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# Expression and Function of Toll-Like Receptor 10 (TLR10) in Diffuse Large B Cell Lymphoma, Acute Myeloid Leukemia, and Glioma

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Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
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**Background:** Toll-like receptor (TLR) family members are part of the major pathogen-recognition system for innate immunity. TLR10, the only remaining orphan receptor with an unknown ligand, has been poorly studied in tumors, and its functional and clinical relevance are unclear.

**Material/Methods:** We analyzed TLR10 expression data in The Cancer Genome Atlas (TCGA) by established computational approaches (UALCAN, GEPIA, CGGA, and TIMER) and confirmed them by immunohistochemistry analysis.





**Results:** Bioinformatics analysis showed that TLR10 was most highly expressed in diffuse large B cell lymphoma (DLBC), acute myeloid leukemia (LAML), and glioblastoma multiforme (GBM) patients. A data-mining study also revealed that TLR10 levels were positively correlated with WHO grade in glioma, and patients with high TLR10 levels showed shorter overall survival (OS) and disease-free survival (DFS) times than patients with low TLR10 levels. TISIDB and TIMER data showed that TLR10 expression was significantly positively correlated with immune infiltrates, especially infiltrating levels of B cells. Importantly, immunohistochemistry analysis revealed that TLR10 expression was a potential biomarker for distinguishing CNS-DLBC (also known as primary central nervous system lymphoma, PCNSL) from GBM.

**Conclusions:** Taken together, these results suggest that TLR10 could serve as a promising theranostic target for patients with glioma and is a potential biomarker for distinguishing PCNSL from GBM.

**MeSH Keywords:** **Glioma • Leukemia, Myeloid, Acute • Lymphoma, Large B-Cell, Diffuse • Toll-Like Receptor 10**

**Abbreviations:** **DAMPs** – endogenous danger-associated molecular patterns; **DFS** – disease-free survival; **DLBC** – diffuse large B cell lymphoma; **GBM** – glioblastoma multiforme; **GEPIA** – gene expression profiling interactive analysis; **LAML** – acute myeloid leukemia; **LGG** – low-grade glioma; **OS** – overall survival; **PAMPs** – pathogen-associated molecular patterns; **PCNSL** – primary central nervous system lymphoma; **PRRs** – pattern-recognition receptors; **TCGA** – The Cancer Genome Atlas; **TIMER** – Tumor Immune Estimation Resource; **TLR10** – toll-like receptor 10

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/921500>

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## Background

Toll-like receptors (TLRs), the most renowned family of pattern-recognition receptors (PRRs), can recognize a broad spectrum of pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns (DAMPs) [1]. Induction of TLRs results in inflammatory signaling cascades, which can play crucial roles in the occurrence and persistence of innate immune inflammatory responses. TLR10, mainly expressed on B cells [2–4], is a recently discovered transmembrane receptor composed of leucine-rich repeat-recognizing domains and a Toll/IL-1 receptor homology (TIR) signaling domain, similar to other TLRs [5]. Although the ligand(s) and downstream signaling pathways of TLR10 remain unclear, it has been identified as an immunomodulatory receptor with inhibitory properties [4,6,7], which distinguishes TLR10 from other TLRs.

In humans, TLR10 has been shown to be involved in osteoarthritis [8], Crimean Congo hemorrhagic fever [9], infections with *Listeria monocytogenes* [10], *Salmonella typhimurium* [11], and *Helicobacter pylori* [12], and influenza [13]. Nevertheless, studies on TLR10 have mainly focused on its immune and inflammatory functions [4,10], and the identification of the involvement of TLR10 in tumors paves the way for future exploratory studies.

Currently, the available data present only a minimal understanding of the relationship between TLR10 and tumors and the associations of TLR10 expression with prognostic significance and immune infiltration levels in DLBC, LAML, and glioma. Considering the unique expression characteristics of B cells and the immunomodulatory properties of TLR10, we investigated TLR10 expression in PCNSL and assessed the potential of TLR10 as a biomarker to distinguish PCNSL from GBM.

## Material and Methods

### Ethics statement

This study involving human tissue specimens was authorized by the Ethics Committee of Tianjin Huanhu Hospital (2019-71). All participants signed informed consent forms and were aware of the study details.

### Clinical tissue specimens and immunohistochemistry

For immunohistochemical analysis, formalin-fixed, paraffin-embedded samples of 20 PCNSL (median age 62, age range 51–68 years) and 20 GBM patients (median age 63, age range 50–70 years) diagnosed at Tianjin Huanhu Hospital were used. The paraffin-embedded brain tissues were sectioned onto slides, and immunohistochemistry was performed on a

YN-05MY automatic immunohistochemical staining system (YongNian, China) with an anti-TLR10 antibody (Cat. No. PAI-46478, Invitrogen, Carlsbad, USA). For counts of percent positivity of TLR10, 3 independent pathologists counted the positive cells in 10 randomized high-power fields (HPFs) of each PCNSL and GBM samples. Cell type specificity of TLR10 expression in PCNSL and GBM were determined using double immunohistochemical labeling technique with TLR10 antibody (red) and anti-Olig2 antibody (brown) (Cat. No. ZA-0561, ZSGB-BIO, Beijing, China).

