Clinical characterization, genetic profile, and epigenetic regulation of TOX in diffuse gliomas

Running title: The characteristics of TOX in diffuse gliomas

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Acknowledgements: none
**Key words:** Glioma; TOX; Inflammatory activity; Immune response; Prognosis
Abstract:

Background:

Multiple studies focusing on immune therapy towards glioblastoma (GBM) have attained great successes. TOX has been found to be closely related to immune environment surrounding tumors, while the expression of TOX has not been fully understood in gliomas.

Methods: Mining data from The Cancer Genome Atlas (TCGA) and the Chinese Glioma Genome Atlas (CGGA), we analyzed 1691 WHO grade I-IV human glioma samples with transcriptome data. R language was used for most statistical analysis. Somatic mutations and somatic copy number variation (CNV) were analyzed using GISTIC 2.0.

Results: TOX was down-regulated in malignant gliomas, especially in the proneural and isocitrate dehydrogenase (IDH) mutant subtypes of GBM. TOX\textsuperscript{low} tumours are related to loss of PTEN and amplification of EGFR, while TOX\textsuperscript{high} tumours harbor mutations in IDH1 (91%). TOX was highly expressed in cellular tumour, leading edge regions. TOX was enriched in multiple immune related processes including lymphocytes migration in GBM in gene ontology and pathway analysis. Finally, TOX had negative association with the infiltration of several immune cell types in tumour microenvironment in cell lineage analysis.

Conclusion: TOX has the potential to be a new target for immune-therapy or a prognostic marker for GBM.
1. Background

Gliomas continue to be the most common and devastating primary brain tumor. Despite multiple conventional therapies, including radiotherapy with adjuvant temozolomide chemotherapy after resection[1,2], patients with LGG (low grade glioma) have a median overall survival of 8-10 years, while patients with GBM (glioblastoma multi-form) only have an overall survival of less than 15 months[3,4]. Given the dismal outcome after standard treatment, potential and new therapeutic approaches are desperately needed.

Recently, strategies eliciting immune responses against tumors have demonstrated breakthroughs in several cancer types[5-7]. One previous study has demonstrated that tumor microenvironment consists of infiltrating immune cells to play critical roles in supporting the progression of gliomas[8], which led to the burgeoning field of glioma immunotherapy research. The subsequent glioma immunotherapy researches pictured a promising future for the treatment of gliomas patients[9,10]. Emerging evidence demonstrates that immune checkpoints interact with GBM and the surrounding immune system, acting as a crucial mediator [11]. Under normal physiological circumstances, immune checkpoints have been proved to be responsible for the self-tolerable immune system [12,13].

B7-H3(CD276), for example, is an immune checkpoint mainly expressed on T cells, and is thought to regulate the T cell-mediated immune response. High expression of CD276 was related to the extent of tumor malignancy[14]. IDO1, another immune checkpoint expressed on T cells, can promote a regulatory phenotype in both T cells and dendritic cells through its activity, facilitating tumor immune escape.[15,16] However, despite multiple studies, the intricate interactions between gliomas and the immune system remain to be elucidated. [17]

TOX (thymocyte selection-associated high mobility group box), a member of a conserved DNA-binding protein family and a founding member of a subfamily including four proteins, is closely associated with the regulation of the development of several immune-relevant cell subsets including CD4 T cells, natural killer cells, and lymphocytes[18,19]. TOX expression is frequently up-regulated in diverse types of cancer including breast cancer, lung cancer, cutaneous lymphoma, gastric cancer, leukemia, central neural lymphoma. Multiple studies have demonstrated that the overregulation of TOX is associated with tumor progression[20].
Deregulation of TOX expression in cancer can be roughly attributed to two mechanisms: genetic alteration[21,22] and epigenetic events[23]. While TOX is proved to be a critical regulator in the differentiation and maturation of immune system, little is known about the immune-related roles of other TOX family members. TOX2 was reported to play potential roles in reproductive organogenesis[24] and cancer[23]. TOX3 is proved to be involved in the regulation of neuron[25] and oligodendrocyte[26] survival while it also plays multiple roles in breast cancer [27]. TOX4, a platinated-DNA interacting protein, interacts with a complex, controlling cell cycle kinetics and chromatin structure[28].

