Original Article

Bromodomain containing 4 transcriptionally activated Deltex E3 ubiquitin ligase 2 contributes to glioma progression and predicts an unfavorable prognosis

Shizhong Wu[1,2#], Shang-Hang Shen[3#], Feng Lu[1,2], Pengfeng Zheng[1,2], Kun Lin[1,2], Jingwei Liao[1,2], Xiaohang Jiang[1,2], Guangming Zeng[1,2], De Wei[1,4,5]

[1]Department of Neurosurgery, Fujian Provincial Hospital South Branch, Fuzhou, China; [2]Department of Neurosurgery, Shengli Clinical Medical College of Fujian Medical University, Fuzhou, China; [3]Department of Neurosurgery, The First Affiliated Hospital of Xiamen University, Medical College of Xiamen University, Xiamen, China; [4]Department of Neurosurgery, Fujian Provincial Hospital, Fuzhou, China; [5]Department of Neurosurgery, Shengli Clinical Medical College of Fujian Medical University, Fuzhou, China

Contributions: (I) Conception and design: S Wu; (II) Administrative support: SH Shen; (III) Provision of study materials or patients: F Lu; (IV) Collection and assembly of data: P Zheng, K Lin, J Liao, X Jiang, G Zeng; (V) Data analysis and interpretation: D Wei; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: De Wei. Department of Neurosurgery, Fujian Provincial Hospital South Branch, Fuzhou 350001, China; Department of Neurosurgery, Fujian Provincial Hospital, Fuzhou 350001, China; Department of Neurosurgery, Shengli Clinical Medical College of Fujian Medical University, Fuzhou 350001, China. Email: weidele@fjmu.edu.cn.

Background: Glioblastoma multiforme (GBM) is the most common type of glioma, and the most aggressive brain malignancy in adults. This study sought to identify novel survival-status related markers, and examine their function in glioma.

Methods: The gene expression, survival heatmaps, and Kaplan-Meier survival plots of the genes were analyzed by using gene expression profiling interactive analysis (GEPIA) dataset, Linked Omics. The single-cell data analysis and tumor immune infiltration analysis was conducted by Tumor Immune Estimation Resource (TIMER) dataset. DBTRG and U251 cells with silenced Deltex E3 ubiquitin ligase 2 (*DTX2*) expression were constructed and used for Cell Counting Kit 8 (CCK-8), and wound healing assay in vitro. Chromatin immunoprecipitation sequencing (ChIP-seq) analysis was used to explore the histone activation marks and transcription factors *DTX2* promoter. Dual-luciferase assays were carried out to detect the luciferase activities of bromodomain containing 4 (*BRD4*) binding to *DTX2*.

Results: We first conducted a survival-status analysis to identify survival status-related genes in The Cancer Genome Atlas GBM and low-grade glioma data sets. A subsequent analysis identified 3 novel prognostic biomarkers; that is, *DTX2*, cytochrome P450 oxidoreductase, and Williams-Beuren syndrome chromosomal region 16 protein. In the validation Chinese Glioma Genome Atlas data sets, *DTX2* showed the best performance, and was examined in a further analysis. Next, 3 short-hairpin ribonucleic acids were designed to silence *DTX2* expression, and CCK-8 and wound-healing assays were applied to study the function of *DTX2*. We found that *DTX2*-silenced glioma cells exhibited a significant decrease in their growth and migration capabilities. Finally, the molecular basis for increased *DTX2* in glioma was investigated via ChIP-Seq analysis and luciferase assays. The analysis revealed that *DTX2* was transcriptionally activated by *BRD4*.

Conclusions: In conclusion, *BRD4* transcriptionally activates *DTX2*, contributes to glioma progression, predicts an unfavorable prognosis, and could provide new options for glioma prognosis prediction and treatment.

Keywords: Glioma; survival status; Deltex E3 ubiquitin ligase 2 (DTX2); bromodomain containing 4 (BRD4)

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Introduction

Gliomas are the most common brain tumors. Under the World Health Organization (WHO) 2016 classification system, there are 5 subtypes of glioma, which are categorized based on tumor morphology and molecular genetics (1). Glioblastoma multiforme (GBM) is the most common type of glioma, and the most aggressive brain malignancy in adults (2,3). Diagnostic (4,5) and therapeutic (6-10) advances have been made, but patients with GBM still have a poor prognosis (2,11). Molecular alterations [e.g., isocitrate dehydrogenase 1 (*IDH1*) mutations, telomerase reverse transcriptase promoter mutations, and 1p/19q co-deletions] have been observed in glioma (12,13), and glioma patients with distinct molecular subtypes show different outcomes and therapeutic responses (14). The identification of novel survival status-related markers and the validation of their functions in glioma could extend current understandings of glioma and shed light on drug development.

It is well established that disturbed signaling transduction mediated by oncogenes is a contributor to cancer cell malignant behaviors and the pathogenesis of glioma (15). Additionally, oncogenes have prognostic significance as biomarkers (16,17). The human Deltex protein family, which functions as ubiquitin E3 ligases, comprises 5 members [i.e., Deltex E3 ubiquitin ligase (*DTX*) 1, *DTX2*, *DTX3*, *DTX3L*, and *DTX4*] (18). The *DTX* family members act as Notch signaling modifiers that control cell fate determination (19), and the pathogenesis and progression of various tumors (18,20,21). The effects of *DTX2* on cell differentiation were demonstrated, *DTX2* changes the methylation status of H3K9 in the distal regulatory region of the MyoD promoter and directly inhibits demethylase activity of Jumonji domain-containing 1C (JMJD1C) by monoubiquitination to reduce MyoD expression (22). However, studies of *DTX2* in cancer are limited, and very few studies of the role of *DTX2* in glioma have been conducted. In colon cancer patients, *DTX2* has been shown to serve as a predictor of relapse in high-risk stage II and III cancer samples (23). A recent study found that *DTX2* recognizes and recruits ADP-ribosylated proteins (24). The role of *DTX2* in glioma is yet unknown.

