BMC Cancer

RESEARCH Open Access

Integrated bioinformatics analysis and experimental validation on malignant progression and immune cell infiltration of LTBP2 in gliomas

Lun Gao[1†], Rui Zhang[2†], Wenbin Zhang[3†], Yanfang Lan[3], Xiangpan Li[3], Qiang Cai[1*†] and Junhui Liu[1*]

Abstract

Background Gliomas are the highly aggressive brain tumor and also the most devastating human tumors. The latent TGF binding proteins (LTBP) had been found to be involved in malignant biological process and could be used as potent biomarkers in several solid tumors. While the role of LTBP family in human glioma remain to be elucidated.

Methods Normalized gene expression and corresponding clinical data of 2407 gliomas samples in public datasets were downloaded from Gliovis. Kaplan–Meier methods and Cox regression analysis was used for survival analyses. Western blot (WB) and Immunohistochemical (IHC) testing were employed to test LTBPs protein level in 154 gliomas samples. Correlation between LTBP2 expression and immune infiltration was evaluated by immunofluorescence (IF) and IHC in glioma tissues. CCK8 and flow cytometric analysis were used to detect the effect of LTBP2 on glioma cells. Orthotopic glioma- mouse models were utilized to evaluate effects in vivo.

Results LTBP2 mRNA level was dramatically higher in glioma samples compared with non-tumor brain tissues in XENA-TCGA_GTEx, Gill and Gravendeel datasets (all $P < 0.01$), and its expression positively correlated with glioma WHO grade, IDH1/2 wildtype and mesenchymal subtypes. These results were confirmed by In-house cohort which was detected by WB and IHC. We found that gliomas patients with high LTBP2 level had shorter OS than those with low LTBP2 level. LTBP2 expression significantly associated with glioma immune score (Spearman $r = 0.68$, $P < 0.01$)) and strongly correlated with infiltration degreee of macrophages both in lower grade gliomas (LGG) and GBM. Knocking down LTBP2 obviously reduced proliferation and enhanced sensitivity to temozolomide in U87 and U251 cells. Nude mice with lower expression of LTBP2 had slower tumor growth, and accompanied by less tumor-associated macrophages (TAMs) infiltration detected by IHC staining in vivo. Finally, low LTBP2 expression glioma patients who received chemotherapy survived longer than patients with high LTBP2 expression.

†Lun Gao, Rui Zhang, Wenbin Zhang and Qiang Cai contributed equally to this work.

*Correspondence:
Qiang Cai
cqno@sina.com
Junhui Liu
liusijia.zu@163.com; doctorhsdqe@whu.edu.cn

Full list of author information is available at the end of the article

Conclusion LTBP2 could be used as a prognostic marker, and high LTBP2 expression related to abundant TAMs infiltration and with a worse response to chemotherapy.

Keywords LTBP family, Prognosis, Biomarker, Tumor-associated macrophages, Chemoresistance

Background

There are 80% of primary malignant tumors in the central nervous system that are gliomas, which is one of the deadly brain tumors [1]. WHO's grading system reflects clinical prognosis, with grade IV gliomas (glioblastomas, GBM) having the worst outcomes. Although GBM accounted for only 15% of brain tumors, it obviously associated with high mortality and disability rate [2]. Previous study had demonstrated that survival proportion for non-GBM astrocytoma patients was 72% (1-year) and 44% (5-year), while survival proportion for GBM adults was 41% (1-year) and 5% (5-year) during 2000–2014 [2, 3]. The poor outcome of patients with GBM was attributed to cellular heterogeneity, therapeutic resistance, and the infiltrative nature of the disease. Chemo- and radioresistance after surgery reduced the effectiveness of standard treatment regimens in GBM patients [4, 5]. Therapy targeting immune microenvironment revolutionizes glioma treatment. Glioma immune checkpoint molecules effectively modulate immune responses and may play an indispensable surrogate role in the treatment of TMZ-resistant gliomas [6]. Uncovering novel genes offered a better understanding of chemoresistance mechanism in glioma and also provided a potential immunotherapeutic target.

The latent TGF binding proteins (LTBP) plays crucial role in mediating the secretion of TGFβ and also acted at the central nexus of a mechanosensing hub that regulated pathways by TGF-beta [7, 8]. Three TGF-beta isoforms and several other matrix constituents could bind to the LTBP family members [9, 10].Previous studies have demonstrated that LTBP1 together with TGF-beta forming a complex that bound to extracellular matrix (ECM) to facilitate the activation of LTBP by integrins [11, 12]. Besides, LTBP2 was also found to be involved in ECM remodeling, elastic fiber aggregation and cell adhesion. While LTBP3 mutations in some patients could lead to the formation of acromicric and geleophysic dysplasia, which indicated the potential role of LTBP3 in promoting long bone growth and lung septation [13]. Recently, a growing body of evidence supported that there were close associations between LTBP family members and cancers formation and progression. Elevated LTBP1 expression significantly positively correlated with lymphatic metastatic ability and inhibition of LTBP1 in Esophageal squamous cell carcinoma (ESCC) cells obviously attenuated TGF-beta induced epithelial–mesenchymal transition (EMT) and cancer-associated fibroblasts (CAFs) transformation [14]. Similarly, LTBP2 expression was increased in gastric cancers (GC) and knocking down LBTP2 reduced the proliferation, invasion and also the EMT in GC cells [15]. However, few studies have explored the relationships between LTBP 1–4 and gliomas. It remains to be demonstrated how LTBPs are expressed and whether they might play a genetic role in gliomas.

Methods

Clinical samples

In this study, we used 118 paraffin-embedded samples which contained 110 glioma tissues and 8 non-tumor brain tissues (NBTs). All paraffin-embedded tissues were collected in the department of neurosurgery of Renmin hospital of Wuhan University from July 2019 to October 2021. Also 12 non-tumor brain tissues (patients who had suffered traumatic brain injuries) and 24 frozen glioma tissues were utilized. All patients agreed and signed informed consents and none of them received any pre-chemo or pre-radiotherapy before surgery. This study was approved by the institutional ethics committee of the faculty of medicine at Wuhan University's Renmin Hospital [approval number: 2012LKSZ (010) H].

Public datasets acquisition and analysis

Normalized gene expression data was acquired from GlioVis website [16]. Gliovis contains the standardization of RNA expression data, clinical baseline information and prognosis of included patients in public datasets. In the course of our research, eight datasets were accessed and downloaded from gliovis: TCGA-GBMLGG, TCGA-GBM, TCGA-LGG, CGGA, Gravendeel [17], Rembrandt [18], Gill [19] and Murat [20]. Pan-cancer group comparison was based on Xiantao academic platform(https://www.xiantao.love/), which mainly obtained data from public databases for standardized analysis. A genomic analysis of the LTBP family genes was conducted using online cBioPortal website (http://www.cbioportal.org/) [21]. The selection of Mesenchymal (MES) related genes was based on the 29 MES_CORE_GENES mentioned in the previous literature [27].

Gene function enrichment analysis

TCGA-GBM was utilized to conduct Gene function enrichment analysis., Gliomas samples were divided into two groups based on LTBP2 mRNA expression (high vs. low group). Then the differential genes between the two groups were screened by DEseq2 procedure on the basis of $\log_2$FC value and p value. Statistically significant

thresholds were adj. *P*-value < 0.01 and $|\log_2 FC| > 2$. Significant differentially expressed genes (DEGs) were then put into the Xiantao academic platform and hypergeometric analysis was performed in GOKEGG database. After zscore was calculated, the results were obtained. Protein–protein interactions of DEGs were performed using STRING platform (http://string-db.org; version 12.0).

Immunohistochemical (IHC) staining

These methods were consistent with our previously published articles [22].

IHC evaluation.

Intensity of staining and the percentage of positive-staining cells were both assessed. Scores ranged from 0 to 3 according to the intensity of staining. The percentage of positive cells was defined as: (0, < 10%;1,10–25%;2,26–50%;3,51–75%; and 4,>75%). We multipled staining intensity times the percentage of positive cells to get final IHC scores. Two individuals independently analyzed the IHC staining results.

Immunofluorescence staining & western blot

The methods were compliance with previous articles we pulicated [23]. The main primary antibodies used for IF/WB were anti-LTBP2 (Bioss, cat#bs-18440R), anti-C68 (Proteintech, cat#28058-1-AP), anti-86 (Cell Signaling Technology, cat#19589), anti-CD11b(biobyt, cat#orb11009), anti-CD163(abcam, cat#ab182422). PCNA (Proteintech, Cat No. 24036-1-AP).

Construction and transfection with shRNA, Cell proliferation detection

The shRNA-treated cells used in this study were stably transfected glioma cells. Specifically, the shRNA (5'-CC AGCAGAAGAGCAAGTGATT-3') targeting LTBP2 was designed and cloned into the lentivirus vector GV493-gcGFP-Puro (GeneChem, Shanghai, P.R. China).In brief, the constructed shRNA lentivirus was co-cultured with glioma cells to facilitate the entry of the lentivirus into the cells. The cells were cultured in 6-well plates and infected with the lentivirus according to the manufacturer's instructions for 24 h. Subsequently, the cells were treated with puromycin (2 μg/ml) for 48 h, and fresh medium was added after washing with PBS buffer.

Cell Counting Kit-8 was used to detect effect of LTBP2 on glioma cell proliferation. 5,000 transfected cells were seeded in 96-well plates, and 3 multiple wells were set in each group. Cells need to be examined at two time points, 24 h and 48 h. 10uL of CCK-8 solution was added to each well, incubated for 1 h, and the absorbance at 450 nm was measured using a microplate reader.

