Neurology Asia 2024; 29(1) : 215 – 228

MAD2L2 as a novel prognostic biomarker and its correlation with immune infiltrates in glioma

[1]Kaiqin Chen, [2]He Li, [3]Yongtai Chen, [4]Hesen Huang

[1]Department of Neurosurgery, Xiang'an Hospital of Xiamen University, Xia Men, Fu Jian, China; [2]Department of Ultrasound. Maternal and Child Health Hospital of Fujian, Fuzhou, Fujian, China; [3]Department of Hepatobiliary Surgery, The Affiliated Longyan First Hospital of Fujian Medical University, Longyan, Fujian, China; [4]Department of Otolaryngology-Head and Neck Surgery, Xiang'an Hospital of Xiamen University, Xia Men, Fu Jian, China

Abstract

Background: The most common type of central nervous system tumor is glioma, the identification of Glioma biomarkers is essential. MAD2L2, which is abbreviated as MAD2-Like2, collaborates with a number of proteins to carry out numerous essential cellular functions. Uncertain is the role of MAD2L2 in glioma. The role of MAD2L2 expression is examined for the first time in relation to gliomas occurrence and development in this study *Methods:* A bioinformatics and clinicopathological analysis, immune infiltration analysis, and enrichment analysis were conducted based on TCGA and additional gene expression analysis. Using ssGSEA and TIMER, the immune response to MAD2L2 expression in glioma was analyzed statistically. This study also analyzed the effect of MAD2L2 overexpression on major chemotherapeutic medicines and the methylation status of glioma patients. CGGA, HPA data analysis, and K-M survival were also utilized to validate the results. *Results:* MAD2L2 was a crucial independent prognostic factor for glioma patients. Correlations were found between MAD2L2 expression and age, IDH status, WHO grades and 1p/19q codeletion. MAD2L2 is intimately connected to the DNA replication, transcription, cell cycle, immune system, and signal transduction pathways, as determined by the GSEA. In glioma, The majority of MAD2L2 DNA methylation sites were hypomethylated, and the degree of methylation was associated with patient outcomes. Chemotherapy would benefit MAD2L2 overexpression patients. according to these results. The expression of MAD2L2 was associated with partial immune cell infiltration and co-expressed with immune-related genes and immune checkpoints.
Conclusion: Glioma is characterized by higher MAD2L2 expression, and high MAD2L2 expression is linked with a poor prognosis. MAD2L2 might participate in tumor development by regulating tumor-infiltrating cells inside the TME. MAD2L2 could possibly be an immunotherapeutic target.

Keywords: MAD2L2, glioma, prognosis, tumor immune cell infiltration, tumor microenvironment, methylation

INTRODUCTION

Glioma is a type of malignant brain tumor that makes up more than 80% of cases and has a complicated outlook for survival.[1,2] Existing treatments, such as surgical resection, chemotherapy, and radiation therapy, have poor efficacy.[3] It has become increasingly apparent that immunotherapy can be an effective cure for malignant tumors, including gliomas.[4] PD-1 inhibitors are novel alternative glioma treatments, due to the importance of the immune system in the development and progression of cancer.[5] Furthermore, cost-benefit analysis suggests that for certain patients with glioma, the therapeutic advantages of immunotherapies may outweigh the costs. However, current immunotherapies are insufficient for treating gliomas because of systemic immunosuppression and the complicated tumor microenvironment (TME) in gliomas.[5,6] The pathophysiological mechanisms underlying the development of gliomas remain poorly understood, thus hindering the development of novel effective therapies for gliomas. Understanding the molecular pathways behind

Address correspondence to: Hesen Huang, Department of Otolaryngology-Head and Neck Surgery, Xiang'an Hospital of Xiamen University, No. 2000, Xiang'an East Road, Xia Men, Fu Jian, 361100, China. Tel:18805067806, Email: hshuang@xah.xmu.edu.cn

Date of Submission: 12 July 2023; Date of Acceptance: 18 October 2023

https://doi.org/10.54029/2024edc

glioma formation can therefore aid the discovery of new potential therapeutic and the development of glioma therapeutics.

MAD2-Like2 (MAD2L2) performs multiple intercellular functions in conjunction with other proteins. MAD2L2 works directly with REV3 and combines with POLD2 and POLD3 to form Polymerase ζ .[7] It plays an crucial role in several physiological processes DNA damage response (DDR), cell cycle checkpoints and mitosis).[8,9] DDR components are a unique target for cancer therapeutics.[10] DDR components and mitotic DDR may be targeted using anti-cancer drugs, such as AURKB and MAD2L2 inhibitors. Inhibition of unchecked cell proliferation can be achieved by deregulating p53 DDR pathways with AURKB and MAD2L2.[9] Many cancers are affected by MAD2L2, and its overexpression has been linked to poor prognosis in colorectal cancer.[11] Nevertheless, how MAD2L2 relates to glioma prognosis is still unknown. Investigating MAD2L2's function in glioma progression will lead to the discovery of novel therapeutic targets and the development of novel approaches for treating gliomas.

We supplemented the Huang *et al*.[12] methodology with research on medication efficacy and gene methylation A. The Cancer Genome Atlas (TCGA) analysis of MAD2L2 expression in gliomas provided the basis for our investigation. The aim is to examine the relationship between MAD2L2 expression and certain clinical indicators and the prognosis of gliomas, we employed R statistical software and Xiantao Tools. To learn more about the biological mechanisms connected to the glioma regulatory network, which may be in charge of glioma development, we also carried out Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. Moreover, we examined the relationship between MAD2L2 and immune cells that infiltrate tumors using tumor immune infiltrates and malignant responses (TIMER) and single-sample GSEA (ssGSEA) Tumor-Infiltrating Immune Cells (TIICs). Additionally, we used Kaplan-Meier (K-M) survival analysis, the Human Protein Atlas (HPA), and Chinese Glioma Genome Atlas (CGGA) to examine the relationship between MAD2L2 and the prognosis of gliomas. Finally, we investigated how popular chemotherapeutic medications and their methylation status in patients with glioma were affected by MAD2L2 overexpression.

METHODS

Data collection

We used TCGA to retrieve glioma-related RNA sequences, as well as clinicopathological and survival data.[13] In order to determine whether cancerous and healthy tissues express MAD2L2, we analyzed normal samples from the Genotype-Tissue Expression (GTEx) database and tumor samples from the TCGA. We excluded patients missing data such as age, WHO stage, Overall Survival (OS) time, Isocitrate Dehydrogenase (IDH), and local invasion. We followed TCGA's recommendations for scientific writing. Most of the information used to generate the validation cohort was derived from CGGA (http://www.cgga.org.cn/)[14] workflow steps are depicted The in Figure 1.

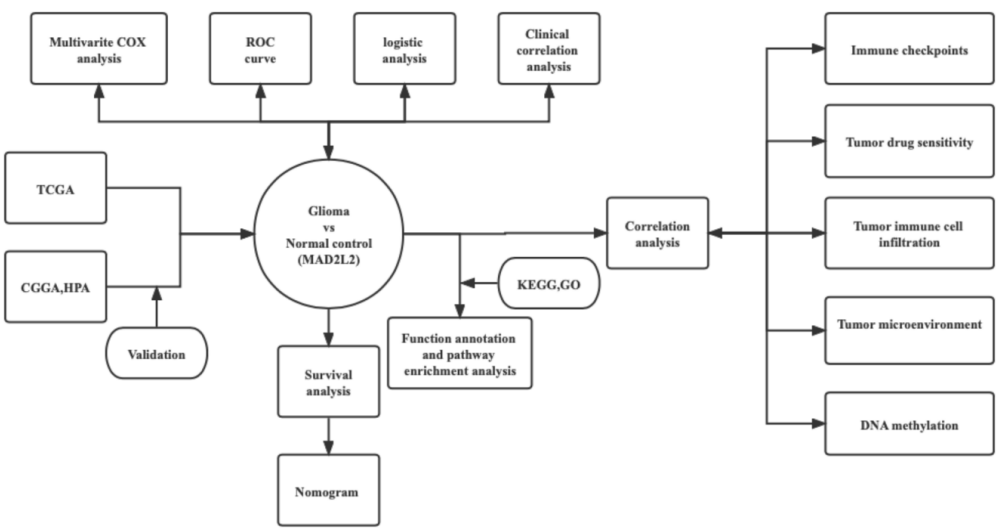

Figure 1. The overview of the workflow

Gene Set Enrichment Analysis (GSEA)

The GSEA is a statistical method for determining whether there are differences in the expression of genes between two biological states. GSEA was initially used to produce an ordered gene list based on the association between all genes and MAD2L2 expression to uncover the mechanism through which MAD2L2 expression affects glioma prognosis. We used GSEA to analyze and explain the substantial survival differences observed between patients with high and low MAD2L2 expression.