In the present study, we first conducted a survival-status analysis to identify survival status-related genes in The Cancer Genome Atlas (TCGA) GBM and low-grade glioma (LGG) data sets. Subsequently, we identified 3 novel prognostic makers, among which *DTX2* had the best performance in the validated Chinese Glioma Genome Atlas (CGGA) data sets. To examine the function of *DTX2* in glioma, short-hairpin ribonucleic acids (shRNAs) were designed to silence *DTX2* expression, and the growth and migration capabilities of 2 cell lines were examined. Finally, the molecular basis for increased *DTX2* in glioma was investigated, and *DTX2* was found to be transcriptionally activated by bromodomain containing 4 (*BRD4*). This study first uncovered the clinical significance, *in-vitro* functions and mechanism of *DTX2* in glioma, which could provide new options for glioma prognosis prediction and treatment. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-555/rc).

Methods

TCGA and CGGA data analysis

The survival-status analysis was conducted with the online tool, LinkedOmics (25). We used the LinkFinder in the LinkedOmics (http://www.linkedomics.org/) module to screen RNA expressions significantly correlated with the clinical parameter of survival status included in TCGA GBM and LGG data sets (http://www.linkedomics.org/login.php#dataSource). The gene expression, survival heatmaps, and Kaplan-Meier survival plots of the genes were analyzed with Gene Expression Profiling Interactive Analysis 2 (GEPIA 2.0) (26). For the CGGA data sets, 3 array data sets (i.e., Array 325, Array 301, and Array693) were used for the analysis (27). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Single-cell data and tumor-immune infiltration analysis

The single-cell data analysis was conducted with the online tool, Single-cell portal (https://singlecell.broadinstitute.org/single_cell). For the tumor-immune infiltration analysis, the Tumor Immune Estimation Resource (TIMER; cistrome.shinyapps.io/timer) was used (28).

Cell lines and cell cultures

DBTRG and U251 cell lines were preserved at our laboratory, and cultured in a humidified atmosphere of 5% carbon dioxide at 37 ℃. DBTRG and U251 were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (FBS). Thawed cells from liquid nitrogen

were used for the first 3 passages.

Real-time PCR

Total RNA was extracted from DBTRG and U251 cells using TRIzol® RNA Isolation Reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Reverse transcription was performed using the PrimeScript™ RT reagent kit (Takara, Dalian, China). All messenger RNA levels were normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The following *DTX2* primers were used in this study: 5'-GTCCACAGCGTCTGGATACAG-3' (F) and 5'-CCTTATTGCCGTTGCAGTACA-3' (R). The following *GAPDH* primers were used in this study: 5'-TGACTTCAACAGCGACACCCA-3' (F) and 5'-CACCCTGTTGCTGTAGCCAAA-3' (R). All samples were treated under the same conditions and analyzed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) using Synergy Brands (SYBR) Premix ExTaq™ (Takara, Dalian, China) in accordance with the manufacturer's protocol.

DTX2 knockdown with shRNAs

The 3 shRNAs targeting *DTX2* were designed and synthesized by Shanghai Sangon Biotech (Shanghai, China). DBTRG and U251 cells were transfected with *DTX2* shRNA lentiviral vectors and their control vectors. The *DTX2* shRNA target sequences were as follows: shRNA1, 5'-GCTTCATCGAGCAGCAGTTTG-3'; shRNA2, 5'-GCAGCAGTTTGTCCAGCAGAA-3'; and shRNA3, 5'-GGTACAACTACACTGTCAACT-3'.

CCK8 assays

Cell Counting Kit 8 (CCK8) (Sigma, product number: 96992) was used to measure cell viability. Briefly, 7500 DTBRG and U251 cells/well were plated in a 96-well plate and incubated overnight. The cells were then washed with phosphate buffered solution, and then underwent shRNA transfection as indicated. Following transfection, all the media were removed and replaced with fresh medium containing CCK8 reagent. The plate was then incubated for 1 h, and absorbance was measured at 450 nm using FLUOstar Omega (BMG Labtech, Offenburg, Germany).

Wound-healing assays

The DBTRG and U251 cells were seeded into 12-well plates with 1% FBS. The cells achieved 100% confluence. The yellow pipette tips were applied for the straight scratch. The wound distance was measured at the indicated time points, and normalized with the starting time point.

Luciferase assays

Luciferase reporter assays were performed according to a standard protocol as previously described (29). Briefly, U251 cells (3×10^4 cells/well) were seeded into 24-well plates in triplicate, and allowed to attach for 24 h. The *DTX2* promoter wild-type (WT) and mutant (MUT) sequences were as follows: WT, 5'-TGGGCCCTACCGGGACTAC ATTTCCC-3'; and MUT, 5'-CCCAAGTACAACTTAC AAGACGTAAGAAA-3'. The indicated plasmids (*DTX2* promoter WT and MUT) and 1.5 ng of pRL-TK Renilla plasmid were transfected using Lipofectamine 3000 Reagent (Thermo Fisher Scientific, Waltham, MA, USA). The cells were treated with dimethyl sulfoxide (DMSO) and JQ1 [1 μM (29)] compounds for 24 h. After 48 h of treatment, the luciferase and Renilla signals were assessed using a Dual-Luciferase Reporter Assay Kit (Promega, cat. No. E1980) in accordance with the manufacturer's instructions.