Flow cytometry analysis

After glioma cells were treated with different concentrations of TMZ, the culture medium was collected in flow tubes. Cells in six-well plates were washed once with PBS, digested with 1 ml of 0.25% trypsin, and when the cells were rounded and partially suspended, the digestion was terminated by adding medium. Cells were suspended by gently blowing with a pipetting gun. The samples were collected in flow tubes, centrifuged at 1500 rpm for 5 min, and the supernatant was discarded. Cells were completely resuspended by adding 3 ml of PBS precooled at 4 ° C, centrifuged at 1500 rpm for 5 min, and the supernatant was discarded. The precipitate was resuspended in 300μL of Binding Buffer. Fluorescence labeling was then performed: after 5μL Annexin V-FITC was added and mixed, 5μL Propidium Iodide was added and mixed. Reactions were carried out at room temperature in the dark for 5–15 min. The green fluorescence of Annexin V-FITC was detected through the FITC channel (FL1), and the red fluorescence of PI was detected through the PI channel (FL2). The flow cytometry parameters were as follows: excitation wavelength Ex = 488 nm and emission wavelength Em = 530 nm, and analyzed by using a FACS-Calibur flow cytometer (Becton Dickinson).

Immune estimation

ESTIMATE (https://bioinformatics.mdanderson.org/estimate/) was used to analyze correlations between LTBP2 expression and ESTIMATE scores. ESIMATE is an online platform for calculating immune infiltration scores based on gene expression. Associations between LTBP2 and immune cells infiltration were assessed by Xiantao academic platform.

Intracranial xenograft model

All nude mice were purchased from Shulaibao (Wuhan, China) Biotechnology Co., Ltd. NC-shRNA or LTBP2-shRNA U87 cells were resuspended in PBS. We stereotacticly injected 3×10^5 cells into the right striatum of Balb/c nude mice. 1% sodium pentobarbital was administered intraperitoneally. In short, we immobilized the nude mice with a stereotactic device. An incision about 1 cm long was cut with a scalpel, and then a puncture needle was used to penetrate the skull with a depth of about 3 mm (2 mm away from midline and before the coronal suture). After stable for 5 seconds, the implanted cells were injected. After waiting for 3 s, the puncture needle was pulled out. The puncture site was closed with bone wax, and then we sutured the skin. The nude mice were placed in a warm tank to prevent death from hypothermia. The nude mice were sacrificed when neurological dysfunction appeared or weight loss was greater than 20%.For euthanasia, cervical dislocation was used on the mice without prior anesthesia. The procedure involved firmly grasping

the back of the head and neck, followed by a rapid, forceful upward and backward motion, causing immediate death. All experiments with animals were approved by the Institutional Animal Care and Use Committee at Renmin Hospital of Wuhan University [approval number: 20231002 A].

Statistical analysis

Data were presented as mean±standard error or deviation (SD).The Chi-square test was used to compare the differences in the number of individuals with high and low gene expression between the groups. Analyzsis of significant differences between two groups was conducted by Student's t-test.We used a two-tailed t-test, and Quantile-Quantile Plot was employed to test for normality. If normality was not met, the Mann-Whitney U test was applied. The one-way ANOVA was used for analysis among more than two groups. We plotted Kaplan-Meier curves based on optimal cut-point for patients with low and high expression of LTBPs. The optimal cut-point was decided by online platform Gliovis. GraphPad Prism 9.5.1 software was used to generate the graphs.

Results

LTBPs expression in gliomas

We used online platform Xiantao academic to explore LTBPs expression in multiple cancers. Preliminary results found that LTBPs were over-expressed in several solid tumors, such as GBM, LGG, pancreatic carcinoma and Thymoma. While in other tumors (Colon adenocarcinoma, rectum adenocarcinoma, testicular germ cell tumors et al.), LTBPs were downregulated in tumor tissues (Fig. 1A). Besides, we focused on LTBPs genetic alterations in gliomas and found that LTBP4 had highest gene altered frequency (2.5%) in LGG. In GBM tissues, LTBP1 and LTBP2 had the highest gene altered frequency (both 28%, Fig. 1B). In order to investigate LTBPs expression in gliomas, we utilized three GEO datasets(Gill, Gravendeel, and Murat). The mRNA levels of LTBP1 in glioma tissues were 9.54±1.07, 7.55±0.96, and 8.65±0.76 in the Gill, Gravendeel, and Murat datasets, respectively, compared to its expression in non-tumor brain tissues (NBTs), which were 8.64±0.66, 6.76±0.25, and 7.55±0.38, with all P-values<0.01 (Fig. 1C). Similarly, LTBP2 expression was significantly higher in glioma tissues compared to NBTs in both the Gill dataset (8.33±1.21 vs. 6.90±0.49, $P<0.001$) and the Gravendeel dataset (6.82±1.04 vs. 5.88±0.17, $P<0.001$) (Fig. 1C). LTBP3 expression followed the same pattern as LTBP1 and LTBP2, showing an elevated mRNA level in glioma tissues. In the Gravendeel dataset, LTBP3 expression was significantly higher in gliomas than in NBTs (8.53±0.64 vs. 7.64±0.48, $P<0.001$), and in the Murat dataset (7.15±0.55 vs. 8.37±0.41, $P<0.01$) (Fig. 1C). These results demonstrate that the expression levels of LTBP1-3 are consistently elevated in glioma tissues compared to

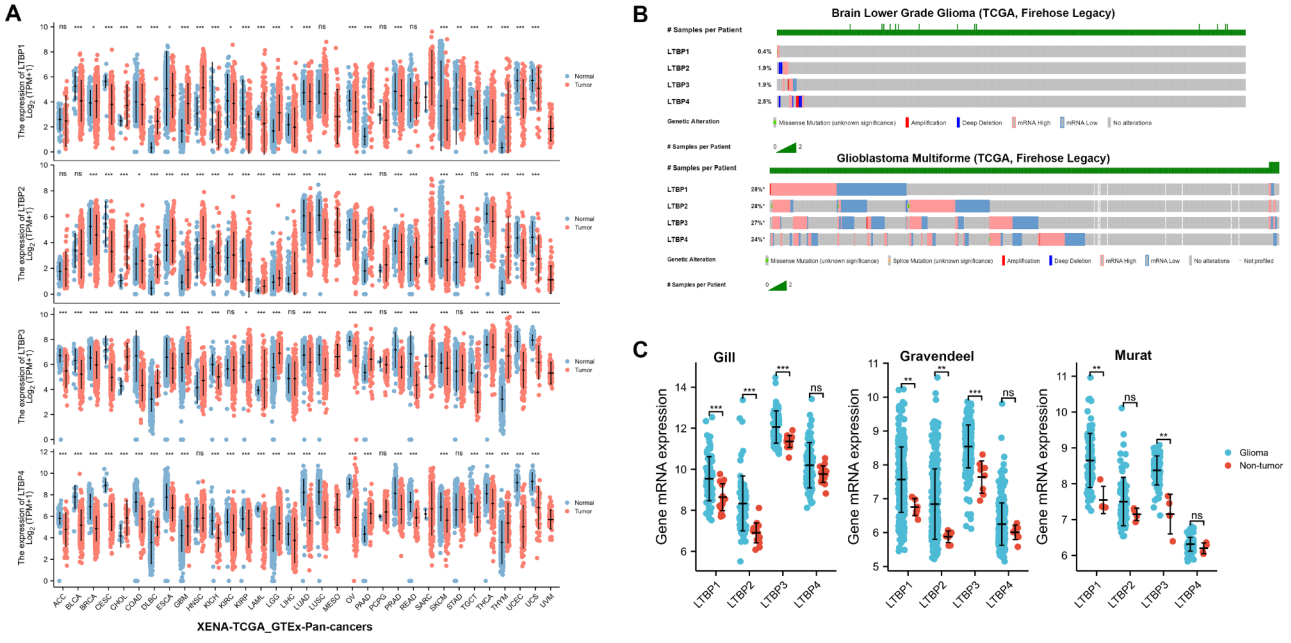

Fig. 1 Gene alterations of LTBPs in gliomas. **A.** The mRNA level of LTBP family in various cancers in TCGA,blue means normal tissue, red refers to tumor tissue. **B.** The genetic alteration of LTBP family in LGG and GBM in TCGA from cBioPortal platform (http://www.cbioportal.org/). The abbreviations for tumors were listed in supplementary material-Abbreviations for fig1A. **C.** Datasets (Gill, Gravendeel & Murat) that contained RNA-sequencing of gliomas and non-tumor brain tissues were all downloaded from GEO platform,red represents the normal tissue, blue refers to tumor tissue. **, $P<0.01$, ***, $P<0.001$. ns, no significance. NBT, non-tumor brain tissues

Table 1 Comparison of clinical characteristics in different LTBP2 expression groups among glioma patients in CGGA

Factors	LTBP2 expression		
	Low	High	P value
Age, n (%)			0.003
≤60	449 (44.5%)	466 (46.2%)	
>60	61 (6%)	33 (3.3%)	
Gender, n (%)			0.063
Female	194 (19.2%)	219 (21.7%)	
Male	316 (31.3%)	281 (27.8%)	
Histology, n (%)			<0.001
Aglio & OAglio & Odglio	241 (24%)	379 (37.7%)	
GBM	264 (26.3%)	121 (12%)	
Grade, n (%)			<0.001
III	129 (12.8%)	202 (20.1%)	
IV	264 (26.3%)	121 (12%)	
II	112 (11.1%)	177 (17.6%)	
Recurrence, n (%)			0.013
Recurrent	185 (18.4%)	146 (14.5%)	
Primary	321 (31.9%)	354 (35.2%)	
Subtype, n (%)			<0.001
Mesenchymal	98 (22.6%)	18 (4.2%)	
Classical	118 (27.3%)	43 (9.9%)	
Proneural	84 (19.4%)	72 (16.6%)	
codel_1p19q, n (%)			<0.001
Non-codel	429 (45.8%)	295 (31.5%)	
Codel	54 (5.8%)	158 (16.9%)	
IDH_codel.subtype, n (%)			<0.001
IDHmut-non-codel	145 (16.4%)	167 (18.9%)	
IDHwt-non-codel	273 (30.8%)	106 (12%)	
IDHmut-codel	49 (5.5%)	133 (15%)	
IDHwt-codel	3 (0.3%)	9 (1%)	
Radio_status, n (%)			0.207
1	390 (42.3%)	373 (40.4%)	
0	73 (7.9%)	87 (9.4%)	
Chemo_status, n (%)			0.403
1	326 (36.1%)	304 (33.7%)	
0	133 (14.7%)	140 (15.5%)	

Aglio,astrocytoma; OAglio, oligoastrocytoma; Odglio, oligodendroglioma; GBM, glioblastoma multiforme; IDH, isocitrate dehydrogenase; Radio_status,radiotherapy; Chemo_status, Chemotherapy

non-tumor brain tissues (Fig. 1C).Our results indicated higher levels of LTBP1-3 expression in gliomas compared with NBTs (Fig. 1C).