### Gene correlation and prognostic significance analysis in GEPIA

The online database Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/index.html>) [14] was used to assess the expression of TLR10 among tumor patients and healthy people. GEPIA is a customized tool based on TCGA and GTEx data. It provides information on differential expression analysis, gene profiling, correlation analysis, and patient survival analysis.

### Gene correlation analysis in UALCAN

UALCAN (<http://ualcan.path.uab.edu/>) [15] was used to clarify the expression of TLR10 in glioma, DLBC and LAML patients and adjacent normal tissues based on in-depth analyses of TCGA gene expression data.

### Immune infiltration level determination in TIMER and TISIDB

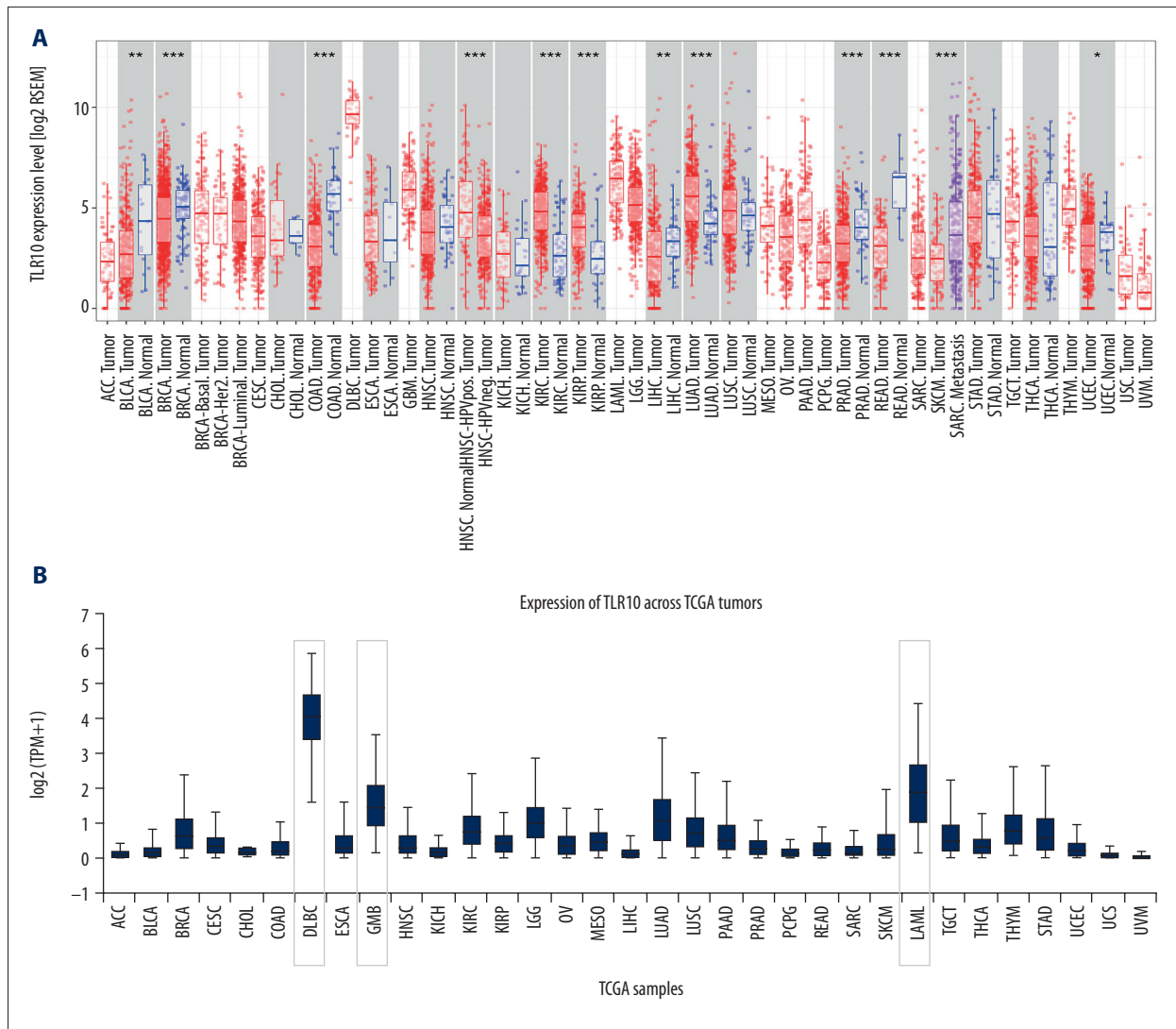
Tumor Immune Estimation Resource (TIMER; <https://cistrome.shinyapps.io/timer/>) was used to thoroughly assess the molecular characteristics of tumor immune interactions [16]. TISIDB is a web portal combined with multiple types of data resources related to immuno-oncology and allows users to perform studies on tumor immune interactions [17].

### Prognosis analysis with the Chinese Glioma Genome Atlas (CGGA)

Survival data for patients who underwent craniotomy in multiple centers in Beijing were collected from CGGA (<http://www.cgga.org.cn/>).

