TEAD4 Serves as a Prognostic Biomarker and Correlates With Immune Phenotype in Lower-Grade Gliomas

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Research Article

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Abstract

Background: Tumor-infiltrating immune cells (TIICs), which play a pivotal role in the tumor microenvironment, are intimately related to tumor progression and clinical outcome. It remains unclear which factors influence tumor immune infiltration in lower-grade gliomas (LGGs). TEAD4 (TEA Domain Transcription Factor 4) is an essential member of the Hippo pathway that is involved in cancer progression, epithelial-mesenchymal transition, metastasis, and cancer stem cell function across multiple types of cancers. However, the prognostic value of TEAD4 and its association with TIICs in LGG have been hardly studied.

Methods: LGG data were obtained from The Cancer Genome Atlas (TCGA) and Chinese Glioma Genome Atlas (CGGA). TEAD4 expression between different groups was compared by R and survival analysis was implemented by Kaplan–Meier curves. *In Virto* experiments were conducted to investigate the role of TEAD4 in glioma cells. Gene set enrichment analysis (GSEA) and protein-protein interaction (PPI) network were used to investigate the differential biological processes and signaling pathways. Multiple computational methods were employed to estimate the association between TEAD4 expression and tumor microenvironment in LGG. Correlations were analyzed by Spearman correlation

Results: TEAD4 expression was up-regulated in higher-grade gliomas and correlated with a poorer clinical outcome. Glioma cell proliferation and migration were promoted by TEAD4 overexpression. GSEA and PPI network indicated that multiple immune-related pathways and hub genes were closely associated with TEAD4 expression in LGG specimens. TEAD4 expression was negatively associated with glioma purity. Multivariate Cox regression analysis indicated that TEAD4 expression and tumor purity were independent prognostic factors in LGG. TEAD4 expression was positively correlated with the infiltration of multiple immune cells, including plasma cells, CD8+ T cells, and macrophages M1 and M2. Correlation analysis showed that the TEAD4 level can predict the efficacy of immune checkpoint blockade therapy.

Conclusions: TEAD4 is highly related to glioma malignancy grades and multiple immune cell infiltration, suggesting TEAD4 can serve as a new biomarker for anti-cancer therapies in LGG.

Background

Glioma is the most prevalent malignant brain tumor in the central nervous system [1]. Gliomas are classified into grade I, II, III, or IV tumors according to the standards of the neuropathological evaluation set by the World Health Organization [2]. WHO II and III gliomas, which are usually referred to as lower-grade gliomas (LGGs), are significant pathologic subtypes of glioma with highly unpredictable clinical behaviors. Some LGGs show relatively moderate tumor characteristics and correlate with a promising prognosis, whereas others share biological similarities with glioblastoma (GBM) and associate with a poor clinical consequence [3]. To arrive at a more complete assessment of the diagnostic criteria, many molecular markers have been taken into consideration, including the IDH mutation, the 1p/19q codeletion,

the TERT promoter mutation, and MGMT promoter methylation [4, 5]. However, additional biomarkers remain to be discovered for the targeted therapy of LGG.

The tumor microenvironment (TME) is a dynamic milieu surrounding a tumor, consisting of blood vessels, infiltrating immune cells, stromal cells, extracellular matrix, and signaling molecules such as cytokines/chemokines [6]. These non-tumor components dilute the tumor purity and collaborate with tumor-regulating biological processes [7]. Among these factors, tumor-infiltrating immune cells (TIICs) are a key part of the complex microenvironment. Although immune cells are considered as an anti-tumor factor, tumor-infiltrating immune cells are usually very pronounced in tumor tissues where they promote tumor development and progression in the TME [8, 9]. Therefore, immunotherapy has emerged as an innovative treatment of many cancers, including gliomas, which is well exemplified by the application of immune checkpoint inhibitors [10]. As another essential constituent of the TME, stromal cells are involved in cell proliferation, tumor invasion, and angiogenesis [11].

TEAD4, also known as Transcriptional Enhancer Factor-3 (TEF-3), is an important member of the TEAD family [12, 13]. TEAD4 contains a TEA DNA binding domain that binds to the promoters of downstream genes and a YAP/TAZ binding domain that associates with transcriptional cofactors [14] TEAD4 cooperates with YAP/TAZ, VGLL, and other transcription factors to actively participate in the regulation of cancer biology via its transcriptional output [12]-[15]. In addition, the molecular mechanisms of TEAD4, such as its subcellular translocation and post-translational modifications, shed new light on an alternative way in which TEAD transcriptional activity may be mediated [16, 17] As the downstream transcriptional factor of the Hippo signaling pathway, TEAD4 has emerged as a novel prognostic marker in a variety of cancers such as gastric cancer [18], breast cancer [19–21], colorectal cancer [22], melanoma [23], bladder cancer [24], prostate cancer [25], and head-neck squamous cell carcinoma [26]. TEAD4 can regulate cancer progression, epithelial-mesenchymal transition, metastasis, cancer stem cell function, and drug resistance [12]. Nonetheless, there is limited knowledge on the potential role of TEAD4 in glioma, especially its correlation with TIICs and glioma progression.

In this study, we analyzed the expression of TEAD4 in LGGs and normal brain tissues and explored the TEAD4 level in different statuses of the 1p/19q codeletion. We found that TEAD4 overexpression was strongly correlated with decreased life span and poor clinical outcome. In addition, we observed several immune-related gene sets enriched in gliomas highly expressing TEAD4. Meanwhile, a protein-protein interaction (PPI) network indicated that various immune-related hub genes were included. Additionally, we discovered that high TEAD4 expression associated with increased immune cell infiltration and lower tumor purity in gliomas, and high TEAD4 expression depended on the malignancy grade. Finally, high TEAD4 expression was related to increased multiple checkpoint genes in LGG. In summary, this study reveals that TEAD4 serves as an immune-associated biomarker for predicting the clinical outcome of LGG patients, demonstrating its potential value as an effective glioma therapeutic target.

Methods

Data acquisition

We obtained two independent datasets of glioma patients with complete clinical information and molecular data from the publicly available Chinese Glioma Genome Atlas (CGGA) (http://www.cgga.org.cn/) [27] and The Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/tcga/) [28,29]. CGGA and TCGA cohorts enrolled 440 and 527 LGG cases, respectively. Additionally, data of 20 patients with normal brains (controls) were acquired from CGGA. Detailed clinicopathological characteristics are described in Table 1 and Additional File 1, 2. Gene expression matrices links can be found in Additional File 3.

Expression analysis

We applied the "ggplot2" package in R to compare TEAD4 RNA expression between LGG and normal brain tissues, different malignancy grades, and different statuses of the 1p/19q codeletion. Boxplots were graphed to visualize the results. The Human Protein Atlas (HPA) (www.proteinatlas.org) is a program that aims to map all human proteins in cells, tissues, and organs using antibody-based imaging, mass spectrometry-based proteomics, transcriptomics, and systems biology [30,31]. We acquired immunohistochemistry images from HPA to determine the distribution, subcellular localization, and protein expression of TEAD4 in normal brain tissues, low-grade gliomas, and high-grade gliomas.

Survival analysis

Kaplan–Meier curves of differential TEAD4 expression and risk tables were generated by the "survival" package to analyze the correlation between TEAD4 expression and LGG patients' overall survival. Moreover, ROC curves were produced by the "pROC" package to evaluate the prognostic power of TEAD4 to predict the outcome.

Cell culture and transfection

Human glioma cell lines (U87 and U251) were purchased from the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco) at 37°C in 5% CO_2.

