High PRMT6 expression Associated With Prognosis and Immune Infiltration in Glioma

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Primary research

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Abstract

Background

Glioma is characterised by easy invasion of surrounding tissues, high mortality and poor prognosis. Moreover, the prognosis of glioma is getting worse and worse with the increase of grade, which is not optimistic. Therefore, biological markers for glioma are needed in clinical to detect and evaluate the situation and prognosis of patients with glioma. In many studies, we have found that the protein arginine methyltransferase 6 (PRMT6) expression is elevated in various tumors, which is associated with prognosis of patient. However, there has been no report or study on the role of PRMT6 in glioma.

Methods

In this study, we used various tumor-related databases to analyze the mechanism of PRMT6 in tumors, especially gliomas, from bioinformatics, and carried out relevant experimental verification with tumor tissues extracted from patients during surgery. Besides, we analyzed the relationship between PRMT6 expression and immune infiltration and immune-related cells, and discussed the possible mechanisms. We also discussed the role of PRMT6 expression in glioma from mutation, clinical indicators, enrichment analysis, and immunohistochemical results.

Results

PRMT6 is significantly differentially expressed in multiple tumors, which is associated with survival and prognosis. Especially in gliomas, the PRMT6 expression gradually increased with the grade increasing. Besides, PRMT6 can be used as an independent prognostic risk factor in the nomogram and has been verified in various databases.

Conclusions

Our results indicate that high PRMT6 expression is a potential biomarker for predicting prognosis and progression of glioma.

Background

Glioma is the most common and aggressive tumors in the central nervous system, accounting for 70–80% among primary malignancy tumors[1–3]. Low grade glioma (LGG) is a kind of glioma with slow growth at the initial stage[4], while glioblastoma multiforme (GBM) is easy to invade with high recurrence rate[5]. Currently, treatments of glioma are mainly surgery and chemoradiotherapy, and the relationship between surgical resection range and prognosis is controversial. However, glioma is more likely to recrudescence and respond less well to treatment, leading to a risk of postoperative seizures[6, 7]. At the molecular level, the molecular markers of glioma mainly include IDH mutation, 1p/19q co-deletion[8], MGMT promoter methylation[9] and TP53 mutation. However, the pathogenesis and molecular of glioma are still poorly understood and need a lot of research. It originates out of considerable significance and the urgent need to study tumor markers at the molecular level. Recently, more and more attention has been paid to the important role of immune cell infiltration in glioma. Some studies have indicated that infiltration of immune cells in gliomas can promote the invasion and progression of gliomas[10, 11]. If macrophages have a high proportion in tumor tissue, the interaction of tumour-infiltrating T cells and multiple signalling pathways remains to be developed, such as the inhibitory effect of PD-1 on inflammatory response[12]. Even though with these immune mechanisms, immunotherapy for gliomas remains to be further explored.

In the nucleus, protein arginine methylation is a post-translational modification involved in signal transduction[13], which is catalyzed by the protein arginine methyltransferase (PRMT). There are eleven known types of PRMT, which can fall into three categories[14]. PRMT6 produces asymmetric dimethylarginine with unique characteristics of self-methylation[15], responsible for the methylation of histone H3R2 and wide expression in various tissues of the body[16]. As a result, PRMT6 had been studied in many tumors. According to PRMT6's unique biological role, many studies have concluded that it can promote the development of cancer through different signaling pathways or inflammatory cells[17]. Recent studies had reported that PRMT6 promotes the proliferation of endometrial cancer cells through the AKT/mTOR pathway, further promoting carcinogenesis[18]. Additionally, there were also related studies on

lung cancer, hepatocellular carcinoma, colon cancer and prostate cancer[19]. Whereas, the mechanism of PRMT6 in gliomas is not precise, with insufficient immune-related studies.

At present, there have no studies being conducted on the role of PRMT6 in glioma. Based on multiple tumor-related public databases, we explored the potential mechanism and role of PRMT6 in gliomas in terms of immune mechanism, epigenetics and clinical prognosis. Our analysis revealed that PRMT6 is a potential tumor marker for glioma, providing important evidence for its epigenetic and immunological correlation.