### Statistical analysis

The expression results generated with established computational approaches are displayed with fold change and P values. Survival curves were estimated by the Kaplan-Meier method. Spearman rank correlation was conducted to evaluate the



**Figure 1.** Expression of TLR10 across TCGA tumors. **(A)** TLR10 mRNA levels in tumors and adjacent normal tissues determined using the TIMER database. **(B)** TLR10 mRNA levels across TCGA tumors, determined using the UALCAN database.

correlation of gene expression.  $P < 0.05$  was considered statistically significant.

## Results

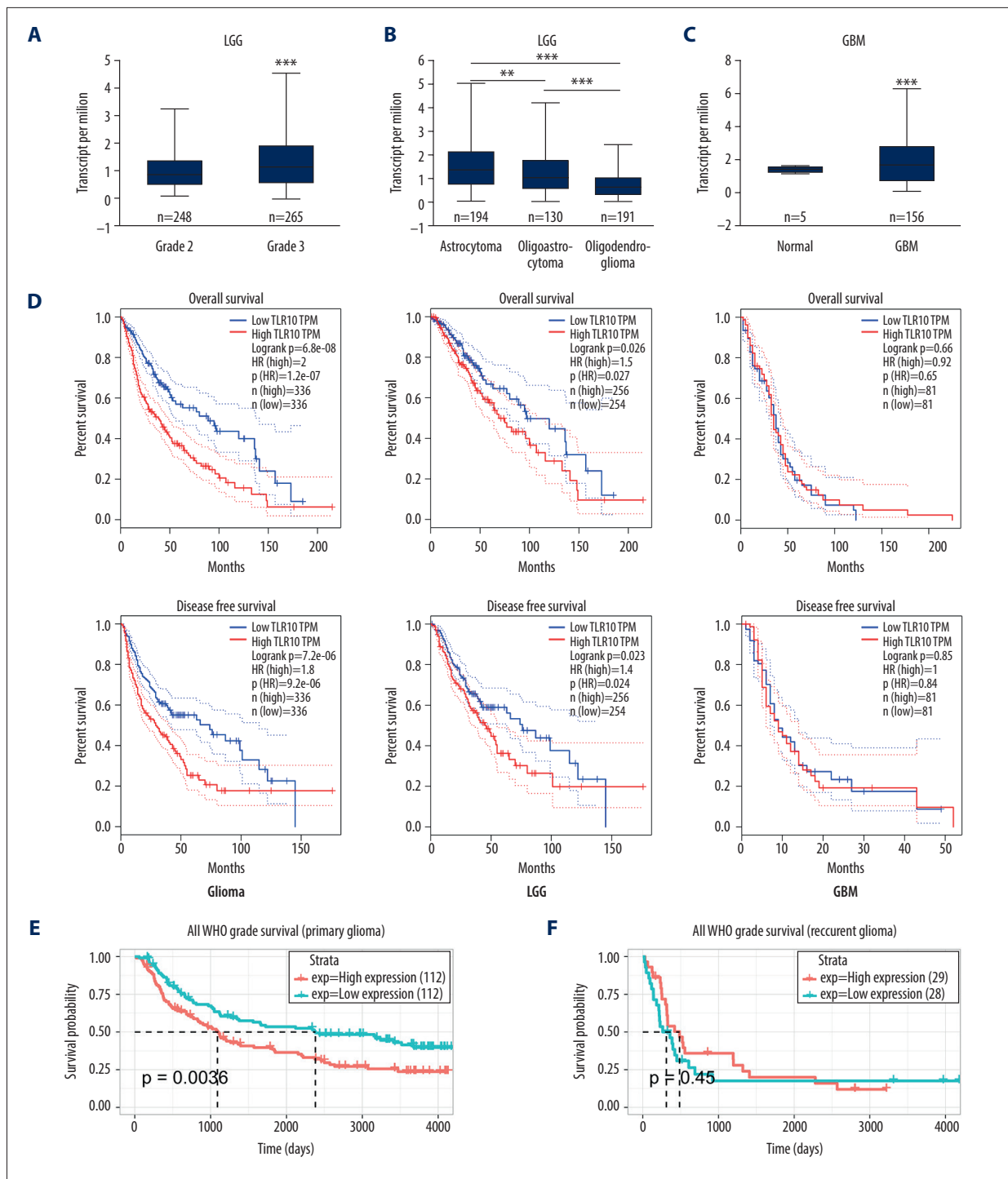
### The mRNA expression levels of TLR10 in various types of human cancers

To evaluate TLR10 mRNA expression, we used established computational approaches (TIMER and UALCAN) to analyze the RNA-seq data from TCGA. The differential expression of TLR10 between tumor and adjacent normal tissues across all TCGA tumors is shown in Figure 1A. The results from TIMER showed that TLR10 expression was significantly lower in BLCA (bladder urothelial carcinoma), BRCA (breast invasive carcinoma),