The human full-length TEAD4 cDNA containing 3× Flag was subcloned into a lentiviral plasmid (TranSheepBio, Shanghai, China) and then verified by direct sequencing. After transfection of the TEAD4 overexpression (OE) plasmid, cells were harvested at 48 h for further experiments. Western blotting was performed to evaluate the transfection efficiency (TEAD4 antibody, DF13283, Affinity Biosciences, Cincinnati, OH, USA; GAPDH antibody, AF9021, Affinity Biosciences).

Cell counting kit-8 assays, colony formation, and transwell migration assays

After transfection, cells were seeded in 96-well plates at 3×10^3 cells per well for cell counting kit-8 (CCK-8) assays. At 0, 24, 48, 72, and 96 h, cells were incubated in fresh medium containing 10% CCK-8 reagent

(MedChemExpress, Monmouth Junction, NJ, USA) for another 2 h under dark conditions. Thereafter, the optical density (OD) was measured at 450 nm.

After transfection, cells were seeded in 6-well plates at 500 cells per well for colony formation assays. After 2 weeks of incubation, cells were fixed with 4% paraformaldehyde and stained with crystal violet at room temperature for 20 min.

After transfection, cells were plated at 2×10^5 per well in the top chamber of transwell inserts (EMD Millipore, Burlington, MA, USA) that were previously coated with Matrigel basement membrane matrix in 200 µl Opti-MEM (Gibco). Inserts were immersed in DMEM supplemented with 10% FBS. After 24 h, cells in the top chamber were washed with PBS and removed with cotton swabs. Invasive cells attached to the underside of the top chamber's membrane were fixed with 4% paraformaldehyde and stained with crystal violet for 15 min. The number of cells in three random fields from each chamber was counted, and the mean number of cells was calculated. The count was determined by ImageJ software. Each experiment was independently performed three times.

Gene set enrichment analysis

GSEA is a computational method that determines if a pre-defined gene set exhibits statistically significant, concordant differences between two biological states. To explore differences in enriched GO/KEGG pathways between TEAD4 low and high expression groups, we performed GSEA (version 4.0.3) to identify the most distinctly enriched gene sets [32,33]. We loaded gene expression profiles and phenotype labels into the software. Thereafter, we successively selected GO and KEGG sets and performed 1000 permutations. Results of enriched pathways were ranked by the normalized enrichment score (NES). Moreover, the significance of each NES was evaluated by the NOM p-value and the FDR q-value.

Visualization of hub genes in the network

We constructed a PPI network of differentially expressed genes (DEGs) between TEAD4 high and low expression groups using the STRING website [34] (https://string-db.org/) and Cytoscape software. Data from Cytoscape were further explored with the MCODE [35] plugin to identify the most interconnected cluster in the PPI network (degree cutoff = 2, node score cutoff = 0.2, K-score = 2, max. depth = 100). Moreover, cytoHubba [36] was performed to select the top hub genes using the MCC algorithm. Thereafter, GenCLiP3 (http://ci.smu.edu.cn/genclip3/analysis.php) [37] was applied to assign functional annotations to the top six hub genes.

Characterization of the tumor microenvironment

The "ESTIMATE" package (https://sourceforge.net/projects/estimateproject/) was applied to calculate the immune and stromal scores of LGG specimens, which further infer tumor purity [7]. The Tumor Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/) is a comprehensive tool for

the systematic analysis of immune infiltrates across various cancer types [38,39]. Levels of six immune TIICs (B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, dendritic cells) were analyzed for 10,897 tumors across 32 cancer types from TCGA. The association between six immune infiltrates and TEAD4 expression or clinical outcome was explored by “gene” modules on the website. TIMER produced scatterplots and survival curves to visualize the results. The identification of cell types by estimating the relative subsets of RNA transcripts x (CIBERSORTx) (https://cibersortx.stanford.edu/) was performed with an online analytical tool that estimated the abundance of member cells based on gene expression data using a deconvolution algorithm [40]. To investigate the fraction of 22 TIICs for each LGG specimen, a mixture file of gene expression data and the signature TIICs matrix “LM22” were input to run CIBERSORTx. Subsequently, a violin plot was generated to present the differential composition of 22 TIICs between TEAD4 high and low expression groups. Correlation analysis between TEAD4 and immune checkpoint gene expression was conducted by the GEPIA2 website(http://gepia2.cancer-pku.cn/).

Statistical analysis

Differences in continuous variables between groups were evaluated by Student’s t-test. The survival curve was graphed to explore survival distributions. Log-rank test was used to determine statistical significance between groups. Univariate and multivariate Cox analyses were performed to investigate the impact of TEAD4 expression and other indicators on the clinical outcome. Multivariate Cox analysis results were depicted as a forest boxplot. A two-sided p-value less than 0.05 was regarded as significant and labeled as *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; and ****, $P < 0.0001$. All statistical analyses were conducted with R (version 4.0.0).

Results

TEAD4 is overexpressed in glioma cancer and correlated with malignancy grades and the 1p/19q codeletion status

According to a previous study, TEAD4 is overexpressed in cancer, and it has emerged as a novel prognostic marker for a variety of cancers, including gastric cancer, breast cancer, colorectal cancer, melanoma, and head-neck squamous cell carcinoma. To identify the novel correlation between TEAD4 expression and clinical outcome, we first assayed the relative TEAD4 mRNA expression level in LGG specimens and compared the level with that in normal brain tissues using the CGGA database. As shown in Figure 1A, the TEAD4 level was significantly up-regulated in glioma tissues compared with respective normal tissues. To investigate the relationship between TEAD4 expression and glioma grade, we examined the TEAD4 mRNA level from grade WHO II and III glioma tissues using data from both CGGA and TCGA databases. We found that the TEAD4 mRNA level was significantly increased in WHO III glioma tissues, indicating increased TEAD4 expression was closely correlated with a higher WHO grade (Figure 1B, C).

In addition, we investigated whether TEAD4 expression was associated with classic genetic alterations in glioma, including the IDH mutation and the 1p/19q codeletion. As there were few LGGs with both the IDH

wildtype and the 1p/19q codeletion, we mainly focused on three molecular subgroups: (1) the IDH mutation and the 1p/19q codeletion (muIDH + Codel); (2) the IDH mutation and the 1p/19q non-codeletion (muIDH + Non-codel); and (3) the IDH wildtype and the 1p/19q non-codeletion (wtIDH + Non-codel). We observed that TEAD4 expression was decreased in muIDH + Codel LGG specimens compared with muIDH + Non-codel LGG specimens in CGGA and TCGA datasets (Figure 1D, E). However, we did not observe significant differences in the TEAD4 mRNA level between muIDH + Non-codel and wtIDH + Non-codel gliomas in both databases. This analysis indicated that the 1p/19q codeletion, but not the IDH mutation, can contribute to the down-regulation of the TEAD4 level.

To verify the data from CGGA and TCGA databases, we collected glioma specimens from the Human Protein Atlas and examined the TEAD4 protein level in glioma and normal tissues using immunohistochemistry data. We found that the TEAD4 level was higher in gliomas compared with respective normal tissues. In addition, TEAD4 displayed significantly stronger staining in high-grade (++) tumor specimens compared with low-grade (+) counterparts in immunohistochemistry assays (Figure 1F). These results indicated that the elevated TEAD4 level is highly associated with the statistically significant probability of an increased glioma malignancy grade.

TEAD4 up-regulation is correlated with decreased life span

To confirm our conclusion, we graphed the survival curve of LGG patients from CGGA and TCGA databases according to the differential expression of TEAD4. The statistical analysis of TEAD4 expression with regard to life span was performed using Kaplan–Meier curves. These results showed that LGG patients with higher TEAD4 expression exhibited a significantly poorer outcome and a shorter median overall survival (Figure 2A, B). Furthermore, as a survival predicting factor, ROC curve analysis provided a good predictive value of TEAD4 expression in LGG specimens. The AUC values of TEAD4, 0.613 and 0.660, respectively, in two cohorts (Figure 2C, D), also confirmed that high TEAD4 expression was associated with a statistically significant probability of increased malignancy and decreased life span.