To date, TOX expression still has not been fully characterized in gliomas with regard to its molecular and clinical pattern. In our study, we investigated the role of TOX expression, aiming to comprehensively delineate the molecular and clinical pattern of TOX. To explore its clinical relevance with LGG and GBM, we mined data from the TCGA dataset and our findings were further well validated in CGGA dataset. This is the first integrative study characterizing TOX expression in LGG and GBM molecularly and clinically. It is undeniable that a better understanding of the TOX feature and expression in gliomas will further promote the research for associated therapies.
2. Materials and methods

2.1 Data collection

This study was ethically approved by Xiangya Hospital, Central South University. From the TCGA and CGGA datasets, we collected TOX data from LGG and GBM samples. RNA-seq data in regard to specific tumor anatomic structure in GBM was downloaded from Ivy Glioblastoma Atlas Project (http://glioblastoma.alleninstitute.org/).

2.2 Biological function and gene set variation analysis

Correlation analysis of TOX was performed using gene expression profiles from the TCGA and CGGA datasets with R language (https://www.r-project.org/). Somatic mutations and somatic copy number alternations (CNAs) which correspond to the cases with RNA-seq data, were downloaded from TCGA database. GSITIC analysis was adopted to determine the genomic event enrichment. CNAs associated with TOX expression and the threshold copy number at alteration peaks were from GISTIC 2.0 analysis (https://gatkforums.broadinstitute.org). The gene sets variation analysis (GSVA) package was used to analyze the differential expression in GO terms of immune related process and immune cell lineages from TCGA and CGGA samples. Correlation analysis was performed by the expression values of risk score and GO term, and the items with p<0.05 and high correlation coefficient were selected. After Spearman correlation analysis, Heatmap was used to construct gene ontology (GO) analysis of the most correlated genes.
2.3 Statistical analysis

Spearman correlation analysis was used to evaluate the correlations between continuous variables. The survival probability was described by Kaplan-Meier survival curves. The Student t-test, $\chi^2$ test or Pearson's Chi-squared test were used to determine the expression levels of TOX with regard to pathological characteristics. The Pearson correlation was applied for evaluating the linear relationship between gene expression levels. Survival package in R project was used for Cox regression analysis. All statistical analyses were performed using R project (version 3.4.1, https://www.r-project.org/). P-values $\leq 0.05$ were considered to be statistically significant. And all tests were two-sided.
3. RESULT

3.1 TOX expression is decreased in malignant gliomas

Mining expression data from publicly available data-bases: TCGA, n = 674; CGGA, n = 1017, we evaluated the mRNA expression levels of TOX in WHO grade I-IV gliomas.

First, we analyzed the expression pattern of TOX across grades and subtypes in TCGA and CGGA datasets. We evaluated TOX levels in various common cancer types including gliomas (Fig. 1A). Compared to normal brain tissues, tumor samples demonstrated significantly up-regulated TOX expression, suggesting its adverse role in glioma development and progression. TOX was significantly elevated in low grade glioma (LGG) samples compared with GBM samples (Fig. 1C). Interestingly, TOX had the highest expression in WHO grade III samples in both the TCGA and CGGA datasets (Fig. 1B).

TOX was down-regulated in 1p/19q non-codeletion pan-glioma analysis, but up-regulated in the 1p/19q codeletion pan-glioma analysis in both TCGA and CGGA cohorts (Fig. 1D). Similarly, IDH mutation, indicating better clinical outcome, had tight association with a high expression level of TOX (Fig. 1E). Furthermore, in WHO grade II glioma samples, the IDH mutation status was significantly related to higher expression of TOX in the TCGA and CGGA cohorts (Fig. 1E). The ROC curve further suggested that TOX could be an valuable predictor for IDH mutation among pan-gliomas analysis, LGG cases, and GBM cases respectively (AUC value = 0.878, P <.001; value = 0.841, P <.001; value = 0.814, P <.001, respectively Fig. 1F).

Notably, in LGG samples, IDH mutation together with 1p/19q codeletion is related to higher expression of TOX in both TCGA and CGGA cohorts (Fig. 1G). In addition, higher expression of TOX was related to methylated glioma in TCGA cohort (Fig. 1H). In CGGA cohort, females had relatively higher expression levels of TOX (Fig. 2A). The different expression level of TOX in glioma in regard to histology was shown in Fig. 2B.