Statistical analysis

GraphPad 8.0 software was employed to analyze the data. The values are shown as the mean ± standard deviation. A one-way analysis of variance and Student's *t*-test were used for comparisons between 2 groups. All assays were replicated 3 times. A P value <0.05 was considered statistically significant.

Results

The survival-status analysis identified 3 novel unfavorable prognostic markers in glioma

To acquire the gene expression signatures associated with glioma prognosis, we applied the online tool, LinkedOmics (25), to generate survival status-related positive and negative genes in TCGA GBM multiforme and LGG data sets, respectively. As a result, 1,748 and 10,754 genes showed significant (P<0.05) survival status-

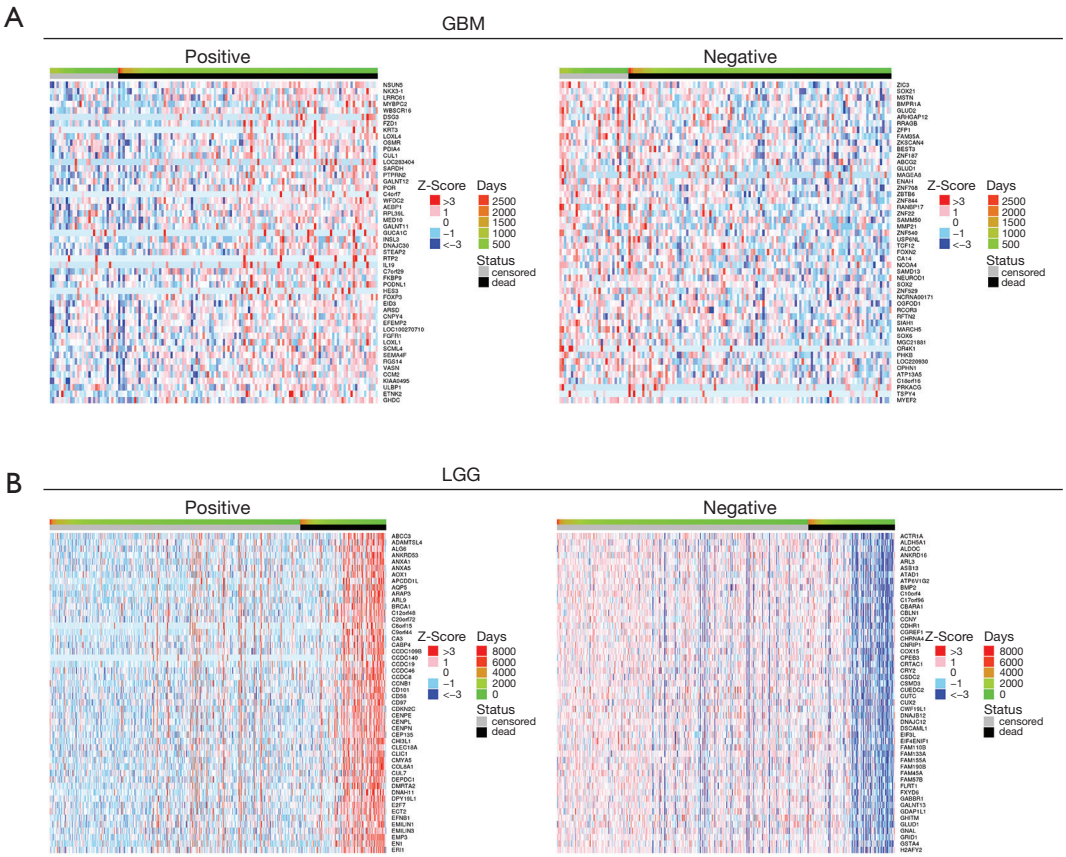

Figure 1 Survival-status revealed prognosis-related genes in glioma. (A) Survival-status analysis showing the expression heatmaps of the top 50 positive and negative correlated genes in TCGA GBM data set; (B) survival-status analysis showing the expression heatmaps of the top 50 positive and negative correlated genes in TCGA LGG data set. GBM, glioma multiforme; LGG, low-grade glioma; TCGA, The Cancer Genome Atlas.

related performance in GBM and LGG, respectively. The top 50 positive and negative genes in GBM and LGG are shown in *Figure 1*. By using a relative value of 0.7 and –0.7, 116 and 3,014 differential genes were acquired for GBM and LGG, respectively. Next, we compared the 116 and 3,014 differential genes, and 24 common genes were found to be related in LGG and GBM (see *Table 1* and *Figure 2A*). Next, we constructed overall survival (OS) map showing the prognostic performance of the 24 common genes in the GBM and LGG data sets (see *Figure 2B*). To further explore the clinical significance of these 24 common genes, the expression of these genes was investigated. GEPIA database was analyzed the expression and OS of 24 common genes in GBM and LGG. The expression of Williams-Beuren syndrome chromosomal region 16 protein (*WBSCR16*), paxillin (*PXN*), cytochrome P450 oxidoreductase (*POR*), membrane-associated RING CH 5 (*MARCH5*), family with sequence similarity 33, member A (*FAM35A*), Deltex E3 ubiquitin ligase 2 (*DTX2*), cleft lip and palate transmembrane 1-like (*CLPTM1L*), and a previously uncharacterized Rho GTPase-activating protein (*ARHGAP12*) were upregulated in GBM and LGG tumor tissues compared with normal tissues (see *Figure 2C*). Finally, Kaplan-Meier survival plots results revealed that the high expression of *AGK*, *CLPTMM1L*, *DTX2*, *POR*, *PXN*, and *WBSCR16* were also correlated with an unfavorable prognosis in GBM and LGG patients; the low expression of *ARHGAP12*, *FAM35A*, MARCH5 and *PPAGB* were also correlated with good prognosis in GBM and LGG patients (see *Figure 2D*) (30).