TEAD4 overexpression promotes glioma proliferation, colony formation, and migration *in vitro*

To elucidate the role of TEAD4 in glioma cells, we overexpressed TEAD4 in U87 and U251 cell lines and compared the findings with those of the negative control (NC) group (Figure 3A). We carried out transwell assays to analyze the effects of TEAD4 overexpression on glioma cell migration. The migratory ability was significantly increased upon TEAD4 overexpression. These results showed that cell migration was enhanced in TEAD4-OE cells (Figure 3B, C).

Next, CCK-8 assay results showed that TEAD4 overexpression promoted glioma cell proliferation (Figure 3D). In addition, colony formation assay results demonstrated that TEAD4 overexpression significantly increased the number of glioma cell colonies (Figure 3E). In conclusion, these results indicated that TEAD4 can promote glioma cell migration, proliferation, and colony formation *in vitro*.

GSEA and PPI of the TEAD4 expression phenotype

In light of the aforementioned findings, we confirmed that TEAD4 plays an oncogenic role in LGGs. To gain insight into the underlying mechanisms, we utilized GSEA to identify GO items and KEGG pathways enriched in TEAD4 high and low expression phenotypes. A NOM p-value < 0.05 and a FDR q-value < 0.05 were taken as the thresholds. We presented the five most enriched GO terms and KEGG pathways in the TEAD4 high expression group (Table 2) and ordered the data according to the NES. As shown in Figure 4A, five KEGG pathways, including those involved in viral myocarditis, glycan biosynthesis, antigen processing and presentation, autoimmune thyroid disease, and the intestinal immune network for IgA production, were significantly enriched in the TEAD4 high expression group. Five GO terms, including antigen processing and presentation, tumor necrosis factor-mediated signaling, antigen processing and presentation of peptide antigens, lymphocyte apoptosis, and response to tumor necrosis factor, exhibited the strongest positive correlation with high TEAD4 expression (Figure 4B). Meanwhile, no gene sets showed significantly differential enrichment in the TEAD4 low expression phenotype.

To further understand the connection between TEAD4 low and high expression phenotypes, we constructed a PPI network of DEGs. “MCODE” (Figure 4C) and “cytoHubba” (Figure 4D) were applied to identify the key cluster and hub genes in the network. The cluster and hub genes in the network included immune checkpoint genes (PDCD1, IDO1, ICOS), cytokines (IL-10, CXCL11), cytokine receptors (CCR2, CXCR3, CXCR6, IL2RA), and some other mediators of immune function (CD19, CD80, CD40LG, GATA3, SLAMF1). We then annotated the biological functions of the top six hub genes and observed that multiple immune processes were significantly enriched (Figure 4E).

Taken together, GSEA showed nearly all the top enriched gene sets associated with immune processes (Table 2) and PPI results revealed the hub genes were highly immune-related.

Association of glioma purity with TEAD4 expression and multivariate analysis

Given the immune phenotype of TEAD4 in LGG specimens, we further investigated the role of TEAD4 in immune cell infiltration. We used the “ESTIMATE” package in R to infer the tumor purity as well as stromal and immune scores of LGG specimens. Stromal and immune scores were positively correlated with the infiltration level of stromal cells and immune cells. The increased TEAD4 level correlated with elevated stromal and immune scores, but reduced tumor purity in LGG patients (Figure 5A), indicating the negative association of glioma purity with TEAD4 expression in LGG.

Next, we investigated whether tumor purity or TEAD4 expression was an independent prognostic biomarker among other factors. We performed correlation univariate analysis using Cox regression. These results revealed the relationship between overall survival and age, grade, tumor purity, and TEAD4 expression (Table 3). Specifically, clinical outcome showed a negative correlation with age, tumor grade, and TEAD4 expression but a positive correlation with tumor purity. Subsequently, multivariate Cox

regression analysis revealed that tumor purity independently correlated with a better survival consequence (HR = 0.076, P = 0.034). Age (HR = 1.059, P < 0.001), grade (HR = 2.422, P < 0.01), and TEAD4 expression (HR = 1.171, P = 0.015) were independent prognostic indicators of LGG (Table 3, Figure 5B).

TEAD4 overexpression affects TIICs in LGG

According to the analysis of glioma purity, we further aimed to explore the connection between TEAD4 expression and the specific types of TIIC by TIMER and CIBERSORT assays. Consistent with the results observed from the ESTIMATE algorithm assay, TEAD4 expression was negatively correlated with glioma purity (r = −0.039, P = 2.93e-01) (Figure 5A). As immune cell infiltration indicated glioma progression and a higher malignancy grade, we tested the correlation between TEAD4 expression and immune cell infiltration by the TIMER assay. These results showed that TEAD4 expression was positively correlated with the infiltration level of multiple immune cells, including B cells (r = 0.326, P = 2.76e-13), CD8+ T cells (r = 0.138, P = 2.48e-03), CD4+ T cells (r = 0.445, P = 1.79e-24), macrophages (r = 0.369, P = 1.14e-16), neutrophils (r = 0.474, P = 5.14e-28), and dendritic cells (r = 0.501, P = 1.41e-31) in LGG (Figure 5A). Meanwhile, an elevated infiltration level of these six types of immune cells was associated with a worse cumulative survival rate in LGG (Figure 5B).

To further confirm our conclusion, we employed CIBERSORT to access the proportions of 22 different types of TIICs in LGG specimens. As presented in Figure 5C and D, plasma cells, CD8+ T cells, and macrophages M1 and M2 exhibited higher proportions with elevated TEAD4 expression compared with counterparts in both CGGA and TCGA databases. Furthermore, the proportion of naive CD4+ T cells was significantly decreased in the TEAD4 high expression group. These results indicated a largely triggered immune cell infiltration in LGG patients with higher TEAD4 expression, especially in those with high malignancy grade tumors, resulting in decreased overall survival.

TEAD4 is positively associated with immune checkpoint genes

Recently, the immune checkpoint blockade has shed light on the novel immune-based therapeutic management of many cancers, including glioma. Stimulated immune checkpoint genes can lead to T cell exhaustion and escape from immune surveillance [41]. Clinical trials targeting CTLA-4 or PD-1/PD-L1 are currently underway, although initial results have been unpromising [1]. Moreover, some immune checkpoint genes were found to be essential components of the hub gene network, as discussed in the “PPI” section. We explored the association between the expression of TEAD4 and multiple immune checkpoint genes, including CTLA4, PDCD1, PD-L1, CD39, TIM-3, and B7-H3 in LGG, and TEAD4 expression was positively correlated with these six genes. These results indicated that LGGs with an elevated TEAD4 level exhibited a higher expression of immune checkpoint genes, suggesting an evasion of immune surveillance. Thus, it would be beneficial for LGGs with an elevated TEAD4 level to block immune checkpoint genes, and we suppose that a combinatorial approach of immunotherapy and targeted therapy can optimize efficacy and reduce chemoresistance.

Discussion

LGG (WHO grades II and III gliomas) is a highly immunosuppressive and malignant type of human brain tumor [1]. Complete resection along with radiotherapy and chemotherapy is the most common strategy in clinical treatment. Although incremental advances have been made in the therapeutic approach, the overall survival of glioma patients remains poor [3, 42]. Therefore, there is a growing need to explore innovative therapeutic strategies such as immunotherapy and precision oncology.