Correlations between LTBPs expression and glioma malignancy

We employed 4 public datasets that in totally contained 2407 different grade gliomas. The baseline information of CGGA and TCGA glioma patients were presented in Tables 1 and 2, respectively. With the increase of glioma grade, LTBP1 and LBTP2 expression level also increased gradually. The median level of LBTP2 expression in WHO grade IV gliomas were (8.44±1.18), (1.46±1.20), (8.14±0.61) and (7.08±1.02)

Table 2 Comparison of clinical characteristics in different LTBP2 expression groups among glioma patients in TCGA

Factors	LTBP2 expression		
	Low	High	*P* value
Age, n (%)			<0.001
<=60	300 (42.9%)	256 (36.6%)	
>60	49 (7%)	94 (13.4%)	
Gender, n (%)			0.519
Female	153 (21.9%)	145 (20.7%)	
Male	196 (28%)	205 (29.3%)	
Race, n (%)			0.003
Asian	11 (1.6%)	2 (0.3%)	
Black or African American	10 (1.5%)	23 (3.4%)	
White	322 (46.9%)	318 (46.4%)	
WHO grade, n (%)			<0.001
G2	148 (23.2%)	76 (11.9%)	
G3	128 (20.1%)	117 (18.4%)	
G4	35 (5.5%)	133 (20.9%)	
Histological type, n (%)			<0.001
Aglio & OAglio & Odglio	314 (44.9%)	217 (31%)	
Glioblastoma	35 (5%)	133 (19%)	
IDH status, n (%)			<0.001
Mut	285 (41.4%)	158 (22.9%)	
WT	62 (9%)	184 (26.7%)	
1p/19q codeletion, n (%)			<0.001
Non-codel	210 (30.3%)	310 (44.8%)	
Codel	136 (19.7%)	36 (5.2%)	

in TCGA, CGGA, Rembrandt and Gravendeel, respectively. While LTBP2 expression in WHO grade III and II were relatively lower (TCGA: 7.67±1.07(III) & 7.39±0.74(II), CGGA: 0.82±1.00(III) & 0.75±0.72(II), Rembrandt:7.75±0.36(III) & 7.80±0.53(II), Gravendeel:6.61±1.07(III) & 6.20±0.71(II)). In GBM tissues, LTBP1 and LTBP2 showed higher expression in all four datasets (all $P<0.01$, Fig. 1A and D).

Considering the pathological molecular characteristics, similarly, the expression of LTBP1 and LTBP2 were obviously elevated in IDH1/2 wildtype gliomas in TCGA, CGGA, and Gravendeel datasets (all $P<0.01$), suggesting that LTBP1 and LTBP2 expression might be related to the malignant degree of gliomas (Fig. 2E and G). However, expressions of LTBP3 and LTBP4 weren't completely consistent in gliomas(Fig. 2E and G), suggesting that the relationship between their expression and glioma malignancy needed to be verified by more samples.

LTBP2 was highly expressed in mesenchymal gliomas

Clinically, GBM could be divided into three major transcriptionally defined subtypes [24]. Mesenchymal GBM always has the worst prognosis of all subtypes [25, 26]. LTBP2 expression was dramatically elevated in ME subtypes than in other two subtypes (all $P<0.001$,Fig. 3A). LTBP1 expression was elevated in ME subtypes compared with PN subtype in TCGA, Rembrandt and

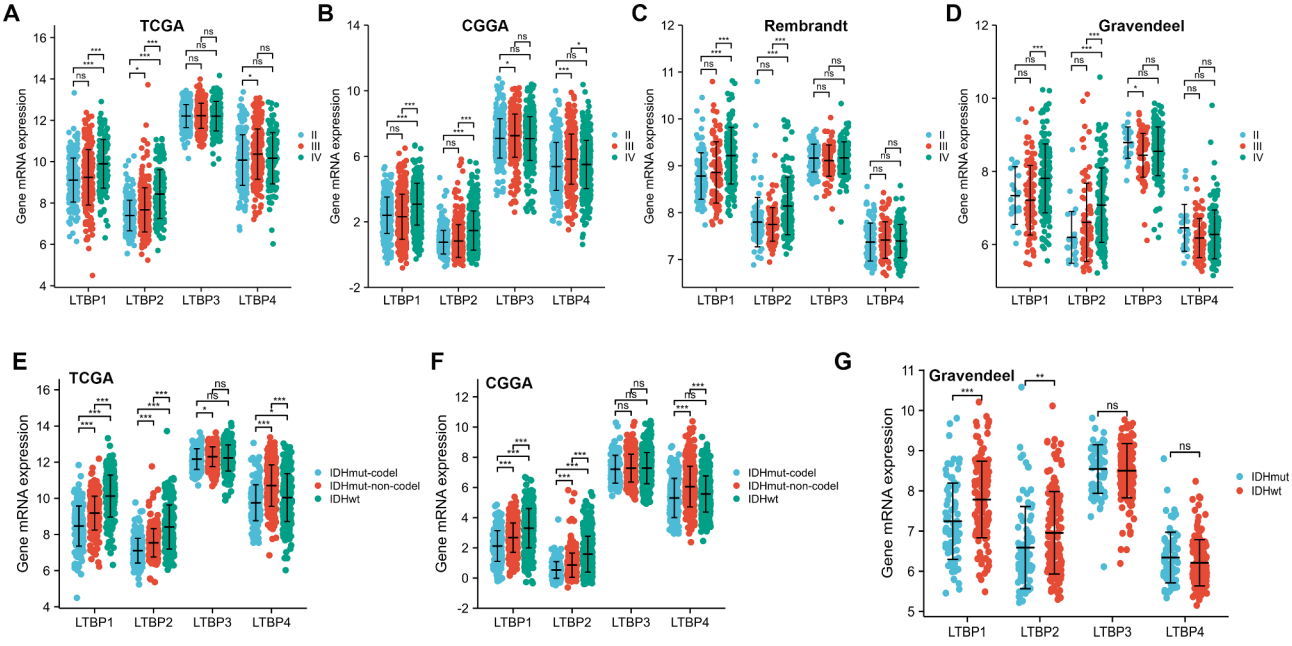

Fig. 2 Correlations between LTBPs expression and glioma malignancy **A-D**. Expression of LTBPs in different gliomas WHO grades in TCGA and CCGA. **E-G**. LTBPs expression in IDH1/2 WT/Mut and 1p19q codel/non-codel of gliomas in TCGA and CCGA. IDH1, isocitrate dehydrogenase 1. WT, wildtype. Mut, mutant. **, $P<0.01$. ***, $P<0.001$. ns, no significance

Gravendeel (all $P<0.001$,Fig. 3A), while no difference was observed between ME and CL subtypes in all four datasets (all $P>0.05$,Fig. 3A). There was no significant difference in the expression of LTBP4 among the three subtypes ($P>0.05$,Fig. 3A), except in the CGGA database. Based on ME signatures associated genes [27], we found that LTBP2 expression positively correlated TNFAIP3 COL1A1, VIM and CHI3L1 in TCGA, CGGA, Rembrandt and Gravendeel datasets (Fig. 3B, all spearman $r>0.5$, all $P<0.05$). These results indicated that among the LTBP family, only LTBP2 might be associated with the malignant progression of epithelial-mesenchymal transition in gliomas.

LTBPs predicted prognosis in gliomas

Gliomas patients with low LTBP1 expression were significantly categorized into longer survival groups according to the TCGA, CGGA, and Rembrandt datasets. GBM patients with high LTBP1 expression showed significantly associated with worse outcome in CGGA, TCGA and Gravendeel, while no difference was found in Rembrandt(Fig. 4A). Overall survival (OS) rates for GBM patients in LTBP2^{high} group were dramatically lower than patients in LTBP2^{low} group in TCGA, CGGA, and Gravendeel (HR=1.24,1.34 and 1.85, all $P<0.05$,respectively). Actually, glioma or LGG patients in LTBP2^{high} group also showed lower OS rates than patients in LTBP2^{low} group (Fig. 4B, Supplementary material 1). However, LTBP3 only showed the ability to discriminate the prognosis of GBM patients in Rembrandt. Even in all-gliomas and LGG patients, the impact of LTBP3 on patients' OS rates showed no difference in TCGA, CGGA, Rembrandt and Gravendeel(Fig. 4C, Figure S1). Moreover, there was obviously higher OS in GBM-LTBP4^{high} group than those expressed low LTBP4 in TCGA (HR=0.81, 1.519, respectively, $P<0.05$). Gliomas and LGG patients with low LTBP4 expression also predicted favor prognosis in TCGA and CGGA (Fig. 4D, Figure S1).

In order to illustrate the ability of LTBPs to predict prognosis in gliomas, we used ROC curves. The results showed that LTBP2 expression has the highest value among LTBP family in predicting survival rates of glioma patients (1 -, 3 - and 5-year, AUC=0.65,0.63 and 0.84, respectively, Supplementary material 2). Considering the relationship between LTBP2 and the malignancy and prognosis of glioma, we would focus on the carcinogenic role of LTBP2 in glioma in the subsequent studies.

