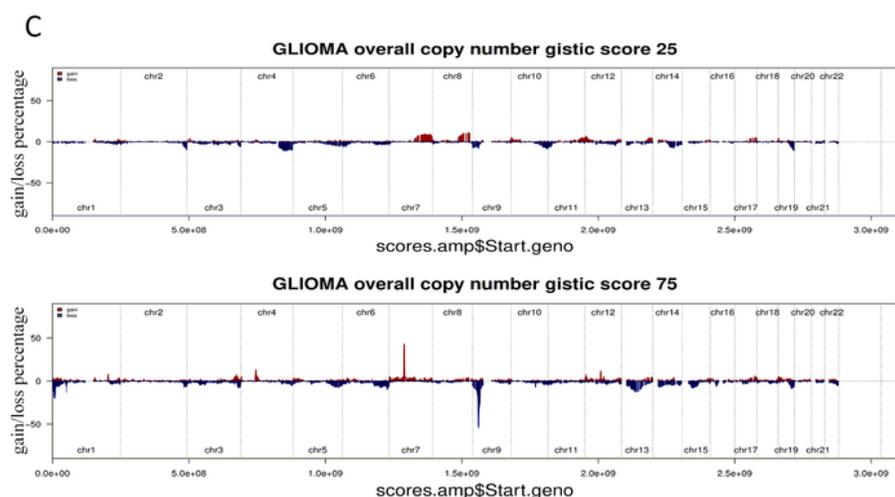
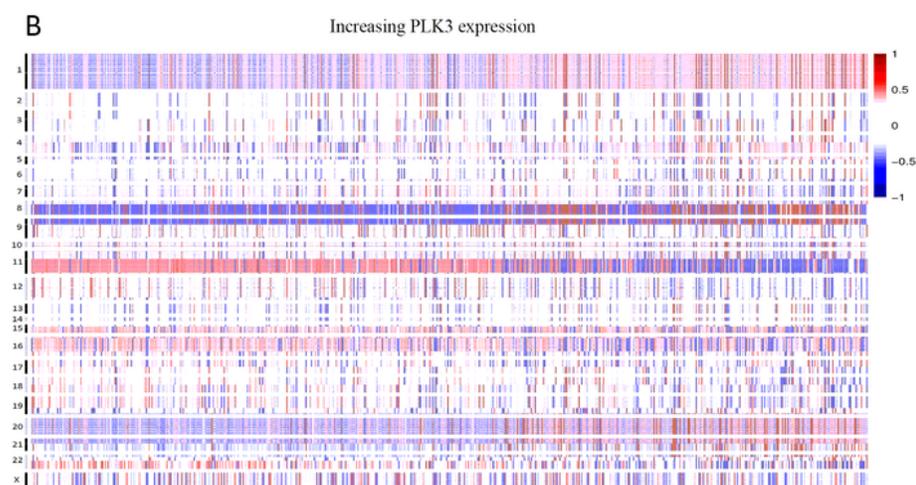
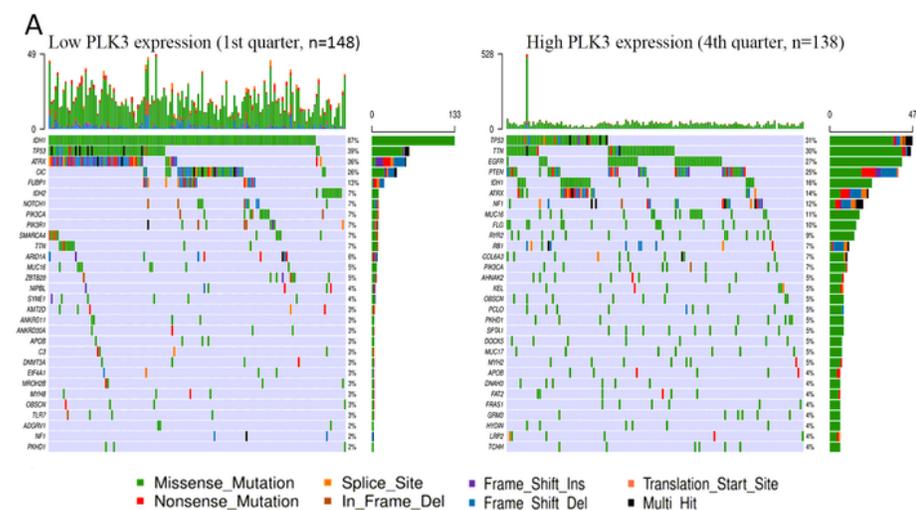


Figure 2

Gnomic profiles associated with different expression level of PLK3. A. Different somatic mutations were found in low and high PLK3 expression in glioma. B. The overall CNAs profile with the increase of PLK3 expression. C. GISTIC 2.0 amplifications and deletions in different PLK3 expression in glioma.