Of the above identified 5 markers, *CLPTM1L* has been reported to predict poor survival outcomes and resistance to temozolomide therapy in glioma (31). Similarly, *PXN* has also been proven to serve as an oncogene in glioma

Table 1 Twenty-four genes commonly correlated with survival status in LGG and GBM

Gene	LGG		GBM	
	Statistic	P value	Statistic	P value
ABCB7	0.794051	0.001293	–0.72152	0.016578
AGK	0.893597	0.000152	0.804206	0.00504
ARHGAP12	–0.98946	3.07E–11	–0.73121	0.000422
CLPTM1L	0.892507	0.000843	0.873131	0.001093
CUL1	1.085598	4.24E–05	1.127012	0.000152
DEFA5	–1.19452	0.000762	–1.96127	0.033578
DTX2	0.914772	3.05E–10	0.768018	0.00157
DUSP21	2.393109	0.000269	1.549411	0.035974
EXOC3	–1.10342	4.59E–05	0.731373	0.034279
FAM35A	–0.90858	8.87E–07	–0.82252	0.000819
IL23R	1.102708	0.002592	1.144196	0.002301
MARCH5	–2.30162	1E–16	–0.82393	0.003978
POR	0.950188	2.89E–05	0.946453	0.000196
PXN	0.91187	2.61E–05	0.704881	0.001214
RNF10	0.795993	0.022542	0.770314	0.049849
RRAGB	–1.30144	1.01E–14	–0.73706	0.000422
RXRB	–1.86159	8.75E–14	–0.75101	0.006494
SAMM50	–1.61489	9.24E–08	–0.76129	0.002265
SCO1	1.4827	4.25E–05	0.723089	0.024169
TFAM	–0.83161	0.000483	–0.70665	0.004929
UBE2Z	0.832866	0.014292	0.865427	0.018523
VPS52	–2.2241	5.75E–12	–0.87765	0.006009
WBSCR16	1.150865	3.37E–07	0.971678	6.72E–05
ZNF705D	1.128865	0.009759	0.773717	0.015574

LGG, low-grade glioma; GBM, glioma multiforme.

progression, and may be a new potential target for therapy (32). As studies on *DTX2*, *POR*, and *RCC1L* (*WBSCR16*) are limited in glioma, we conducted a further analysis of these 3 genes.

DTX2 is a novel prognostic marker and is positively correlated with immune cells in LGG

Next, we validated the expression of these 3 genes in the CGGA database (27). Of the 3 data sets included in the CGGA database, DTX expression level was correlated with WHO grade, IDH mutation status and 1p/19q co-deletion status (see *Figure 3A*). Additionally, glioma patients with higher *DTX2* expression also had worse survival outcomes, which suggests that *DTX2* is an unfavorable prognostic marker (see *Figure 3B*). An examination of the expression of *DTX2* in the single-cell data of the glioma samples revealed that *DTX2* was highly expressed in both malignant and immune cells (see *Figure 3C*). As the tumor immune microenvironment plays an important role in glioma progression and therapeutic responses, we analyzed the expression significance of tumor immune infiltrating