DNA methylation analysis

In the interest of discovering the mechanism underlying MAD2L2 function in glioma, the methylation status of MAD2L2 in glioma patients was analyzed using the UALCAN. The MAD2L2 methylation level was evaluated by using the MethSurv database.[15]

Drug sensitivity estimation

In the study, we utilized the Genomics of Drug Sensitivity in Cancer data to identify drug-sensitive genes. Using ridge regression, half maximal inhibitory concentration (IC50) of the samples was determined.[16]

Analysis of immune infiltration

The TIMER database is a significant resource for studying immune infiltration in cancer. We analyzed the connection between MAD2L2 expression and TIICs[17] using TIMER database data. TIMER estimates the frequency of TIICs by the deconvolution of gene expression data.[18]

Immune checkpoints and Tumor Mutation Burden (TMB) analysis

A total of seven transcripts associated with immunological checkpoints (*SIGLEC15, TIGIT, CTLA4, HAVCR2, CD274, LAG3,* and *PDCD1LG2*) were selected. We analyzed their co-expression with MAD2L2.[19] The relationship between MAD2L2 and TMB in gliomas was evaluated using data from TCGA.[20]

Immunohistochemistry (IHC) staining

Expression variations of MAD2L2 protein were evaluated by IHC staining of normal and glioma tissues from HPA (http://www.proteinatlas.org/) using the antibody CAB008110.[21]

Comprehensive analysis

Based on the K-M plotter, both hazard ratios and log-rank p-values were calculated for MAD2L2 expression and glioma patient survival rates.[22]

In order to determine the role of MAD2L2 expression in relation to other pathological and clinical parameters (age, WHO stage, OS time, and IDH), we performed multivariate Cox regression analysis. Based on the results of multivariate Cox proportional risk model, nomograms prediction graph was established.

Statistical method

The R programming language was used for all statistical analysis (version 3.6.3). Log2 transformations were used to standardize gene expression levels. A two-sample t-test was used to evaluate differences between normal and cancerous tissue types. Kruskal–Wallis one-way ANOVA was used to compare three or more groups. Cox proportional hazards model, K–M analysis, and log-rank testing were used for survival analyses. Spearman's test was used to analyze pairwise correlations, and $p < 0.05$ is significant.

RESULTS

Survival results and variable evaluation

We found that the expression of MAD2L2 is higher in glioblastoma (GBM) than in low-grade glioma (LGG), and the expression of MAD2L2 is also higher in low-grade glioma than in normal tissue. (Figure 2A). Unpaired glioma and normal tissue samples from TCGA and GTEx were also analyzed. MAD2L2 expression differed between glioma and normal tissues, with expression in tumors being significantly upregulated ($p < 0.001$, Figure 2B). To evaluate MAD2L2's diagnostic utility, we plotted its Receiver operating characteristic (ROC) curve. MAD2L2's Area Under Curve (AUC) was 0.941 (Figure 2C), showing that it has diagnostic potential as a biomarker.

Patient characteristics

In May 2022, baseline clinical and gene expression data for 696 glioma were acquired from TCGA. (Table 1). The median age of the glioma patients was 45 years. The patients were divided into two groups based on age: those under 60 years old (79.5%) and those over 60 years old (21.5%). There were 298 (42.8%) women and 398 (57.2%)

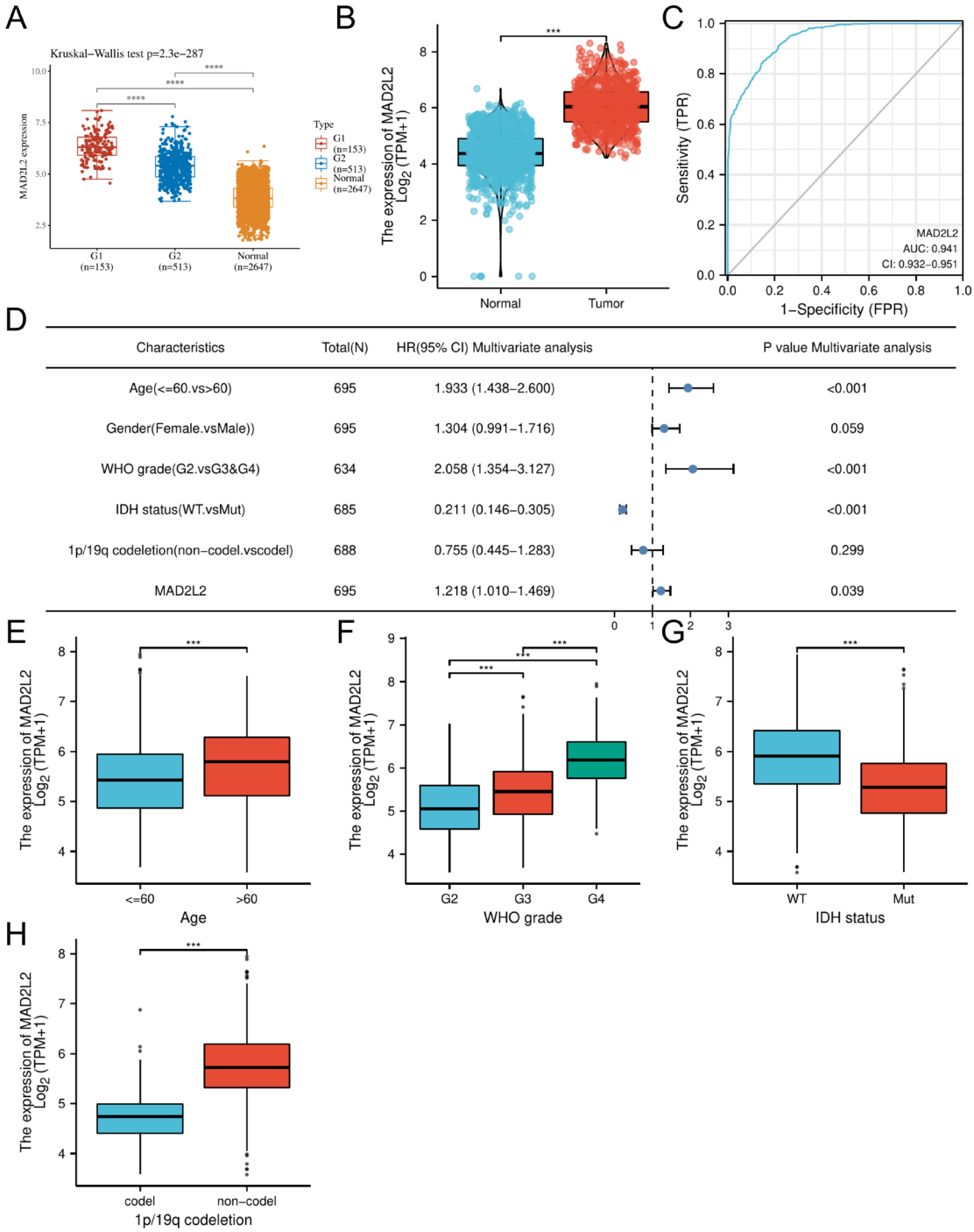

Figure 2. The expression level of MAD2L2 in GBM(G1), LGG(G2) and normal tissues(G3) (A). Expression of MAD2L2 in glioma and normal tissues (B). ROC curve of MAD2L2 for glioma (C). Forest plot of multivariate Cox analysis of MAD2L2 expression and other clinicopathological variables (D). Expression of MAD2L2correlated significantly with Age (E) WHO grade (F) IDH status (G) and 1p/19q codeletion (H).