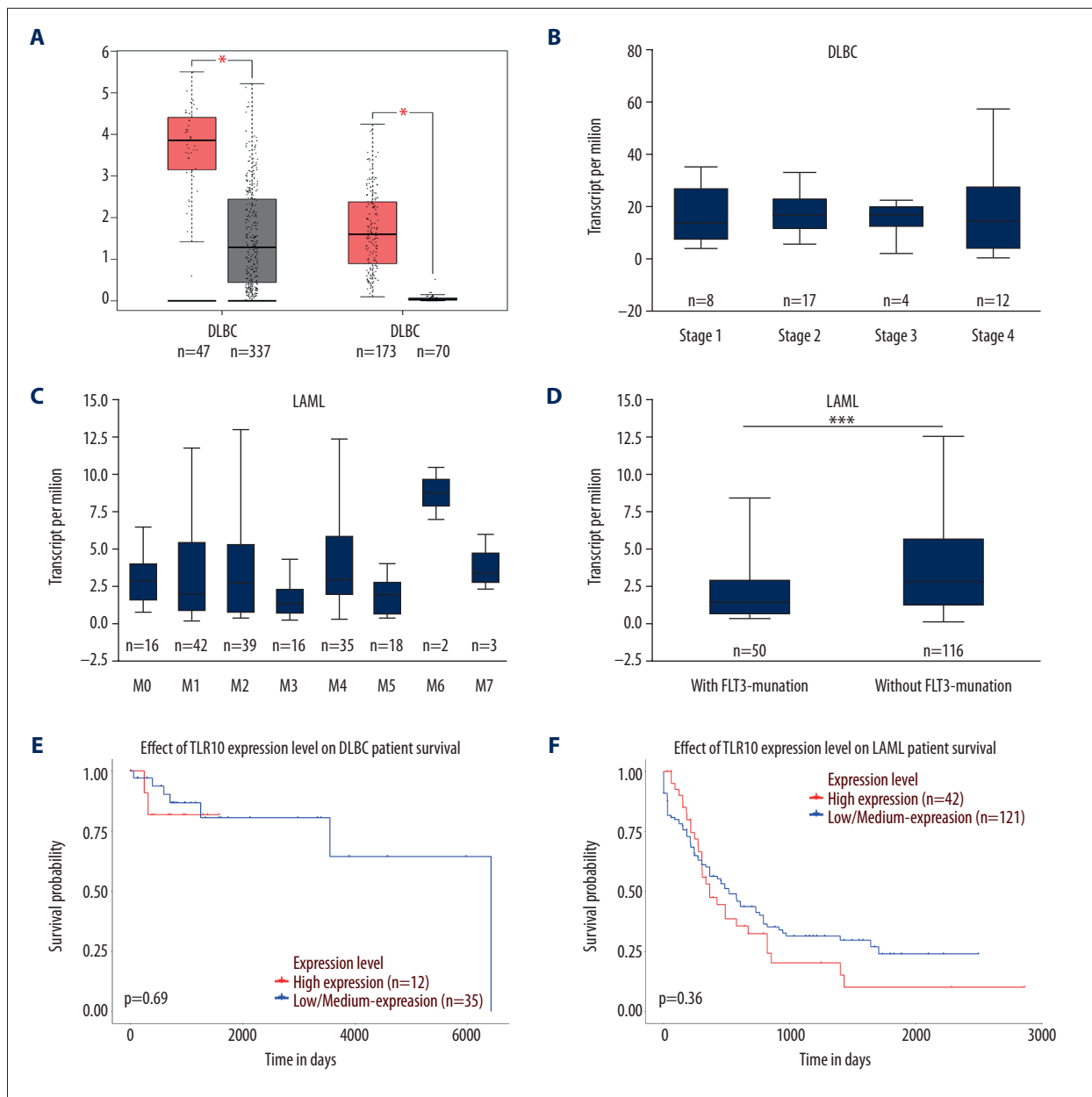
COAD (colon adenocarcinoma), LIHC (liver hepatocellular carcinoma), PRAD (prostate adenocarcinoma), and UCEC (uterine corpus endometrial carcinoma) than in adjacent normal tissues. However, TLR10 expression was significantly higher in KIRC (kidney renal clear cell carcinoma), KIRP (kidney renal papillary cell carcinoma), and LUAD (lung adenocarcinoma) than in adjacent normal tissues. Moreover, data from UALCAN (Figure 1B) revealed that TLR10 was more highly expressed in DLBC, GBM, and LAML than in other human cancers.

### The expression level and prognostic significance of TLR10 in DLBC, glioma, and LAML

As TLR10 had the highest expression in DLBC, GBM, and LAML compared with other human cancers, we explored the expression pattern and estimated the clinical significance of TLR10 in



**Figure 2.** Expression level and prognostic significance of TLR10 in glioma patients determined using data mining. **(A)** Expression of TLR10 in LGG based on tumor grade determined using UALCAN data. **(B)** Expression of TLR10 in LGG based on histological subtypes determined using UALCAN. **(C)** Expression of TLR10 in GBM determined using UALCAN data. **(D)** The prognostic significance of TLR10 expression in glioma patients. The **left panel** shows the relationship between TLR10 expression and OS/DFS in glioma patients. The **middle panel** shows the relationship between TLR10 expression and OS/DFS in LGG patients. The **right panel** shows the relationship between TLR10 expression and OS/DFS in GBM patients. **(E, F)** CGGA analysis showed that TLR10 expression was negatively associated with survival in primary glioma patients **(E)** but was not negatively associated with survival in recurrent glioma patients **(F)**.