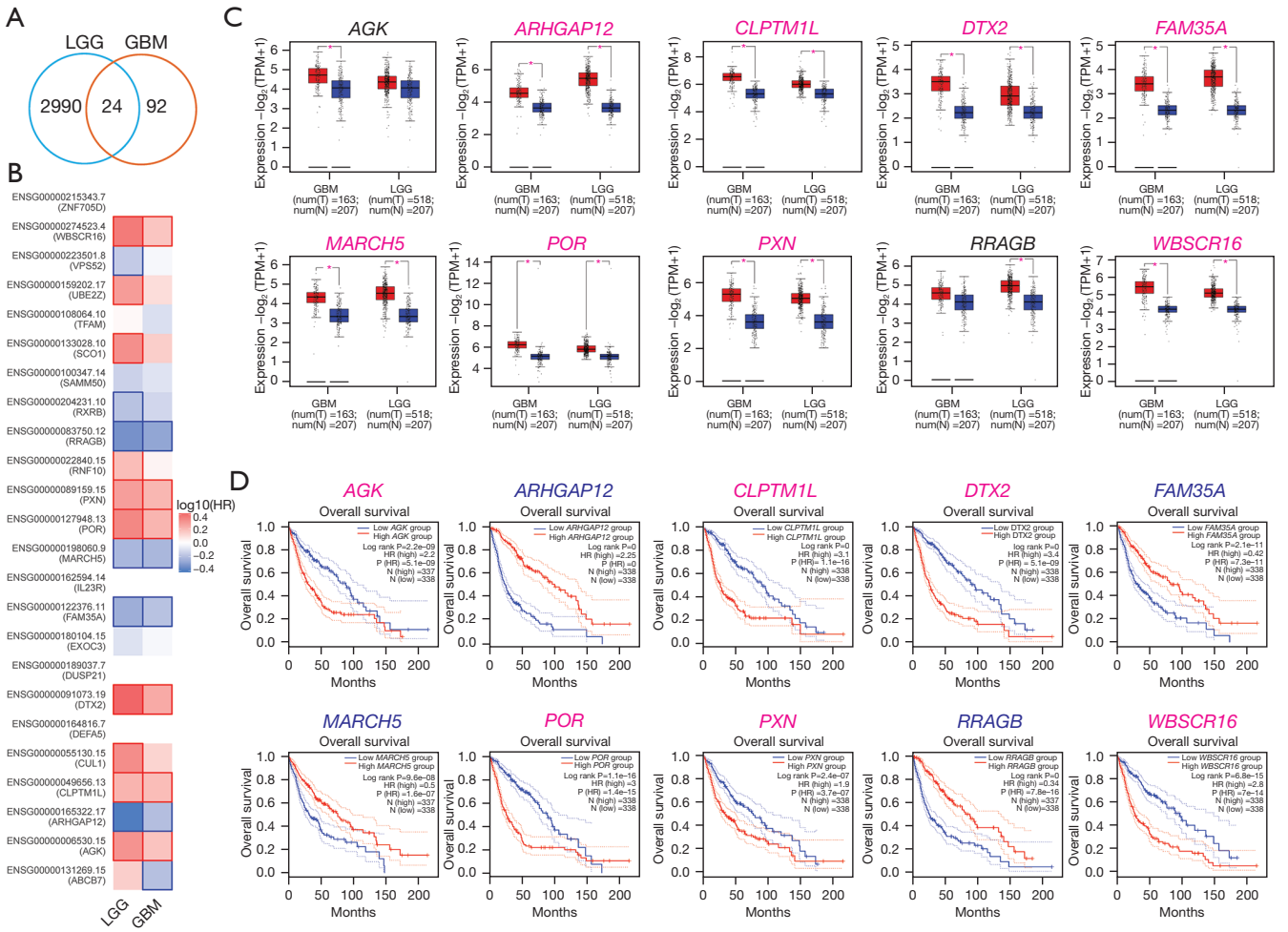

Figure 2 The survival and expression analysis revealed that DTX2 was a high-risk prognostic marker. (A) All 24 genes were found to be related to survival status in the LGG and GBM groups; (B) OS map showing the prognostic performance of the 24 common genes in the GBM and LGG data sets; (C) all 10 genes showed differential expression in TCGA GBM and LGG data sets; (D) Kaplan-Meier survival plots of the 10 genes in TCGA GBM and LGG data sets using GEPIA dataset (30). Genes with a higher expression and an unfavorable prognosis are labeled in red, and genes with a lower expression and favorable prognosis are labeled in blue. *, P<0.05. LGG, low-grade glioma; GBM, glioma multiforme; DTX2, Deltex E3 ubiquitin ligase 2; OS, overall survival; TCGA, The Cancer Genome Atlas.

cells using TIMER. No strong correlation was observed in GBM, but the correlation coefficients in LGG were higher in B cells (Corr =0.429), $CD4^+$ T cells (Corr =0.647), macrophages (Corr =0.573), neutrophils (Corr =0.495), and dendritic cells (Corr =0.563) (see *Figure 3D*).

Silencing DTX2 significantly repressed glioma cell growth and migration in vitro

To study the function of *DTX2* in glioma cell malignant behavior, the expression of *DTX2* was silenced with 3 shRNAs (shRNA 1–3) in the 2 glioma cell lines of DBTRG and U251 cell lines. The knockdown efficiency was examined with qRT-PCR, and shRNA1 had the highest knockdown efficiency (see *Figure 4A*), and thus was used in subsequent studies. Next, CCK8 assays were conducted to study the growth difference between the control and *DTX2*-silenced glioma cells. As *Figure 4B* shows, the cell growth capability of *DTX2*-silenced glioma cells was significantly lower at 72 h in both cell lines compared with control, which suggests that repressing *DTX2* expression may provide a glioma growth repressing