LTBP2 related to macrophage infiltration in gliomas

According to the level of LTBP2 in TCGA-GBM dataset, we divided glioma samples into two groups to screen differential genes. Functional enrichment analysis of the DEGs was performed using Database for Annotation, Visualization, and Integrated Discovery (DAVID, https://david.ncifcrf.gov/.). The results revealed enrichment in genes related to "extracellular matrix organization (GO:0030198),humoral immune response (GO:0006959)" (Supplementary material 3). In order to further uncover its potential functions of LTBP2, we used public platform. ESTIMATE that was designed to download

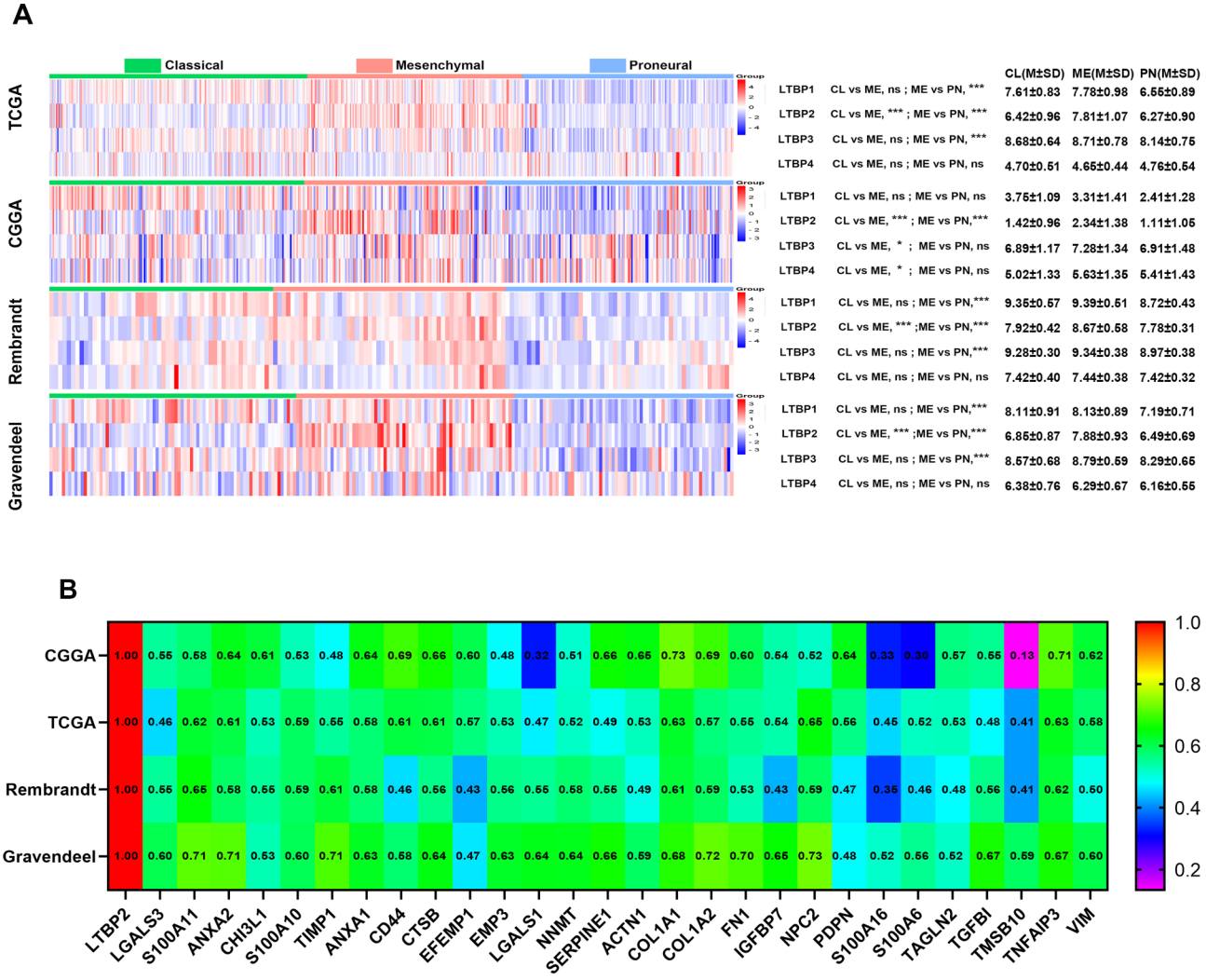

Fig. 3 LTBP2 was highly expressed in mesenchymal gliomas. **A**. Heat maps showed mRNA level of LTBPs in three glioma subtypes in 4 public datasets. **B**. Correlation between LTBPs and 29 ME-related markers in TCGA, CGGA, Rembrandt and Gravendeel datasets, respectively. Spearman correlation analysis was employed. *, $P < 0.05$. ***, $P < 0.001$. ns, no significance

stromal, immune, and ESTIMATE scores for each sample of all tumor types. Our preliminary results showed that LTBP2 expression positively correlated with glioma stromal, immune, and ESTIMATE scores (Spearman r=0.68,0.56 and 0.63, respectively, all P<0.01, Fig. 5A-5 C)). Next, we found the strongest relationship between LTBP2 and the degree of macrophage infiltration (Fig. 5D & Supplementary material 3). Moreover, LTBP2 level positively correlated with the level of macrophage markers CD163, CD68, CD86, CD11b and CD206 in TCGA and CGGA datasets (Table 3). These results indicated that LTBP2 might be crucial gene in mediating the infiltration of immune cells, especially for macrophages. In order to verify these findings, we performed tissue IHC and IF staining. We found that high LTBP2 expression always accompanied by high level of macrophage infiltration, just as the results of macrophage markers (CD163, CD11b, CD68) staining shown in IHC (Fig. 6A) and IF (Fig. 6B).

LTBP2 significantly associated with glioma malignancy in In-house cohort

Further experimental validation was conducted in order to illustrate the expression pattern of LTBP2 in gliomas. Baseline information of in-house cohort patients was presented in Table 4. Western blot analysis revealed elevated levels of LTBP2 in glioma tissues compared with NBT (Fig. 7A and B). In parallel, IHC analysis revealed significantly higher LTBP2 expression in glioma tissues, which was associated with higher WHO grades in gliomas (Fig. 7C and E). Interestingly, we found LTBP2 expression was obviously elevated in IDH1/2 wildtype both in LGG and GBM tissues using IHC staining (Fig. 7F and G).

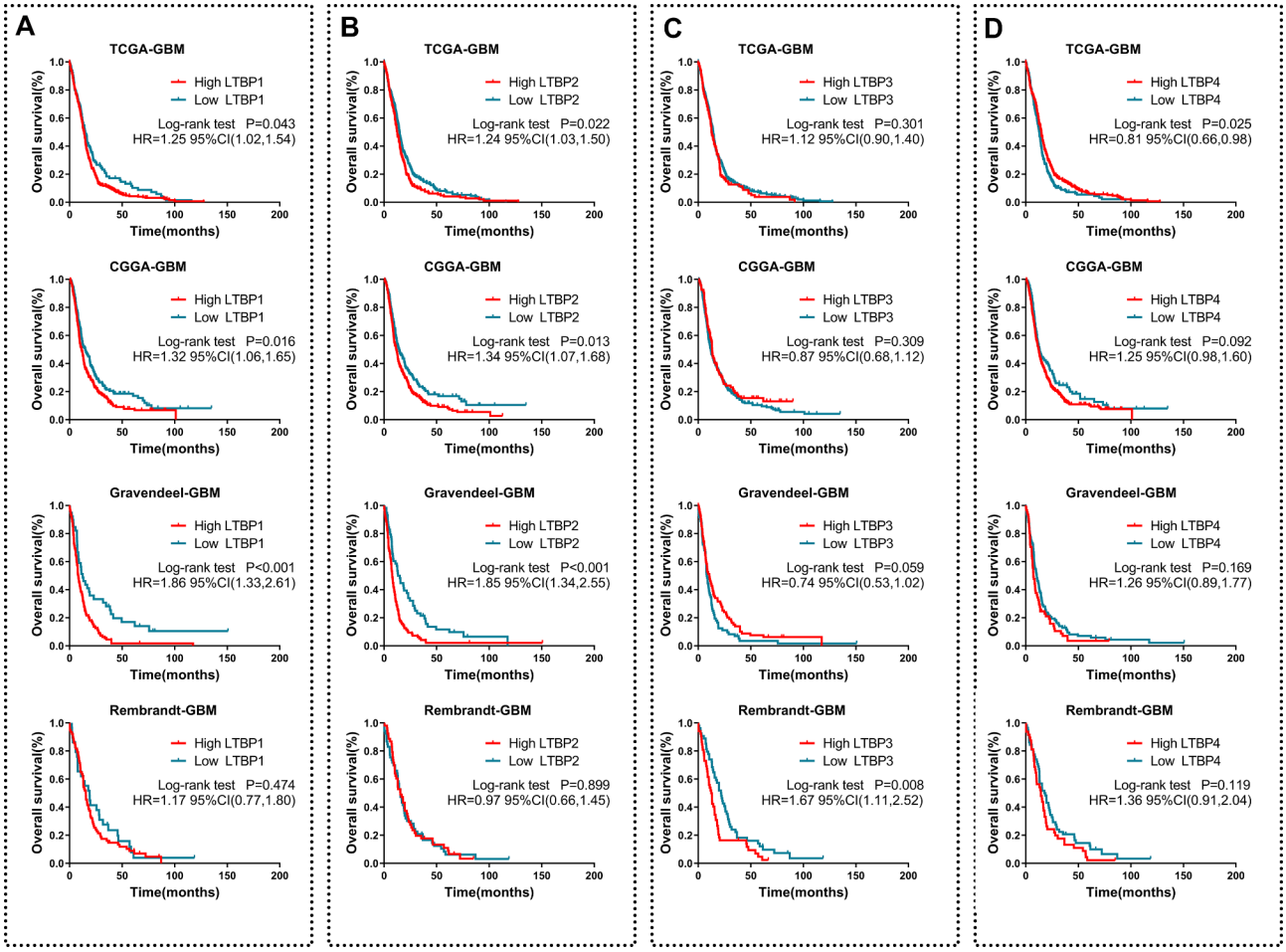

Fig. 4 LTBPs predicted prognosis in glioma Relationship between LTBP2 expression and prognosis of glioma patients. Kaplan–Meier analysis was employed. Overall survival data of LGG (WHO I–III) and GBM (WHO IV) patients were analyzed. We used optimal cutoff points which were determined by Gliovis. Data were acquired from Gliovis

LTBP2 enhanced glioma cell proliferation and induced TMZ resistance

We used shRNA-LTBP2 to knockdown its expression in U87 and U251 and LTBP2 expression was dramatically reduced after transfecting with shRAN-LTBP2 (Fig. 8A C, all $P<0.01$). Besides, the results of CCK8 showed that sh-LTBP2 effectively reduced proliferation ability of U87 and U251 cells (Fig. 8D and E, all $P<0.01$). TMZ resistance contributed to glioma progression and tumor recurrence. We found that LTBP2 knockdown resulted in enhanced more apoptotic cells to TMZ in U87 and U251 cells, which was accompanied by reduced BCL2 expression (Fig. 8A,8 F-8 H).