Table 1: Baseline data on MAD2L2 expression in glioma patients

Characteristic	levels	Overall
n		696
Age, n (%)	<=60	553 (79.5%)
	>60	143 (20.5%)
Gender, n (%)	Female	298 (42.8%)
	Male	398 (57.2%)
Race, n (%)	Asian	13 (1.9%)
	Black or African American	33 (4.8%)
	White	637 (93.3%)
WHO grade, n (%)	G2	224 (35.3%)
	G3	243 (38.3%)
	G4	168 (26.5%)
IDH status, n (%)	WT (Widetype)	246 (35.9%)
	Mut (Mutant)	440 (64.1%)
1p/19q codeletion, n (%)	codel	171 (24.8%)
	non-codel	518 (75.2%)
Age, median (IQR)		45 (34, 59)

men. The majority of the patients (670; 98.1%) were non-Asian, while 13 (1.9%) were Asian. WHO grade was divided into G2 grade (224 cases (35.2%)) and G3 and G4 grades (411 cases (64.8%)). IDH status was divided into Mut (440 (64.1%))and WT (246 cases (35.9%)). 1p/19q codeletion was divided into codel (171 (24.7%)) and non-codel (518 (75.3%)). We used Cox regression analysis to determine the correlation between MAD2L2 expression and OS and other multivariate features of patients with glioma (Figure 2D). Multivariate stepwise cox regression analysis showed that MAD2L2 expression in glioma and paracancerous tissues differed based on age (HR = 1.933, p < 0.001), WHO grade (HR = 2.508, p < 0.001), IDH status (HR = 0.211, p < 0.001), and MAD2L2 expression (HR = 1.218, p = 0.038) (Figure 2E–H).

Logistic regression analysis of the correlation between MAD2L2 expression and clinicopathological factors

Correlation analysis between MAD2L2 expression and clinicopathological and prognostic features showed that age (>60 vs. ≤60, p = 0.004), WHO grade (G3 and 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) correlated with MAD2L2 expression in gliomas. The results indicate that MAD2L2 expression is associated with a higher incidence of high-grade 1p/19q co-deletion tumors in gliomas than low-expression MAD2L2. (Table 2).

GSEA of MAD2L2

We next analyzed KEGG pathways and GO terms to determine the possible biological activities associated with MAD2L2. KEGG pathway analysis showed the strongest positive links between MAD2L2 expression and five pathways: SYSTEMIC LUPUS ERYTHEMATOSUS, CELL CYCLE, RIBOSOME, DNA REPLICATION, and GRAFT VERSUS HOST DISEASE (Figure 3A). LONG TERM POTENTIATION, PHOSPHATIDYLINOSITOL SIGNALING SYSTEM, CARDIAC MUSCLE

Table 2: Logistic analysis of the association between MAD2L2 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
WHO grade (G3&G4 vs. G2)	635	3.714 (2.632-5.284)	<0.001
IDH status (Mut vs. WT)	686	0.309 (0.222-0.429)	<0.001
1p/19q codeletion (non-codel vs. codel)	689	32.400 (17.097-69.731)	<0.001

CONTRACTION, NEUROACTIVE LIGAND RECEPTOR INTERACTION, and CALCIUM SIGNALING PATHWAY had the strongest negative correlations with MAD2L2 expression (Figure 3B). GO analysis identified five annotations that were positively correlated with high MAD2L2 expression: PROTEIN DNA COMPLEX, MITOTIC SISTER CHROMATID SEGREGATION, CHROMATIN ORGANIZATION INVOLVED IN REGULATION OF TRANSCRIPTION, NEGATIVE REGULATION OF GENE EXPRESSION, and EPIGENETIC and DNA PACKAGING COMPLEX (Figure 3C), while PRESYNAPTIC MEMBRANE, VOLTAGE-GATED ION CHANNEL ACTIVITY, NEUROTRANSMITTER TRANSPORT, SYNAPTIC MEMBRANE, and VOLTAGE-GATED CATION CHANNEL ACTIVITY were negatively correlated with MAD2L2 expression (Figure 3D). Consistent with these results, MAD2L2 function is linked to glioma development and progression, and MAD2L2 interferes with the occurrence and development of glioma by affecting DNA replication, RNA transcription, cell cycle, the immune system, and signal transduction pathways.

