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BPTF promotes glioma development through USP34-mediated de-ubiquitination of FOXC1

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Summary. Glioma is the most prevalent malignant tumor of the brain, and the study of the molecular mechanisms associated with its development has important clinical significance. Our previous study found that BPTF promotes the malignant phenotype of glioma and is significantly associated with poor prognosis; the downstream regulatory mechanisms are explored in this study. Western blot and immuno-histochemical staining were used to detect protein expression in cells or tissues. BPTF knockdown as well as FOXC1-overexpressing lentiviruses were used in combination for the construction of the U251 cell model, leading to functional rescue experiments. CCK8 assay, flow cytometry, scratch assay, and Transwell assay were used to detect cell proliferation, apoptosis, and migration, respectively. Finally, immuno-precipitation assays, combined with western blot (WB), were used to detect the interaction between proteins as well as the level of ubiquitination modification. The obtained results suggested that BPTF knockdown may inhibit the malignant behavior of glioma cells by downregulating FOXC1 expression. Moreover, FOXC1 expression was significantly higher in glioma tissues than in normal brain tissues and was significantly associated with higher tumor stage and worse patient prognosis. Finally, the mechanism of FOXC1 regulation by BPTF was found to result from the affected protein stability of FOXC1 through USP34-mediated de-ubiquitylation. In conclusion, the BPTF/FOXC1 axis was identified as a key promotor in glioma development and may be a potential target in the inhibition of glioma development.

Key words: Glioma, BPTF, FOXC1, Ubiquitination

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Introduction

Glioma is the most common brain malignancy arising from the neuroepithelium of the central nervous system (Xia et al., 2022; Siegel et al., 2023). According to the classification of central nervous system tumors defined by the World Health Organization (WHO), gliomas can be divided into grades I-IV. With the increase in grade, the degree of tumor malignancy increases, and the prognosis deteriorates (Bai et al., 2020; Ostrom et al., 2020). Statistics showed that about 2% of adult malignant tumors are brain tumors, of which glioma accounts for up to 80% (Siegel et al., 2023). At present, the preferred treatment for glioma is still an individualized comprehensive treatment strategy consisting of surgical treatment combined with various treatment methods such as radiotherapy, chemotherapy, and molecular targeted therapy (Yan et al., 2020). Despite the rapid development of medical technology and the emergence of various comprehensive treatments, there is still no good curative treatment for glioma, resulting in a high mortality rate, especially for high-grade (WHO grade III and IV) glioma. One of the most important reasons for the very poor survival of patients with high-grade glioma after surgery is its highly invasive and metastatic characteristics. The vast majority of high-grade gliomas will progress quickly after initial treatment, with recurrence and metastasis, ultimately leading to the patient's death (Zanders et al., 2019; Ruff et al., 2020; Tan et al., 2020). Therefore, elucidating the key molecular mechanism of glioma proliferation, invasion, and metastasis is helpful in understanding glioma recurrence and metastasis in-depth, and, based on this, new prevention and treatment strategies could be developed to improve the clinical diagnosis and treatment of glioma, which has important clinical significance.

BPTF (Bromodomain PHD-finger transcription factor) is an important epigenetic regulator located on chromosome 17q24.2, which encodes a transcription factor that consists of 2781 amino acids and mainly contains two PHD zinc fingers and a glutamine-rich

bromodomain acidic domain (Jones et al., 2000). Previous studies have shown that BPTF is involved in key signaling pathways that manage embryonic development and is an indispensable gene during embryonic development. It can regulate embryonic trophoblast, nervous system, and oral thymus development (Goller et al., 2008; Landry et al., 2008). As the largest nucleosome remodeling factor subunit, BPTF is involved in constituting nucleosome remodeling factors, which use the energy provided by ATP hydrolysis to slide nucleosomes and improve chromatin accessibility, providing preconditions for the progression of transcription (Frey et al., 2017). Several studies have shown that BPTF plays an important role in the progression of various human tumors such as colorectal cancer (Xiao et al., 2015) and renal cell carcinoma (Yang et al., 2020). Previous studies by our group revealed the regulatory role of BPTF in the glioma cell phenotype and its clinical potential as an independent prognostic factor for glioma (Pan et al., 2019). On the other hand, studies reported by our group and by Green et al. both identified MYC signaling as an important downstream mechanism in the function of BPTF in regulating glioma (Green et al., 2020). This study aimed to investigate whether BPTF promotes glioma progression through FOXC1, one of the known MYC signaling activators.

