

CD48 is a key molecule of immunomodulation affecting prognosis in glioma

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Purpose: Glioma is a refractory disease associated with immune cell infiltration, and the effectiveness of checkpoint blockade remains suboptimal. As an adhesion and costimulatory molecule, CD48 plays a significant role in immunomodulation. As such, studying CD48 may provide additional understanding of the immune and inflammation response of glioma.

Methods: Using R language and GraphPad Prism 7, RNA sequencing data of 946 patients from Chinese Glioma Genome Atlas and The Cancer Genome Atlas cohorts were analyzed.

Results: CD48 was highly expressed in the malignant progression of glioma. As an independent risk factor, high-CD48 patients were associated with poor prognosis. CD48 influenced glioma purity and the local immune cell subpopulation. CD48 was closely related to immune function in glioma. Patients with an enhanced immune phenotype, high CD48, were associated with immune suppressive molecules and checkpoints. In addition, CD48 correlated with the immune and inflammatory response. A checkpoint risk score including CD48, SLAMF8 and PD-L1 was used to assess the role of checkpoints. Risk score was particularly high in a malignant subtype of glioma and was an independent predictive indicator of unfavorable outcome. Additionally, age, IDH subtype and MGMT promoter status influenced the predictive significance of checkpoint risk score.

Conclusion: CD48 exhibits a crucial role in reduced survival and immunomodulation in glioma. In addition, we found that checkpoints play a greater role in patients older than 40 years old with IDH wild-type and MGMT methylated status. These findings suggest that combining CD48 blockade with PD-L1 may be a promising approach to glioma immunotherapy for specific subpopulations of patients.

Keywords: CD48, glioma, prognosis, immunomodulation, immunotherapy

Introduction

Diffuse gliomas are common and progressive primary brain tumors that are highly lethal for patients, even for those receiving maximal therapy.¹ The median overall survival of glioblastoma (GBM), the most aggressive and invasive type, remains at 14.6 months despite radical radiotherapy and chemotherapy treatment regimes.² Although novel strategies of glioma immunotherapy are emerging sequentially, such as dendritic cell (DC) therapy and checkpoint blockade, the effectiveness of these novel treatments remains suboptimal at present.³ Therefore, in-depth studies on the immunity status of glioma are urgently needed, in order to identify more effective therapies against this refractory disease.

In glioma, various immune cells and stroma compose the non-tumor component of the tumor parenchyma, such as T cells, tumor-associated macrophages (TAMs) and natural killer (NK) cells. By cross-talking via secretion of cytokines or ligand-receptor interaction, they form a hotbed for the malignant progression of gliomas.⁴

Moreover, they generate a strong immunosuppressive response by developing multipronged approaches.⁵ It has been reported that TAMs interact with glioma stem cells to promote tumor progression and immunosuppression.^{6,7} In addition, immune checkpoints play a significant role in suppressing the antitumor immune response. PD-1/PD-L1 are key regulatory coinhibitory molecules associated with the escape of T and B cell-mediated antitumor immunity in cancer.⁸ However, the efficacy of glioma immunotherapy against PD-1 has been shown in only a subset of glioma patients and is often accompanied with inflammation and immune-related side effects.³ This indicates that aside from PD-1/PD-L1, there remain other checkpoints that may play an important role in tumor immunity of glioma that are yet to be identified.

Signaling lymphocytic activation molecule family 2 (SLAMF2, CD48) is an adhesion and costimulatory molecule expressed constitutively on most hematopoietic cells, particularly in antigen presenting cells (APC). CD48 binds to CD2 and is involved in a wide variety of innate and adaptive immune responses, ranging from granulocyte activity and allergy to T cell activation and autoimmunity, and CTL or NK function and antimicrobial immunity.⁹ CD48 expression is increased in autoimmunity and allergy diseases, and anti-CD48 monoclonal antibodies have been shown to attenuate experimental autoimmune encephalomyelitis.¹⁰ Recently, it has been demonstrated that CD48 interaction with its high-affinity receptor 2B4 (CD244) leads to monocyte/macrophage-elicited NK cell dysfunction in hepatocellular carcinoma.¹¹ These results suggest that CD48 may play an important role in mediating the immune response in both immune activation or suppression. Nevertheless, there has been little investigation into the immunomodulatory role of CD48 in glioma. Here, we explore the clinical and functional role of CD48 in glioma using RNA sequencing (RNA-seq) data of 946 patients in Chinese Glioma Genome Atlas (CGGA) and The Cancer Genome Atlas (TCGA), which may provide deeper insight into immune checkpoints in glioma treatment.

Material and methods

Patient samples

Our study included 976 glioma samples with detailed clinical information, 310 from the CGGA database and 636 from TCGA database. TCGA cohort was downloaded from public databases (<https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp>). Overall survival (OS) was estimated from date of

diagnosis to death or final follow-up. Methods for sequencing, detecting IDH mutation and MGMT promoter methylation state were described previously.^{12,13} Patient characteristics are described in [Table S1](#). There were no duplicate samples from the same patient.

Bioinformatics analysis

Using methods described previously we calculated stromal and immune scores, glioma purity and microenvironment cell populations.^{14,15} After Pearson correlation analysis, genes ($r > 0.4$) were chosen for gene ontology (GO) analysis via DAVID (<http://david.abcc.ncifcrf.gov/home.jsp>). We performed GSEA (<http://www.broadinstitute.org/gsea/index.jsp>) to find differences in phenotypes attributed to low or high CD48 expression. Functional gene sets were obtained from the Amigo2 Web portal (<http://amigo.geneontology.org/amigo/landing>) and reference articles.¹⁶ Principal components analysis (PCA) was used to profile patterns of the transcriptome and immune function between low- and high-CD48 glioma. Inflammation status was calculated using gene set variation analysis (GSVA).

Statistical analysis

Statistical analysis was carried out using SPSS, GraphPad Prism 7 and R 3.3.3 (<https://www.r-project.org/>). Student's *t*-tests were applied to evaluate differences in expression, and Pearson correlation was analyzed to calculate correlations. Low- and high-expression groups were classified based on median expression. Survival distribution was assessed via Kaplan–Meier survival analysis, and the log-rank test was used to estimate significance between stratified groups. Univariate and multivariate Cox regression analyses were used to identify independent prognostic factors. Receiver operating characteristic (ROC) curves were made using Medcalc software. Other statistical computations and figures were built with R (`ggplot2`, `corrplot`, `pheatmap`). Statistical significance was defined as a 2-tailed *p*-value < 0.05 .