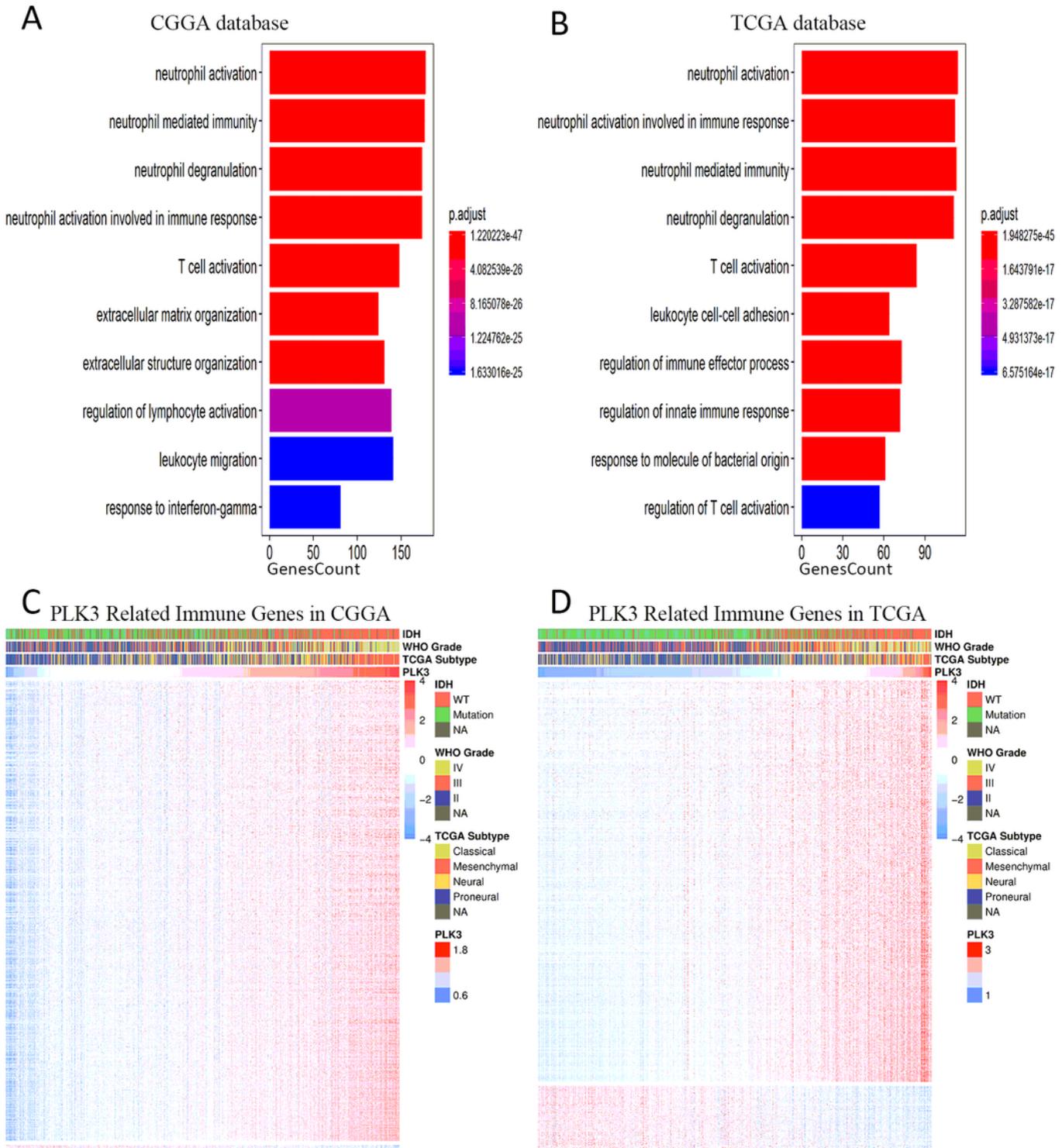


Figure 3

PLK3-related biological process and immune genes in glioma. A and B. Gene ontology analysis demonstrated that PLK3 was mostly associated with inflammatory response and immune response in CGGA and TCGA datasets. Heatmap analysis demonstrated that PLK3 was positively correlated with most immune genes in CGGA and TCGA datasets.