LTBP2 slicing suppressed glioma cell proliferation and macrophage infiltration in vivo

We employed LTBP2 shRNA to stable knockdown GBM cells and to generate an in vivo orthotopic xenograft glioma model. We found that the mice implanted with LTBP2 shRNA developed smaller brain tumors than the mice in control groups. Besides, IHC staining revealed that macrophage infiltration in LTBP2 knockdown glioma tissues was dramatically reduced when compared with NC-shRNA group (Fig. 9).

LTBP2 associated with chemotherapy response to gliomas

According to TCGA datasets, we found that LTBP2 expression was obviously higher in gliomas with unmethylated MGMT promoter than in those with methylated MGMT promoter (Fig. 10A). Interestingly, gliomas patients with methylated MGMT promoter in low LTBP2 group survival longest (median survival: 114.1 months) and patients with unmethylated MGMT promoter in high LTBP2 group had the worst prognosis among the four groups (median survival: 16.6 months) (Fig. 10B). In TCGA datasets, GBM patients received chemoradiotherapy (alkylating agents combined with IR) with unmethylated MGMT promoter in high LBTP2 group had lower OS than those in low LBTP2 group (Fig. 10C), while this was not the case for GBM patients received

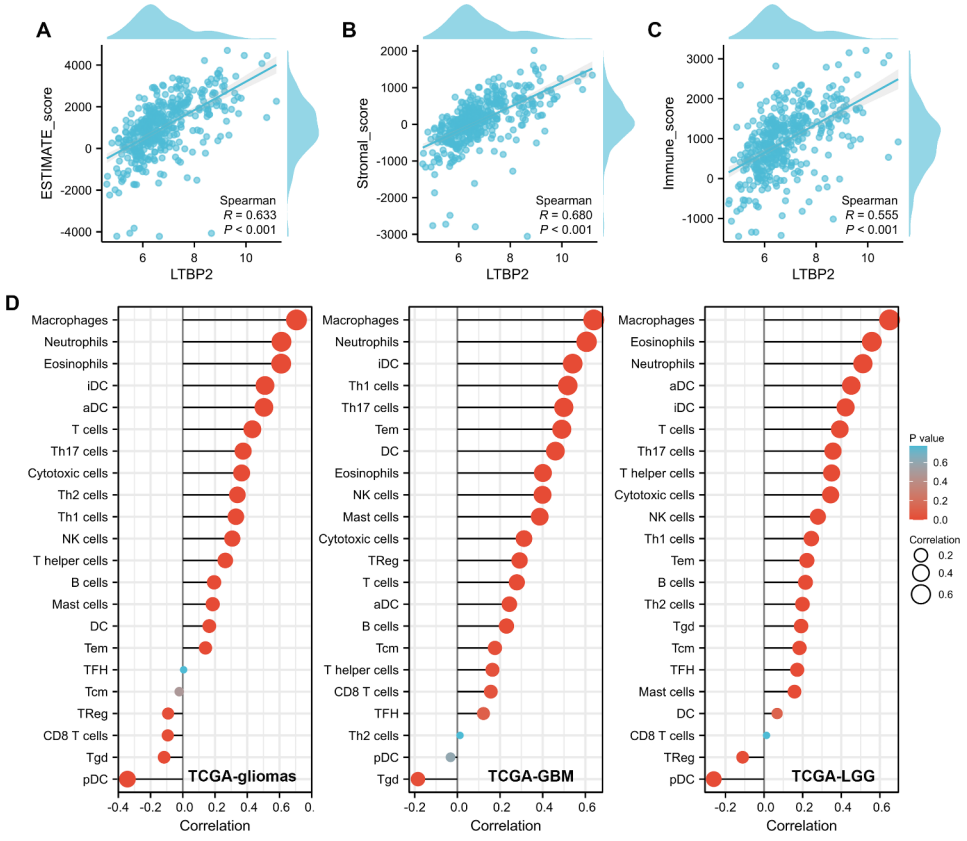

Fig. 5 LTBP2 correlated with immune cell infiltration in gliomas. **A-C**. Relationship between LTBP2 expression and glioma stromal, immune, and ESTIMATE scores, respectively. Spearman correlation analysis was employed. **D**. Correlations between immune cells infiltration in glioma tissues and LTBP2 expression in TCGA database

Table 3 Correlations between LTBP2 and immune cell markers in public datasets

Immune Cells	TCGA				CGGA		
	Markers	Cor	95%CI	p Value	Cor	95%CI	p Value
CD8 + T cell	CD8A	0.37	0.30–0.44	< 0.001	0.51	0.46–0.55	< 0.001
	CD8B	0.33	0.35–0.40	< 0.001	0.52	0.47–0.56	< 0.001
T cell	CD3D	0.51	0.45–0.56	< 0.001	0.43	0.37–0.48	< 0.001
	CD3E	0.55	0.48–0.59	< 0.001	0.61	0.56–0.65	< 0.001
B cell	CD86	0.56	0.51–0.61	< 0.001	0.59	0.55–0.63	< 0.001
	CD79A	0.24	0.16–0.31	< 0.001	0.25	0.19–0.31	< 0.001
	CSF1R	0.47	0.41–0.52	< 0.001	0.53	0.48–0.57	< 0.001
Monocyte	CCL2	0.61	0.55–0.65	< 0.001	0.60	0.56–0.64	< 0.001
	CD68	0.58	0.52–0.63	< 0.001	0.62	0.58–0.65	< 0.001
Macrophage	NOS2	0.01	0.00–0.09	0.83	0.07	0.01–0.14	0.156
	CD163	0.56	0.51–0.61	< 0.001	0.69	0.66–0.72	< 0.001
	IRF5	0.51	0.45–0.56	< 0.001	0.53	0.48–0.57	< 0.001
	PTGS2	0.40	0.33–0.46	< 0.001	0.56	0.41–0.51	< 0.001
	MS4A4A	0.56	0.52–0.62	< 0.001	0.64	0.60–0.68	< 0.001
	ITGAM	0.58	0.53–0.64	< 0.001	0.63	0.59–0.67	< 0.001
Neutrophil	CCR7	0.40	0.34–0.47	< 0.001	0.600	0.55–0.63	< 0.001
	PDCD1	0.44	0.37–0.50	< 0.001	0.54	0.49–0.58	< 0.001
T cell exhaustion	CTLA4	0.38	0.31–0.44	< 0.001	0.40	0.34–0.45	< 0.001
	LAG3	0.17	0.08–0.24	< 0.001	0.12	0.06–0.19	< 0.001
	HAVCR2	0.55	0.50–0.61	< 0.001	0.60	0.56–0.64	< 0.001
	BTLA	0.40	0.33–0.47	< 0.001	0.40	0.35–0.45	< 0.001

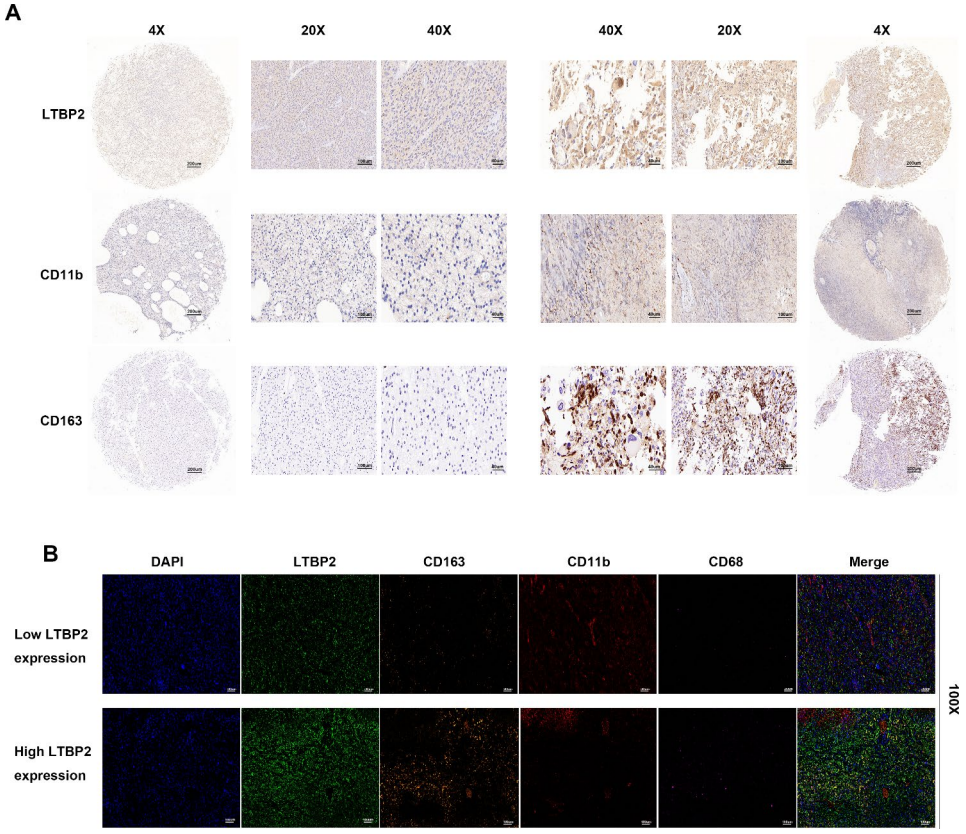

Fig. 6 LTBP2 related with immune cells infiltration in gliomas tissues. **A**. We employed IHC to detect macrophage markers, namely CD11b and CD163 in high and low LTBP2 expression glioma tissues. **B**. multi-IF staining of CD68, CD11b and CD163 in glioma tissues. Nuclei were stained by DAPI

Table 4 Comparison of clinical characteristics in different LTBP2 expression groups among glioma patients in in-house cohort

Factors	LTBP2 expression		
	Low	High	P value
Age (mean ± SD)	44.72 ± 14.68	49.13 ± 13.20	0.43
Gender			0.70
Female	21	31	
Male	26	32	
Karnofsky score			0.99
> 80	45	61	
≤ 70	2	2	
WHO Grade			0.008
I-II	23	15	
III-IV	24	48	
Tumor type			0.99
Primary	43	58	
Recurrence	4	5	
MGMT promoter methylation			0.99
Positive	42	56	
Negative	5	7	
IDH1/2 status			0.14
Wildtype	16	27	
Mutant	10	6	
Chemotherapy	45	51	0.02
Radiotherapy	39	46	0.26

only IR (Fig. 10D). Moreover, in CGGA datasets, both for glioma patients and GBM patients, we found that patients with high LTBP2 expression who received only IR or combined with chemotherapy always had shorter survival time than patients in low LTBP2 group (Fig. 10E H). These results indicated that LTBP2 may influence chemoradiotherapy response and might act as a potential biomarker for chemoradiotherapy response predicting in patients with gliomas.