Results

CD48 is enriched in glioblastoma and the mesenchymal subtype

To explore the expression pattern of CD48 in glioma, we examined the RNA-sequencing data in CGGA cohorts. The expression level of CD48 increased along with glioma grade and was most enriched in grade IV glioma, GBM. An investigation of the different molecular subtypes of glioma revealed higher CD48 expression in wild-type

IDH, compared to lower grade glioma (LGG) or GBM (Figure 1A). Moreover, we obtained a consistent result in the TCGA database, suggesting that CD48 expression was associated with malignant progression (Figure S1A).

Mesenchymal glioma is the most malignant TCGA subtype. Consistent with this, CD48 was the most enriched in the mesenchymal subtype (CGGA, Figure 1A; TCGA, Figure S1A). Analysis of ROC curves demonstrated that CD48 was a good predictor of mesenchymal subtype in CGGA (AUC=85%) (Figure 1B) and TCGA (AUC=91.9%) datasets (Figure S1B).

CD48 is an independent risk factor for poor outcome

To uncover whether CD48 expression determined prognosis of glioma patients, we compared OS between the low- and high-CD48 groups. In the CGGA cohort, high-CD48 patients had a shorter OS compared to low-CD48 patients in glioma, including high-grade glioma (HGG) (Figure 1C and D). The same result was validated in TCGA cohort (Figure 1E and F). Considering the prognostic importance of CD48, we then assessed the predictive value of CD48 in the CGGA dataset. Results of this

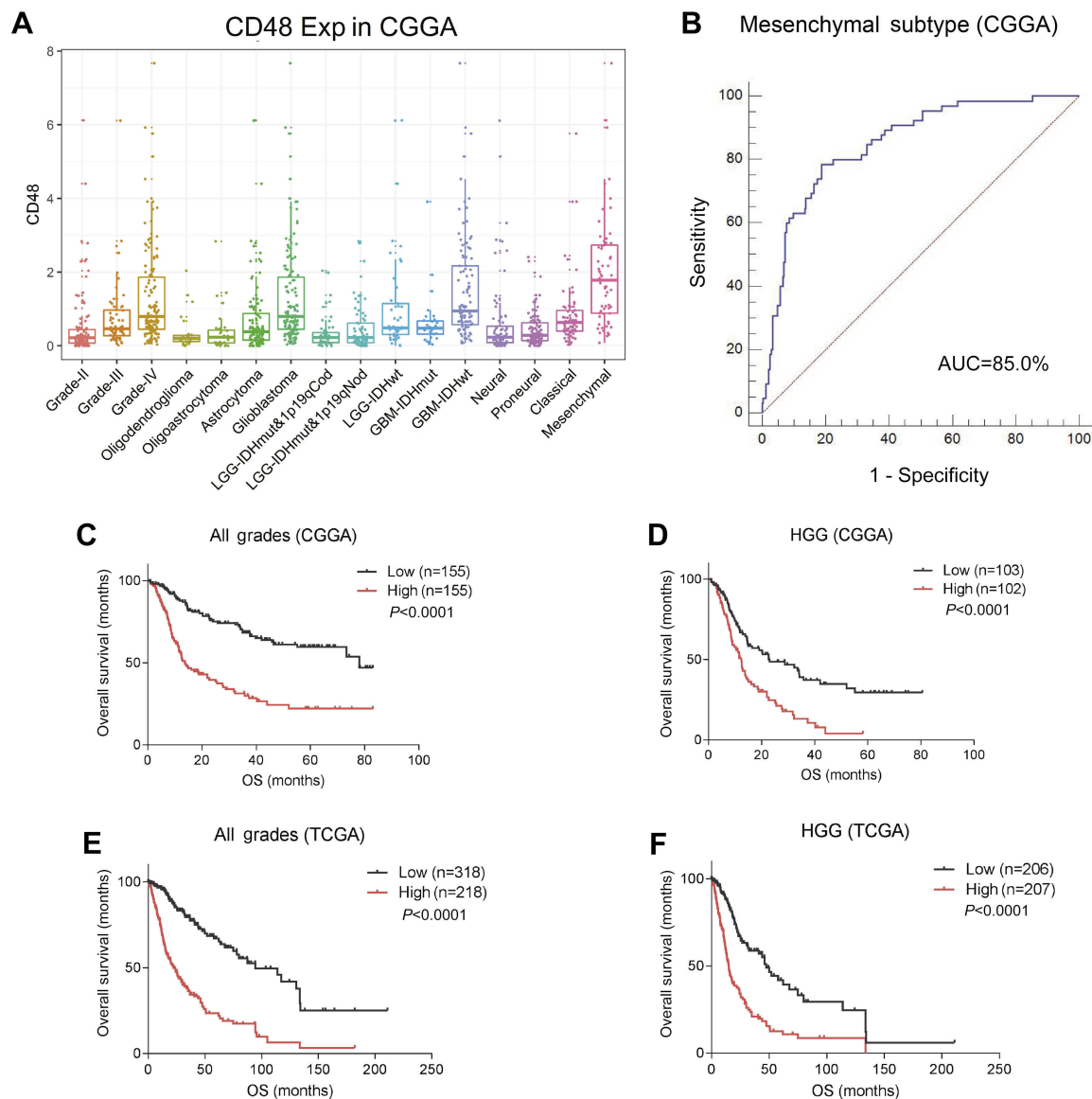


Figure 1 Expression profile and prognostic value of CD48 in glioma. CD48 was differentially expressed in different grades, histopathologic classifications, molecular and TCGA subtypes (A). CD48 was a good predictor of the mesenchymal subtype (B). Expression of CD48 determined prognosis of glioma in CGGA (C and D) and TCGA (E and F) cohorts. **Abbreviations:** Exp, expression; LGG, lower grade glioma; GBM, glioblastoma multiforme; IDHwt, IDH wild-type; IDHmut, IDH mutant; Ip19qCod, Ip19q codeleted; Ip19qNod, Ip19q non-codeleted; AUC, area under curve; OS, overall survival; HGG, high grade glioma.