Figure 4

Relationships between PLK3 and immune checkpoints. A and B. Correlations among PLK3, PD-1, PD-L1 and CD80 in whole gliomas, low-grade gliomas and GBM. C and D. Associations between PLK3 and other immune checkpoints.

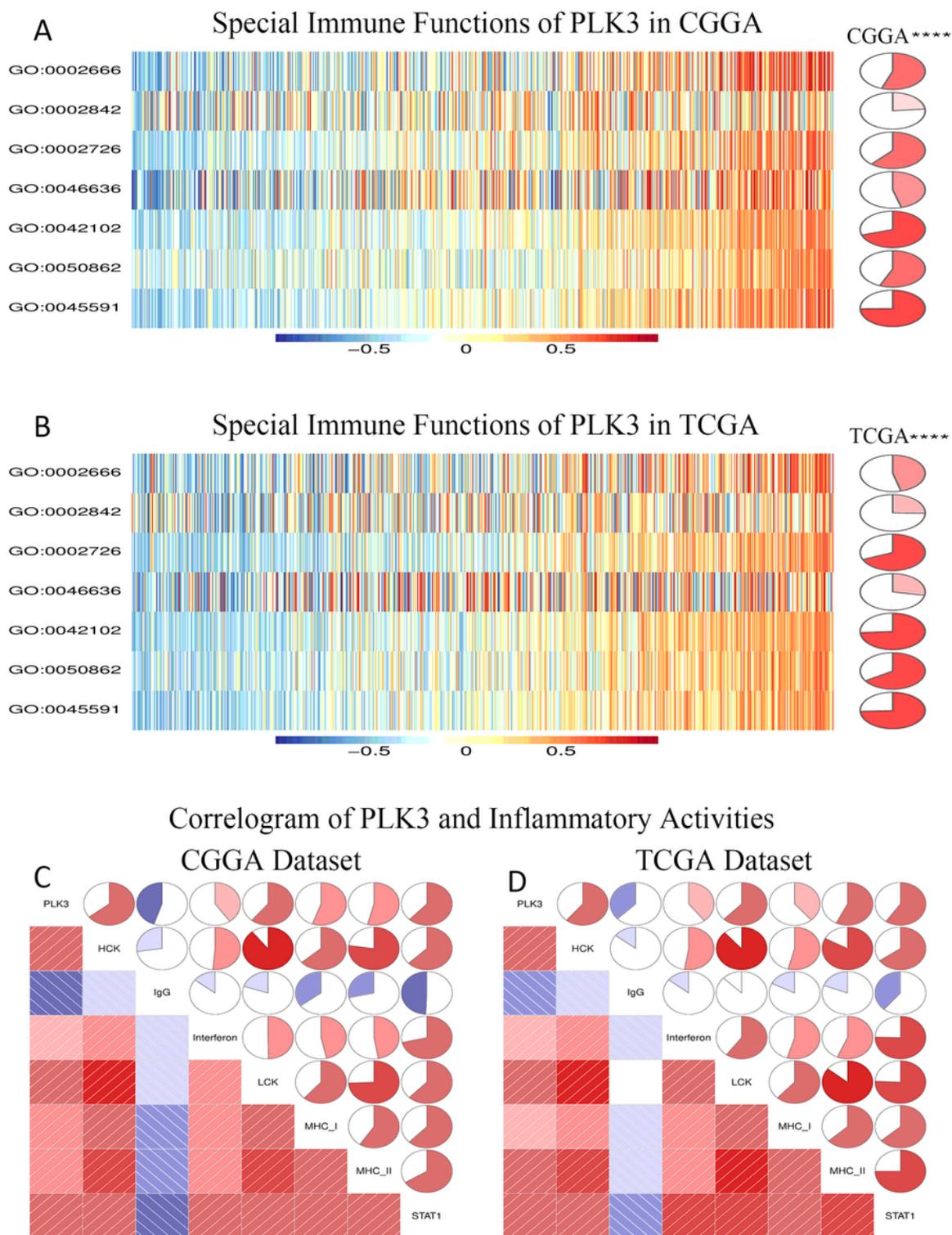


Figure 5

PLK3-related T cell immunity and inflammatory activities in glioma. A and B. PLK3 related T cell immunity. ns, *, *** indicated no significant difference, $P < 0.05$, $P < 0.001$, respectively. GO:0002666, positive regulation of T cell tolerance induction. GO:0002842, positive regulation of T cell mediated immune response to tumor cell. GO:0002726, go positive regulation of T cell cytokine production. GO:0046636, go negative regulation of alpha-beta T cell activation. GO:0042102, go positive regulation of

t cell proliferation. GO:0050862, go positive regulation of T cell receptor signaling pathway. GO:0045591, go positive regulation of regulatory T cell differentiation. C and D. relationship between correlogram of PLK3 and inflammatory activities. Red color indicated positive correlations and blue color indicated negative correlations.