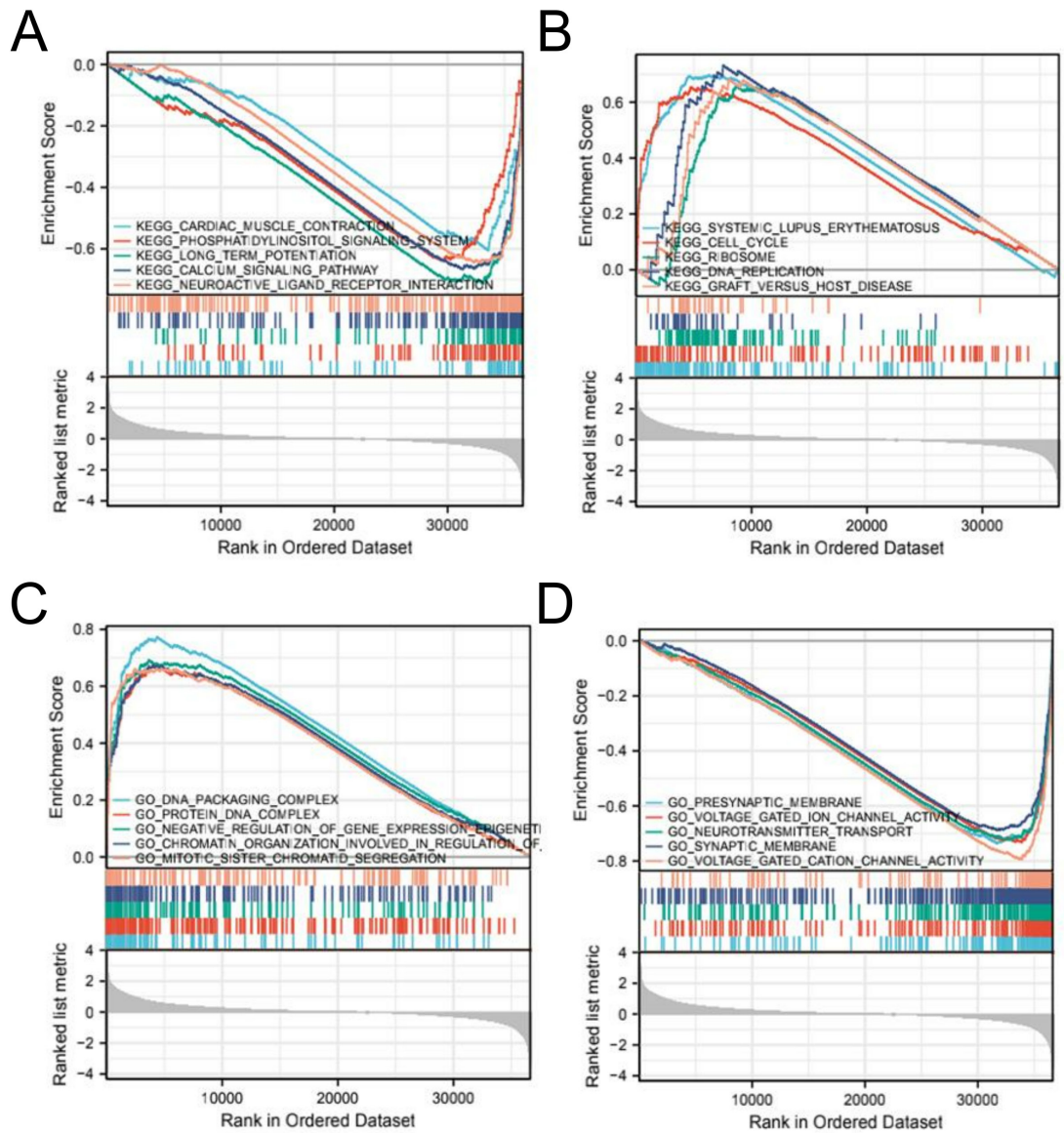

Figure 3. KEGG pathway showed five positively correlated groups (A), and five negatively correlated groups(B). GO pathway showed five positively correlated groups (C), and five negatively correlated groups(D).

Correlation between methylation and expression of MAD2L2

To elucidate the mechanisms generating MAD2L2 overexpression in glioma tissues, we examined the relationship between MAD2L2 expression and its methylation state. UALCAN database data demonstrated that GBM tissues have considerably decreased DNA methylation in the promoter region of MAD2L2 compared to normal brain tissues. ($p < 0.001$) (Figure 4C). Patients with glioma tended to have hypomethylated MAD2L2 DNA sequences, and there was a correlation between the degree of methylation and patient outcomes; low glioma methylation was associated with worse overall survival compared to high MAD2L2 methylation. (Figure 4A, B). cg11857246, cg26807243, cg15012981, and cg06938333 methylation sites were indicative of poor LGG prognosis, while cg11857246, cg26572388, cg26807643, cg13030948, and cg19911179 methylation sites were indicative of poor GBM prognosis (Figure 4D–L).

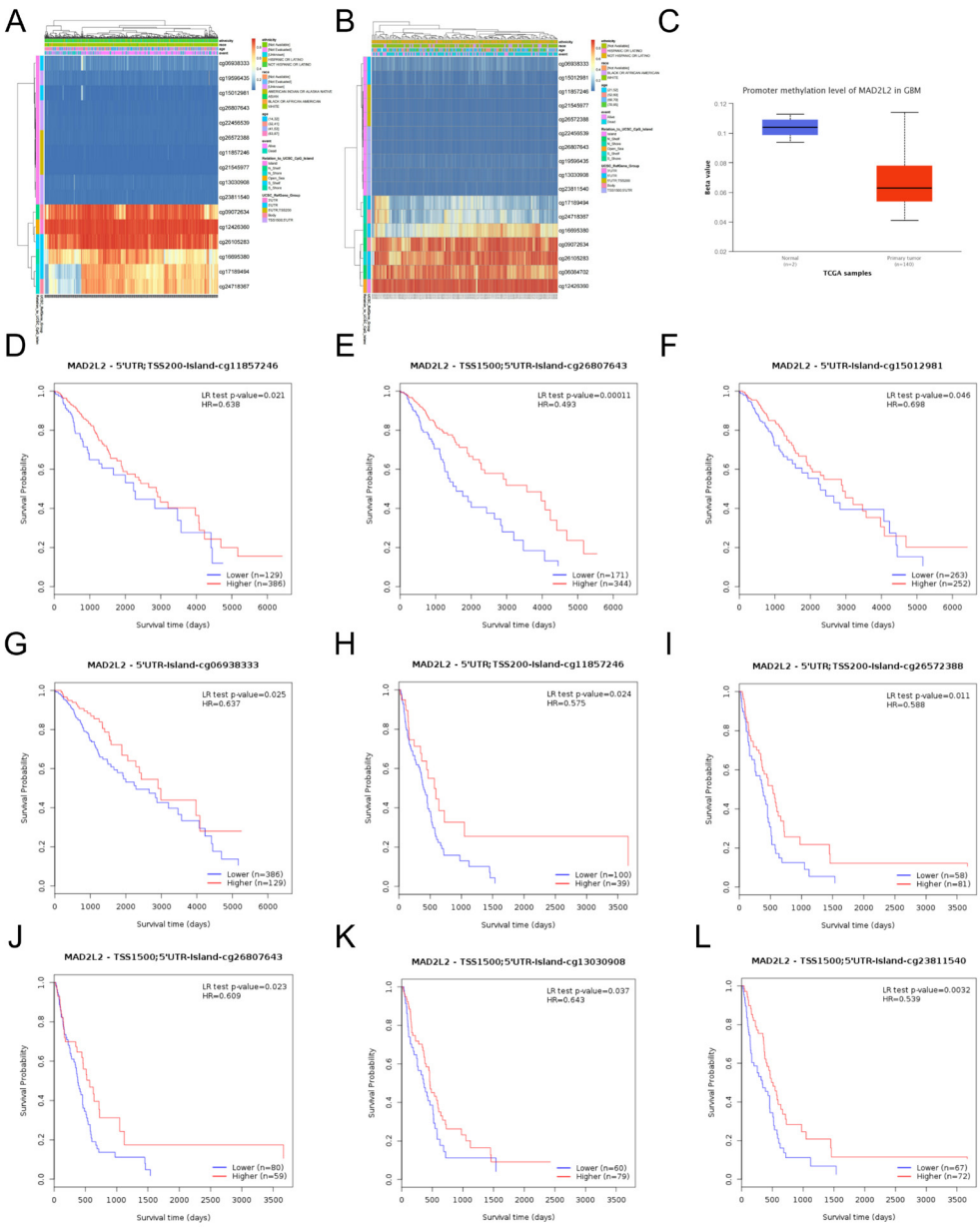

Figure 4. DNA methylation level of MAD2L2 and its effect on prognosis of patients with glioma. Correlation between MAD2L2 mRNA expression level and methylation level. (LGG:A, GBM:B) The promoter methylation level of MAD2L2 in GBM was obtained from the UALCAN database. (C). Kaplan-Meier survival curves for several methylation sites of MAD2L2. (LGG:D-G, GBM:H-L)

Comprehensive analysis

Patients with increased MAD2L2 expression had poor OS, as shown by Kaplan-Meier plots. ($p < 0.001$, Figure 5A), Disease Specific Survival (DSS) ($p < 0.001$, Figure 5B), and Progression-Free Interval (PFI) ($p < 0.001$, Figure 5C). Using the correlation with glioma prognosis, we constructed a nomogram to assess patients' OS independent of risk factors (Figure 5D), and a significant predictive value was shown by the calibration curve. (Figure 5E).

Predicting the potential effects of chemotherapy

We predicted the chemotherapeutic response of each patient using data from Genomics of Drug Sensitivity in Cancer (GDSC). IC_{50} predictions for four main chemotherapeutics (temozolomide, cisplatin, paclitaxel, and docetaxel) revealed that all high-risk patients had significantly low IC_{50} values (Figure 6A–D). Thus, these chemotherapeutic drugs would benefit patients overexpressing MAD2L2.