TEAD4 is an important member of the TEAD family. As a final effector of the Hippo pathway, TEAD4 plays essential roles in cell proliferation, epithelial-mesenchymal transition, metastasis, cancer stem cell maintenance, and drug resistance [12, 13]. It is noteworthy that TEAD4 strongly correlates with the poor survival of patients with various cancers [18–26]. Furthermore, a previous study has focused on how a dysfunctional Hippo pathway can impact crosstalk between tumor cells and the host immune system [43]. TEAD4 has been identified as a novel prognostic molecular marker associated with immune cell infiltration in bladder cancer [24]. In a mouse model of liver cancer, high YAP activity in cancer cells stimulated the recruitment of macrophages M2 to create an immunosuppressive environment in a TEAD-dependent manner via the expression of cytokines [44]. In another mouse model of prostate cancer, the activated YAP-TEAD complex induced CXCL5 expression to promote the recruitment of myeloid-derived suppressor cells to the TME to allow immune tolerance and inhibit the immune response [45]. However, the role of TEAD4 in glioma, as well as its interaction with TME, has been hardly studied. Here, we report the prognostic impact of TEAD4 in LGG. We found that TEAD4 expression was significantly up-regulated in LGG and positively correlated with the glioma grade at both RNA and protein levels. In addition, high TEAD4 expression suggested a poorer survival outcome. TEAD4 could promote glioma proliferation and migration *in vitro*. GSEA partially revealed the potential mechanism, and it indicated that signaling pathways related to antigen processing and presentation, lymphocyte apoptosis, and TNF-mediated signaling were differentially enriched in the TEAD4 high expression group. Coincidentally, TNFα treatment has been demonstrated to mediate the formation of the YAP/TEAD/p65 transcriptional complex and regulate gene transcription and breast cancer cell migration [46].

This is the first study to show that TEAD4 can serve as a novel prognostic marker of different tumor-infiltrating immune cells in glioma. Using the ESTIMATE algorithm, we observed decreased tumor purity in the TEAD4 high expression group as stromal and immune scores increased. Meanwhile, glioma purity, TEAD4 expression, tumor grade, and age were independent prognostic factors in LGG. We then further explored the association between TEAD4 expression and TIICs and arrived at some interesting observations. TEAD4 expression was found to positively correlate with the infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in LGG. In addition, CIBERSORTx analysis revealed that plasma cells, CD8+ T cells, and macrophages M1 and M2 showed higher percentages, whereas naive CD4+ T cells showed a lower proportion in the TEAD4 high expression group. According to our knowledge, macrophages and CD8+ T cells are enriched in the TME of highly malignant gliomas and correlate with poor prognosis [47]·[48]. Furthermore, PPI analysis indicated that the interaction of some chemokines/cytokines can mediate important biological processes in the TEAD4

high expression phenotype. Previous studies have reported that glioma cells secrete various chemokines/cytokines to drive immune cell infiltration [8],[49], which might explain the increased infiltration of immune cells in gliomas with elevated TEAD4 expression. Another intriguing finding was that the 1p/19q codeletion was linked to decreased TEAD4 expression. A recent study has revealed that LGGs with the 1p/19q codeletion exhibited less immune cell infiltration and lower immune checkpoint gene expression than LGGs with the 1p/19q non-codeletion [50]. This study partly ascribed this interesting phenomenon to the copy number deletion of more than 40 genes encoding cytokines/chemokines that are located on chromosome 1p/19q. Therefore, it is reasonable to speculate that a low level of immune-related genes in gliomas with the 1p/19q codeletion can affect TEAD4 expression.

Immunotherapy has revolutionized the management of many cancers and improved our knowledge of tumor immunology. There are several immune-based therapeutic approaches for glioma, including oncolytic virus therapies, cancer vaccines, chimeric antigen receptor (CAR) T-cell therapy, and immune checkpoint inhibitors [1, 9]. To date, some of these treatments have generated impressive clinical responses; however, due to uncertain efficacies, they are not propagable yet. The main impediment is the highly heterogeneous and ever-changing nature of the TME. Recently, accumulating evidence has prompted an increased emphasis on understanding the interplay between TIICs and LGGs [51–53]. Moreover, our analysis indicated that TEAD4 expression is positively associated with multiple immune checkpoint genes, which could result in immune evasion in tumors. Therefore, LGGs with a high TEAD4 level may benefit from immune checkpoint blockade therapy. In a previous review, we proposed that targeting the TEAD4-YAP/TAZ complex was an attractive therapeutic approach for cancer [15]. Given the evidence above, individualized immunotherapy combined with TEAD4-YAP/TAZ complex-targeted therapy can be an optimal solution for LGG.

Certainly, our study still has some limitations. We used algorithm analysis to acquire tumor purity and the fraction of TIICs based on gene expression profiles, which might not be accurate enough. Hence, *in vivo* models are needed to further verify the relationship between TEAD4 expression and TIICs. To date, organoid culture models [54] and three-dimensional bioprinting technology [55, 56] have been used to mimic the TME in the human brain. On the other hand, given that TEAD4 is a key transcriptional factor in the Hippo pathway, it would be important to identify its upstream signals, transcriptional output, and co-factors in LGG.

Conclusions

In this study, we systematically documented the abundance of tumor-infiltrating immune cells for specimens in TCGA and CGGA databases and analyzed the correlation with the glioma grade. We identified the prominent role of TEAD4 in LGG progression and elucidated its correlation with glioma purity and TIICs. High TEAD4 expression is associated with poor overall survival, lower tumor purity, and higher immune infiltration levels in LGG. Our results demonstrated that TEAD4 can serve as a prognostic marker in LGG and may provide insights for novel therapeutic strategies.

Abbreviations

TIICs: Tumor-infiltrating immune cells; LGG: Lower-grade glioma; TEAD4: TEA Domain Transcription Factor 4; TCGA: The Cancer Genome Atlas; CGGA: Chinese Glioma Genome Atlas; GSEA: Gene set enrichment analysis; PPI: Protein-protein interaction; YAP: Yes1 Associated Transcriptional Regulator; TAZ: Tafazzin; VGLL: Vestigial Like Family Member; GBM: Glioblastoma; TME: The tumor microenvironment; TEF-3: Transcriptional Enhancer Factor-3; HPA: Human Protein Atlas; OS: Overall survival; FBS: Fetal bovine serum; OE: Overexpression; CCK-8： Cell counting kit-8; NES: Normalized enrichment score; NOM: Nominal; FDR: False discovery rate; DEGs: Differentially expressed genes; TIMER: The Tumor Immune Estimation Resource; NC: Negative control; PDCD1: Programmed Cell Death 1; IDO1: Indoleamine-Pyrrole 2,3 Dioxygenase; ICOS: Inducible T Cell Costimulator; IL-10: Interleukin 10; CXCL11: C-X-C Motif Chemokine Ligand 11, CCR2: C-C Motif Chemokine Receptor 2; CXCR3: C-X-C Motif Chemokine Receptor 3; CXCR6: C-X-C Motif Chemokine Receptor 6; IL2RA: Interleukin 2 Receptor Subunit Alpha; CD19: CD19 Molecule; CD80: CD80 Molecule; CD40LG: CD40 Ligand; GATA3: GATA Binding Protein 3; SLAMF1: Signaling Lymphocytic Activation Molecule Family Member 1; HR: Hazard ratio; CTLA4: Cytotoxic T-Lymphocyte Associated Protein 4; PD-L1: Programmed Cell Death 1 Ligand 1; CD39: Cluster of Differentiation 39; TIM-3: T Cell Immunoglobulin Mucin 3; B7-H3: CD276 Molecule; CXCL5: C-X-C Motif Chemokine Ligand 5.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed in this study are available in TCGA (https://portal.gdc.cancer.gov) and CGGA (http://www.cgga.org.cn). The data that support the findings of this study are available from the corresponding author upon reasonable request

Competing interests

The authors declare no conflicts of interest.