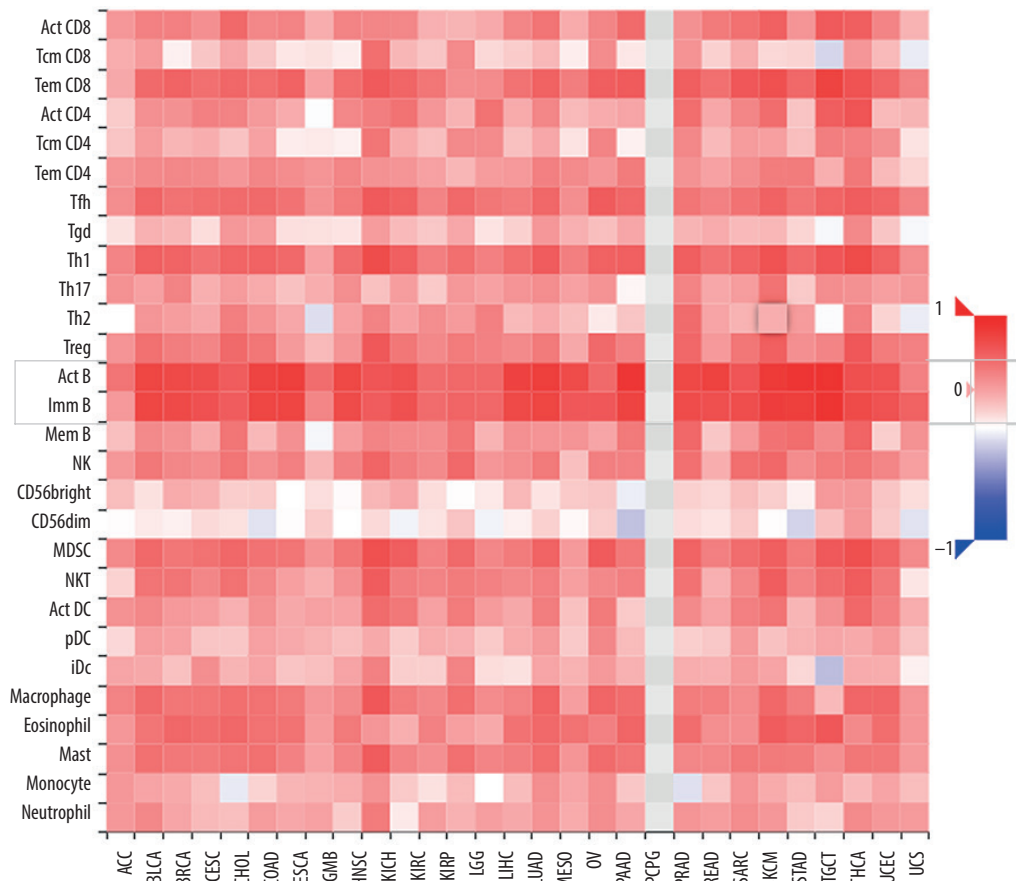


**Figure 3.** Expression level and prognostic significance of TLR10 in DLBC and LAML patients determined using data mining. **(A)** TLR10 mRNA levels in DLBC and LAML determined using GEPIA data (red, tumor; gray, adjacent normal tissues). **(B)** Expression of TLR10 in DLBC based on individual cancer stages. **(C)** Expression of TLR10 in LAML based on FAB classification. **(D)** Expression of TLR10 in LAML based on FLT3 mutation status. **(E, F)** Effect of TLR10 expression levels on DLBC **(D)** and LAML **(E)** patient survival.

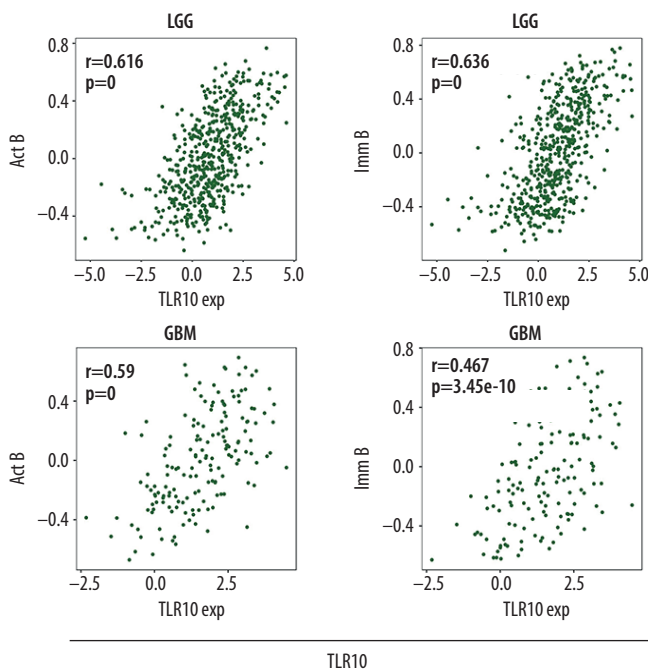
LGG, GBM, DLBC, and LAML. As shown in Figure 2, the UALCAN results revealed that TLR10 was much more highly expressed in the GBM group than in the normal group (Figure 2A) and was positively correlated with WHO grade in LGG (Figure 2B). TLR10 expression was different in the 3 histological types of LGG. TLR10 expression was the highest in astrocytoma and the lowest in oligodendroglioma subtypes (Figure 2C). To evaluate the prognostic significance of TLR10 expression in glioma