Table 1 Univariate and multivariate cox analysis of CD48 in CGGA

Variable	Univariate analysis		Multivariate analysis	
	HR	p-value	HR	p-value
CD48 expression	1.342	<0.0001	1.202	0.006
Age at diagnosis	1.038	<0.0001	1.014	0.072
WHO grade	3.480	<0.0001	2.456	<0.0001
KPS score	0.972	<0.0001	1.001	0.498
IDH status	0.228	<0.0001	0.997	0.992
1p19q codeletion	0.135	<0.0001	0.314	0.002
MGMT promoter status	0.526	0.0003	1.205	0.560
Radiotherapy	0.429	<0.0001	0.632	0.149

Abbreviations: HR, give space hazard ratio; WHO, World Health Organization; KPS, Karnofsky Performance Status.

analysis indicated that CD48 was an accurate predictor of 1–3-year survival (AUC =77.7%, 79.2% and 81.3%, respectively) (Figure S1C–E).

After univariate and multivariate cox analysis of key clinical and molecular factors, we found that CD48 expression was an independent prognostic factor of glioma in the CGGA dataset (HR =1.202, $P=0.006$) (Table 1). Consistent with this, CD48 also independently indicated unfavorable prognosis in TCGA dataset (HR =1.144, $P=0.006$) (Table S2). These finding indicated that CD48 might play a significant role in determining glioma prognosis.

CD48 influences glioma purity and local microenvironment cell populations

As an immune costimulatory molecule, we investigated whether CD48 influenced the composition of the tumor environment using glioma purity. We found CD48 had strongly positive correlation with glioma immune score ($r=0.717$) and stromal score ($r=0.571$), but negative correlation with glioma purity ($r=-0.685$) (Figure 2A). Additionally, local microenvironment cell population analysis showed that CD48 was positively associated with an enrichment of myeloid dendritic cells ($r=0.470$), B lineage ($r=0.397$), CD8 T cells ($r=0.343$) and monocytic lineage ($r=0.236$) (Figure 2A). Taken together, these results indicated that CD48 exerted an important function in influencing the local immune microenvironment of glioma.

CD48 is related to immune function

To explore the biological process of CD48, we first made PCA to study transcriptomic features. Based on CD48

expression level, whole transcriptome expression profiles were found to be different between high and low groups (Figure S2A and B), suggesting that different levels of CD48 expression resulted in distinct biological phenotypes.

After correlation analysis, genes strongly correlated with CD48 ($r>0.4$) in both CGGA and TCGA datasets were selected for functional annotation. These genes were predominately enriched in the interferon-gamma-mediated signaling pathway, T cell costimulation, T cell receptor signaling pathway, chemotaxis and antigen processing and presentation (Figure 2B). GSEA further confirmed that in the high-CD48 group an activated phenotype of antigen processing and presenting, the IFN- γ -mediated signaling pathway, T cell activation and T cell receptor signaling pathway were present (CGGA, Figure 2C–H; TCGA, Figure S2C–H). Moreover, CD48 was more correlative with antigen processing and presenting via MHC I, suggesting that CD48 was associated with the CD8 T cell immune response.

CD48 correlates with immune suppressive molecules

Using PCA, we found high-CD48 patients had distinct phenotypes of innate immunity, T cell-mediated immune response and immune response to tumor cells from low-CD48 patients (CGGA, Figure 3A–C; TCGA, Figure S3A–C). GASE analysis showed high expression of CD48 was accompanied with increased active innate and T cell-mediated immune responses (CGGA, Figure S3D–I; TCGA, Figure S3J–O). However, after analysis of correlation between CD48 and immune suppressive factors involved in immunomodulation,¹⁷ we found CD48 had a strong and positive association with most immune suppressive factors (Figure 3D and E). We suspected that although high-CD48 patients had an activated immune phenotype, they seemed to be immunosuppressive.

To confirm our speculation, we further performed analysis of correlation. CD48 was significantly correlated with immune suppressive molecules in all grades of glioma, as well in GBM of CGGA and TCGA cohorts (Figure 3F–I). Moreover, a strong association between CD48 and immune checkpoints, which suppressed antitumor immunity, was seen in glioma and GBM (Figure 3J–M). However, CD48 did not correlate with CTLA-4 and PD-L1. These results implied that CD48 exerted an immunosuppressive role in glioma immunity.