Relationship between MAD2L2 expression, TIIC, and TMB

The immune system cannot be separated from cancer because of its role as a defense mechanism. Total survival and the health of sentinel lymph nodes are heavily influenced by the presence or absence of tumor-infiltrating lymphocytes.[23] Using data from the TIMER database, The link between MAD2L2 expression and the degree of immune infiltration in glioma was studied. We identified significant correlations between MAD2L2 expression and B cells (GBM: r = -0.034, p = 7.03e-01; LGG: r = 0.395, p = 2.68e-19), CD8+ T cells (GBM: r = -0.044, p = 6.19e-01; LGG: r = -0.065, p = 1.57e-01), CD4+ T cells (GBM: r = -0.1, p = 2.53e-01; LGG: r = -0.065, p = 1.57e-01), macrophages (GBM: r = -0.214, p = 1.41e-02; LGG: r = 0.29, p = 1.21e-10), neutrophils (GBM: r = -0.139, p = 1.15e-01; LGG: r = 0.339, p = 2.87e-14), and dendritic cells (GBM: r = -0.044, p = 6.15e-01; LGG: r = 0.459, p = 3.47e-29) (Figure 7A). These results

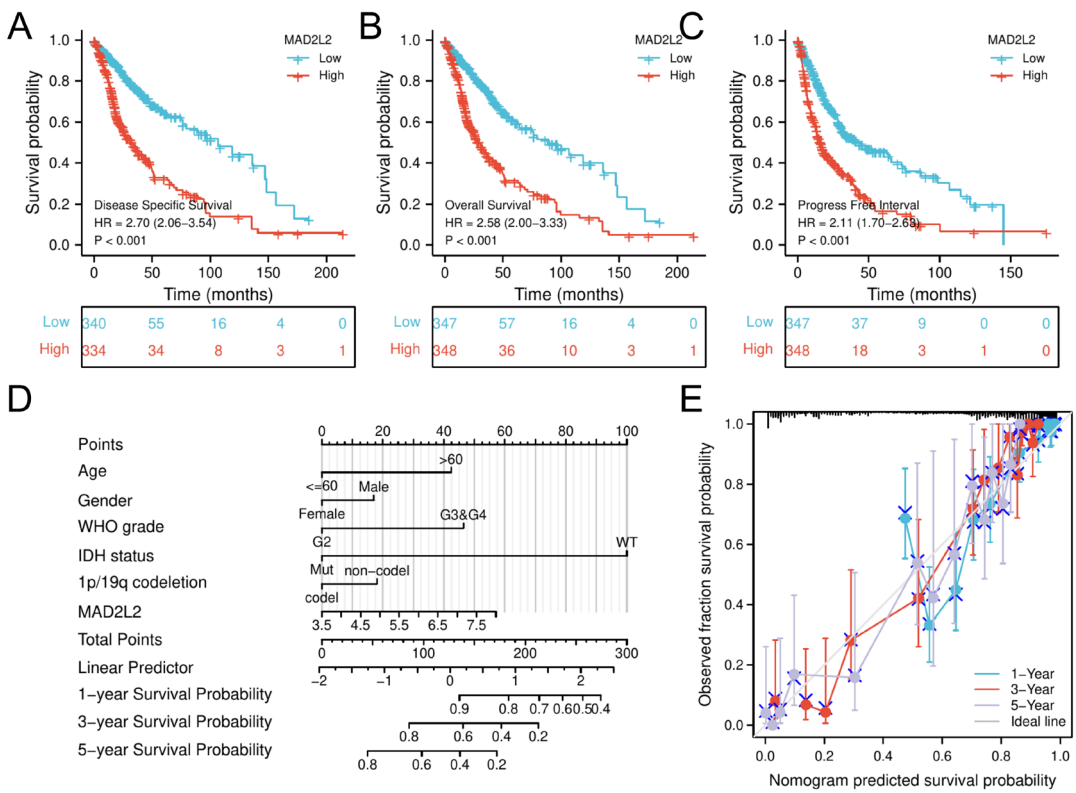

Figure 5. Prognostic analysis of MAD2L2 expression. Compared with patients with low MAD2L2 expression, patients with high MAD2L2 expression had poorer prognosis, including Overall-Survival (OS) (A), Disease-Specific-Survival (DSS) (B), Progress-Free-Interval (C) (three log-rank P<0.05). Multivariate analysis nomogram of clinical features based on MAD2L2 expression (D). Calibration plot showing the predictive performance of the model constructed using multivariate Cox regression analysis (E).

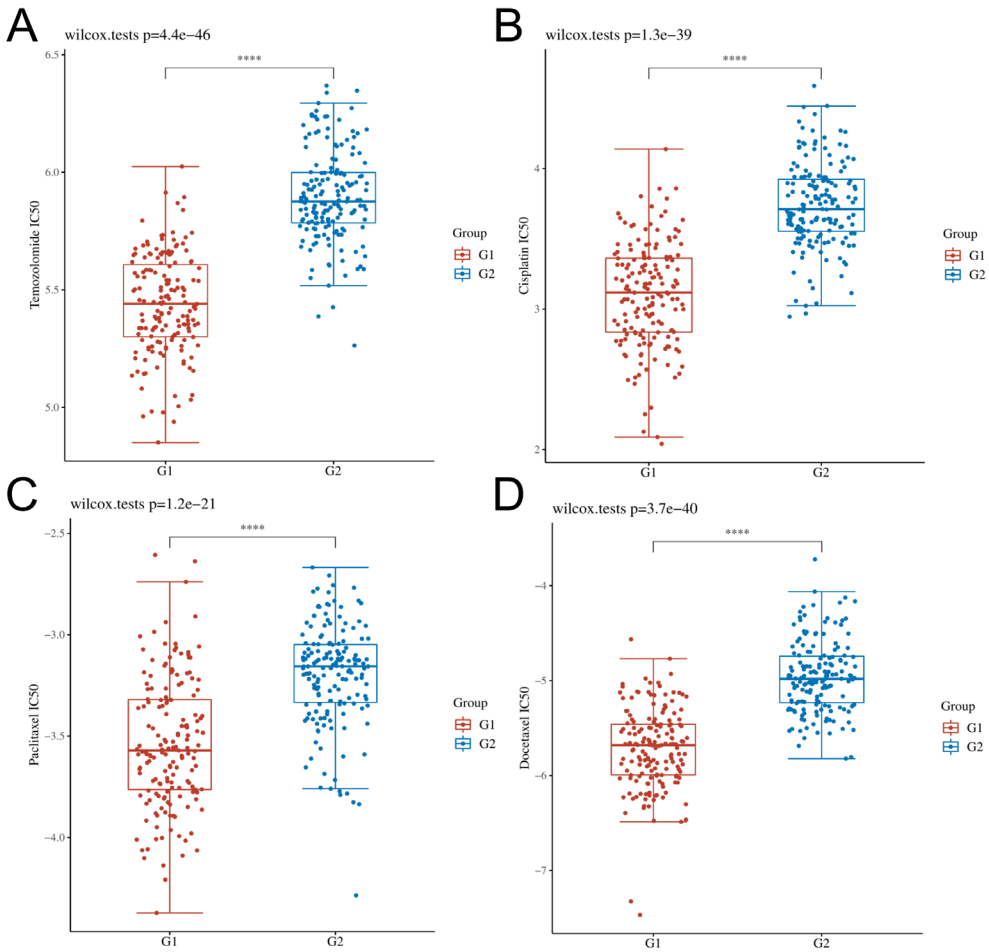

Figure 6. Drug sensitivity estimation. chemotherapy IC50 estimation for temozolomide (A) cisplatin (B), paclitaxel (C), docetaxe (D). (high (G1) and low (G2) MAD2L2)

indicate that MAD2L2 plays a crucial function in glioma immune infiltration. Next, we evaluated the correlations between MAD2L2 expression and 24 distinct types of immune cells in glioma. MAD2L2 expression was positively correlated with B cells, CD8+ T cells, DC, mast cells, CD56^{bright} natural killer cells, pDC, Tcm, Tem, TFH, and Treg (Figure 7B). Moreover, MAD2L2 expression levels were shown to be considerably different across invading immune cells such as Tcm, Tgd, Th2 cells, Th1 cells, aDCs, B cells, CD8 T cells, cytotoxic cells, eosinophils, iDCs, macrophages, neutrophils, CD56^{bright}, pDC and NK cells. The high-risk group had considerably higher numbers of T cells, neutrophils, aDC, eosinophils, iDC, macrophages, T helper cells, and Th2 cells. When comparing the high MAD2L2 expression group to the low MAD2L2 expression group. Levels of pDC, CD56^{bright} NK cells, Treg, TFH, Tcm, Tem, DC, and mast cells were significantly lower in the high MAD2L2 expression group than in the low MAD2L2 expression group ($p < 0.05$, Figure 7C). Additionally, We evaluated the relationships between the 24 immune cells using a heatmap and found that the ratios of different TIIC subgroups were moderately to highly associated. (Figure 7D).