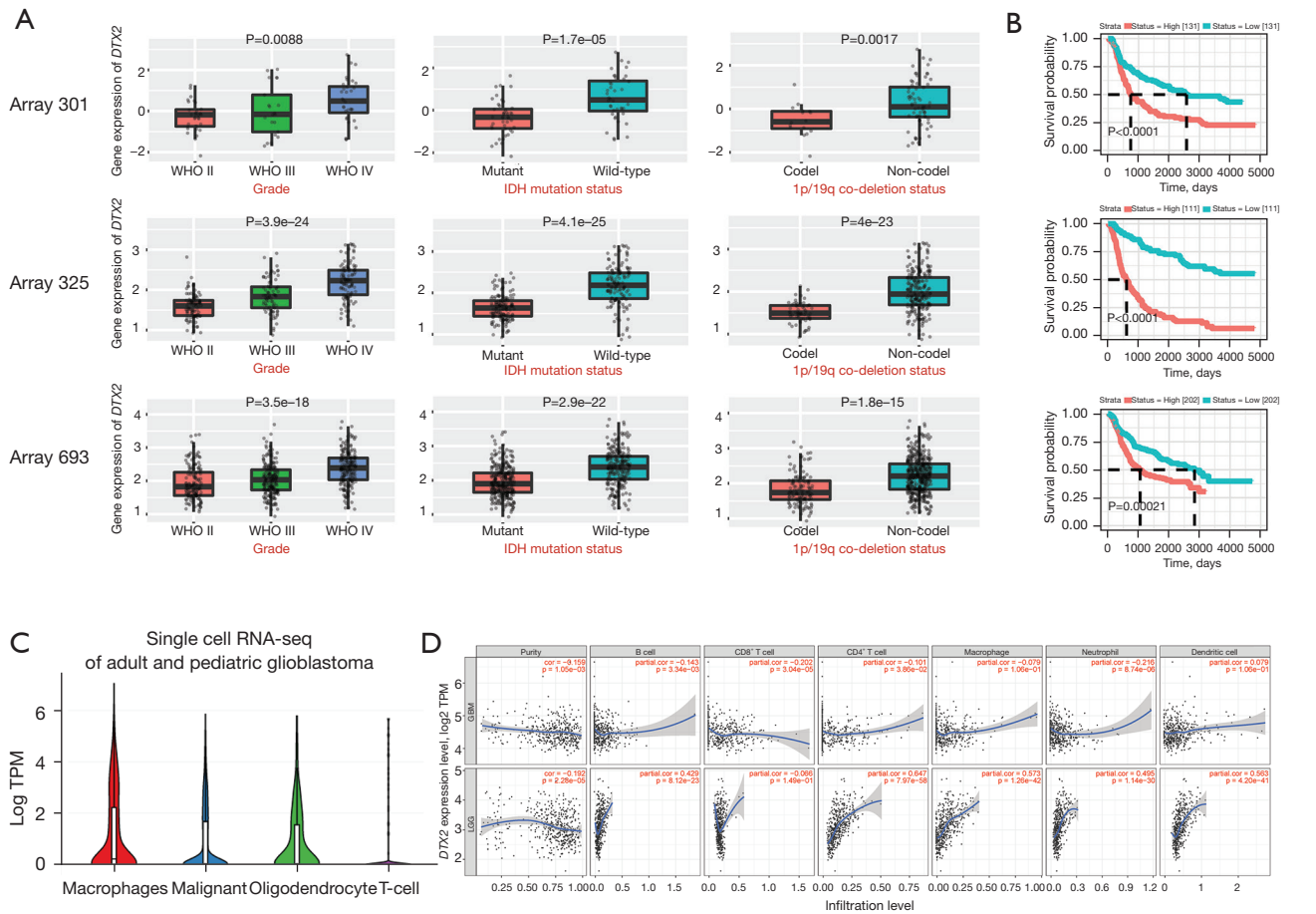

Figure 3 *DTX2* was highly expressed in malignant glioma cells. (A) *DTX2* showed increased expression in the CGGA glioma data sets of higher grades, and the IDH WT and 1p/19q co-deletion groups; (B) Kaplan-Meier survival plots of *DTX2* expression in the 3 CGGA array data sets; (C) expression of *DTX2* in the single-cell RNA-Seq of adult and pediatric glioblastoma patients; (D) correlation analysis of *DTX2* with tumor-infiltrating immune cells in GBM and LGG. TPM, transcripts per million; *DTX2*, Deltex E3 ubiquitin ligase 2; CGGA, Chinese Glioma Genome Atlas; IDH, isocitrate dehydrogenase; WT, wild type; LGG, low-grade glioma.

strategy. Finally, the migration capabilities of the cells were examined using wound-healing assays, *DTX2* silencing was found to significantly decrease the migration distance compared to that of the control cells (see *Figure 4C*). These *in-vitro* data suggest that *DTX2* contributes to cancer cell growth and migration, and *DTX2* could serve as a therapeutic target in glioma.

DTX2 is transcriptionally activated by BRD4 in glioma

Finally, to investigate the transcriptional reasons for the significantly higher expression of *DTX2* in glioma, we first analyzed transcriptional factors with the chromatin immunoprecipitation (ChIP)-sequencing data provided in ChIP-atlas. The transcription activation signals (DNase-Seq, RNA polymerase II, histoneH3lysine 4 trimethylation (*H3K4me3*), andhistoneH3lysine 27 acetylation (*H3K27ac*) in the *DTX2* promoter region suggested a transcriptional activation status in glioma tissues and cell lines (see *Figure 5A*). Of all the transcription factors included, 3 transcriptional regulons (i.e., *MAX*, *BRD4*, and *BRD2*) had binding signals at the *DTX2* promoter. Next, the expression correlations of *MAX*, *BRD4*, and *BRD2* with *DTX2* were validated with TCGA and the CGGA data sets. As *Figure 5B,5C* show, the expression level *BRD4* was positively correlated with *DTX2* expression in both data sets (Corr =0.42 for TCGA and Corr =0.514 for the CGGA).