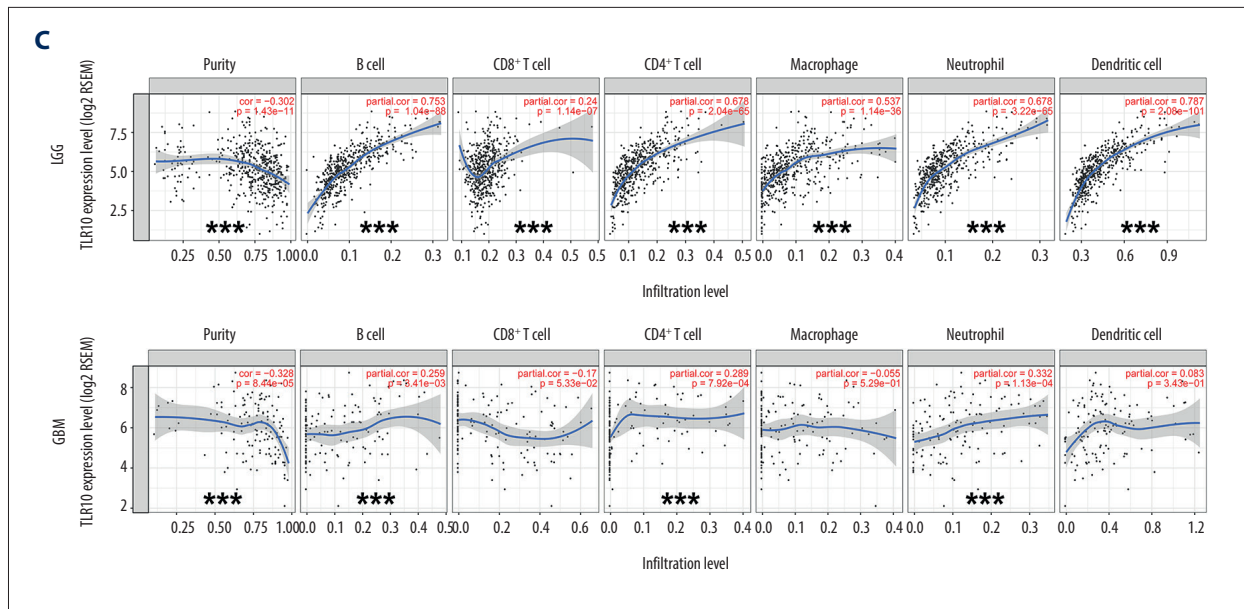
patients, we analyzed the relationship between TLR10 expression and the TCGA glioma cohort, including GBM (n=162) and LGG (n=510) patients, using GEPIA and the CGGA cohort, including glioma (n=281) patients. The GEPIA results (Figure 2D) showed that a high TLR10 expression level was associated with poor overall survival (OS,  $P=6.8 \times 10^{-8}$ ) and poor disease-free survival (DFS,  $P=7.2 \times 10^{-6}$ ) in glioma patients. Furthermore, we conducted subgroup analysis on the TCGA GBM cohort and the

**A**



**B**





**Figure 4.** Correlation of TLR10 expression with immune infiltration level. (A) TLR10 expression was significantly positively correlated with infiltrating levels of B cells in almost all human tumors according to TISIDB data. (B) Correlation analysis between TLR10 expression and infiltrating levels of B cells in LGG and GBM determined using TISIDB data. (C) TIMER analysis of the correlation of TLR10 expression with the immune infiltration level in LGG and GBM.

TCGA LGG cohort. We observed that TLR10 expression in the TCGA LGG cohort was negatively associated with OS ( $P < 0.05$ ) and DFS ( $P < 0.05$ ) but had no significant relationship with OS and DFS in the TCGA GBM cohort. Moreover, the CGGA analysis showed that TLR10 expression was negatively associated with survival ( $P = 0.0036$ ) in primary glioma patients (Figure 2E) but not in recurrent glioma patients (Figure 2F). Taken together, these findings show that TLR10 was highly expressed in both LGG and GBM, and high TLR10 expression was associated with a poor prognosis in glioma patients.

In addition, we evaluated the impact of TLR10 expression on clinical significance in DLBC and LAML by data mining the GEPIA and UALCAN databases. Figure 3A shows that TLR10 was remarkably increased in DLBC and LAML tissues compared with normal tissues and had a significant relationship with FAB classification/FLT3 mutation in LAML (Figure 3C, 3D) but not with DLBL tumor grade (Figure 3B). Although TLR10 was highly expressed in DLBC and LAML, there was no correlation between TLR10 expression and the prognosis of DLBC (Figure 3E) and LAML (Figure 3F).