3.2 Molecular characteristics of TOX in gliomas

The molecular categorization of human gliomas has four distinct sub-classes: mesenchymal (MES), classical (CL), neural (NE), and proneural (PN). MES and CL subtypes are related to
more aggressive behavior of gliomas and more dismal clinical outcome of patients compared with PN or NE subtypes[29] [30]. Therefore, we subsequently analyzed the expression level of TOX among these four molecular subtypes on the basis of VERHAAK_2010 classification scheme [31].

In the TCGA dataset, higher TOX expression was seen in MES and CL subtypes of GBM compared to NE and PN subtypes, while the distinction was conspicuous in LGG samples and pan-glioma analysis (Fig. 2C). The ROC curve further indicated that TOX might serve as a predictor for CL and MES subtypes in pan-gliomas analysis, LGG, and GBM samples (AUC value = 0.883, P < 0.001; value = 0.860, P < 0.001; value = 0.695, P < 0.001, respectively Fig. 2E). Moreover, the highest TOX expression was seen in the PN molecular subtype (Fig. 2C).

We next evaluated the intra-tumour distribution of TOX in GBM samples. Based on the TCGA dataset, the analysis of RNA sequencing data revealed the high expression of TOX in cellular tumour, leading edge and infiltrating tumour (Fig. 2D). To further confirm the upregulation of TOX expression at the protein level, we downloaded the results of IHC staining for TOX from the The Human Protein Atlas (https://www.proteinatlas.org) (Fig. 2F). TOX has higher expression in LGG and GBM compared to normal brain tissue. The expression of TOX is also higher in LGG than GBM, which is consistent with our results.

3.3 TOX expression predicts better survival probability

We further investigated the prognostic value of TOX in human gliomas. Based on the calculated median values of TOX expression in gliomas, we generated Kaplan-Meier survival curves. In TCGA GBM dataset, TOX\textsuperscript{high} patients exhibited significantly longer overall survival (OS), disease specific survival (DSS), and progressive free survival (PFS) compared with TOX\textsuperscript{low} patients (P < 0.05, respectively; Fig. 3A,3B,3C). In addition, in TCGA LGG datasets, TOX\textsuperscript{high} patients exhibited significantly longer overall survival (OS), disease specific survival (DSS), and progressive free survival (PFS) compared with TOX\textsuperscript{low} patients (P < 0.001, respectively; Fig. 3D,3E,3F). This result was further confirmed in pan-glioma analysis (P
In CGGA dataset, TOX$^{\text{high}}$ patients were associated with longer OS in pan-glioma, LGG, and GBM analyses ($P < 0.001$, $P < 0.001$ and $P < 0.05$ respectively; Fig. S1A, S1B, S1C). Furthermore, Cox regression analysis was performed to explore the clinical prognostic value of TOX in gliomas. In the Univariate analysis, TOX, together with high WHO Grade, age at diagnosis, 1p19q codeletion, and IDH mutations were significantly related to OS in both TCGA and CGGA databases (Table 1,2). In the multivariate analysis, TOX was also proved to be a valuable predictor in both cohorts. These results revealed that TOX might serve as a predictor for the better prognosis of glioma patients.

3.4 The association between TOX expression levels and distinct genomic alterations

We next performed somatic mutation analysis and copy number variation (CNV) using the TCGA dataset to determine whether TOX expression levels were associated with specific genomic characteristics. By comparing the TOX$^{\text{low}}$ ($n = 158$) and the TOX$^{\text{high}}$ ($n = 158$) clusters (Fig. 4C), we obtained an overall CNV profile. The amplified chromosome 7 and the deleted chromosome 10, two most common genomic events in GBM, both were frequently associated with the TOX$^{\text{low}}$ cluster (Fig. 4A). The genomic hallmark of oligodendroglioma, the deletion of 1p and 19q, was more frequently occurred in the TOX$^{\text{high}}$ cluster (Fig. 4A).