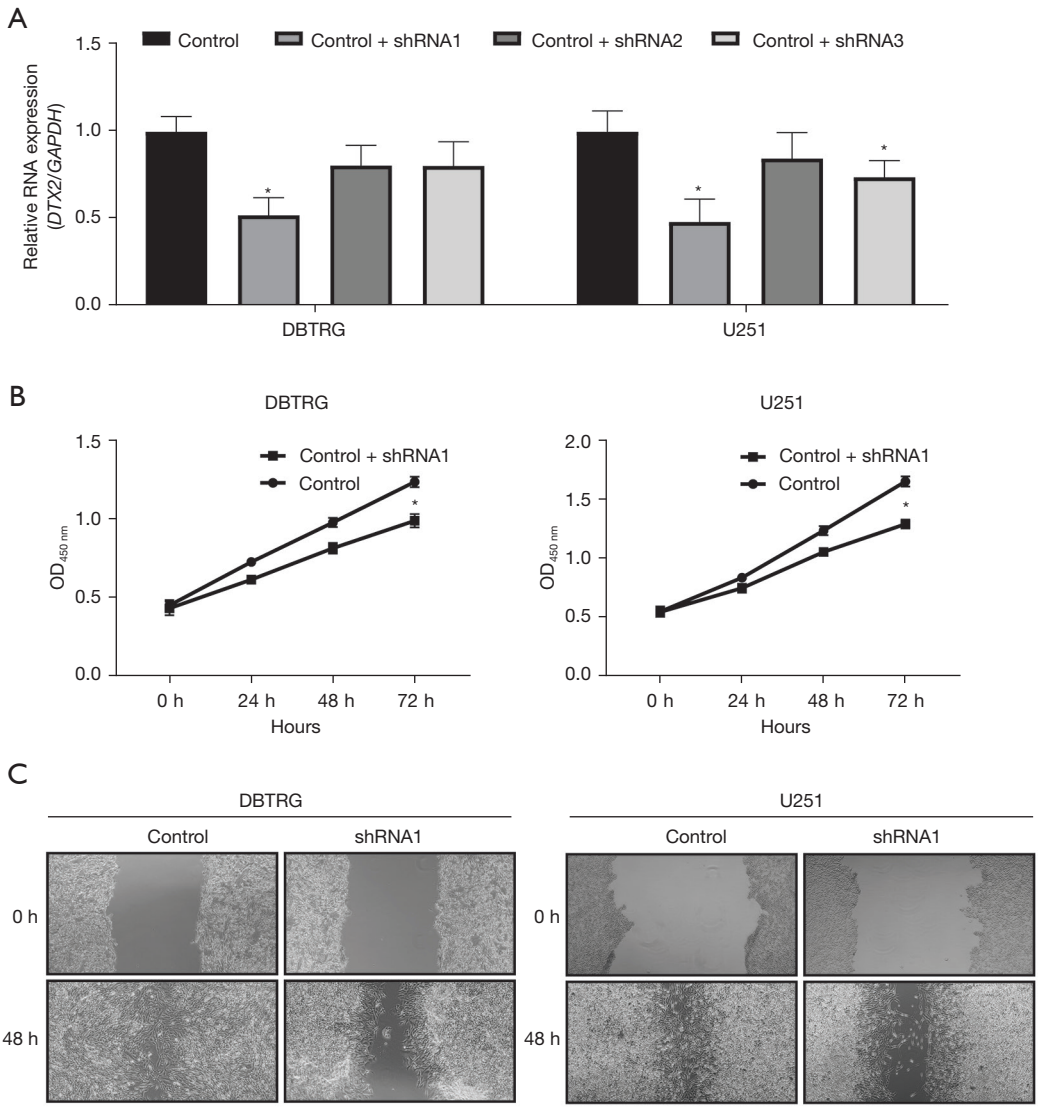

Figure 4 *DTX2*-silenced glioma cells showed decreased proliferation and migration capabilities. (A) The expression of *DTX2* was examined by RT-qPCR in the control and 3 shRNA groups; (B) CCK8 assays showed the proliferation capability of the *DTX2*-silenced glioma cells; (C) wound-healing assays showed that the migration capability of the *DTX2*-knocked down cells were observed under electron microscope (magnification, 40×). All assays were replicated 3 times. *, P<0.05. *DTX2*, Deltex E3 ubiquitin ligase 2; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; shRNA, short-hairpin ribonucleic acid; RT-qPCR, real-time quantitative PCR; CCK8, Cell Counting Kit 8.

Furthermore, thus, we used *BRD4* in the subsequent assays.

Multiple studies have proven that *BRD4* serves as an oncogene and a potential therapeutic target in glioma (33,34). JQ1 is one of the most widely used *BRD4* inhibitors (34). Thus, the expression of *DTX2* was examined with qRT-PCR after *BRD4* treatment, and JQ1 treatment was found to decrease the abundance of *DTX2* in both cell lines (*see Figure 5D*). Finally, a luciferase assay was conducted to validate the transcriptional regulation of *BRD4* in *DTX2*, and while *BRD4* treatment significantly reduced luciferase intensity in the *DTX2* promoter WT group, no significance was observed in the MUT group (*see Figure 5E*). In conclusion, these results suggest that *DTX2* contributes to cell growth and migration, serves as an unfavorable marker,