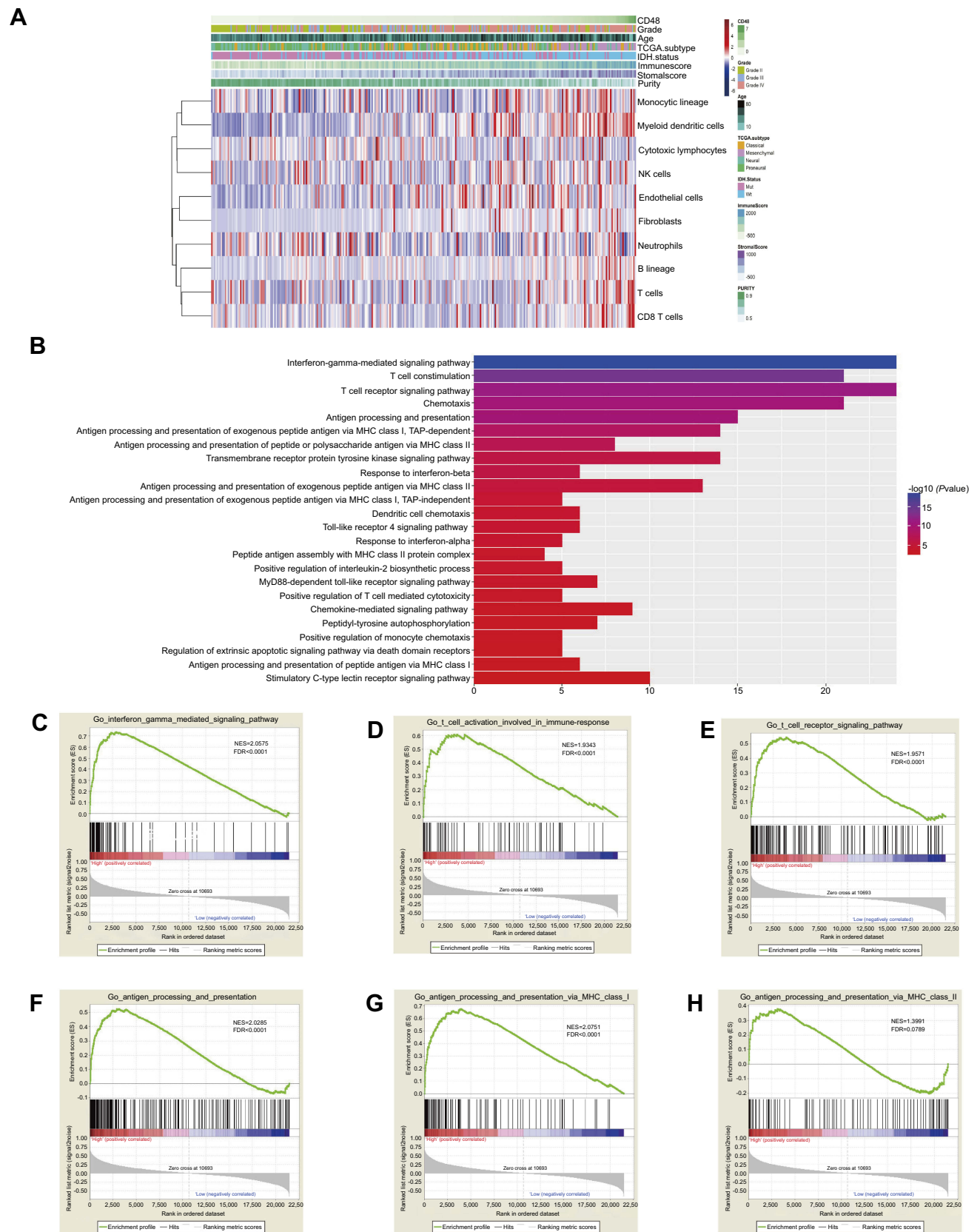


Figure 2 Microenvironment and biological function of CD48. High CD48 was associated with low purity, enrichment of myeloid dendritic cells, B lineage, CD8 T cells and monocytic lineage (A). Biological function was analyzed by DAVID among correlative genes (B). Biological processes relative to CD48 were validated using GSEA (C–H). **Abbreviations:** NK, natural killer; GO, gene ontology.

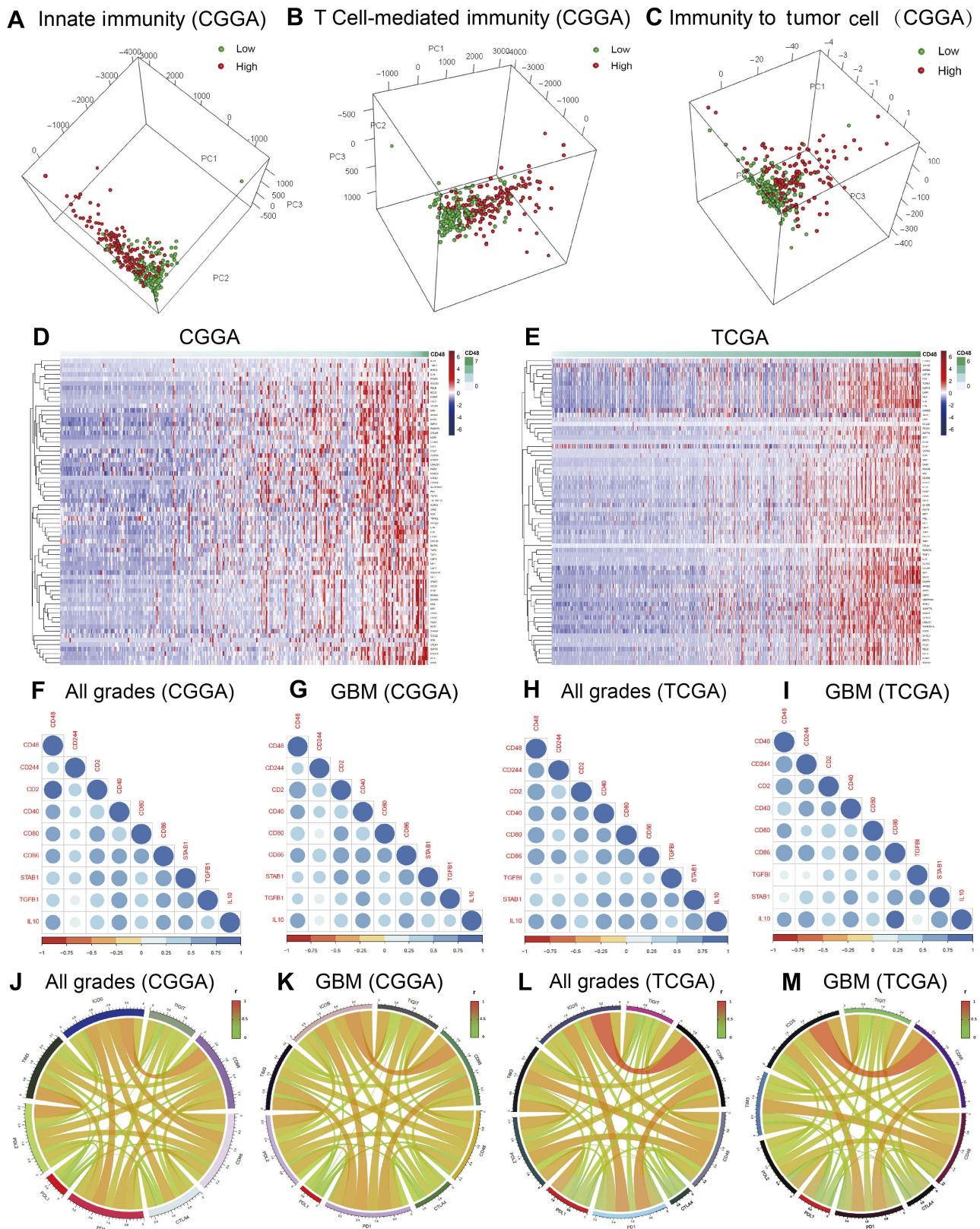


Figure 3 Immune response is influenced by CD48. Innate immunity, T cell-mediated immunity and immune response to tumor cell were dissimilar between low- and high-CD48 groups in CGGA (A–C). CD48 was closely associated with most immune suppressive factors (D and E). Immune suppressive molecules were positively associated with CD48 (F–I). CD48 was strongly correlated with immune checkpoints (J–M).