In this study, the capacity of BPTF to regulate the expression of FOXC1 and the dependence of BPTF-induced regulation of glioma cell phenotypes on FOXC1 were explored. Moreover, the expression pattern of FOXC1 in glioma was investigated through immunohistochemical staining of a tissue microarray consisting of glioma tissues and normal brain tissues. The correlation between FOXC1 and glioma characteristics was calculated accordingly. Furthermore, the mechanism, at the molecular level, by which FOXC1 expression was regulated by BPTF was also studied.

Materials and methods

Cell culture

Human malignant glioma U251 cells were obtained from the BeNa Culture Collection (Beijing, China) and cultured in 90% DMEM-H supplemented with 10% fetal bovine serum (FBS). The cells were treated with Penicillin-Streptomycin (100×) and maintained in a humidified 37°C incubator with 5% CO_2.

Gene knockdown or overexpression cell models and cell infection

The negative control (sh-NC or OE-NC) and lentiviruses containing BPTF or USP34 knockdown sequences and FOXC1 overexpression sequences were designed by the Shanghai Yibeirui Biomedical Science and Technology Co., Ltd. (Shanghai, China). Briefly, shRNAs and overexpression sequences were cloned into the linearized BR-V-108 vector using Fermentas T4 DNA Ligase (Thermo Fisher Scientific, USA). Verified lentiviruses were transfected into the Top 10 E. coli recipient cells for amplification. The recombined lentiviral vector plasmid, along with the auxiliary plasmids (Helper 1.0 and Helper 2.0), was co-transfected into 293T cells. The plasmids were then extracted using the EndoFree Maxi Plasmid Kit (Tiangen, China), and the qualified plasmid was packaged with the virus. The lentivirus quality and titer were determined.

For lentiviral infection, logarithmic growth phase cells were seeded in a 6-well plate at 5×10^3 cells per well. The lentiviral vector was added at a multiplicity of infection of 15. After 72h, cells infected with the lentiviral vectors were selected using 1 μg/mL puromycin (Beyotime Institute of Biotechnology). Knockdown and overexpression infection efficiency were evaluated using qPCR and WB analysis.

Western blot (WB) analysis

To investigate the expression levels of BPTF, USP34, and FOXC1, U251 cells were collected and lysed using RIPA lysis buffer (Millipore). The protein concentration was determined using the BCA Protein Assay kit (Cat. 23225, HyClone-Pierce). Subsequently, 20 μg of total cellular proteins was separated on a 10% SDS-PAGE gel. After transfer onto polyvinylidene difluoride (PVDF) membranes, the blots were blocked with TBST solution containing 5% non-fat milk at room temperature for 1 hour. The membranes were then incubated overnight at 4°C on a rocker with the following primary antibodies: FOXC1 (1:50/1:1000, Cat. ab227977, Abcam); BPTF (1:1000, Cat. bs-11641R, Bioss); USP34 (1:1000, Cat. 18827-1-AP, Proteintech); GAPDH (1:30000, Cat. 60004-1-lg, Proteintech); and Ubiquitin (1:2000, Cat. sc-8017, Santa Cruz). After washing with TBST three times, the membranes were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG polyclonal secondary antibody (1:3000, Cat. A0208, Beyotime) or HRP-conjugated goat anti-mouse IgG polyclonal secondary antibody (1:3000, Cat. A0216, Beyotime) at room temperature for 1h. The bands of cellular proteins were visualized using enhanced chemiluminescence, and band quantification was performed using a Chemi-luminescence imaging system (GE, USA). GAPDH was used as the internal control to normalize the expression of the other proteins.