Materials And Methods

Analysis on gene expression level

Genotype-Tissue Expression (GTEx) database mainly includes gene expression in healthy human tissues and organs [20]. We obtained the PRMT6 expression in various normal tissues from this database. In Figure 1a, the X-axis lists 31 kinds of tissues and the Y-axis represents the PRMT6 expression calculated by $\text{Log}_2(\text{TPM}+1)$.

The Cancer Genome Atlas (TCGA) database is a comprehensive database, containing not only the molecular level data of dozens of primary tumors, but also the pertinent clinical data. First, we selected the data set from the TCGA database with the number of normal samples greater than equal 5, with a differential analysis among the 18 tumors (Figure 1b) performed. Then, we combined the normal samples from the GTEx database with those from the TCGA database for further difference analysis of PRMT6. 24 tumor samples were taken as X-axis, and the PRMT6 expression calculated by $\text{Log}_2(\text{TPM}+1)$ as Y-axis (Figure 1c).

Cancer Cell Line Encyclopedia (CCLE) database mainly records the genetic characteristics of cell lines, sequencing more than 900 cancer cells from more than 30 human tissues. In this study, the data from multiple tumor cell lines were downloaded from the CCLE database, compared with PRMT6 expression levels in 21 tissues (Additional file 1: Figure S1). 1019 samples and 21 tissues were taken as X-axis, and the PRMT6 expression calculated by $\text{Log}_2(\text{TPM}+1)$ as Y-axis.

Analysis on gene expression and survival prognosis

In our study, the relationship between PRMT6 expression and clinical prognostic indicators, including overall survival (OS), disease-specific survival (DSS) and progression-free survival (PFS), was analyzed with gene expression profiles from the TCGA database in 33 tumors. We used Kaplan-Meier survival analysis curve and forest map respectively to visualize the relationship between them (Figure 2).

Analysis on gene expression and immune estimation

CIBERSORT is a software to analyze immune cell infiltration[21]. We downloaded markers for gene expression of 22 immune cells from the website. In this study, scores of immune infiltration were collected and the correlation between the PRMT6 expression in these tumors and the immune cell infiltration (Additional file 2: Figure S2). The immune cell infiltrate scores of CIBERSORT were taken as the X-axis, and the PRMT6 expression calculated by $\text{Log}_2(\text{TPM}+1)$ as Y-axis (Figure 3a).

We performed a comprehensive analysis of the total number of immune and stomatal cells in each type of tumor using the estimation package in R software (Additional file 3: Figure S3). The corresponding scores was taken as X-axis, and the PRMT6 expression calculated by $\text{Log}_2(\text{TPM}+1)$ as Y-axis, with the distribution of each score described by a density curve (Figure 3c-f).

We obtained the information of more than 40 immune checkpoint genes and investigated the relationship between the PRMT6 expression and the immune checkpoint gene expression (Figure 3b).

TISIDB database is an open online tool to integrate various tumor immunology resources[22], which we used to analysis the relationship between PRMT6 expression in various tumors and immune or molecular subtypes (Additional file 4: Figure S4).

Analysis on gene expression and mutation

The number of gene mutations in each tumor was obtained from the TCGA database, which was corrected by comparing the total length of exons. We tested the correlation between PRMT6 expression and tumor mutational burden (TMB) and visualized the relationship between them through radar map (Figure 4a).

Microsatellite instability (MSI) is a change in the length of a microsatellite due to insertion or deletion of duplicate units in the tumor compared to normal tissues. We visualized the correlation between the PRMT6 expression in tumors and MSI by radar map (Figure 4b).