The immune checkpoints (*IGLEC15, TIGIT, CTLA4, HAVCR2, CD274, LAG3*, and *PDCD1LG2*) were positively correlated with MAD2L2 expression (Figure 7E). MAD2L2 expression in glioma positively correlated with TMB, and the difference was statistically significant (Figure 7F). Higher Tumor Immune Dysfunction and Exclusion (TIDE) scores, worse ICB responses, and shorter life times after ICB treatment were observed in glioma patients expressing high amounts of MAD2L2 compared to those expressing low levels of MAD2L2. (Figure 7G).

Data verification

MAD2L2 expression levels in glioma and

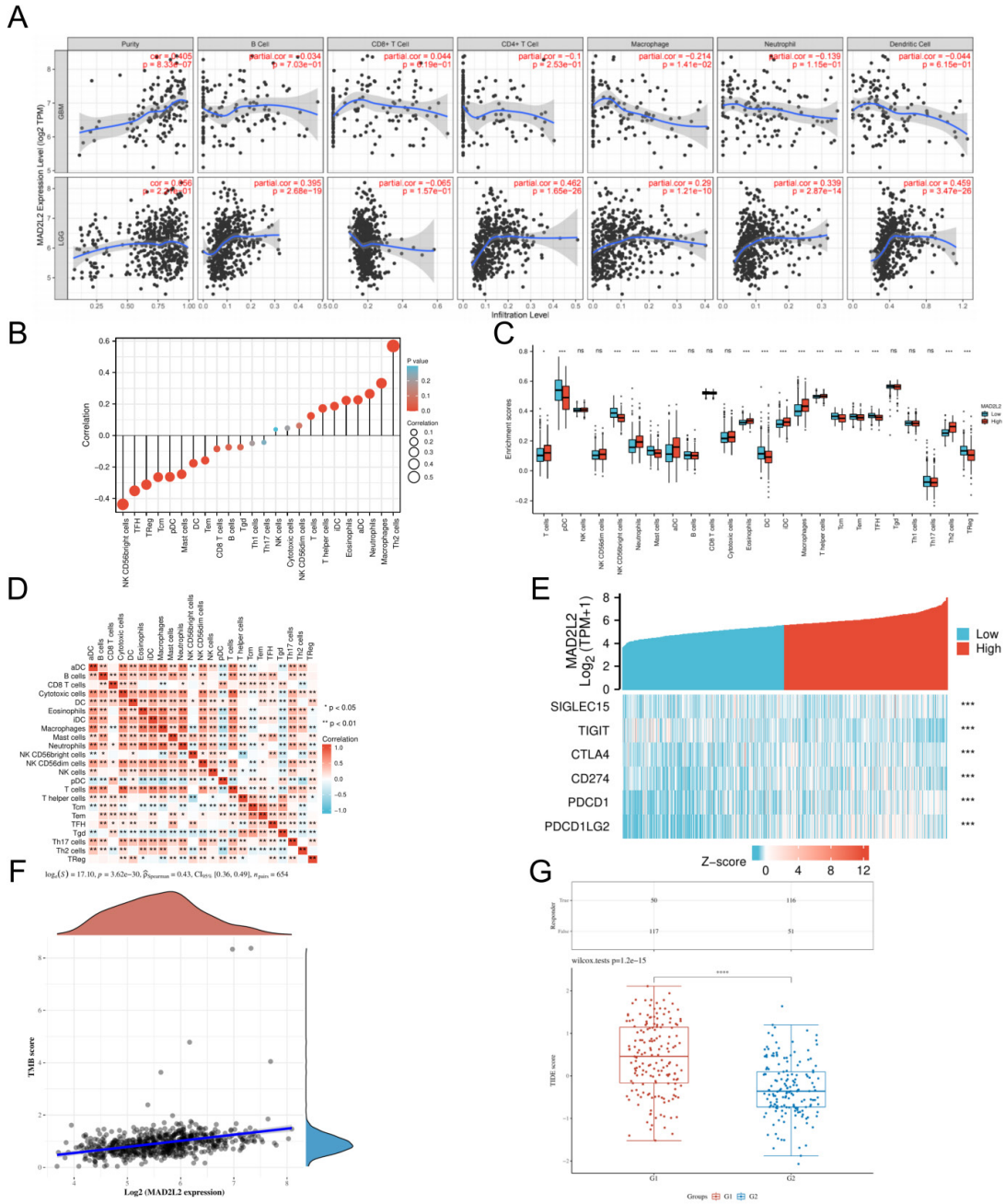

Figure 7. Correlation of MAD2L2 expression in the tumor microenvironment with immune cell infiltration and expression of immune checkpoints in gliomas. The relationship between MAD2L2 expression and the infiltration level of six types of immune cells (A). The relationship between MAD2L2 expression and 24 types of immune cells (B). Change ratio of 24 immune cell subtypes in high and low MAD2L2 expression groups in tumor samples (C). Heat map of 24 immune infiltrating cells in tumor samples (D). Immune checkpoints with low and high MAD2L2 expression different expressions (E). Correlation analysis between MAD2L2 gene expression and TMB was performed using Spearmans method (F). Differential responses of low (G1) and high (G2) MAD2L2 expression to immune checkpoint blockade (G).

paracancerous tissues differed considerably depending on patient age, WHO grade (Figure 8A–B). The expression of MAD2L2 in glioma in CGGA database and MAD2L2 in TCGA have different relationship with IDH (Figure 8C). K–M survival analysis showed that high MAD2L2 expression was associated with poor overall survival of patients with glioma (Figure 8D, $p < 0.001$). Furthermore, analysis of immunohistochemical data from the Human Protein Atlas (HPA) database showed no MAD2L2 staining in the normal cerebral cortex (Figure 8E), whereas moderate MAD2L2 staining was observed in glioma tissues (Figure 8F).