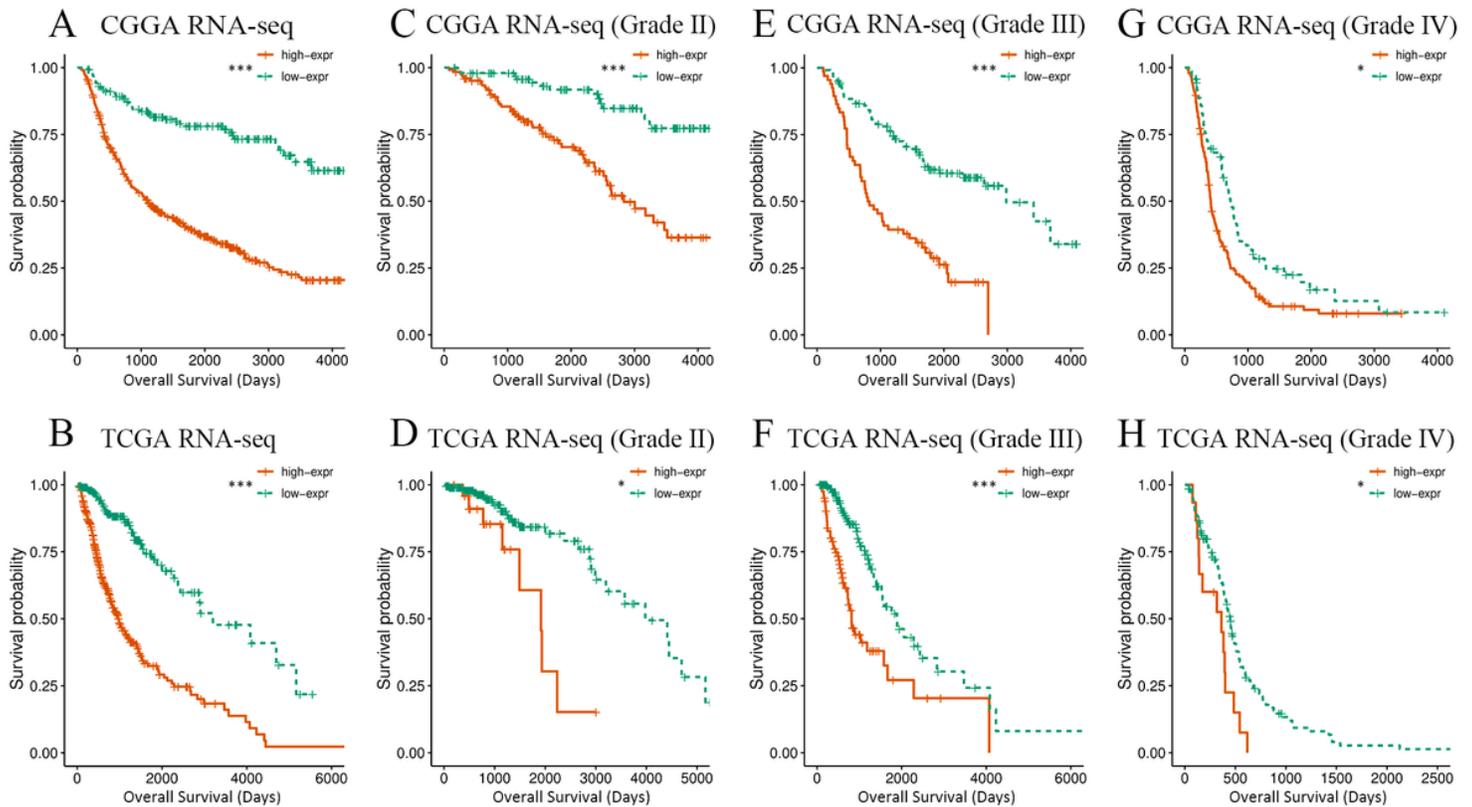


Figure 6

Overall survival in patients with glioma in low and high PLK3 expression. Kaplan–Meier survival analysis for patients with gliomas in whole grades (A and B), gliomas in grade II (C and D), gliomas in grade III (E and F), and gliomas in grade IV (G and H) in CGGA and TCGA datasets. ns, *, *** indicated no significant difference, $P < 0.05$, $P < 0.001$, respectively.

Supplementary Files

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