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Author's contributions

Conception and design: CZ, JZ and KZ, Acquisition and analysis of data: MC, BH, HL and ML. Experiment performance: MC and BH. Figures illustration: MC. Manuscript writing: MC, BH, LZ, KC and HL. Discussion and revision: CZ, JZ and KZ. Final approval of manuscript: All authors.

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Tables

Due to technical limitations, table 1 to 3 is only available as a download in the Supplemental Files section.

Figures

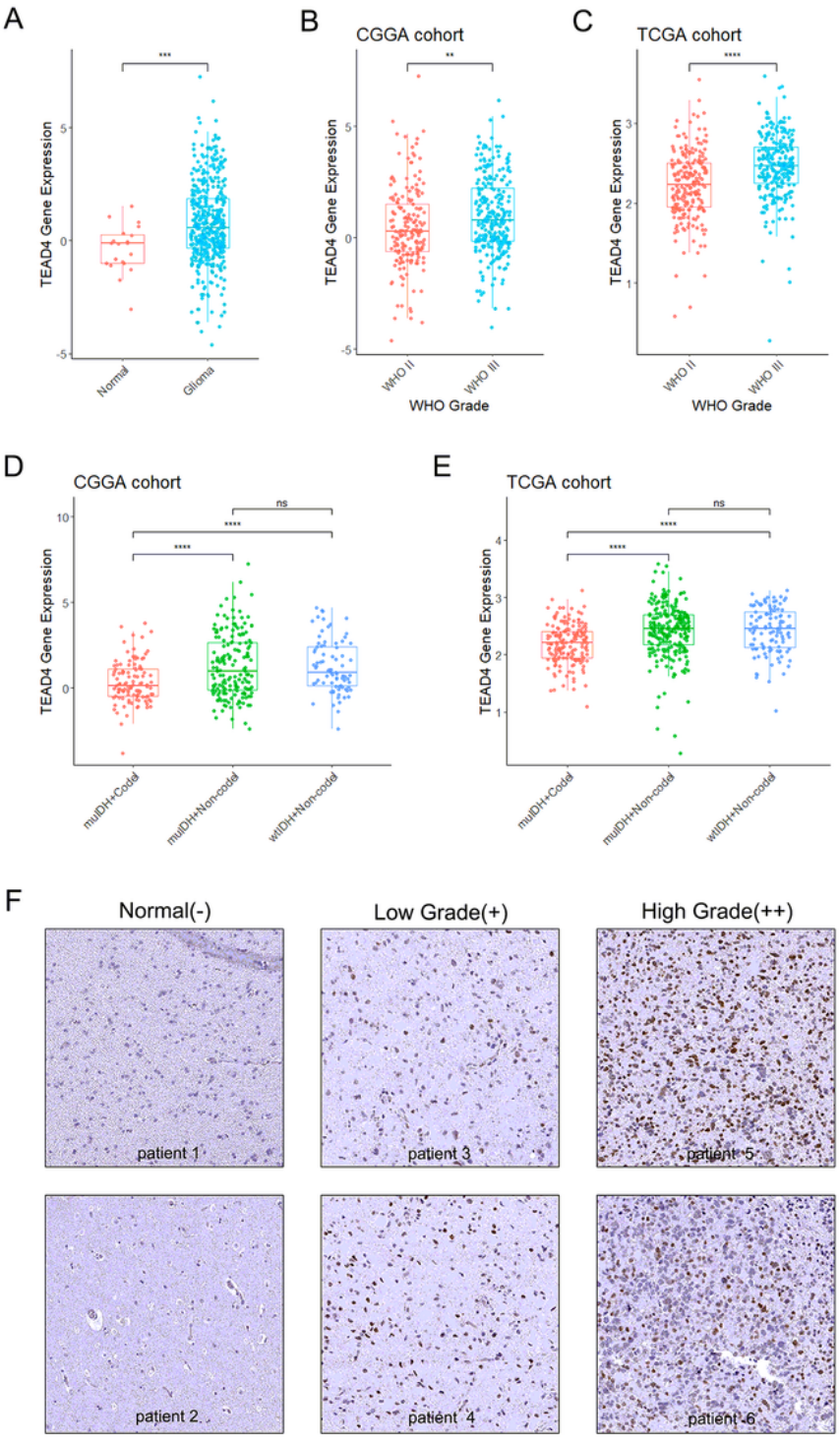

Figure 1

Expression analysis of TEAD4 based on the data obtained from the CGGA and TCGA database. (A) Differential TEAD4 expression in LGG and normal brain tissue. (B, C) Differential TEAD4 expression in grade II and III LGG. (D, E) Differential TEAD4 expression in LGG of different 1p/19q codeletion and IDH mutation statuses. (F) Representative immunohistochemistry images of TEAD4 in normal brain tissues, low-grade gliomas, and high-grade gliomas. Negative (-); Weak expression (+); Moderate expression (++). ** P < 0.01, **** P < 0.0001.