We next identified 43 and 61 genomic events enriched in either the TOX$^{\text{high}}$ or TOX$^{\text{low}}$ group using GSITIC analysis (Fig. 4B). In TOX$^{\text{low}}$ samples, oncogenic driver genes including PIK3C2B (1q32.1), PDGFRA (4q12), EGFR (7p11.2), and CDK4 (12q14.1) were frequently amplified genomic regions. Meanwhile, frequently deleted genomic regions included tumour suppressor genes such as PARK7 (1p36.23), CDKN2A (9p21.3), and PTEN (10q23.3). In TOX$^{\text{high}}$ samples, 8q23.3 and 12p32.32 were two significant amplified peaks, while significant deletion showed peaks in 2q37.3, 4q35.2, 9p21.3, 11p15.5, and 19q13.43. Notably, a 4q12 peak was detected in both TOX$^{\text{high}}$ and TOX$^{\text{low}}$ samples. However, the G score in TOX$^{\text{high}}$ samples was obviously higher than that in TOX$^{\text{low}}$ samples. Based on TOX expression levels, the somatic mutation profiles revealed that mutations in IDH1 (91%), CIC
(28%), and ATRX (37%) were significantly enriched in GBM samples with high TOX expression (Fig. 4C). In addition, frequently observed mutations were EGFR (27%), IDH1 (20%), PTEN (18%), and MUC16 (16%) in gliomas with low TOX expression (n = 158; Fig. 4C).

3.5 TOX is involved in complicated immune processes

We further investigated the potential immune-related functions of TOX in glioma using GSVA analysis in TCGA dataset. In GBM alone, we found that TOX was positively associated with B cell activation, T cell receptor signaling pathway, B cell homeostasis, and T cell proliferation. In contrast, TOX had negative association with lymphocyte migration, natural killer cell activation, and lymphocyte chemotaxis. (Fig. 5B) In pan-glioma analysis, TOX had negative association with T cell migration, Negative T cell selection, Natural killer cell mediated immunity, Regulation of T cell cytokine production, Positive regulation of T cell apoptotic process, B cell mediated immunity, Lymphocyte migration, and Lymphocyte chemotaxis. (Fig. 5A) In LGG alone, TOX was negatively related to T cell migration, Lymphocyte migration, Regulation of T cell cytokine production, lymphocyte mediated immunity, and regulation of αβ T cell proliferation. Similar results were seen in CGGA dataset. (Fig. 5C)

A previous study has demonstrated that TOX is essential in the development of Innate Lymphoid Cells [32]. Consequently, we paid special attention to two pathways mentioned above: lymphocyte migration and lymphocyte chemotaxis. As the threshold was set as logFC>2 and adjust P ≤0.01, a total number of 2778 differentially expressed genes (DEGs) were detected between high expression of TOX sample and low expression of TOX sample (Fig. 5D). As for lymphocyte migration, eight genes were found involved in both DEGs and lymphocyte migration gene sets. SAA1, CXCL11, CXCL10, CCL2, CCL20, CXCR3, and MYO1G were related with high expression of TOX, whereas RET was related with low TOX expression. As for lymphocyte chemotaxis, expression of TOX was negatively related to SAA1, CXCL11, CXCL10, CCL2, CCL20, CXCR3 (Fig. 5E,5F).

3.6 TOX is irrelevant to inflammatory activities
We examined the association between the molecules and various molecules related to inflammatory activity in both TCGA and CGGA datasets. TOX expression was negatively associated with inflammatory activity signatures including HCK, LCK, MHC-I, MHC-II, STAT1, and interferon metagenes, but positively associated with the IgG metagene in pan-glioma analysis, LGG alone and GBM alone (Fig. 6A, 6B, 6C; Fig. S1D, S1E, S1F, S1G).

These results indicated that TOX was not involved in signaling transduction of T cell activation, macrophage activation, or antigen presenting cells (APCs). However, TOX might have interaction with B lymphocytes in the process of immune-activation and subsequent glioma suppression.