Discussion

Latent TGFβ binding protein 4 (LTBP-4) belongs to a family of four extracellular matrix (ECM) proteins (LTBP-1 to −4) that are structurally similar to the fibrillins. In this study, we found that LTBP1-2 expression was higher in gliomas compared to normal tissues, and its high expression predicted worse prognosis in LGG and GBM., which was a new finding. Further WB and IHC staining substantiated the notion that high LTBP2 correlated not just with OS, but also with even worse pathologic characteristics of the glioma. Moreover, glioma tissues with high LTBP2 expression always accompanied by high level infiltration of macrophage. Slicing LTBP2 not only reduced glioma cell proliferation and TMZ resistance in vitro, but also inhibited tumor growth

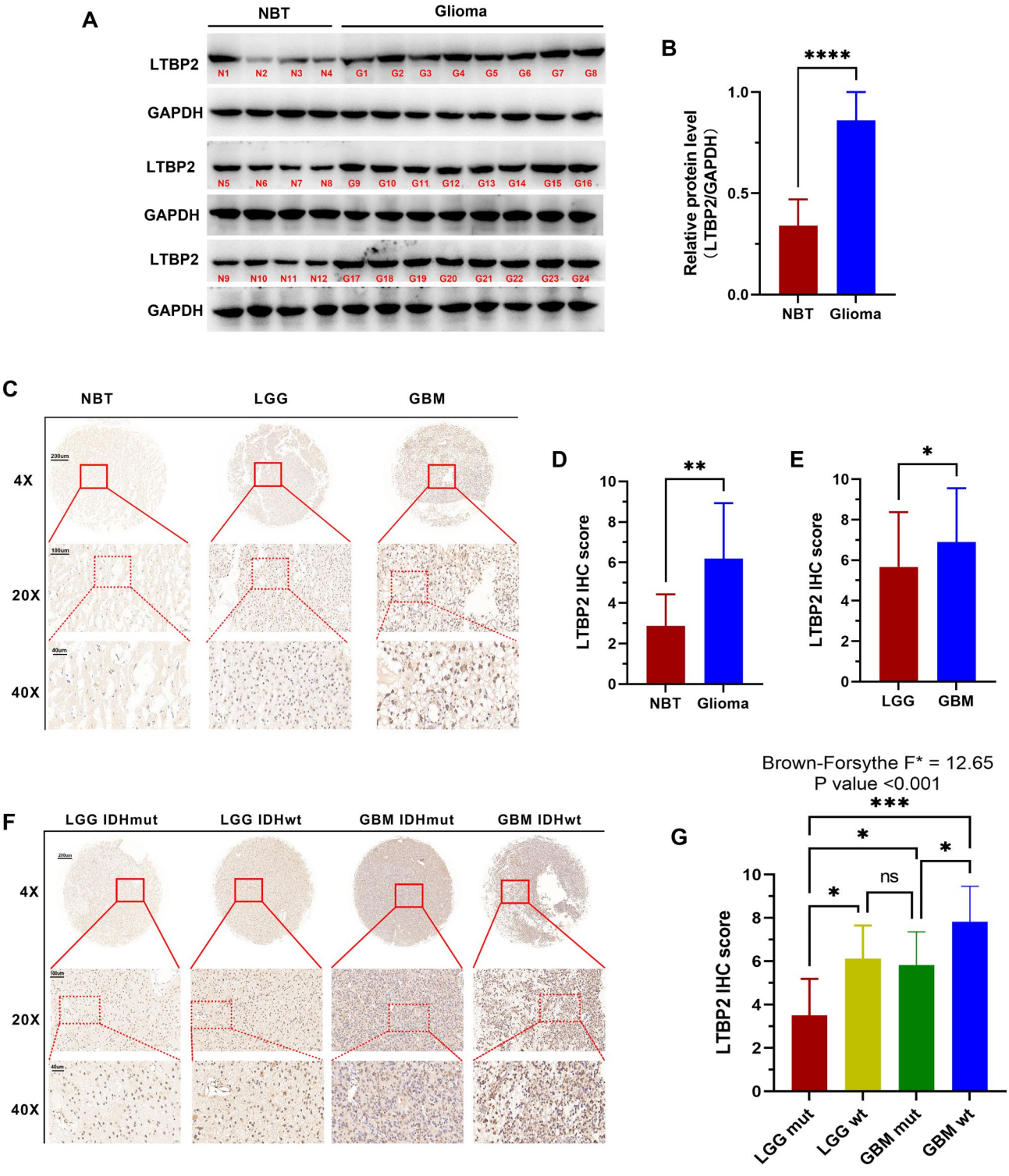

Fig. 7 LTBP2 significantly associated with glioma malignancy in In-house cohort. **A-B**. LTBP2 level in gliomas and NBT detected by WB. GAPDH was used as loading control. **C-E**. IHC staining of LTBP2 in all grades glioma tissues. LTBP2 protein detection in different IDH1/2 status. **F-G**. Representative IHC staining of LTBP2 were presented. *, $P < 0.05$. **, $P < 0.01$

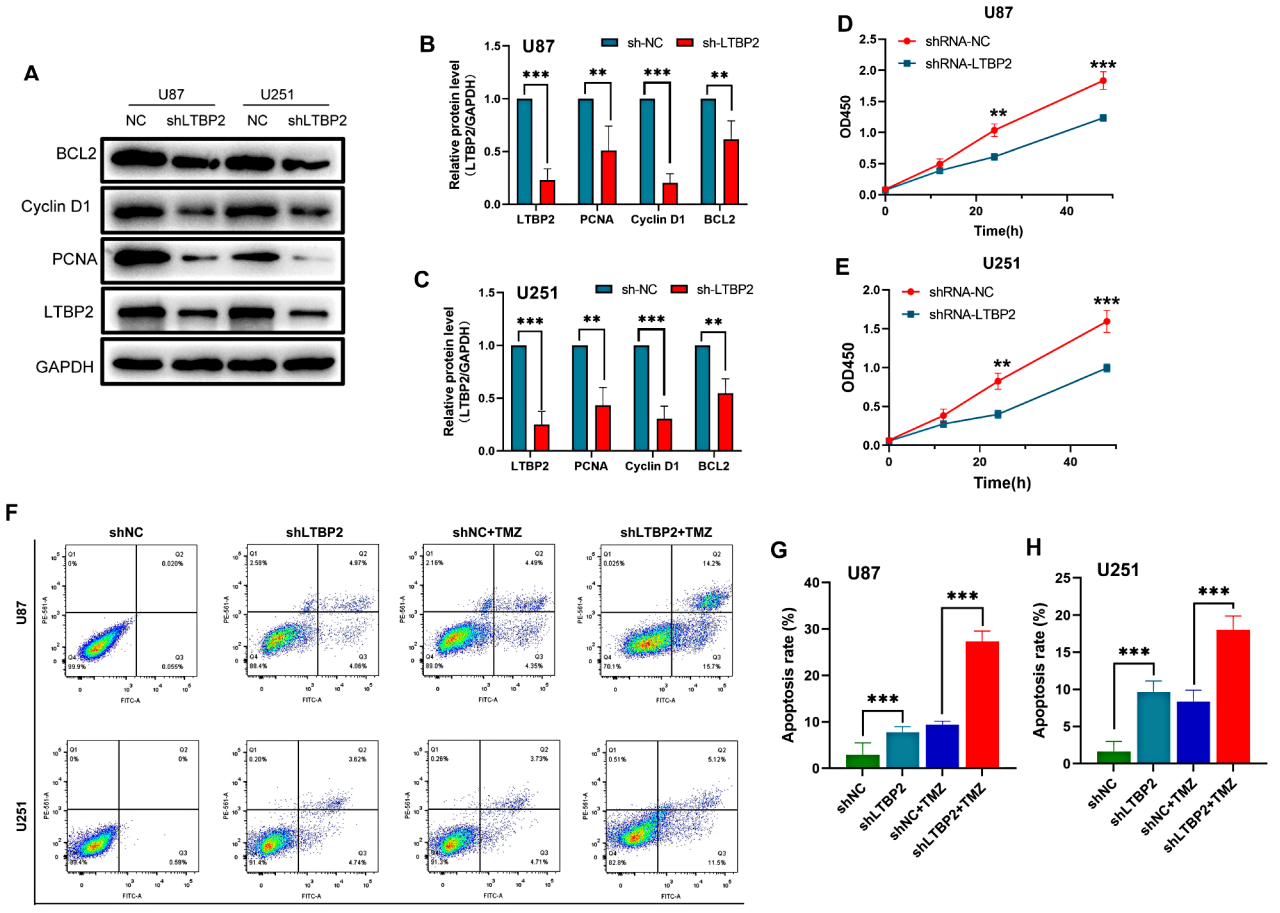

Fig. 8 LTBP2 promoted glioma cell proliferation and induced TMZ resistance. **A-C**: shRAN-LTBP2 stable transfected U87 and U251 were constructed. WB was used to detect level of LTBP2,PCNA,BCL2 and cyclinD1. **D-E**: CCK8 was performed to detect the proliferation of U251 and U87. the OD value was measured with a microplate reader at 450 nm. **F-H**: glioma cells were treated with TMZ (200uM) and then cells were stained with Annexin V-FITC followed by flow cytometric analysis. **, $P < 0.01$. ***, $P < 0.001$