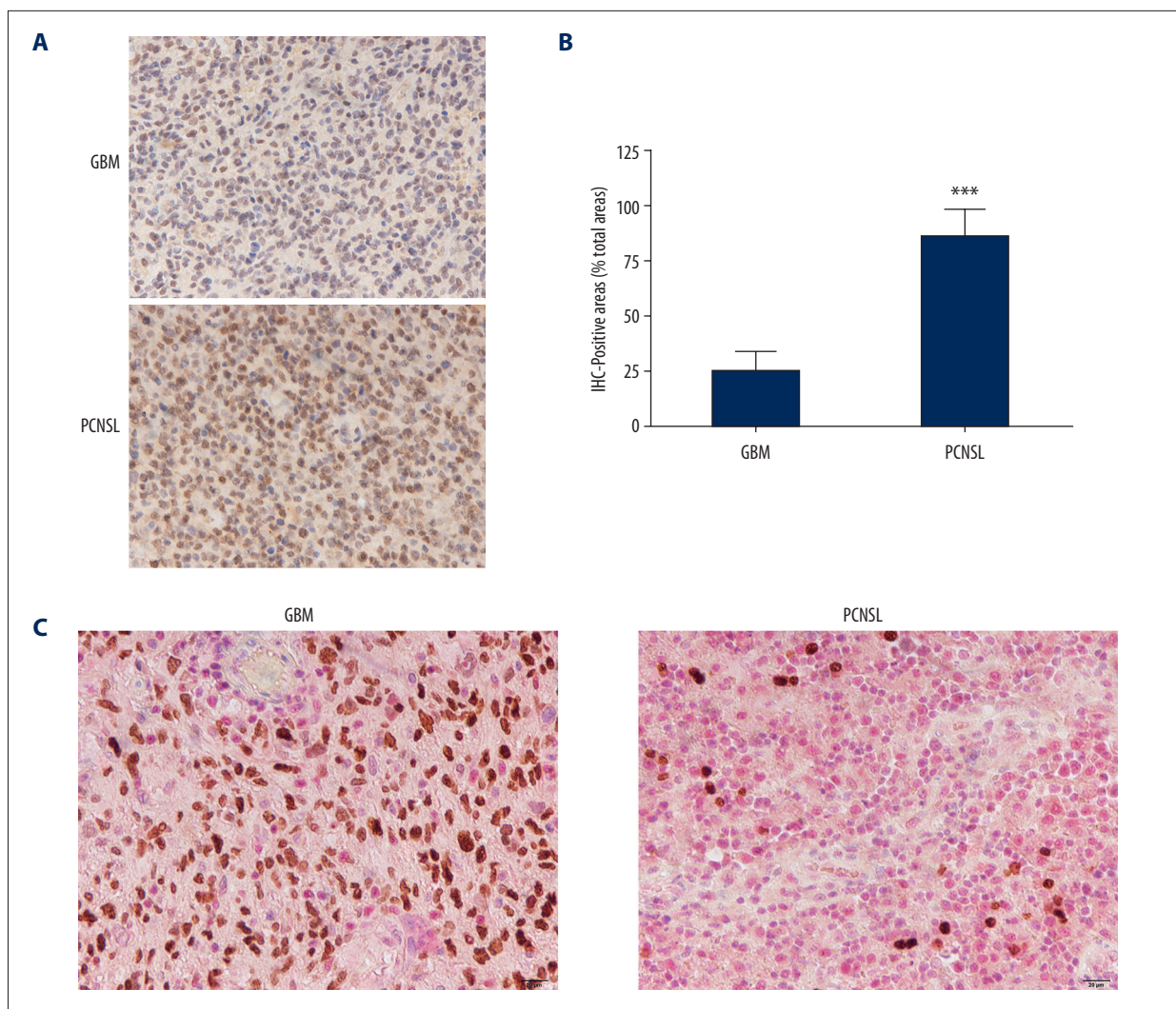
### TLR10 expression is correlated with immune infiltration levels, especially infiltrating levels of B cells

Tumor-infiltrating lymphocytes (TILs) can be correlated with either better or worse prognosis in cancers [18]. Therefore, we analyzed whether TLR10 expression was correlated with immune infiltration levels with TISIDB and TIMER data (Figure 4).

The data obtained from TISIDB showed that TLR10 expression was significantly correlated with infiltrating levels of B cells in almost all human tumors (Figure 4A, 4B). The data from the “gene” module of TIMER demonstrated that TLR10 mRNA expression was negatively correlated with tumor purity (LGG,  $r = -0.302$ ,  $P = 1.43E-11$ ; GBM,  $r = -0.328$ ,  $P = 8.44E-5$ ) and positively correlated with the infiltration level of B cells (LGG,  $r = 0.753$ ,  $P = 1.04E-88$ ; GBM,  $r = 0.259$ ,  $P = 3.41E-03$ ), CD4+ T cells (LGG,  $r = 0.678$ ,  $P = 2.04E-65$ ; GBM,  $r = 0.289$ ,  $P = 7.92E-04$ ), and neutrophils (LGG,  $r = 0.678$ ,  $P = 3.22E-65$ ; GBM,  $r = 0.332$ ,  $P = 1.13E-4$ ) in LGG and GBM (Figure 4C). According to the above results, we predicted that TLR10 expression is correlated with the immune infiltration level, especially the level of B cells.