3.7 The tight associated between TOX and immune cells in the tumour microenvironment

We further examined the significance of increased TOX in immune-related microenvironment in gliomas via performing GSVA analysis. We identified the immune cell types in the microenvironment of gliomas to see if they are influenced by TOX and to evaluate its presumed role in the interaction between gliomas and immune cells. We first investigated the relationship between TOX and 28-immune cell populations using cell type gene set variation analysis[33]. In both TCGA and CGGA cohorts, we found that TOX was positively associated with Eosinophil in pan-glioma analysis, whereas multiple immune cell types with infiltration characteristics, macrophages, monocytes, CD4+ TEM, CD8+ T effector memory cells (TEM), neutrophils, Myeloid-derived suppressor cells (MDSC), and natural killer (NK) cells were negatively associated with TOX in the pan-glioma analysis and in the LGG analysis (Fig. 7D, 7F; Fig. S3, S4). For GBM samples, DCs, MDSC, macrophages, mast cell, NK cells, CD8+ TEM, and CD4+ TEM etc. were found to be negatively associated with TOX (Fig. 7B; Fig. S3, S4). We further validated these results in a 24-immune cell lineage analysis, confirming the rejection of multiple immune cell types[34] in TOX\textsuperscript{high} glioma samples. In 24-immune cell lineage analysis, Neutrophils, Eosinophils, Macrophages, NK cells, and DCs were negatively associated with TOX. TFH (follicular helper cells) and tumor growth delay (TGD) were positively associated with TOX in the pan-glioma analysis and the LGG group (Fig. 7C, 7E; Fig. S3, S4), while TFH and B cells were positively associated with TOX and Macrophages...
and DCs were negatively associated with TOX in GBM samples (Fig. 7A; Fig. S3, S4).
Altogether, our data revealed that the high expression of TOX tend to reject the infiltration of
immune cells in the microenvironment of gliomas.

3.8 TOX is synergistic with other immune checkpoint members

Given that the immune checkpoint molecules vitally regulate immune processes, we assessed
the correlation between TOX and several crucial immune checkpoints in glioma samples. TOX
was strongly correlated with CD276, IDO1, PDCD1LG2, and VTCN1 in pan-glioma analysis
and GBM alone in both TCGA and CGGA cohorts (Fig. 8A,8B; Fig. S5), which the correlation
was significantly better in LGG samples alone (Fig. 8C; Fig. S5). The analysis of TOX family
showed favorable inter-relationship between TOX, TOX2, TOX3, and TOX4 in pan-glioma
analysis, LGG samples, and GBM samples (Fig. 8D,8E,8F; Fig. S4).

4. Discussion:

After many years of research, gliomas, especially GBM, stay as the most devastating brain
tumor with dismal outcomes. Strategies eliciting an immune response against tumor have
demonstrated breakthroughs in preclinical and clinical trials in many malignant tumors,
which makes immune therapy promising and appealing. TOX together with other classical
immune checkpoints including PD1 and CD270 are closely related to the development of
several immune-relevant cell subsets, affecting the tumor progression. Consequently, a better understanding of the TOX feature in glioma is undoubtedly significant in treatment strategy development.

Based on an integrative and large-scale bioinformatic analysis, we delineated the clinical and molecular landscape of TOX among gliomas. TOX was found to be highly elevated in gliomas based on its mRNA expression levels, especially in the LGG. TOX was up-regulated in methylated glioma, glioma with IDH mutation, and glioma with 1p/19q codeletion. In addition, women tended to have a higher expression level of TOX. TOX high was closely related with the CL and MES molecular subtypes, which made it a sensitive diagnostic marker for gliomas. TOX was localized to Cellular Tumour, Leading Edge, and Pseudopalisading Cells Around Necrosis. Moreover, high expression of TOX was associated with better survival in pan-glioma analysis, LGG samples or GBM samples. We also explored the expression level of TOX with regard to distinct genomic alternations. We found that multiple events of somatic mutations had negative association with TOX expression, which suggested that TOX expression was irrelevant to the malignant biological process. All these results indicated that TOX expression was in the wake of the glioma occurrence, and TOX was critical in suppressing the oncogenic process and progression in gliomas. The oncogenic drivers including PIK3C2B, EGFR, and CDK4 were amplified in the gliomas with low TOX expression[35]. Meanwhile, tumor suppressor genes, CDKN2A and PTEN, were found to have a deletion peak in the cases with low TOX expression[36]. Given that genomic alternations may promote the progression of tumor through transforming the tumor microenvironment[37], these results suggested that TOX expression was associated with benign biological processes.