Abbreviations: CGGA, Chinese Glioma Genome Atlas; TCGA, The Cancer Genome Atlas; GBM, glioblastoma multiforme.

High-CD48 glioma is associated with an enhanced inflammatory response

An inflammatory response is always accompanied by an immune response and plays an important role in the malignant progression of glioma. We chose 7 metagenes of 104 genes representing different types of inflammatory and immune responses;¹⁸ CD48 was positively associated with six of these clusters except IgG (Figure 4A and B). Using GO terms involved in the inflammatory response, we performed GSEA to further explore the relation between CD48 and inflammation. We found that CD48 was correlated with inflammation, with no differences observed between chronic or acute inflammatory signatures in glioma (Figure 4C and D). These suggested that CD48 was crucial in promoting inflammation in glioma.

Checkpoint risk signature of CD48, SLAMF8 and PD-L1 reflects glioma prognosis

SLAMF8, a family member molecule of CD48, plays a significant role in both the immune and inflammatory response.¹⁹ CD48 and SLAMF8 both were strongly correlated with classical checkpoints, with little association with PD-L1. Therefore, we assembled a risk signature including CD48, SLAMF8 and PD-L1 to assess the role of checkpoints in glioma (Risk score = $0.2029 * PD-L1$

+ $0.2942 * CD48$ + $0.4562 * SLAMF8$). The risk score was much higher in malignant progression, such as GBM, IDH-wild type of LGG and GBM, and the mesenchymal subtype (CGGA, Figure 5A; TCGA, Figure S4A); the accuracy of risk scores of the mesenchymal subtype was 94.2% in CGGA and 94.8% in TCGA cohorts (CGGA, Figure 5B; TCGA, Figure S4B).

In all grades of glioma, higher risk score was associated with shorter OS. As such, a higher risk score was correlated with an unfavorable prognosis in LGG and GBM of CGGA datasets (Figure 5C–E). Additionally, these results were validated in the TCGA dataset (Figure 5F–H). After univariate and multivariate cox analyses, we demonstrated that the checkpoint risk score was an independent prognostic factor in CGGA (Table 2) and TCGA (Table S3) cohorts.

Checkpoint risk signatures have different determined values in different subtypes in GBM

GBM is the most malignant and refractory type of glioma. Recent research has focused on immune checkpoint studies and immunotherapy, which may provide new avenues for treatment of glioma. We identified that checkpoint risk scores had predictive value in both female and male groups (Figure 6A–D). Additionally,

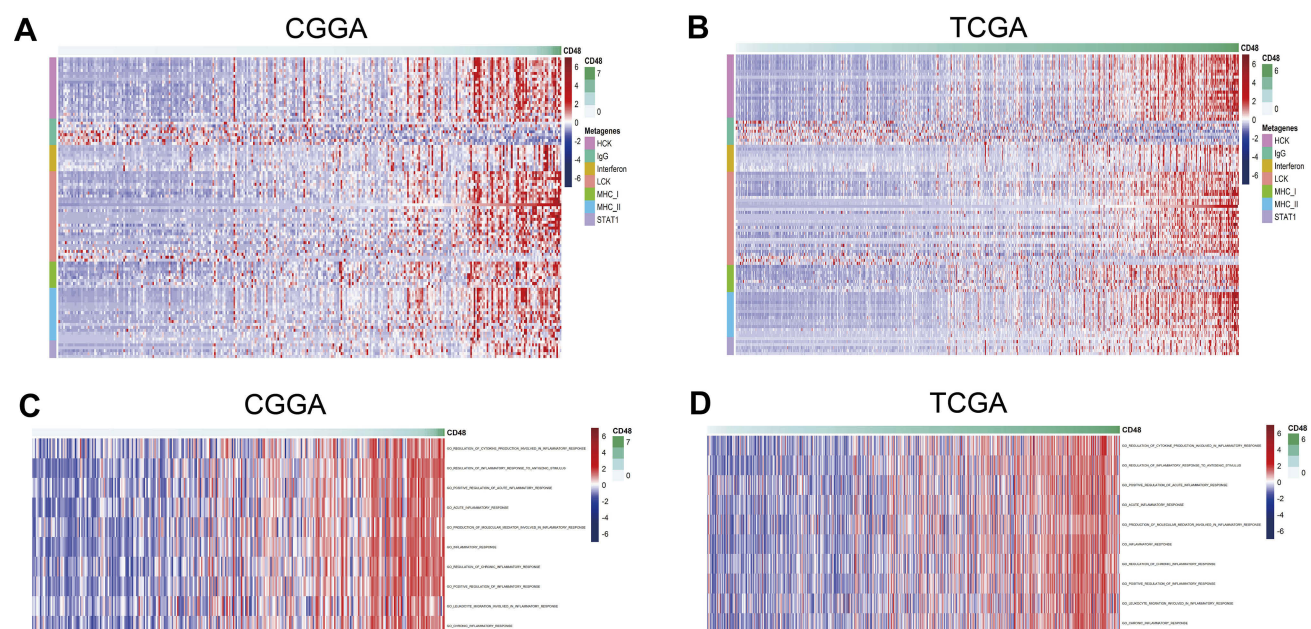


Figure 4 Association between CD48 and the inflammatory response. Genes involved in the inflammatory response correlated with CD48 in CGGA and TCGA cohorts (A and B). GSEA confirmed correlation between CD48 and the inflammation process, including acute and chronic inflammation (C and D).

Abbreviations: CGGA, Chinese Glioma Genome Atlas; TCGA, The Cancer Genome Atlas; GSEA, gene set variation analysis.