### The expression level of TLR10 in PCNSL and GBM

PCNSL (primary central nervous system lymphoma, also known as CNS-DLBC) is a malignant brain tumor with limited treatment measures and shows prominent infiltration of tumor-infiltrating lymphocytes [19]. Based on the role of TLR10 in immune infiltration, especially in infiltrating B cells, we further analyzed the potential of TLR10 as a biomarker to distinguish PCNSL from GBM to address the problem of difficult diagnosis in PCNSL. Immunohistochemistry analysis was used to examine the expression of TLR10 in PCNSL and GBM. We found that the expression levels of TLR10 in PCNSL were significantly higher than those in GBM tumors (Figure 5A, 5B). Further, cell type specificity of TLR10 expression in PCNSL and GBM was determined using double immunohistochemical labeling



**Figure 5.** Expression of TLR10 in GBM and PCNSL. **(A)** Immunohistochemical analysis of TLR10 expression in PCNSL and GBM tissues. **(B)** Statistical quantitative analysis of TLR10 percentage. Data are presented as the mean±SD. \*\*\*  $p < 0.001$ , one-way ANOVA. **(C)** Double immunohistochemical labeling technique with TLR10 antibody (red) and anti-Olig2 antibody (brown). Scale bar 20 µm.

technique with TLR10 antibody (red) and anti-Olig2 antibody (brown), a highly specific GSC marker. Immunohistochemistry data showed that TLR10 is rarely expressed in olig2-positive glioma cells in GBM, but almost all cells in PCNSL express TLR10 (Figure 5C). These results confirm that TLR10 can be used as a biomarker for distinguishing PCNSL from GBM.

## Discussion

TLR10 was previously regarded as the only pattern-recognition receptor with unknown ligand specificity and biological function [5]. TLR10 is mainly expressed by immune cells [20], especially B cells [4]. Recent studies have tended to regard TLR10 as an anti-inflammatory pattern-recognition receptor.

Accordingly, Hess reported that TLR10-differentiated dendritic cells presented a decreased ability to activate T cells as measured by IL-2 and IFN- $\gamma$  production [21]. A similar effect was observed in the macrophage cell line THP-1, wherein the levels of the chemokines CCL20, CCL1, and IL-8 were decreased following TLR10 knockdown [13]. These studies demonstrated the inhibitory function of TLR10 on inflammation in both immune and nonimmune cells. Moreover, TLR10 has been shown to be involved in osteoarthritis [8], Crimean Congo hemorrhagic fever [9], infections with *Listeria monocytogenes* [10], *Salmonella typhimurium* [11], and *Helicobacter pylori* [12], and influenza [13]. Nevertheless, studies on TLR10 have mainly focused on its immune and inflammatory functions [4], and the identification of the involvement of TLR10 in tumors paves the way for future exploratory studies.



The present study reveals the expression and function of TLR10 in glioma, DLBC, and LAML, highlighting the importance of TLR10 in tumor processes. We found that TLR10 levels were positively correlated with WHO grade in glioma, and patients with high TLR10 levels showed shorter overall survival and disease-free survival times than patients with low TLR10 levels. TISIDB and TIMER data showed that TLR10 expression was significantly positively correlated with immune infiltrates, especially infiltrating levels of B cells. These results indicate that TLR10 can serve as an independent novel prognostic factor in glioma.

Another important highlight of our study is the potential of TLR10 as a biomarker to distinguish PCNSL from GBM. PCNSL is extranodal lymphoma confined to the CNS with aggressive clinical manifestations and poor outcomes [22]. One recent study reported that PCNSL can initiate from B cells, which play a physiological role in producing IL-10 to suppress detrimental inflammation in the CNS [23,24]. The diagnosis of PCNSL is often difficult because of its similarity to other brain tumors, such as GBM [19]. Based on the inhibitory effect of TLR10 on inflammation and its role in B cell infiltration, we assessed the role of TLR10 in PCNSL. We found that the expression levels of

TLR10 in PCNSL were significantly higher than those in GBM tumors. These results confirm that TLR10 can be used as a biomarker for distinguishing PCNSL from GBM.

Taken together, our data reveal for the first time that TLR10 is involved in tumor development, especially in tumor-associated immune infiltration. Furthermore, TLR10 was found to act as a biomarker for distinguishing PCNSL from GBM. Although the real biological function of TLR10 may not be completely revealed until its natural ligand is discovered, we believe that the data shown in the present study advance our knowledge of TLR10 and its potential function in tumors.

## Conclusions

TLR10 could serve as a promising theranostic target for patients with glioma and acts as a potential biomarker for distinguishing PCNSL from GBM.

## Conflict of interest

None.

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