DISCUSSION

Glioma is a common type of brain cancer with a convoluted pathophysiology involving altered gene expression and malfunctions and alterations

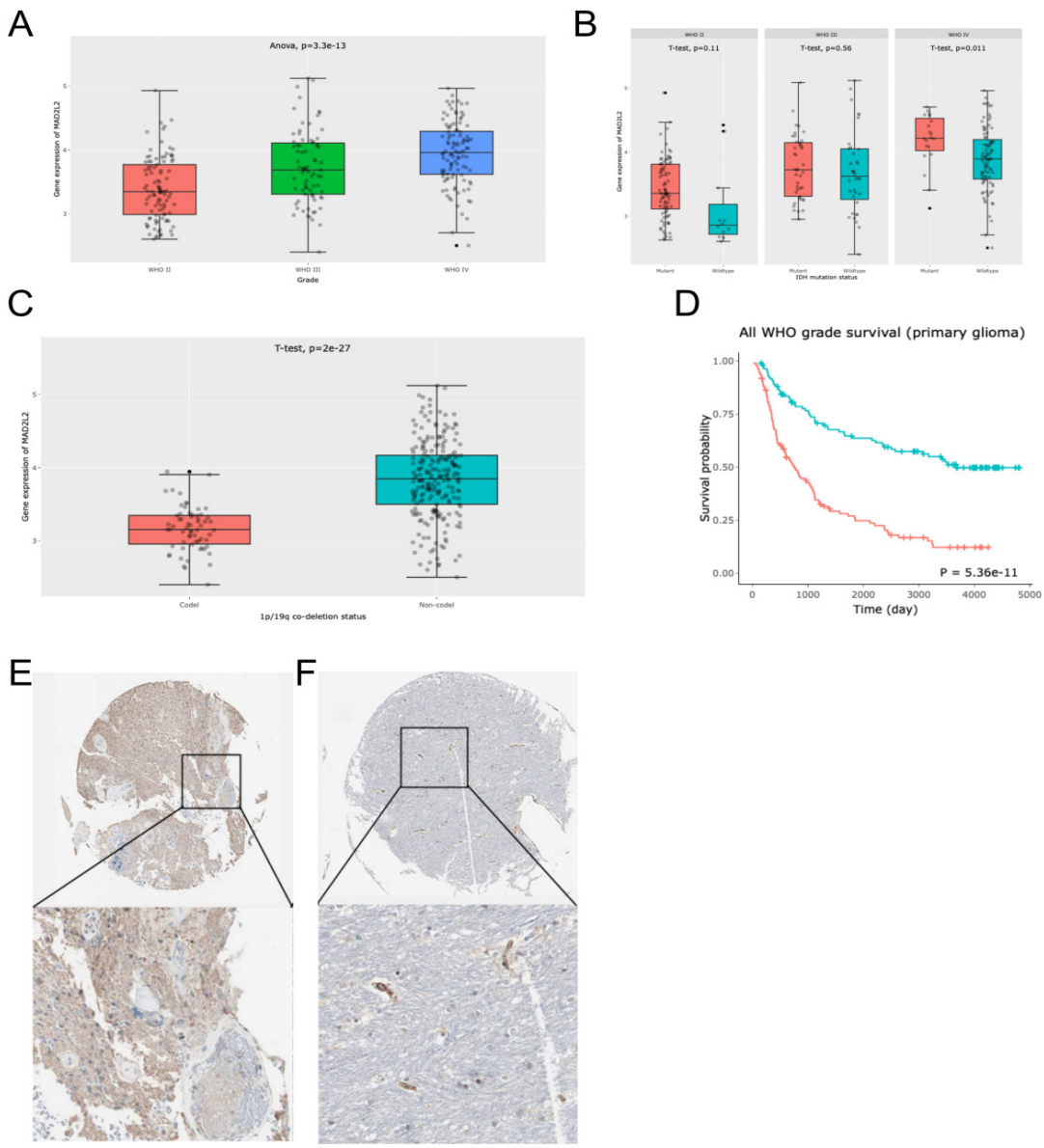

Figure 8. Expression of MAD2L2 in each Clinical information in the CGGA database (A, B, C) Kaplan-Meier estimate of overall survival of patients in the CGGA database (Blue: Low MAD2L2 and Red: High MAD2L2)(D). The expression of MAD2L2 protein in glioma and normal tissues was observed by HPA immunohistochemistry (E,F). Normal tissue (E); glioma tissue (F).

in several signaling pathways. Although various prognostic markers for gliomas have been described, only a few are currently used in clinical practice, making it important to identify new prognostic indicators. The prognostic value of MAD2L2 in gliomas has not been studied before.

The cell cycle is the series of events that occurs as a cell grows and divides by duplicating its constituent parts and correctly dividing them into daughter cells[24], and the inheritance of a correct and stable genome requires error-free DNA replication and chromosomal transmission from parent to daughter cells.[25] Genomic instability, one of the hallmarks of malignancies, is primarily caused by replication stress brought on by cancer genes in tumor cells. The cell cycle checkpoints that normally preserve DNA integrity may be bypassed in the face of these replication stresses, thus increasing genetic variability. MAD2L1 has received considerably more attention than MAD2L2, which plays a role in cell cycle control and DDR. This DDR-mitotic crosstalk is poorly understood, and no glioma-associated studies have been conducted, yet it holds great promise as a target for cancer treatment.[26]

Using public available datasets of clinically described patients with glioma, several clinical, pathological, mutational, and immune parameters were evaluated in relation to MAD2L2 expression in gliomas. We found that upregulated MAD2L2 expression is a reliable predictor of clinical characteristics associated with gliomas and can be used as an immunotherapy target. We conducted a survival analysis to assess the predictive significance of MAD2L2 in gliomas. KEGG pathway and GO analyses showed that systemic lupus erythematosus, cell cycle, ribosome, DNA replication, graft-versus-host disease, protein-DNA complexes, mitotic sister chromatid segregation, chromatin organization involved in transcriptional regulation, negative regulation of gene expression, and epigenetic and DNA packaging complexes were promoted by high MAD2L2 levels, whereas phosphatidylinositol signaling system, myocardial contraction, calcium signaling pathway, neuroactive ligand receptor, presynaptic membrane, voltage-gated ion channel activity, neurotransmitter transport, synaptic membrane, and voltage-gated cation channel activity were suppressed by upregulated MAD2L2 expression. The results of our study shed light on MAD2L2 biology and function, and suggest that MAD2L2 overexpression might increase glioma risk. MAD2L2 inhibitors are currently not being studied or considered for glioma treatment. Our findings may facilitate the development and use of MAD2L2 inhibitors in the treatment of gliomas.

Gene expression is often silenced by the epigenetic process of DNA methylation. Our findings suggest that DNA hypomethylation may be associated with MAD2L2 overexpression in glioma as patients with glioma with hypomethylated MAD2L2 levels had worse disease prognosis.

Tumor occurrence and growth depend heavily on the immunological microenvironment.[27] Besides serving as predictors of responses to tumor cell immunotherapy, TME profiles may also influence tumor prognosis.[28] Anti-PD-1 treatment, an example of immunotherapy, has shown promising results in preclinical studies of patients with glioma.[29,30] However, most immunotherapy-based clinical trials have not achieved optimal therapeutic results.[31] The complex immunosuppressive TME is a key contributor to the low success rates of glioblastoma immunotherapies. It is also possible that glioma-specific peripheral immunosuppression dampens the efficacy of immunotherapy. By studying single-cell datasets, we discovered that MAD2L2 is a significant immune-related prognostic marker in the glioma TME. We found that MAD2L2 expression in glioma tissues is correlated with immune infiltration. Based on these associations, we showed that MAD2L2 is crucial for regulating the immunology of glioma tumors. MAD2L2 expression levels in different types of immune cells varied significantly. Our research indicates that MAD2L2 is essential for the modulation of glioma immunological activity.

Tumor immunotherapy has garnered increased interest and is the subject of several recent studies. Tumor immunotherapy enhances survival and provides lasting therapeutic advantages with minimal adverse effects. Several immunotherapeutic methods, particularly those that activate anti-cancer immunity through immune checkpoint inhibition, have emerged as potential anti-tumor modalities, modifying and encouraging the patient's immune system to destroy cancer cells.[32]

However, our study had several limitations. First, the data analyzed here were obtained from online databases that are regularly updated and expanded, thus the results of this study are subject to change. Second, our study lacks information on difficult and in-depth treatment options. Third, we did not perform in vivo and in vitro studies to verify the molecular mechanism of action of MAD2L2 and its role in glioma immunity.

We aim to focus on the baseline information of patients and conduct additional tests to confirm these findings in future studies. Finally, the mechanism through which MAD2L2 promotes tumor growth and metastasis in glioma also needs further investigation.