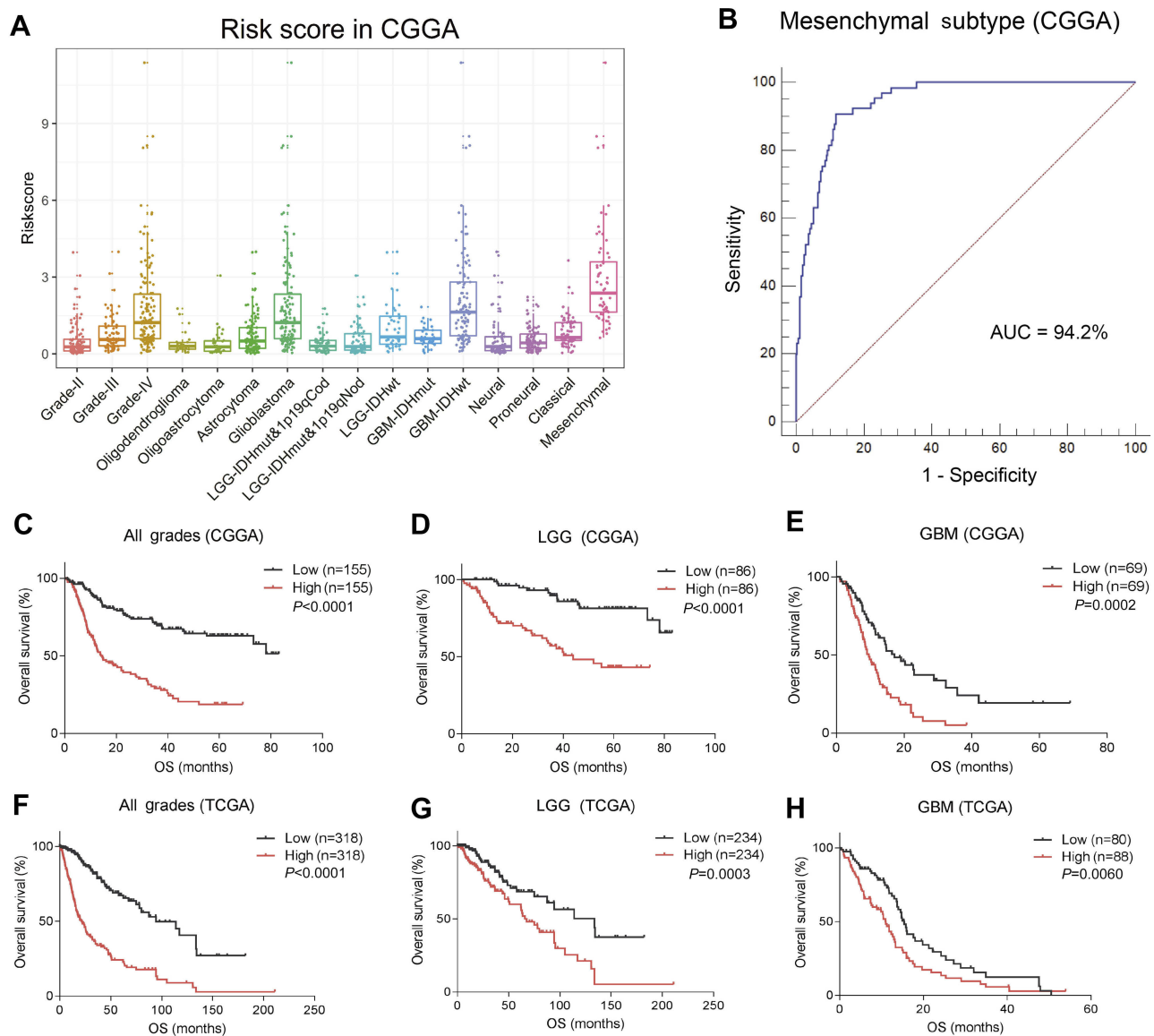


Figure 5 Distinct distribution and prognosis of checkpoint risk score. Checkpoint risk score exhibited various distributions among grades, histopathologic classifications, molecular and TCGA subtypes (A). Checkpoint risk score was especially high in the mesenchymal subtype (B). High checkpoint risk score was associated with unfavorable outcomes in glioma in CGGA (C–E) and TCGA (F–H) cohorts.

Abbreviations: CGGA, China Glioma Genome Atlas; AUC, area under curve; TCGA, The Cancer Genome Atlas; OS, overall survival; LGG, lower grade glioma; GBM, glioblastoma multiforme.

similar outcomes were observed in patients younger than 40 between low- and high-risk scores. However, survival differed among patients older than 40 (Figure 6E–H), suggesting that checkpoint factors might influence prognosis in older patients. Considering ethnicity, we found increased risk score indicated shortened survival time in Asian of CGGA (Figure 5E) and in white of TCGA (Figure S4C). But there were not enough patients to analyze the predictive value of risk score in black.

Next, we investigated the prognostic role of risk score in the different molecular subtypes of GBM. In IDH wild-type

GBM, prognosis was significantly different compared to other subtypes, which was attributed to variable risk scores. However, no difference in survival was observed in IDH mutant GBM (Figure 6I–L). Moreover, we observed obvious differences in survival time between low and high-risk score groups in MGMT-methylated patients, but not in GBM with unmethylated MGMT promoter (Figure 6M–P).

Discussion

Malignant glioma is a heterogeneous disease accompanied by the infiltration of multiple immune cells which mount

Table 2 Univariate and multivariate cox analysis of checkpoint risk score in CGGA

Variable	Univariate analysis		Multivariate analysis	
	HR	p-value	HR	p-value
Risk score (high vs low)	3.651	<0.0001	1.733	0.007
Age at diagnosis (>40 vs <40)	1.879	<0.0001	1.148	0.474
WHO grade (II, III and IV)	3.480	<0.0001	2.365	<0.001
KPS score (increasing)	0.972	<0.0001	1.002	0.450
IDH status	0.228	<0.0001	0.790	0.305
1p19q codeletion	0.135	<0.0001	0.362	0.007
MGMT promoter status	0.526	0.0003	0.827	0.295
Radiotherapy	0.429	<0.0001	0.457	<0.001

Abbreviations: HR, hazard ratio; vs, versus; WHO, World Health Organization; KPS, Karnofsky Performance Status.