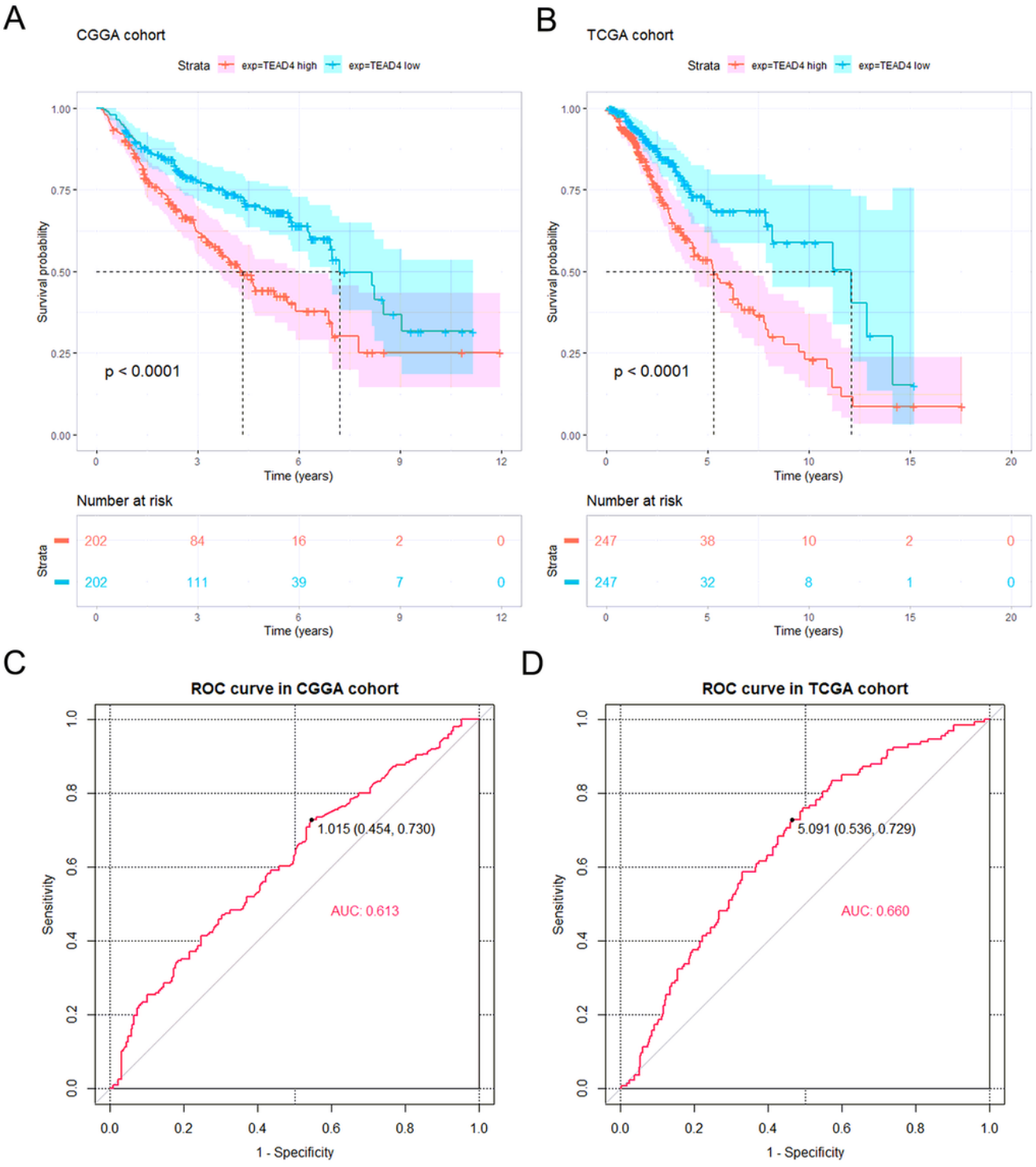

Figure 2

The survival analysis and prognostic value of TEAD4 in LGG. (A, B) Survival curve and risk table of differential TEAD4 expression groups. (C, D) ROC curve analysis of TEAD4 expression in LGG to determine its predictive power of survival.

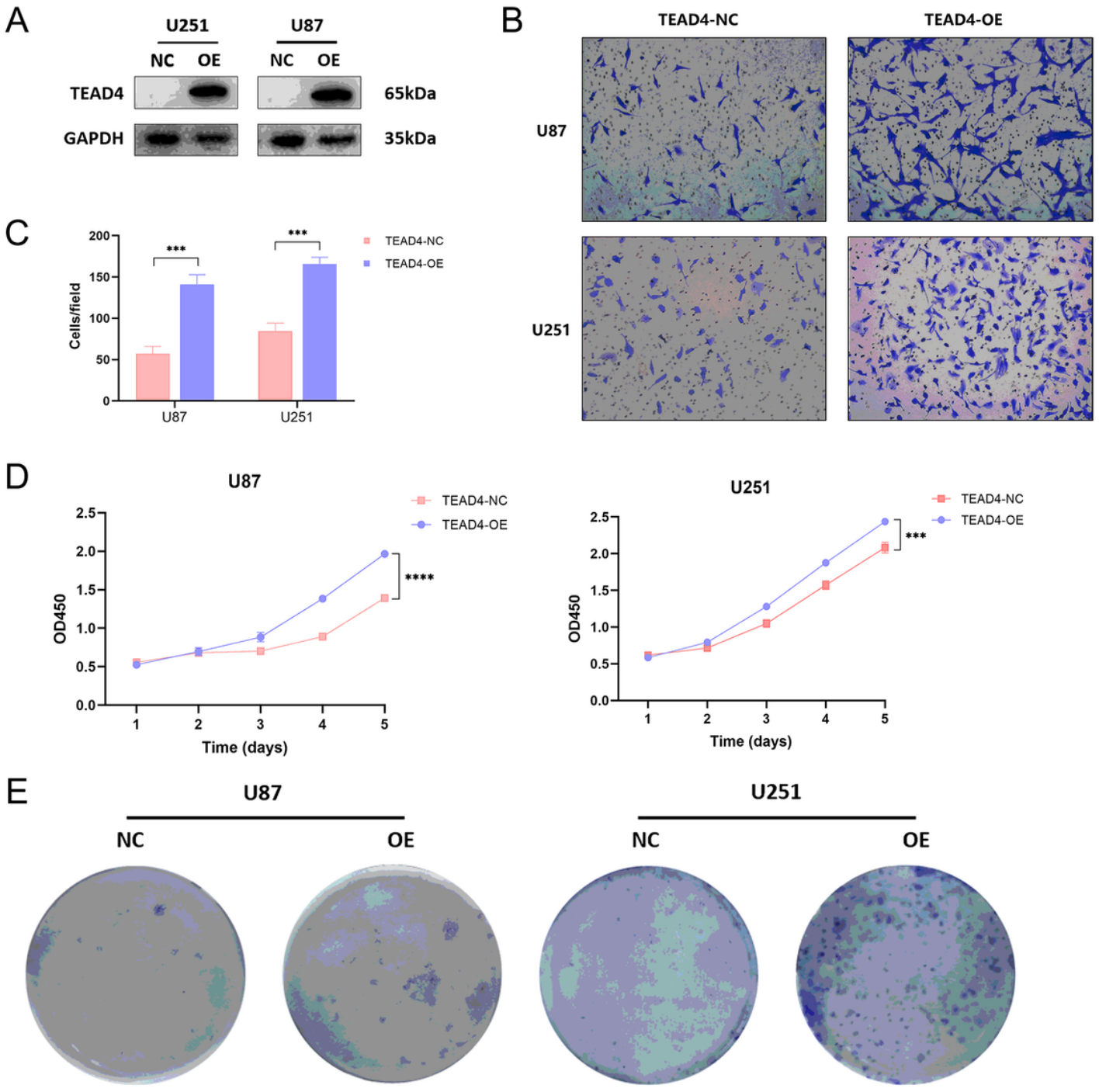

Figure 3

TEAD4 overexpression promotes proliferation, colony formation, and migration in glioma cells. (A) Up-regulation of TEAD4 in U87 and U251 cell lines via transfection. Full-length gels are presented in Additional File 4. (B, C) Transwell analysis of U87 and U251 cells with or without TEAD4 OE. (D) CCK-8 assays of U87 and U251 cells with or without TEAD4 OE. (E) Colony formation assays of U87 and U251 cells with or without TEAD4 OE. Representative staining images are presented. Data represent mean ± standard error of the mean for three independent experiments. *** P < 0.001, **** P < 0.0001.