Analysis on gene expression and clinical indicators

Chinese Glioma Genome Atlas (CGGA) is a complete genome sequencing database for patients with glioma in China. We obtained the clinical information and PRMT6 gene expression information of 325 patients with glioma from the CGGA database[23, 24]. Then, we analyzed the clinical information of CGGA and TCGA databases respectively, and the PRMT6 expression and its correlation (Figure 5a-j). Kruskal–Wallis test and Mann–Whitney test were used to determine whether gene expression was different among various clinical indicators. On the X axis is the classification of various clinical indicators, and on the Y axis is the level of gene expression.

Construct and verify the nomogram

We used the high and low PRMT6 expression as an independent prognostic risk factor and constructed a nomenclature diagram combined with other common risk factors (Figure 6c). Risk factors analyzed in this study included age, gender, tumor grade, IDH mutation status, 1p19q co-deletion status, chemotherapy status, and PRMT6 expression level. To verify whether PRMT6 can act as an independent prognostic risk factor, we first plotted the survival analysis curve with Kaplan-Meier method (Figure 6a-b). Then, we applied univariate Cox and least absolute shrinkage and selection operator (LASSO) regression model to screen all the variables. We took TCGA database as the training set and CGGA database as the verification set, and consistency index (C-index) and calibration curve to evaluate the nomogram (Figure 6d-e).

Gene Set Enrichment Analysis(GSEA)

GSEA is a computational method to determine whether a predefined data set has significant consistent differences between two biological states[25], which can be downloaded from the website. The expression dataset was the PRMT6 expression, the experimental group and the control group were set to high PRMT6 expression and low PRMT6 expression, and the number of permutations was set to 1000. In the results obtained, NOM p-val and FDR q-VAL value less than 0.05 were considered to be significantly different.

Immunohistochemical analysis

The specimens used in this study were collected from 32 glioma tissues and 4 glioma paracancerous tissues surgically from the First Affiliated Hospital of China Medical University between 2012 and 2014. The clinical information of all patients was obtained, including survival time and survival status through telephone follow-up. Of the 32 cases of glioma, there were 20 males and 12 females aged from 29 to 80, with a mean age of 51; according to the grading standards of the World Health Organization (WHO), there were 12 cases of WHO II, 7 cases of WHO III and 13 cases of WHO IV; 21 deaths, with a mortality rate of 65.6%; the mean follow-up time was 43.65 months.

PRMT6 antibody used in this study is rabbit anti-human polyclonal antibody from Novus, USA (No. NB 110-40713). SP hypersensitive immunohistochemical kit was used for the secondary antibody, which was purchased from Fuzhou Maixin Biotechnology Development Limited Liability Company, China.

The experimental procedure was strictly carried out in accordance with the instructions of the immunohistochemical kit. Firstly, the paraffin sections were dehydrated and then microwaved to repair the antigen. The primary antibody (dilution concentration of PRMT6 was 1:500) was added for incubation for 1 hour, and then the secondary antibody was dropped to incubate DAB for color development. The blank control group was set to replace primary antibody with phosphate buffer saline. Through the identification of pathologists in our hospital, the staining intensity and positive cell rate of each section were graded by semi-quantitative integration method, and then the positive intensity was obtained by the product of two fractions (Table 1). Immunohistochemical scores: 0-1 is negative expression, 2-4 is low expression, and 6-9 is high expression.

Table 1: Grading criteria

Immunohistochemical score	Staining intensity of positive cells	Percentage of positive cells
0	No staining	<5%
1	Weak staining	5%-25%
2	Medium staining	26%-75%
3	Strong staining	>75%

Data analysis and processing

We used the Log-rank test and Cox analysis to calculate OS, DSS and PFS. Spearman test, Wilcox test and Kruskal test were used to determine the correlation coefficient between the PRMT6 expression and other indicators, and the corresponding P value, hazard quantitation (HR) and 95% confidence interval (95%CI) were calculated. All P values less than 0.05 were examined statistically significant. In this study, R-3.6.3 was used for data analysis and visualization, and Adobe illustrator CC 2019 was used for figures processing. Immunohistochemical data processing and graphics rendering was done with GraphPad Prism 8.