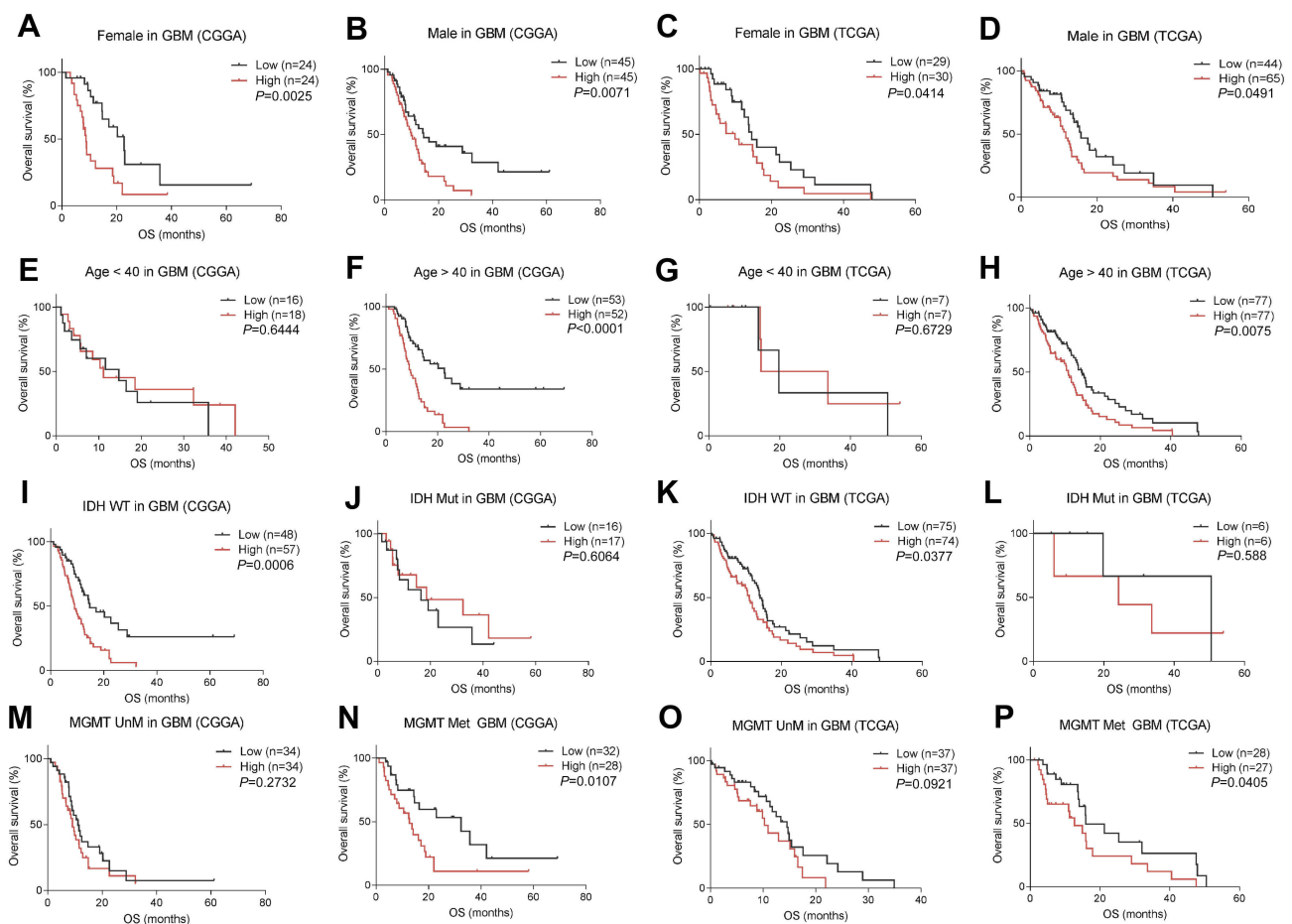


Figure 6 Prognostic value of checkpoint risk score in different subtypes of GBM. The predictive value of risk score was equal in male and female groups (A–D). Risk score correlated with prognosis among patients older than 40, but not in the group of younger patients (E–H). High-risk score indicated poor prognosis with IDH wild type and MGMT methylation status, but not IDH mutation and MGMT methylation status (I–P).

Abbreviations: GBM, glioblastoma multiforme; OS, overall survival; CGGA, Chinese Glioma Genome Atlas; TCGA, The Cancer Genome Atlas; WT, wild-type; Mut, mutant; Met, methylated; unM, unmethylated.

a complex immune response. Immunomodulation among immune cells or between tumor cells and immune cells plays a significant role in promoting progression of

neoplasm.²⁰ Checkpoints are key molecules in immunomodulation, and checkpoint blockade shows significant potential for immune therapy of glioma.²¹ Here, we

demonstrate that the expression of the adhesion and costimulatory molecule of immune cells, CD48, correlated with malignant phenotype and unfavorable prognosis. Identification and characterization of such molecules may aid in our understanding of immune mechanisms in glioma, paving the way for novel treatments.

After analysis of glioma RNA-seq data of 946 patients, we identified that CD48 expression was increased along with glioma grade and was particularly enriched in GBM. CD48 has been reported to be highly expressed in breast cancer, with high tumor-infiltrating lymphocytes (TIL), as one molecule of eight-gene TIL signature (including CD19, CD3D, CD48, GZMB, LCK, MS4A1, PRF1 and SELL),²² which suggested that CD48 is commonly enriched in more malignant tumors associated with TIL. We found CD48 was enriched in IDH-wild glioma and mesenchymal subtypes, which indicates involvement in an active immune response.^{23,24} In addition, it has been reported that CD48 had a positive correlation with the disease status of primary Sjögren's syndrome, in both serum and salivary glands.^{25,26} Taken together, these findings indicate that CD48 is highly expressed in the immune and inflammatory response, as well as in glioma. As such, the detection of CD48 in serum may be an indicator to reflect the immune and inflammation status of glioma patients; however, further study is needed.

Previously, we found that immune-related factors, STAB1, TGF- β and IL-10 play crucial roles in controlling the development and prognosis of glioma cells.^{27,28} Jiang's team has reported that coinhibitory checkpoints, PD-1,²⁹ PD-L1³⁰ and TIM-3¹⁶ are independent prognostic risk factors with immune-related function in glioma. Here, we confirmed that the costimulatory molecule CD48 also predicted prognosis of glioma independently, with high-CD48 expressing glioma associated with shorter OS. These results suggest that immune-related molecules play a crucial role in promoting the malignant progress of gliomas and despite the presence of the blood-brain barrier, glioma still could promote tumor development by immunomodulation.