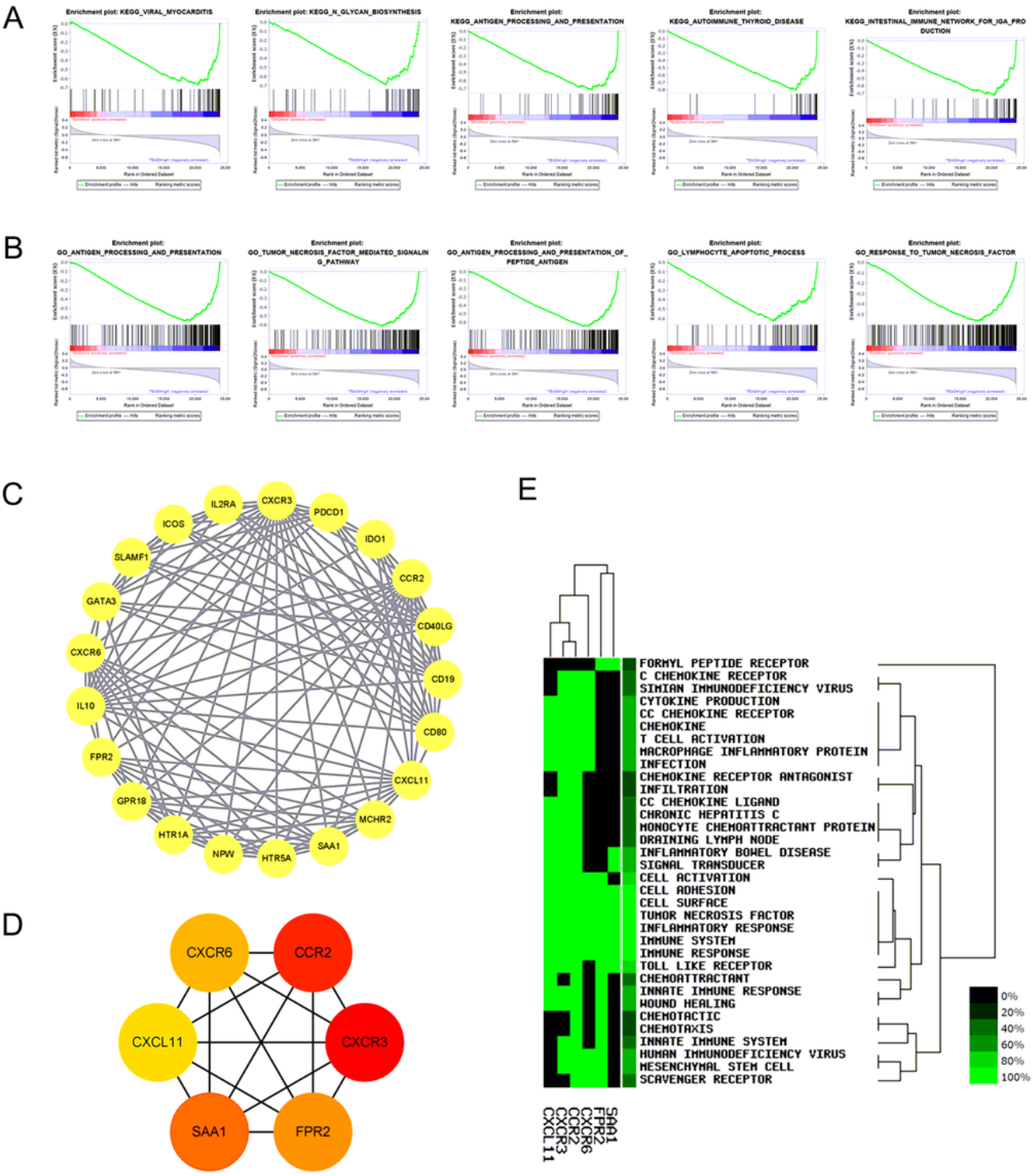

Figure 4

GSEA and PPI of the TEAD4 expression phenotype. (A) The five most enriched GO items in the TEAD4 high expression group. (B) The five most enriched KEGG pathways in the TEAD4 high expression group. (C) A key cluster of the PPI network identified by “MCODE”. (D) Six hub genes of the PPI network identified by “cytoHubba”. (E) Function Enrichment of the six hub genes.

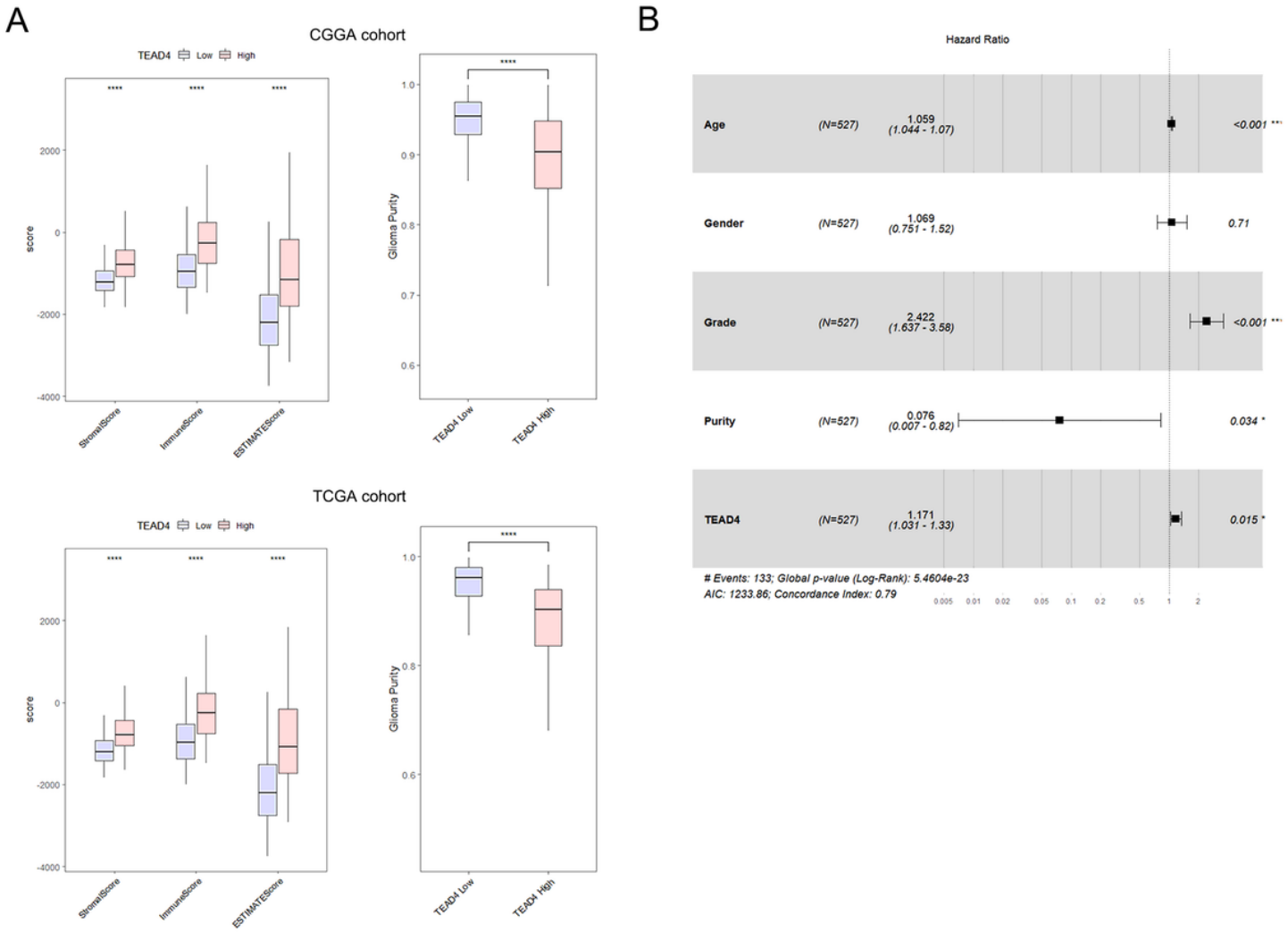

Figure 5

Association of glioma purity with TEAD4 expression and multivariate analysis. (A) Distinct distribution of stromal score, immune score, and glioma purity between the TEAD4 high and low expression groups in LGG. (B) Multivariate Cox analysis of TEAD4 expression, glioma purity, and other factors. **** P < 0.0001.