Results

PRMT6 expression in different human tissues and cancers

To elucidate the differences between PRMT6 in healthy tissues and tumor tissues, we performed a visual analysis of the PRMT6 expression in healthy tissues. As observed in Figure 1a, PRMT6 is expressed at low levels in healthy brain tissues. Then we also analyzed the PRMT6 expression in different tumors and normal tissues. Tumors with significant positive associations included: Adrenocortical carcinoma (ACC), Bladder Urothelial Carcinoma (BLCA), Breast invasive carcinoma (BRCA), Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), Colon adenocarcinoma (COAD), Esophageal carcinoma ESCA, Glioblastoma multiforme (GBM), LGG, Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma(LUAD), Lung squamous cell carcinoma (LUSC), Ovarian serous cystadenocarcinoma (OV), Pancreatic adenocarcinoma (PAAD), Prostate adenocarcinoma (PRAD), Skin Cutaneous Melanoma (SKCM), Stomach adenocarcinoma (STAD), Testicular Germ Cell Tumors (TGCT), Thyroid carcinoma (THCA), Uterine corpus endometrial carcinoma (UCEC), Uterine Carcinosarcoma (UCS). There were also some negatively correlated tumors: Kidney renal clear cell carcinoma (KIRC), Acute Myeloid Leukemia (LAML), Kidney Chromophobe KICH, Kidney renal papillary cell carcinoma (KIRP).

Analysis of survival prognosis

We used clinical information from the TCGA database to investigate the correlation between PRMT6 and survival prognosis. Forest maps of OS, DSS, and PFI showed that the PRMT6 expression is a risk factor in BLCA, LGG and UCEC, as well as a protection factor in BRCA. In addition, PRMT6 expression was divided into high and low groups, with survival analysis curves. In OS analysis, the high and low PRMT6 expression was significant in LGG and UCEC. In DSS analysis, the high and low PRMT6 expression was significant in LGG, LUAD and UCEC. In PFI analysis, the high and low PRMT6 expression was significant in LGG, COAD and LUAD. These evidence suggest that PRMT6 has important reference for prognosis in tumors.

Multidimensional immune correlation analysis

In order to investigate the effect of PRMT6 on immune level, we analyzed its interaction with different immune infiltrating cells, immune microenvironment, immune subtype and immune checkpoint genes from different perspectives. As we can see from Additional File 1 Figure S1, PRMT6 expression in BRCA, CESC, Head and Neck squamous cell carcinoma(HNSC), KIRC, LGG, LIHC, PAAD, SKCM, Thymoma (THYM), UCEC are significantly correlated with the immune cells existed, indicating that PRMT6 expression is strongly correlated with immune cells in tumors.

Then, we analyzed the relationship between PRMT6 expression and stromal cell and immune cell score in tumors. The stronger the correlation, and the higher the score, the more significant the proportion of stromal cells and immune cells in the tumor tissue. As we can see from the Additional file 2 Figure S2, gene expression was negatively correlated with immune cells or stromal cells of score in BLCA, CESC, GBM, LAML, LUSC, OV, Pheochromocytoma and Paraganglioma (PCPG), PRAD, Sarcomav(SARC), STAD, TGCT, THCA,

THYM and UCEC. Interestingly, it's only positive in LGG, which can further prove the intrinsic relationship between PRMT6 expression in g and immune cells.

Also, we analyzed the expression relationship between PRMT6 and 47 common immune checkpoint genes. From Figure 3b, we can see that the PRMT6 expression in various tumours is strongly correlated with immune checkpoint genes.

Finally, we used the TISIDB online tool to analyze the relationship between gene expression and immune or molecular subtypes (Additional file 3 Figure S3). The results show that the expression of LGG is significantly higher than others’.

Mutation correlation analysis

We calculated TMB in each cancer tumor, and we can see that from the radar map in ACC, THCA, PRAD, PAAD, OV, LGG, COAD and CESC have significantly correlation. In the MSI radar map, SKCM, PAAD, LUAD, KIRC, Lymphoid Neoplasms Diffuse Large B-cell Lymphoma (DLBC) and COAD have significant differences. In the MSI, COAD, DLBC KIRC, LUAD, PAAD, SKCM have statistically significant differences.