GBM elicits the activation of multiple immune cell types from immune systems. While GBM has also been proved to rely on tumor infiltrating macrophages which produce numerous cytokines, growth factors, and interleukins that directly created a permissive tumor microenvironment, promoting glioma cell growth and proliferation[38]. In our study, TOX was found to negatively associate with macrophages, suppressing the permissive tumor microenvironment of GBM. Furthermore, the correlation analysis suggested that TOX high GBM cells are inclined to reject the infiltration of immune cells (Cytotoxic lymphocytes, Neutrophils, Monocytic lineage, NK cells, B cells, Fibroblasts, and T cells) into the tumour
microenvironment. These data support that TOX contributes to the anti-tumour immunity in the GBM microenvironment. Accumulating evidence has proved that TOX1 is critical in the generation and development of CD4 T cells[39], NK cell, and NKT cell[40,41]. Therefore, our results showed great consistency with the previous studies. Furthermore, the pan-glioma analysis indicated that the negative correlation between TOX and immune infiltrating cells was much more significant in LGG samples than in GBM samples.

Previous studies have proved that APCs can present antigens to T cells in the central nervous system (CNS), which the activated tumor-specific T cells (TST) can subsequently respond to CNS tumor. Additionally, tumour progression influences the integrity of the blood brain barrier (BBB), which further enables a direct explosion of GBM to immune system[42]. TOX, regulating the differentiation of TST cells, was critical for the exhaustion of CD8 T cells by translating continuous stimulation into a distinct exhausted T (TEx) cell epigenetic and transcriptional developmental program[39,43], preventing the overstimulation of T cells and the subsequent activation-induced cell death under the stimulation of chronic antigen such as cancer[44]. In our study, activated CD8 T cell was negatively associated with the TOX expression, which is also consistent with the reported function of TOX.

Through GSVA analysis, we revealed that TOX function was positively associated with immune related pathways including T cell receptor signaling pathway, T cell proliferation, and B cell activation, while negatively associated with lymphocyte migration, natural killer cell activation, and lymphocyte chemotaxis. These results suggested that TOX was correlated with the development of B cell and T cell, and suppressed the development of lymphocyte and natural killer cell in GBM. In addition, the pan-glioma analysis indicated that TOX had negative association with T cell migration, Negative T cell selection, Regulation of T cell cytokine production, Natural killer cell mediated immunity, Positive regulation of T cell apoptotic process, B cell mediated immunity, Lymphocyte migration, and Lymphocyte chemotaxis, which further confirmed the more obvious lymphocyte-suppressing role of TOX involved in immune system of LGGs. Notably, the negative relation with lymphocyte migration and lymphocyte chemotaxis indicated that TOX inclined to prohibit the formation of immune infiltrating environment conducive to glioma. The interaction between TOX and immune system was illustrated in Fig. 9.
Preclinical benefits were seen in several immune checkpoint inhibitor treatments. Therefore, we also investigated the correlation between TOX and other immune checkpoint members. TOX had high correlation with CD276, IDO1, PDCD1LG2, and VTCN1 in both pan-glioma analysis and GBM alone. The results that TOX was highly correlated with multiple well-known immune checkpoint members suggested that targeting TOX and other immune checkpoint molecules could be a novel approach to treat gliomas.

5. Conclusion:

Taken together, this study illuminates the role that TOX plays in the development of human gliomas. Notably, TOX seems to have a more significant correlation with LGG than with GBM. Future studies are warranted to explore TOX as a new prognostic marker or even immune-therapeutic target for GBMs and LGGs, which the subsequent pharmaceutical research in regard to TOX is expected to demonstrate promising results.
Declaration

Ethics approval and consent to participate:
Not applicable

Consent for publication:
Not applicable

Availability of data and material:
The datasets generated and analyzed during the current study are available in the Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/), TCGA data source (https://xena.ucsc.edu) and CGGA data portal (http://www.cgga.org.cn).

Competing interests:
The authors declare that they have no competing interests

Funding:
This work was supported by the National Natural Science Foundation of China (NO.81703622, NO. 81472693, NO. 81873635), China Postdoctoral Science Foundation(NO.2018M633002), Hunan Provincial Natural Science Foundation of China(NO.2018JJ3838), Hunan provincial health and Health Committee Foundation of China(C2019186), and Science and Technology Department of Hunan Province (NO.2015SK2032-2).