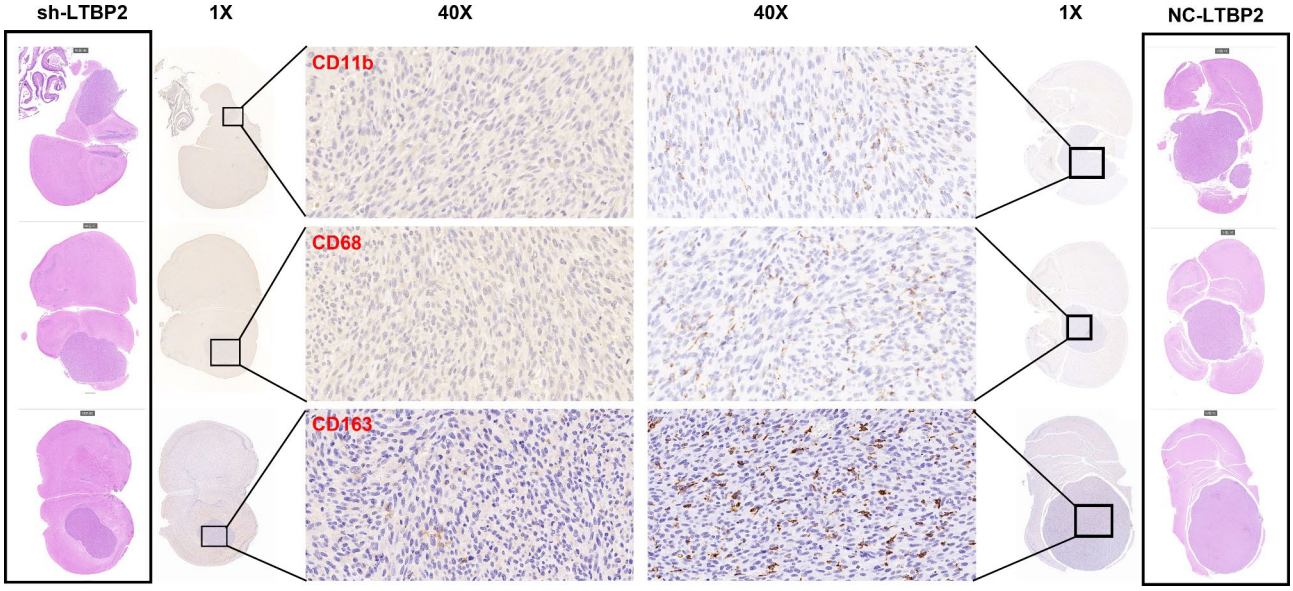

Fig. 9 LTBP2 related to macrophage infiltration in glioma tissues in vivo LTBP2 shRNA GBM cells was used and generated an intracranial xenograft model in vivo. Macrophage markers, CD11b, CD68 and CD163 were detected by IHC in xenograft model tissues

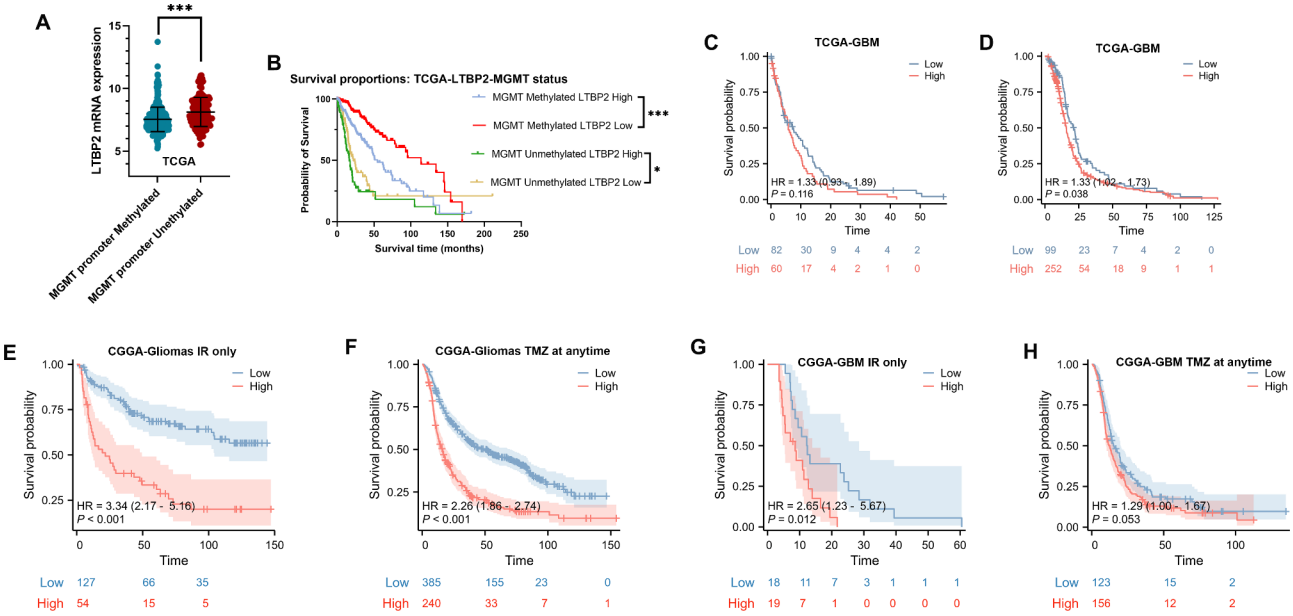

Fig. 10 LTBP2 associated with chemoradiotherapy response to gliomas. **A.** LTBP2 expression in different MGMT promoter methylated status in TCGA dataset. **B.** Based on MGMT promoter methylated status and LTBP2 expression, glioma patients were divided into four groups and log-rank tests were used to compare survival curves. Patients in TCGA-GBM cohort were separated by treatments (IR **(C)** or combined with chemotherapy **(D)**). KM curves according to LTBP2 expression are generated in TCGA-GBM dataset. In CGGA dataset, glioma and GBM patients who received only IR (**E, G**) or combined with chemotherapy (**F, H**) were separated. Association between LTBP2 expression and prognosis was evaluated and KM analysis was employed for survival evaluation

and macrophage infiltration in vivo. Our study revealed that LTBP2 could be used as a reliable prognostic marker in gliomas and LTBP2 might be involved in remodeling tumor microenvironment, especially the infiltration of macrophage.

LTBP family has long been explored for its role in extracellular matrix remodeling because it is an important glycoprotein that activates TGFβ [28]. LTBP could initiate the TGF-beta signaling and promoted the migration/invasion of tumor cells [29]. TGFβ was found to bind the promoter of LTBP3 and enhanced the transcriptional activity, which subsequently increased LTBP3 mRNA and MEK/erk pathway [30]. Recently, emerging studies have found that the LTBPs participated in the malignant biological progression of tumors. Ying Huang, et al. found that LTBP2 level was elevated in Colorectal cancer tissues than its paired normal colorectal tissues. Moreover, increased LTBP2 level significantly accompanied by higher Tumor, node, metastasis (TNM) stage, mesenchymal subtypes and also worse OS time in colorectal cancer patients [31]. Interestingly, LTBP2 was also presented to be obviously over-expressed in both gastric cancer and Pancreatic Carcinoma, and LTBP2 could be used as a prognostic indicator and potential therapeutic target [15, 32]. Previous studies have shown that different members of LTBP family might play different roles (promoting or suppressing cancer) in different tumors. By analyzing the expression of LTBP family in glioma in public databases and our own cohort, we uncovered LTBP2 might be the family member more crucial in promoting the biological process of glioma.

GBM could be divided into three molecular subtypes, namely proneural, classical, and mesenchymal). Proneural glioma always accompanied by IDH1/2 mutations and off course had better prognosis. While on the contrary, mesenchymal GBM frequently exhibits therapy-resistance characteristics with adverse prognosis. Our preliminary findings showed that LTBP2 was mostly enriched in mesenchymal GBM in all four public datasets. Previous studies showed Inhibition of LTBP2 reduced the expression of mmp2 and mmp9, and also reduced the invasion by regulating NF-kb signaling in endometrial stromal cell [33]. circEPSTI1 significantly promoted Oral squamous cell carcinoma (OSCC) cell proliferation and accelerated EMT by increase LTBP2 expression via PI3K/Akt/mTOR signaling pathway [34]. Combined with the previous results, we hypothesized that LTBP2 might play key role in mediating epithelial-mesenchymal transition (EMT). While in our study the validation of LTBP2 on cellular EMT was lacking.

The initiation and progression of gliomas always accompanied by tumor cell-immune cell interaction in glioma microenvironment. Stromal cells and glioma cells cooperated to remodel immune suppressive and also drug resistance micro- environment [35]. Using ESTIMATE platform, we found LTBP2 expression in glioma

positively correlated with stromal and immune score, which indicated its potential role of LTBP2 in modulating microenvironment in gliomas. Subsequent analysis further revealed that LTBP2, most likely, was closely related to the infiltration of macrophages in LGG and GBM tissues. Among LTBP family members, LTBP1 seemed closely associated with immune cell infiltration according to previous studies. WGCNA identified 15 co-expression modules in Pulmonary arterial hypertension (PAH) and LTBP1 has the highest diagnostic efficacy for PAH. Moreover, LTBP1 was found to be colocalized with CD4+cells and positively correlated with CD4+T cell infiltration in lungs [36]. In this study, LTBP2 expression positively correlated with many immune cell markers, especially macrophages, neutrophil, and monocytes. In vivo experiments confirmed that interfering with LTBP2 expression in glioma cells could significantly inhibit macrophage infiltration in gliomas tissues. Through the validation of these glioma samples and in vivo experiments, we confirmed that LTBP2 was involved in the regulation of the glioma immune microenvironment and might be a potential immunotherapeutic target. The infiltration of macrophages, especially M2 type, in glioma tissues was a key factor causing TMZ resistance and malignant progression. LTBP2 could be confirmed to mediate TMZ resistance in vitro and was found to induce more M2 macrophage infiltration in animal models. Our further research may focus on investigating whether LTBP2 can induce TMZ resistance by inducing macrophage infiltration or M2 polarization, which may bring new therapeutic targets for glioma immunotherapy.