Various clinical indicators correlation analysis

To further elucidate the potential clinical value of PRMT6 in glioma, we analyzed various clinically common indicators including age, tumor grade, IDH mutation status, 1p19q co-deletion status, chemotherapy status. As can be seen from Figure 5, the PRMT6 expression level in TCGA and CGGA databases was significantly correlated with various clinically common risk factors.

Independent prognostic risk factor analysis

We considered high and low PRMT6 expression as an independent risk factor. First, survival analysis curves were performed based on TCGA and CGGA data, showing that the high and low PRMT6 expression significantly affected the prognosis of patients. Then, we constructed the nomogram by screening for various independent risk factors. We used TCGA database as the training set and CGGA database as the validation set for external validation. As from the results of the nomogram, PMRT6 is potentially valuable as an independent prognostic risk factor. In order to test the accuracy of the nomogram model, we calculated the C-index, which was 0.84 (95%CI:0.865-0.815) in the nomogram of TCGA, and 0.772 (95%CI:0.801-0.743) in the nomogram of CGGA. The calibration curves of the 1-year and 3-year survival rates of the two models were drawn respectively, and the results showed that the two models had better validation performance.

Gene enrichment results

According to the results of KEGG and GO enrichment analysis, the role of PRMT6 in gliomas is mainly related to the regulation of cell cycle, the involvement of DNA damage and repair, and the conduction of some signaling pathways. Table 2 reveals some representative pathways and related functions of enrichment.

Table 2: Gene enrichment results

Gene set names	NOM p-val	FDR q-val
KEGG gene set		
KEGG_BLADDER_CANCER	0	0.038
KEGG_SMALL_CELL_LUNG_CANCER	0.002	0.038
KEGG_PANCREATIC_CANCER	0.006	0.044
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	0.002	0.046
KEGG_P53_SIGNALING_PATHWAY	0	0.031
KEGG_MISMATCH_REPAIR	0	0.045
KEGG_NUCLEOTIDE_EXCISION_REPAIR	0.006	0.043
KEGG_ECM_RECEPTOR_INTERACTION	0.008	0.047
KEGG_PYRIMIDINE_METABOLISM	0.002	0.034
KEGG_GLUTATHIONE_METABOLISM	0.002	0.049
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	0	0.045
GO gene set		
Cell cycle regulation		
GO_CELL_CYCLE_G1_S_PHASE_TRANSITION	0	0.029
GO_POSITIVE_REGULATION_OF_CELL_CYCLE_PHASE_TRANSITION	0	0.033
GO_REGULATION_OF_CELL_CYCLE_PHASE_TRANSITION	0.002	0.032
GO_REGULATION_OF_DNA_TEMPLATED_TRANSCRIPTION_IN_RESPONSE_TO_STRESS	0	0.032
GO_REGULATION_OF_POSTTRANSCRIPTIONAL_GENE_SILENCING	0	0.037
GO_REGULATION_OF_TRANSCRIPTION_FROM_RNA_POLYMERASE_II_PROMOTER_IN_RESPONSE_TO_HYPOXIA	0.002	0.038
GO_SIGNAL_TRANSDUCTION_INVOLVED_IN_CELL_CYCLE_CHECKPOINT	0	0.041
GO_MITOTIC_CELL_CYCLE_CHECKPOINT	0.002	0.035
DNA damage and repair		
GO_DNA_DAMAGE_RESPONSE_DETECTION_OF_DNA_DAMAGE	0	0.042
GO_DNA_DAMAGE_RESPONSE_SIGNAL_TRANSDUC	0	0.042
GO_DNA_SYNTHESIS_INVOLVED_IN_DNA_REPAIR	0.002	0.041
GO_G1_DNA_DAMAGE_CHECKPOINT	0.004	0.047
GO_NUCLEOTIDE_EXCISION_REPAIR_DNA_GAP_FILLING	0.004	0.045
GO_SIGNAL_TRANSDUCTION_IN_RESPONSE_TO_DNA_DAMAGE	0	0.04
Signal transduction		
GO_REGULATION_OF_SIGNAL_TRANSDUCTION_BY_P53_CLASS_MEDIATOR		
GO_SIGNAL_TRANSDUCTION_BY_P53_CLASS_MEDIATOR	0.004	0.042
	0.002	0.039
GO_TUMOR_NECROSIS_FACTOR_MEDIATED_SIGNALING_PATHWAY	0	0.042