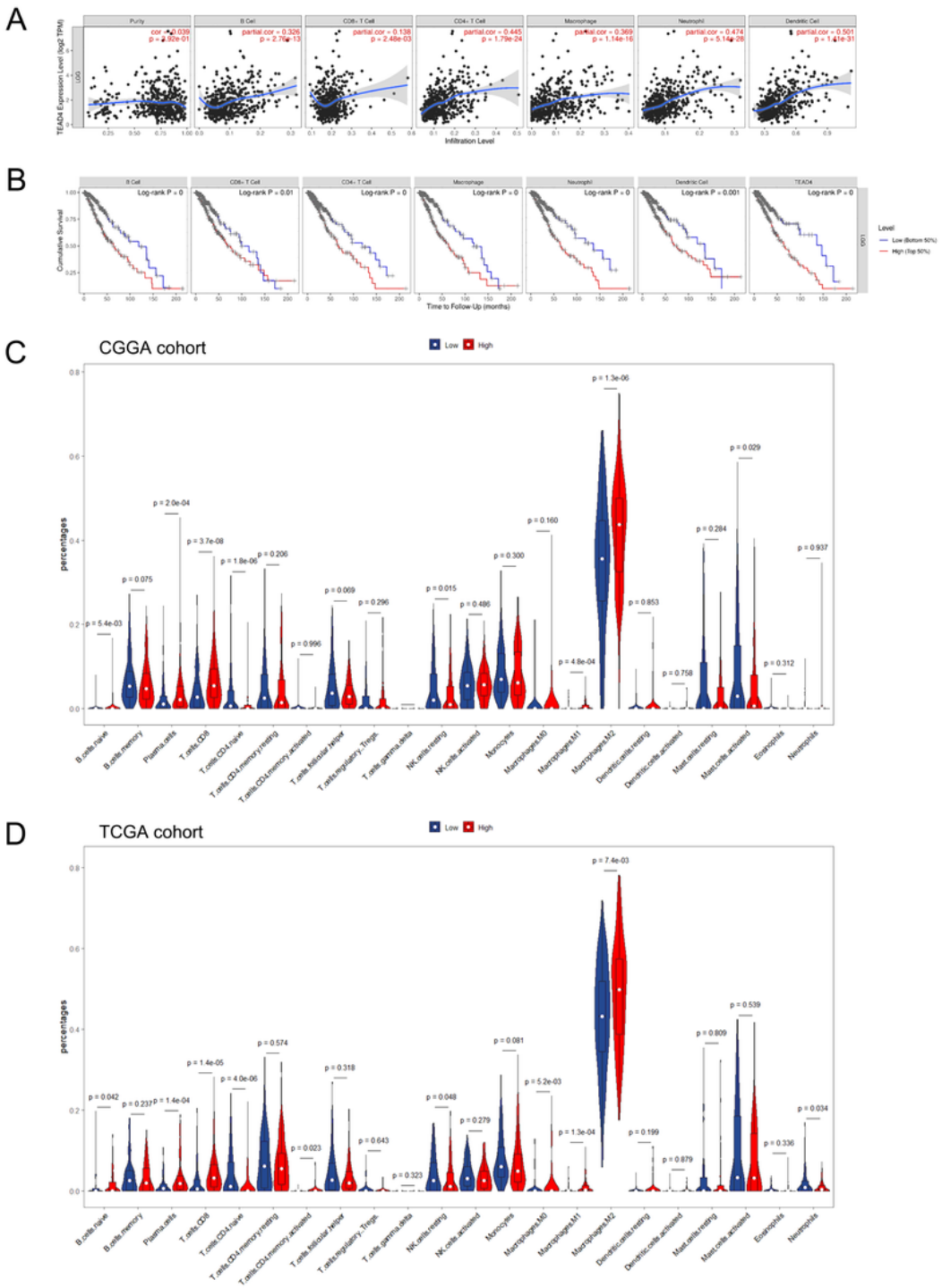

Figure 6

TEAD4 expression affects TIICs in LGG. (A) Correlations between TEAD4 expression and immune infiltration levels of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells in LGG. (B) Survival curves of different infiltration levels of immune cells. (C, D) The percentages of 22 subpopulations of immune cells in high and low TEAD4 expression groups in LGG specimens from CGGA and TCGA.

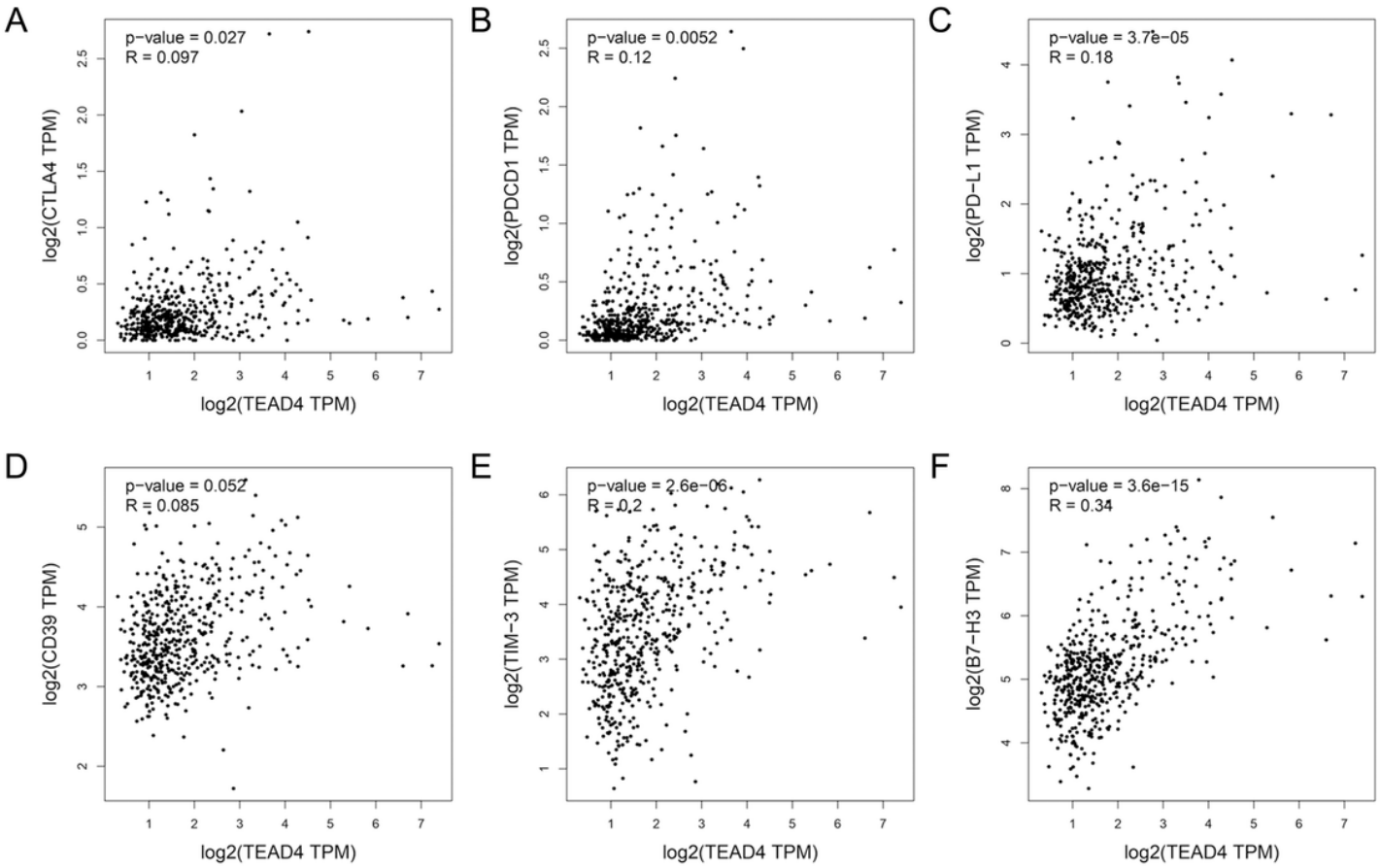

Figure 7

Correlation between TEAD4 and immune checkpoint genes expression. (A-F) Correlation analysis of TEAD4 expression and six immune checkpoint genes (CTLA4, PDCD1, PD-L1, CD39, TIM-3, and B7-H3) in LGG.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
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- Table3.xlsx
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- AdditionalFile2.xlsx
- AdditionalFile3.docx
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