Glioma is a heterogeneous tumor with a complex microenvironment. Infiltrating stromal and immune cells form the major fraction of non-tumor cells, such as endothelial cells, T cells and TAMs and so on, diluting the purity of tumor cells. Higher levels of immature blood vessels and TAMs serve as robust indicators of poor prognosis.^{31,32} Glioma purity is an intrinsic characteristic of this tumor in the development of a suitable

microenvironment. Macrophages, microglia and neutrophils are enriched in low purity glioma. Low glioma purity means malignant entities, reduced survival time and intensive immune phenotypes.¹⁴ A positive correlation exists between CD48 and immune score and stromal score, and a negative correlation exists between CD48 and glioma purity. This suggests that CD48 expression in glioma may influence the composition of the tumor microenvironment to boost the advancement of glioma.

Myeloid dendritic cells, B lineage, CD8 T cells and monocytic lineage, which are the main functional immune cells during the anti-tumor immune response, were enriched in high-CD48 glioma. T cells are functional immune cells of adaptive immunity that can kill tumor cells with the costimulatory signal from APC through MHC molecules. Dendritic cells and monocyte-macrophage lineages are the main APC. As a costimulatory signaling molecule, CD48 may interact with its ligand to regulate immunological activity of these immune cells. GO analysis confirmed that CD48 was involved in antigen processing and presentation, T cell costimulation and the T cell receptor signaling pathway. High-CD48 glioma correlated with antigen processing and presenting via MHC I, which interacts with CD8 T cells. Considering the local microenvironment cell population, these results verified that CD48 plays a significant role in immunomodulation of CD8 T cell-mediated immunity and may be involved in anti-tumor immunity.

Although CD48 has little correlation with the enrichment of NK cells in glioma, CD48 is reported to regulate activity and NK cell function.³³ NK cells can control innate immunity and also adaptive immunity through receptor/ligand-specific cross talk, mainly CD48/2B4.³⁴ Additionally, it has been reported that CD48 prolonged the time frame of effective T cell activation by supporting mature DC survival.³⁵ In glioma, high-CD48 glioma followed a more activated innate immunity and T cell-mediated adaptive immunity, consistent with our previous finding that malignant gliomas are associated with a more enhanced immune phenotype.³⁶ However, other research has demonstrated that CD48 also inhibits the activity of NK cells.¹¹ Therefore, we suggest that high-CD48 glioma is associated with stronger immunosuppression at the same time.

Studies on immunosuppression have revealed that ligation of CD80 and CD86 on T cells by the cell surface molecule CTLA-4 on suppressor cells results in suppression of T cell activation.³⁷ Suppression of primed immune responses by DCs correlates with RelB nuclear binding activity and CD40 expression, with immature DCs also inducing CD8+

T regulatory cells to produce high levels of IL-10.³⁸ Regulatory CD4+ T cells induced by the DCs transfer tolerance to primed recipients in an IL-10-dependent fashion.³⁹ TGF- β could suppress T-cell proliferation and suppression of CD8+ T cells that mediate tumor rejection by CD4+CD25+. T cells require TGF- β to interact with CD8+ T cells.⁴⁰ These suppressive molecules are positively associated with CD48 in our study, suggesting that CD48 works with them to promote immunosuppression.

Immune checkpoints have been shown to play immunosuppressive roles in cancer, such as CTLA-4, PD-1, PD-L1/2, Tim-3, ICOS, TIGIT and CD96. These checkpoint blockades are under investigation, and new inhibitory pathways are being investigated.^{41,42} CD48 has a strong association with most checkpoints, but little with CTLA-4 and PD-L1, which demonstrates that CD48 may have a different immunomodulating role from CTLA-4 and PD-L1. While therapies targeting CTLA-4 and PD-1 have shown remarkable success in glioma mouse models, clinical patients benefit little from these treatments,⁴³ and some patients experience serious immune-related side effects or even fatal cerebral edema and myocarditis.^{44,45} High-CD48 gliomas revealed close association with the inflammatory response. Therefore, we suggest that multiple checkpoint blockades may promote antitumor immunity without excessive inflammation. As such, CD48 may be an appropriate target, potentially by use of an inhibitor against PD-L1 or CTLA-4.

Immunotherapy, in particular checkpoint blockade, has become the current focus of cancer research. Checkpoint risk signature including PD-L1, CD48 and SLAMF8 was confirmed as an independent risk factor of glioma, correlating with malignant progression. These results emphasize that risk factor immune checkpoints exert significant influence in determining development and outcome of glioma. Moreover, checkpoint risk score was an accurate predictor of the mesenchymal subtype, further indicating that the mesenchymal subtype is accompanied by a more complex immune microenvironment and immune response. This suggests that the mesenchymal subtype of GBM may be more suitable for immunotherapy.

Considering the factors of gender and age, we found checkpoint risk score had a crucial influence on prognosis in groups of patients older than 40, but not in groups of young patients. This indicates that age is an important factor in influencing tumor immunity, and older patients might have a better response to checkpoint blockade immunotherapy than young patients, which may be due to the increased expression

of regulatory immune checkpoints on T cells with age in human and mice.^{46,47} However, patients older than 75 are associated with a decline in the immune system, which theoretically can reduce the efficacy of immunotherapy.⁴⁸ In addition, our result confirmed that checkpoint risk score plays an important role in IDH wild-type GBM rather than mutant IDH. IDH wild-type GBM presents with a more complex microenvironment and stronger immune response,²⁴ which results in an unfavorable outcome.⁴⁹ Therefore, checkpoint blockade may be the optimal approach to block immunosuppression and activate effective antitumor immunity.

Conclusion

Through multiple analysis of large glioma cohorts, we found the costimulatory and adhesion molecule CD48 correlated with malignant progression and poor prognosis. By influencing local immune microenvironments, CD48 plays an important role in antigen presenting and T cell activation. With an active immune phenotype, CD48 was strongly associated with immunosuppression and inflammatory responses. Further studies will need to further investigate the immunomodulatory role of CD48 in antitumor immunity of glioma. Due to its role in immunomodulation, CD48 may become an ideal target by checkpoint blockade, which is most suitable for IDH wild-type GBM patients above 40 years old.

Ethical approval

This article does not contain any studies with human participants or animals.

Acknowledgments

We thank all the members of Dr Anhua Wu's laboratory for help with our study. This work was supported by grants from the National Natural Science Foundation of China (grant numbers: 81172409, 81472360, 81402045, and 81872054) and the Science and Technology Department of Liaoning Province (grant number: 2011225034).

Disclosure

The authors report no conflicts of interest in this work.

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