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Reviewing and approving the final vision: All authors
Data availability sharing:
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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**Figure legends**

Fig.1. TOX expression is upregulated in malignant gliomas. A. Analysis of TOX mRNA levels (log2) in CNS tumours based on the 2016 WHO classification from TCGA. B. Analysis of TOX mRNA levels in WHO grade II-IV gliomas from TCGA and CGGA. C. Analysis of TOX mRNA levels in LGG and GBM in TCGA and CGGA datasets. D. TOX expression was upregulated in the 1p/19q codeletion gliomas compared with the 1p/19q non-codeletion gliomas from TCGA and CGGA. E. TOX expression was upregulated in the IDH mutant gliomas compared with the IDH wild-type gliomas from TCGA and CGGA. F. Receiver operating characteristic (ROC) curve assessing the sensitivity and specificity of TOX expression as a valuable predictor in IDH mutant gliomas from TCGA. G. TOX expression was upregulated in the 1p/19q codeletion as well as IDH mutant gliomas from TCGA and CGGA. H. TOX expression was upregulated in the methylated gliomas from TCGA.

Fig.2. A. TOX expression was upregulated in female patients with gliomas from CGGA. B. The expression levels of TOX based on the histopathologic classification. C. The TOX expression pattern in the TCGA molecular subtype in pan-glioma analysis, LGG and GBM samples. D.
TOX expression was detected in different anatomic location of GBM in IVY GBM database. E. ROC curves predicted TOX as a biomarker of classical and mesenchymal subtype glioma.

Fig.3. TOX expression predicts better survival in glioma patients. Kaplan-Meier analysis of overall survival (OS), disease specific survival (DSS) and progressive free survival (PFS) based on high vs low expression of TOX in pan-glioma analysis, LGG and GBM alone in TCGA dataset. The median value of TOX expression was used as the cut-off value. P-values were obtained from the log-rank test.

Fig.4. Distinct genomic profiles associated with TOX expression. A. The overall CNAs profile in regular sequence of increasing TOX expression. B. GISTIC 2.0 amplifications and deletions in gliomas with low and high TOX expression. Chromosomal locations of peaks of significantly recurring focal amplification (red) and deletions (blue) were presented. C. Differential somatic mutations were detected in gliomas with low and high TOX expression.

Fig.5. TOX related immune process in pan-glioma analysis (A), and LGG (B) and GBM (C) patients in TCGA dataset. D. Volcano plot for differentially expressed genes (DEGs). E. genes involved in both lymphocyte chemotaxis gene set and DEGs. F. genes involved in both lymphocyte migration gene set and DEGs.

Fig.6. Heatmaps illustrating TOX related inflammatory activities in GBM (A), LGG (B) and pan-glioma analysis (C) in TCGA dataset, respectively. Expression values are z-transformed and are colored red for high expression and blue for low expression, as illustrated in the scale bar. Correlation-grams illustrate P values for analysis between TOX and inflammatory metagenes in GBM (D), LGG (E) and pan-glioma analysis (F) in TCGA dataset, respectively.
Fig. 7. TOX is related to immune cells in the tumour microenvironment. Heatmaps illustrating the relationship between TOX and 24 immune cell populations based on TCGA GBM (A), LGG (C) and pan-glioma analysis data (E), respectively. Heatmaps illustrating the relationship between TOX and 28 immune cell populations based on TCGA GBM (B), LGG (D) and pan-glioma analysis data (F), respectively. The z-transformed expression values are colored red for high expression and blue for low expression, as indicated in the scale bar.

Fig. 8. TOX is correlated with classic immune checkpoint molecules in gliomas. Correlation of TOX and immune checkpoint members in pan-glioma analysis (A), GBM (B) and LGG (C) samples in TCGA. Correlation of TOX and other family members in pan-glioma analysis (D), GBM (E) and LGG (F) samples in TCGA.

Fig. 9. Working model of the effect of TOX in the immune system in glioma. TOX is highly expressed in glioma, regulating B cell activation and suppressing NK cell mediated immunity and lymphocyte chemotaxis and migration. TOX also mediated T cell proliferation and T cell exhaustion, while suppressed T cell migration and cytokine production.

Table Legends

Table 1: Univariate and multivariate cox analyses in gliomas in CGGA.

Table 2: Univariate and multivariate cox analyses in gliomas in TCGA.