Gene sets with NOM p-val and FDR q-value<0.05 are considered as significant.

Immunohistochemical results

Immunohistochemical results showed that PRMT6 protein was positively expressed in the nucleus and was brownish yellow or brown in color. The positive rate was 87.5% in glioma and 25% in normal tissue, with statistical difference, and a P value of 0.0055. Additionally, the PRMT6 expression in normal tissues, LGG and GBM was significantly different, with a significant upward trend. Based on the obtained clinical information, we drew a survival analysis curve, which further verified the significant correlation between high and low PRMT6 expression and prognosis.

Table 3: Expression of PRMT6 in glioma and normal tissues

Tissue types	Number of cases	PRMT6			P value
		Negative	Low	High	
		Expression	Expression	Expression	
Glioma tissue	32	4	16	11	0.0055
Normal tissue	4	3	1	0	

Discussion

PRMT6 is a key epigenetic enzyme in the PRMT's family[26], which is a histone modification associated with transcriptional activation with unique self-methylation activity[27, 28]. PRMT6 involves in various regulatory processes, including signal transduction and transcriptional activation[29]. At present, there have been more and more studies found that PRMT6 plays an important role in many tumors, but there is still a lack of relevant research in glioma. Hence, considering these conditions, we investigated the PRMT6 expression in pan-cancer and analyzed the correlation of PRMT6 expression from multiple perspectives such as immunity and mutation. At the same time, we found the close relationship and potential value of PRMT6 and gliomas. Moreover, the potential clinical value of PRMT6 was verified from the perspective of clinical indicators., so the potential value of PRMT6 as a tumor marker of glioma and its role in immunity were firstly discussed in this study..

The results of our study suggest that the expression level of PRMT6 plays an important role in various tumors, which is consistent with some known studies, for example, PRMT6 could be used as a target for colon cancer in the intestinal tract[30]; the PRMT6 expression was up-regulated in endometrial cancer samples and promoted the growth and metastasis of tumor cells by activating related pathways. Additionally, it affected aerobic glycolysis through signalling pathways in hepatocellular carcinoma[31], and also showed special value in gliomas. The gene expression analysis showed that PRMT6 expression was low in normal brain tissue, but increased significantly in glioma. And both gene expression profile and immunohistochemical results have been verified.

At present, people pay more and more attention to the role of immune cells in tumor, and the type of immune cell infiltration and the subtype of immune cells are closely related to the occurrence and development of tumor[32, 33]. This study found that the PRMT6 expression in various tumor was mainly related to T cell and macrophage infiltration. In glioma, the primary immunoinfiltrating cell are T cells, CD4 memory resting cells and dendritic cells. People also pay more and more attention to the immunotherapy for tumors, which is an emerging direction for the immunotherapy that stimulates the immune system to enhance the anticancer ability[34]. A number of studies have shown that immunotherapy on autologous dendritic cells can improve patient survival[35]. Referring to our results, we speculated that there is an intrinsic relationship between PRMT6 expression and immunotherapy targeting dendritic cells. Recent studies have also shown that regulatory T cells (Treg) function in immune regulation is associated with PRMT. And there was an autoimmune response in mice with PRMT5 deficiency when Treg cells were infiltrated[36]. The results of this study showed that in glioma, the up-regulated PRMT6 expression was positively correlated with the infiltration of CD4 T cells, suggesting that PRMT6 is a valuable therapeutic target in immunotherapy for gliomas.