In conclusion, in this study, through the analysis of public database data, it was discovered that MAD2L2 might serve as a prognostic biomarker for glioma, with strong potential as a critical tool in the diagnosis and treatment of this formidable disease. The results of this investigation have added weight to the argument that MAD2L2's affiliation with immune infiltration may hold the key to understanding the complex biology of glioma, and immunotherapy may thus prove to be a highly promising treatment option for this currently intractable disease.

ACKNOWLEDGEMENTS

The authors would like to thank all the medical staff who contributed to the maintenance of the medical record database.

DISCLOSURE

Data availability: The data used to support the findings of this study are included within the article, and the datasets and code can be obtained from the corresponding author upon reasonable request.

Financial support: This work was supported by Guidance in Medical and Health Program of Xiamen, China(3502Z20224ZD1159), Guidance in Medical and Health Program of Xiamen, China (3502Z20224ZD1155).

Conflicts of interest: None

REFERENCES

1. Lin TK, Chang CN, Tsai CS, *et al*. The long non-coding RNA LOC441204 enhances cell growth in human glioma. *Sci Rep* 2017; 7:5603. doi: 10.1038/s41598-017-05688-0
2. Bouckaert C, Germonpre C, Verhoeven J, *et al*. Development of a rat model for glioma-related epilepsy. *Int J Mol Sci* 2020; 21(19):6999. doi: 10.3390/ijms21196999
3. Jiang T, Mao Y, Ma W, *et al*. CGCG clinical practice guidelines for the management of adult diffuse gliomas. *Cancer Lett* 2016; 375: 263-73. doi: 10.1016/j.canlet.2016.01.024
4. Voorwerk, L.; Slagter, M.; Horlings, HM. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. *Nat Med* 2019; 25:920-8. doi: 10.1038/s41591-019-0520-5
5. Jahan N, Talat H, Curry WT. Agonist OX40 immunotherapy improves survival in glioma-bearing mice and is complementary with vaccination with irradiated GM-CSF-expressing tumor cells. *Neuro Oncol* 2018; 20: 44-54. doi: 10.1093/neuonc/nox125
6. Cai X, Yuan F, Zhu J. Glioma-associated stromal cells stimulate glioma malignancy by regulating the tumor immune microenvironment. *Front Oncol* 2021; 11: 672928. doi: 10.3389/fonc.2021.672928
7. Martin SK, Wood RD. DNA polymerase zeta in DNA replication and repair. *Nucleic Acids Res* 2019; 47: 8348-61. doi: 10.1093/nar/gkz705
8. Lok TM, Wang Y. Xu WK, Xie S, Ma HT, Poon RYC. Mitotic slippage is determined by p31(comet) and the weakening of the spindle-assembly checkpoint. *Oncogene* 2020; 39: 2819-34. doi: 10.1038/s41388-020-1187-6
9. Marima R, Hull R, Penny C, Dlamini Z. Mitotic syndicates Aurora Kinase B (AURKB) and mitotic arrest deficient 2 like 2 (MAD2L2) in cohorts of DNA damage response (DDR) and tumorigenesis. *Mutat Res Rev Mutat Res* 2021; 787: 108376. doi: 10.1016/j.mrrev.2021.108376
10. Ma L, Li X, Zhao X. Oxaliplatin promotes siMAD2L2induced apoptosis in colon cancer cells. *Mol Med Rep* 2021; 24(3):629. doi: 10.3892/mmr.2021.12268
11. Rimkus C, Friederichs J, Rosenberg R, Holzmann B, Siewert JR, Janssen KP. Expression of the mitotic checkpoint gene MAD2L2 has prognostic significance in colon cancer. *Int J Cancer* 2007; 120; 207-11. doi: 10.1002/ijc.22155
12. Huang HS, Du Y, Zhao D. The relationship between the prognostic marker LIMA1 in head and neck squamous cell carcinoma and immune infiltration. *J Oncol* 2022:1040116. doi: 10.1155/2022/1040116
13. Wang Z, Jensen MA. Zenklusen JC. A practical guide to the cancer genome atlas (TCGA). *Methods Mol Biol* 2016; 1418:111-41. doi: 10.1007/978-1-4939-3578-9_6
14. *s* 2021; 19: 1-12. doi: 10.1016/j.gpb.2020.10.005
15. Modhukur VIT, Metsalu T, Lokk K, Laisk-Podar T, Vilo J. MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data. *Epigenomics* 2018; 10: 277-88. doi: 10.2217/epi-2017-0118
16. Jiang Q, Sun J, Chen H. Establishment of an immune cell infiltration score to help predict the prognosis and chemotherapy responsiveness of gastric cancer patients. *Front Oncol* 2021; 11: 650673. doi: 10.3389/fonc.2021.650673
17. Li T, Fan J, Wang B. TIMER: A web server for comprehensive analysis of tumor-infiltrating immune cells. *Cancer Res* 2017; 77: e108-e110. doi: 10.1158/0008-5472.CAN-17-0307
18. Li B, Severson E, Pignon JC. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biol* 2016; 17: 174. doi: 10.1186/s13059-016-1028-7
19. Jiang P, Gu S, Pan D. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy

response. *Nat Med* 2018; 24: 1550-8. doi: 10.1038/s41591-018-0136-1
20. Thorsson V, Gibbs DL, Brown SD. The immune landscape of cancer. *Immunity* 2018; 48: 812-30. e814. doi: 10.1016/j.immuni.2018.03.023
21. Lanczky A, Nagy A, Bottai G. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. *Breast Cancer Res Treat* 2016; 160: 439-46. doi: 10.1007/s10549-016-4013-7
22. Uhlen M, Fagerberg L, Hallstrom BM. Proteomics. Tissue-based map of the human proteome. *Science* 2015; 347: 1260419. doi: 10.1126/science.1260419
23. Trabelsi, M, Farah, F, Zouari, B. An immunoscore system based on CD3+ and CD8+ infiltrating lymphocytes densities to predict the outcome of patients with colorectal adenocarcinoma. *Onco Targets Ther* 2019; 12: 8663-73. doi: 10.2147/OTT.S211048
24. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. *Environ Mol Mutagen* 2017; 58: 235-63. doi: 10.1002/em.22087.
25. Barnum KJ, O'Connell MJ. Cell cycle regulation by checkpoints. *Methods Mol Biol* 2014; 1170: 29-40. doi: 10.1007/978-1-4939-0888-2_2.
26. Petsalaki E, Zachos G. DNA damage response proteins regulating mitotic cell division: double agents preserving genome stability. *FEBS J* 2020; 287: 1700-21. doi: 10.1111/febs.15240.
27. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol* 2013;14:1014-22. doi: 10.1038/ni.2703
28. Wu T, Dai Y. Tumor microenvironment and therapeutic response. *Cancer Lett* 2017; 387:61-8. doi: 10.1016/j.canlet.2016.01.043
29. Kim JE, Patel MA, Mangraviti A. Combination therapy with anti-PD-1, anti-TIM-3, and focal radiation results in regression of murine gliomas. *Clin Cancer Res* 2017; 23: 124-36. doi: 10.1158/1078-0432.CCR-15-1535
30. Reardon DA, Gokhale PC, Klein SR. Glioblastoma eradication following immune checkpoint blockade in an orthotopic, immunocompetent model. *Cancer Immunol Res* 2016; 4: 124-35. doi: 10.1158/2326-6066.
31. Yang T, Kong Z, Ma W. PD-1/PD-L1 immune checkpoint inhibitors in glioblastoma: clinical studies, challenges and potential. *Hum Vaccin Immunother* 2021; 17: 546-53. doi: 10.1080/21645515.2020.1782692
32. Ding Y, Wang Y, Hu Q. Recent advances in overcoming barriers to cell- based delivery systems for cancer immunotherapy. *Exploration* 2022; 2(3):20210106. doi: 10.1002/EXP.20210106