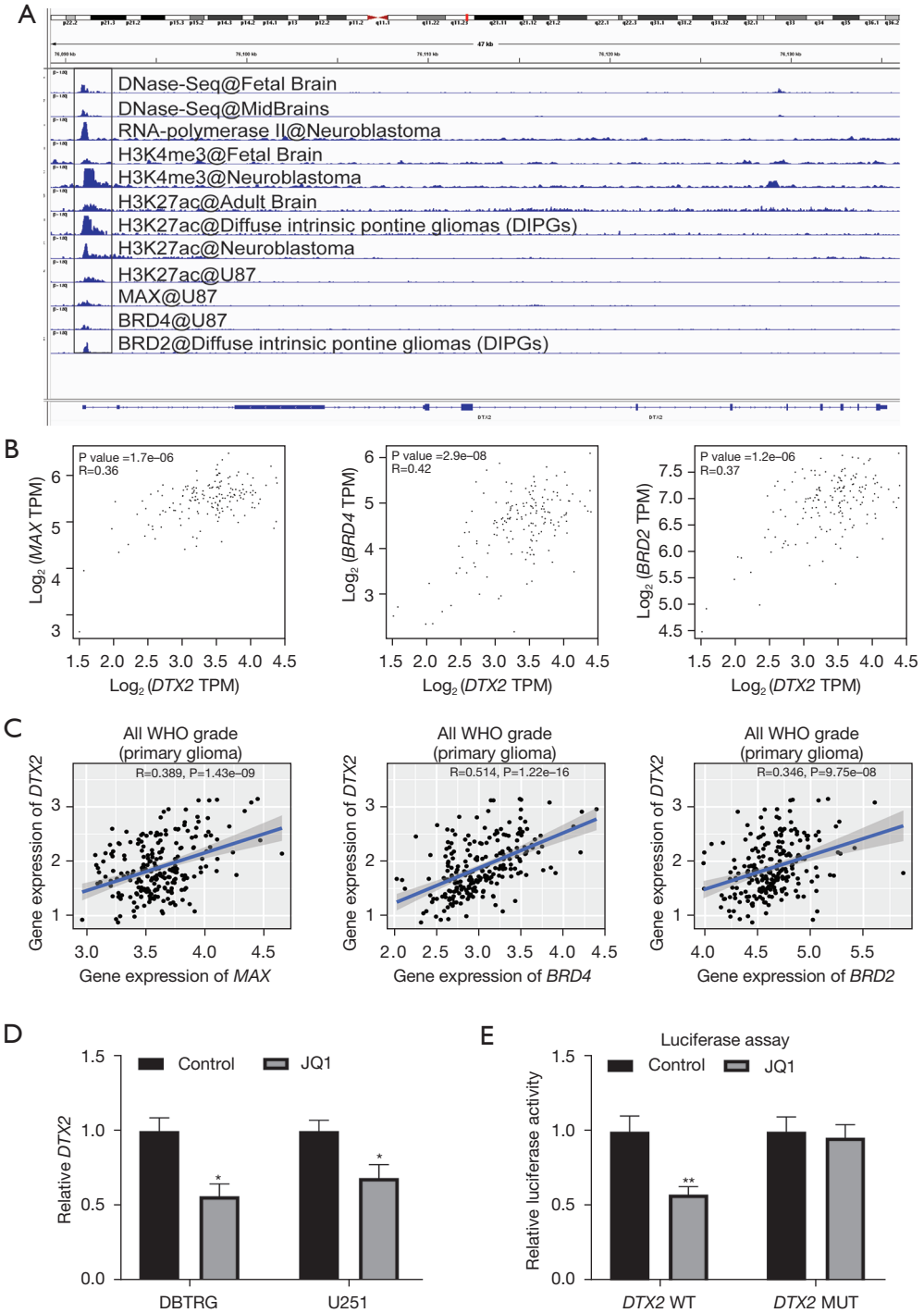

Figure 5 *DTX2* is transcriptionally activated by *BRD4* in glioma. (A) ChIP-sequencing peaks of histone activation marks and transcription factors were analyzed, and binding peaks within the *DTX2* promoter are shown; (B) the expression correlation of *DTX2* with transcription factors *MAX*, *BRD4*, and *BRD2* in TCGA GBM and LGG data sets; (C) the expression correlation of *DTX2* with transcription factors *MAX*, *BRD4*, and *BRD2* in the CGGA data sets; (D) *BRD4* inhibitor JQ1 repressed *DTX2* expression in glioma cells; (E) dual-luciferase assays showing that JQ1 reduced the luciferase activities. *, P<0.05; **, P<0.01. TPM, transcripts per million; *DTX2*, Deltex E3 ubiquitin ligase 2; BRD4, bromodomain containing 4; WT, wild type; MUT, mutant; ChIP, Chromatin immunoprecipitation; TCGA, The Cancer Genome Atlas; GBM, glioma multiforme; LGG, low-grade glioma.

and is transacted by *BRD4* in glioma.

Discussion

Glioblastoma patients have a poor prognosis, and the identification of novel makers may benefit patients by predicting their survival status and time. By conducting a survival-status and time analysis, we identified 10 genes related to prognosis. A subsequent expression analysis revealed 3 novel genes; that is, *DTX2*, *POR*, and *RCC1L*. *POR*, also known as *CYPOR*, encodes an endoplasmic reticulum membrane, oxidoreductase, which is essential in multiple metabolic processes (35). In breast cancer, *POR* has been revealed to be a novel and independent prognostic biomarker (36). *WBSCR16* is located in a large deletion region of Williams-Beuren syndrome (WBS), and encodes a protein containing regulator of chromosome condensation 1-like repeats. Studies have shown that *WBSCR16* is essential for mitochondrial function (37-39), and is involved in the regulation of oxidative phosphorylation (40,41). In cancer, *WBSCR16* has been found to be significantly correlated with the survival of adrenal cortical carcinoma (42). The role of *DTX3L* in glioma remains largely unexplored (18). *DTX3L* has been shown to be upregulated in glioma, and is associated with glioma progression (43). This study investigated the role of *DTX2* in glioma, and our team intends to examine the role of the other 2 genes in the future.

We first characterized *DTX2* as a novel prognostic marker in the GBM and LGG samples. Notably, *DTX2* showed higher expression with advanced WHO grades III and IV compared with WHO grades II, which also suggests that it has progression-related clinical significance. The results of the subsequent cellular assays also supported this finding, as *DTX2*-silenced cell lines exhibited repressed growth and migration capabilities. Additionally, the expression of *DTX2* was also increased in IDH WT and 1p/19q co-deletion glioma samples, which implies that its role in glioma may be maintained irrespective of these molecular changes. The initial functional evaluation of *DTX2* in the 2 glioma cell lines suggests that *DTX2* could be a therapeutic target for glioma, but further *in-vivo* studies and more gain-of-function studies need to be conducted to validate this *in-vitro* phenotype. Besides, it is better to validate the expression of *DTX2* by real world data, and explore the effects of *DTX2* on apoptosis. We will carry out these experiments in the future. Recently, Ahmed *et al.* showed that the C-terminal domain harbors an adenosine diphosphate (ADP)-ribose-binding pocket and recruits poly-ADP-ribose (PAR)-modified proteins (e.g., *PARP1*) for ubiquitination (24). It has been revealed that ubiquitination of NOTCH2 by DTX3 suppresses the proliferation and migration of human esophageal carcinoma (44). Given that the substrates of E3 ligases differ with cancer types and pathological conditions, the specific substrate of DTX2 in glioma still needs to be explored.

Finally, *BRD4* was proposed to be a transcriptional regulator of *DTX2* in glioma. JQ1, which is one of the most widely used *BRD4* inhibitors (34), reduced the expression of *DTX2*. Notably, *BRD4* inhibitors may overcome resistance to *PARP* inhibitors in multiple cancer types (45-47). Given that *DTX2* is transcriptionally activated by BRD4, and may mediate the ubiquitination of *PARP1*, we propose the regulation axis of *BRD4-DTX2-PARP1* in glioma, which may provide an alternative option for *BRD4* and *PARP* inhibitors in glioma.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-555/rc).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-555/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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