At the gene level, we also compared the correlation between the expression level of PRMT6 and the expression of some immune checkpoint genes in tumors. In the immune checkpoint genes analogous to LGG, the genes significantly connected with the activation of T and B lymphocytes and macrophages, such as CD40, CD44, CD80 and CD86. Besides, NRP-1 gene was associated with immune regulation and cell migration and interacted with Tregs[37]. The HAVCR2 gene encoded TH1-specific cell surface proteins, which was

associated with regulating macrophage activation[38]. All these evidence was further expressed that PRMT6 is strongly associated with infiltrating immune cells in LGG. Moreover, this study linked arginine methylation with immunity building a bridge between epigenetics and immunology.

TMB is a promising biomarker, and there have been studies shown that high TMB is positively correlated with immunotherapy[39]. Furthermore, TMB is closely related to the overall survival rate and tumor proliferative activity of patients with glioma[40], which is an important reference factor in the treatment and prognosis of glioma. This association may be closely related to PRMT's involvement in cell cycle regulation and DNA damage, such as catalyzed cyclin expression, leading to spontaneous DNA damage, checkpoint deletion, and chromosome instability. These factors will lead to gene coding errors, base substitutions, and gene insertion or deletion errors in somatic cells, further increasing TMB and thereby promoting glioma proliferation.

In the GSEA results, we found that PRMT6 expression is related to the regulation of cell cycle, DNA damage and repair, and signal transduction in gliomas, acting as an important role in DNA repair and regulation of DNA polymerase β (Pol- β), and an enzyme involved in basic repair[41]. PRMT6 is co-activated in staining as a nuclear factor to facilitate the transcription process[42]. From this evidence, we can infer that PRMT6 can promote the transcription of tumor cells in gliomas and thus increases the proliferation of tumor cells.

To verify the clinical value of PRMT6, we analyzed its association with clinically common risk factors and studied it as an independent prognostic risk factor. One of the most noteworthy was the correlation with chemotherapy. Studies have shown that PRMT6 can lead to dysfunctions of P21^{CDKN1A} (P21) in cancers, causing it to be methylated and promoting phosphorylation of threonine 145 on P21, making cancer cells more resistant to anti-tumor drugs[43, 44]. At present, the imbalance of arginine or lysine methyltransferase in cancer has been gradually noticed[45], and new anticancer drugs targeting these sites are in clinical trials[46]. The development prospects of PRMT6 inhibitor chemotherapeutic drugs in the treatment of glioma are considerable.

Conclusions

According to the above evidence, we can conclude that high PRMT6 expression is a potential tumor marker for gliomas with important predictive value for the prognosis of patients. Additionally, PRMT6 may be involved in the regulation of glioma cell cycle through signal transduction, promote the RNA transcription process, and further improve the proliferation and invasion of glioma cells. In clinical treatment, immunotherapy and chemotherapy are both areas to be developed.

Abbreviations

LGG: low-grade glioma; PRMT6: protein arginine methyltransferase 6; PRMT: protein arginine methyltransferase; GTEx: Genotype-Tissue Expression; CCLE: Cancer Cell Line Encyclopedia; TCGA: The Cancer Genome Atlas; OS: overall survival; DSS: disease-specific survival; PFS: progression-free survival; TMB: tumor mutational burden; MSI: microsatellite instability; MMR: mis-match repair; HR: hazard ration; 95%CI: 95% confidence interval; NES: normalized enrichment score; FDR: false discovery rate; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CHOL: Cholangiocarcinoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; PRAD: Prostate adenocarcinoma; STAD: Stomach adenocarcinoma; KICH: Kidney Chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; THCA: Thyroid carcinoma; UCEC: Uterine corpus endometrial carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; HNSC: Head and Neck squamous cell carcinoma; LIHC: Liver hepatocellular carcinoma; PAAD: Pancreatic adenocarcinoma; SKCM: Skin Cutaneous Melanoma; THYM: Thymoma; LAML: Acute Myeloid Leukemia; OV: Ovarian serous cystadenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; SARC: Sarcomav; TGCT: Testicular Germ Cell Tumors; ACC: Adrenocortical carcinoma; COAD: Colon adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; P21:P21^{CDKN1A}