Conclusion

LTBP2 was a novel oncogene that closely associated with glioma malignancy. LTBP2 could be used as a prognostic biomarker and a potential chemotherapy response marker in gliomas.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-12976-2.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Acknowledgements
Not applicable.

Author contributions
LJH, GL and ZR : Designed the study, Review and language polishing; LYF, LXP and CQ: Validation and writing; ZWB and LJH: Contributed to preparing the figures and tables.

Funding
This investigation was financially supported by the National Natural Science Foundation of China (grant no. 82203136, 82200391).
Authors' contributions and materials: LJH, GL and ZR : Designed the study, Review and language polishing; LYF, LXP and CQ: Validation and writing; ZWB and LJH: Contributed to preparing the figures and tables.

Data availability
The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate
This study was approved by the institutional ethics committee of the faculty of medicine at Wuhan University's Renmin Hospital [approval number: 2012LKSZ (010) H].

Consent for publication
Not applicable.

Animal ethics
This study passed the Ethical Approval for Research Involving Animals of Renmin Hospital of Wuhan University [approval number: 20231002 A].

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Neurosurgery, Renmin Hospital of Wuhan University, Wuhan 430060, China
[2]Department of Critical Care Medicine, Renmin Hospital of Wuhan University, Wuhan 430060, China
[3]Department of Oncology, Renmin Hospital of Wuhan University, Wuhan 430060, China

Received: 1 May 2024 / Accepted: 23 September 2024
Published online: 10 October 2024

References

1. Ostrom QT, Gittleman H, Truitt G, Boscia A, Kruchko C, Barnholtz-Sloan JS. Cbtrus statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2011–2015. Neurooncology. 2018;20:iv1–86.
2. Ostrom QT, Cote DJ, Ascha M, Kruchko C, Barnholtz-Sloan JS. Adult glioma incidence and survival by race or ethnicity in the United States from 2000 to 2014. JAMA Oncol. 2018;4:1254–62.
3. Molinaro AM, Taylor JW, Wiencke JK, Wrensch MR. Genetic and molecular epidemiology of adult diffuse glioma. Nat Rev Neurol. 2019;15:405–17.
4. Rong L, Li N, Zhang Z. Emerging therapies for glioblastoma: current state and future directions. J Exp Clin Cancer Res. 2022;41:142.
5. Tan AC, Ashley DM, Lopez GY, Malinzak M, Friedman HS, Khasraw M. Management of glioblastoma: state of the art and future directions. CA-Cancer J Clin. 2020;70:299–312.
6. Gonzalez-Tablas PM, Otero A, Arandia GD, Pascual-Argente D, Ruiz ML, Sousa-Casasnovas P, Garcia-Martin A, Roa MDOJ, Villasenor-Ledezma J, Torres CL, et al. Tumor cell and immune cell profiles in primary human glioblastoma: impact on patient outcome. Brain Pathol. 2021;31:365–80.
7. Munger JS, Sheppard D. Cross talk among tgf-beta signaling pathways, integrins, and the extracellular matrix. Cold Spring Harbor Perspect Biol. 2011;3:a5017.
8. Rifkin DB, Rifkin WJ, Zilberberg L. Ltbps in biology and medicine: ltbp diseases. Matrix Biol. 2018;71–72:90–9.
9. Saharinen J, Keski-Oja J. Specific sequence motif of 8-cys repeats of tgf-beta binding proteins, ltbps, creates a hydrophobic interaction surface for binding of small latent tgf-beta. Mol Biol Cell. 2000;11:2691–704.

10. Saharinen J, Taipale J, Keski-Oja J. Association of the small latent transforming growth factor-beta with an eight cysteine repeat of its binding protein ltbp-1. Embo J. 1996;15:245–53.
11. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, et al. The integrin alpha v beta 6 binds and activates latent tgf beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell. 1999;96:319–28.
12. Yang Z, Mu Z, Dabovic B, Jurukovski V, Yu D, Sung J, Xiong X, Munger JS. Absence of integrin-mediated tgfbeta1 activation in vivo recapitulates the phenotype of tgfbeta1-null mice. J Cell Biol. 2007;176:787–93.
13. Mcinerney-Leo AM, Le Goff C, Leo PJ, Kenna TJ, Keith P, Harris JE, Steer R, Bole-Feysot C, Nitschke P, Kielty C, et al. Mutations in ltbp3 cause acromicric dysplasia and geleophysic dysplasia. J Med Genet. 2016;53:457–64.
14. Cai R, Wang P, Zhao X, Lu X, Deng R, Wang X, Su Z, Hong C, Lin J. Ltbp1 promotes esophageal squamous cell carcinoma progression through epithelial-mesenchymal transition and cancer-associated fibroblasts transformation. J Transl Med. 2020;18:139.
15. Wang J, Liang WJ, Min GT, Wang HP, Chen W, Yao N. Ltbp2 promotes the migration and invasion of gastric cancer cells and predicts poor outcome of patients with gastric cancer. Int J Oncol. 2018;52:1886–98.
16. Bowman RL, Wang Q, Carro A, Verhaak RG, Squatrito M. Gliovis data portal for visualization and analysis of brain tumor expression datasets. Neurooncology. 2017;19:139–41.
17. Gravendeel LA, Kouwenhoven MC, Gevaert O, de Rooi JJ, Stubbs AP, Duijm JE, Daemen A, Bleeker FE, Bralten LB, Kloosterhof NK, et al. Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. Cancer Res. 2009;69:9065–72.
18. Madhavan S, Zenklusen JC, Kotliarov Y, Sahni H, Fine HA, Buetow K. Rembrandt: helping personalized medicine become a reality through integrative translational research. Mol Cancer Res. 2009;7:157–67.
19. Gill BJ, Pisapia DJ, Malone HR, Goldstein H, Lei L, Sonabend A, Yun J, Samanamud J, Sims JS, Banu M, et al. Mri-localized biopsies reveal subtype-specific differences in molecular and cellular composition at the margins of glioblastoma. Proc Natl Acad Sci U S A. 2014;111:12550–5.
20. Murat A, Migliavacca E, Gorlia T, Lambiv WL, Shay T, Hamou MF, de Tribolet N, Regli L, Wick W, Kouwenhoven MC, et al. Stem cell-related self-renewal signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. J Clin Oncol. 2008;26:3015–24.
21. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, et al. The cbio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401–4.
22. Liu J, Zhu X, Gao L, Geng R, Tao X, Xu H, Chen Z. Expression and prognostic role of glia maturation factor-gamma in gliomas. Front Molec Neurosci. 2022;15:906762.
23. Liu J, Gao L, Zhu X, Geng R, Tao X, Xu H, Chen Z. Gasdermin d is a novel prognostic biomarker and relates to tmz response in glioblastoma. *Cancers* 2021, *13*.
24. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, Cahill DP, Nahed BV, Curry WT, Martuza RL, et al. Single-cell rna-seq highlights intratumoral heterogeneity in primary glioblastoma. Science. 2014;344:1396–401.
25. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Soroceanu L, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell. 2006;9:157–73.
26. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in pdgfra, idh1, egfr, and nf1. Cancer Cell. 2010;17:98–110.
27. Chanoch-Myers R, Wider A, Suva ML, Tirosh I. Elucidating the diversity of malignant mesenchymal states in glioblastoma by integrative analysis. Genome Med. 2022;14:106.
28. Saharinen J, Hyytiainen M, Taipale J, Keski-Oja J. Latent transforming growth factor-beta binding proteins (ltbps)--structural extracellular matrix proteins for targeting tgf-beta action. Cytokine Growth Factor Rev. 1999;10:99–117.
29. Taipale J, Saharinen J, Keski-Oja J. Extracellular matrix-associated transforming growth factor-beta: role in cancer cell growth and invasion. Adv Cancer Res. 1998;75:87–134.
30. Kantola AK, Keski-Oja J, Koli K. Induction of human ltbp-3 promoter activity by tgf-beta1 is mediated by smad3/4 and ap-1 binding elements. Gene. 2005;363:142–50.
31. Huang Y, Wang G, Zhao C, Geng R, Zhang S, Wang W, Chen J, Liu H, Wang X. High expression of ltbp2 contributes to poor prognosis in colorectal cancer patients and correlates with the mesenchymal colorectal cancer subtype. *Dis. Markers* 2019, *2019*, 5231269.
32. Wang C, Wang G, Zhang L, Pan J, Wei Y. Latent transforming growth factor beta binding protein 2 (ltbp2) as a novel biomarker for the diagnosis and prognosis of pancreatic carcinoma. Med Sci Monit. 2017;23:3232–9.
33. Wang D, Zhang Y, Cui L, Yang Q, Wang J. Elevated latent transforming growth factor beta binding protein 2 in endometriosis promotes endometrial stromal cell invasion and proliferation via the nf-kb signaling pathway. Mol Cell Endocrinol. 2022;550:111647.
34. Wang J, Jiang C, Li N, Wang F, Xu Y, Shen Z, Yang L, Li Z, He C. The circepsti1/mir-942-5p/ltbp2 axis regulates the progression of oscc in the background of osf via emt and the pi3k/akt/mtor pathway. Cell Death Dis. 2020;11:682.
35. Patnaik A, Swanson KD, Csizmadia E, Solanki A, Landon-Brace N, Gehring MP, Helenius K, Olson BM, Pyzer AR, Wang LC, et al. Cabozantinib eradicates advanced murine prostate cancer by activating antitumor innate immunity. Cancer Discov. 2017;7:750–65.
36. Lian G, You J, Lin W, Gao G, Xu C, Wang H, Luo L. Bioinformatics analysis of the immune cell infiltration characteristics and correlation with crucial diagnostic markers in pulmonary arterial hypertension. BMC Pulm Med. 2023;23:300.

Publisher's note