Declarations

Acknowledgement

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Authors' contributions

Guangyu Li selected the research topic and conducted the guidance of the process of the topic. Yi Yang wrote the article, conducted the immunohistochemical experiment and analyzed the experimental results. Zhenshuang Wang, Jinhai Huang, Chengran Xu and Shengrong Long performed data processing and image analysis. Lun Li and Minze Yao collected survival information through telephone follow-up.

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Availability of data and materials

The data used in this study are all from public databases, including GTEx database (<http://commonfund.nih.gov/GTEX/>), CCLE database (<http://portals.broadinstitute.org/ccle/>), TCGA database (<https://portal.gdc.cancer.gov/>), CIBERSORT database (<https://cibersortx.stanford.edu/>), TISIDB database (<Http://cis.hku.hk/TISIDB>), CGGA database (<http://www.cgga.org.cn/>).

Ethical approval and consent to participate

Our study was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University. All participants offered written informed consent before surgery. The study conforms to the provisions of the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors have declared that no competing interest exists.

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Figures

Figure 1

Expression and comparison of PRMT6 in different normal tissues and tumors. a. PRMT6 Expression in 31 normal human tissues. b-c. Differences in PRMT6 expression between normal tissues and paracancer. (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

Figure 2

Analysis on PRMT6 expression and prognosis paracancer. (a.d.h). Forest map of the relationship between PRMT6 expression and prognosis in 33 tumors, OS,DSS and PFI, respectively. (b-c). In OS, the survival analysis curve consisted of high and low expression of PRMT6, and the tumors with statistical significance included LGG and UCEC. (e-g). In DSS, the survival analysis curve consisted of high and low PRMT6 expression, and the tumors with statistical significance included LGG, LUAD and UCEC. (i-k). In PFI, the survival analysis curve consisted of high and low expression PRMT6, and the tumors with statistical significance included LGG, COAD and LUAD.

Figure 3

The relationship between PRMT6 expression and immune. a. Relationship between PRMT6 expression and immune cells in LGG. b. Correlation between PRMT6 expression and immune checkpoint. c-f. Correlations between PRMT6 expression and immunescore or stromascore in LGG and GBM.

Figure 4

The relationship between PRMT6 expression and mutation. a. PRMT6 expression in 33 tumors was correlated with TMB. b. PRMT6 expression in 33 tumors was correlated with MSI.

Figure 5

The relationship between PRMT6 expression and common clinical risk factors. a. c. e. g. i. The relationship between the two in the TCGA database. b. d. f. h. j. The relationship between the two in the CGGA database.

Figure 6

a. Survival analysis curve based on TCGA data. b. Survival analysis curve based on CGGA data. c. Nomogram based on TCGA data. d. Calibration curve of nomogram based on TCGA data. e. Calibration curve of nomogram based on CGGA data.

Figure 7

Results of gene enrichment analysis. a. Gene enrichment KEGG analysis. b. Gene enrichment GO analysis on the enrichment results of cell cycle regulation. c. Gene enrichment GO analysis on the enrichment results of DNA damage and repair. d. Gene enrichment GO analysis on the enrichment results of signal transduction.

Figure 8

Immunohistochemical results. a. Histograms of PRMT6 scores and tissue types in immunohistochemical experiments. b. Survival analysis of high and low expression of PRMT6 in immunohistochemical experiments. c-d. Immunohistochemical staining sections of normal paracancer tissue with PRMT6 were 200 - and 400 - fold microscopic images, respectively. e-f. Immunohistochemical staining sections of LGG with PRMT6 were 200 - and 400 - fold microscopic images, respectively. g-h. Immunohistochemical staining sections of GBM with PRMT6 were 200 - and 400 - fold microscopic images, respectively.

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