CCK-8 assay

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) assay. A total of 2×10^3 cells was seeded and cultured in 96-well plates at 37°C with 5% CO_2 for 0, 24, and 48h. Ten microliters of CCK-8 reagent (Sigma, Germany) were added to each well and incubated for 4 hours until a visible color change occurred. After the incubation period, the optical density (OD) values were measured by determining the

absorbance at 450 nm using a microplate reader (Tecan Infinite, Switzerland).

Apoptosis assay

Lentivirus-infected U251 cells were seeded in 6 cm dishes. After trypsin digestion, the cells were resuspended in the same medium. For apoptosis analysis, cells were collected in tubes at a concentration of 1×10^6 cells/ml, following the protocol of the Annexin V/PI apoptosis kit (Southern Biotech, USA). Then, 5 µl of Annexin V-allophycocyanin and 5 µl of propidium iodide (PI) were separately added to stain the cells for 15 min at room temperature in the dark. The PI staining allowed the identification of cells in the late apoptotic stage and dead cells as their membranes become permeable to PI. Cells stained with both Annexin V and PI were recorded as Annexin V (+) PI (+). The stained cells were analyzed using an MM High-throughput Flow Cytometer (Guava, Germany), measuring the fluorescence emission at 530 nm and >575 nm. The percentages of cells in each group were determined by comparing them to the total number of cells.

Wound healing assay

For the wound healing assay, U251 cells were seeded in a 6-well plate and cultured at 37°C with 5% CO_2. When the cells reached approximately 90% confluency, a wound was created by gently dragging a 200 µl sterile pipette tip across the cell monolayer. The cells were then washed twice with PBS to remove any cellular debris. Subsequently, the cells were cultured in 0.5% PBS with 5% CO_2 at 37°C. The process of wound healing was captured using Cellomics (ArrayScan VT1, Thermo) at two time points (0 and 24h), and the migration area was analyzed using Cellomics software. Each experiment was repeated three times.

Transwell assay

Lentivirus-infected U251 cells (6×10^4) suspended in serum-free medium were seeded in the upper chamber of a Transwell migration assay, while 30% FBS medium was added to the bottom chamber. After 40h of incubation, non-migratory cells in the upper chamber were removed by wiping, and the migrating cells were fixed with formaldehyde for 15 min. Following a wash with PBS, the migrating cells were stained with crystal violet for 10 min. Finally, the stained cells were observed and captured under an inverted fluorescence microscope (Olympus IX 73) at a magnification of ×200.

Immunohistochemistry (IHC) assay

Formalin-fixed and paraffin-embedded (FFPE) sections of 144 cases of glioma cancer tissue and 29 cases of para-carcinoma tissues were employed in this study. This research obtained ethical approval from the Ethical Committee of Haikou Affiliated Hospital of Central South University Xiangya School of Medicine (No. SC20180231). All patients provided informed consent, and their clinicopathological data were collected and summarized. The microarray was deparaffinized and rehydrated using graded concentrations of ethanol. Antigen retrieval was performed using 1× EDTA buffer, and endogenous peroxidase activity was blocked with 3% H_2O_2. The slides were then incubated overnight at 4°C with a primary antibody targeting FOXC1 (1:100, Cat. ab227977, Abcam) and a biotinylated secondary antibody. All slides were stained with DAB and counterstained with hematoxylin. The immunohistochemistry (IHC) scores were determined by multiplying the positive cell score (percentage of highly stained cells in the cancer area: negative, 0; 1-25%, 1; 26-50%, 2; 51-75%, 3; and 76-100%, 4) by the staining intensity score (negative, 0; weak, 1; moderate, 2; and strong, 3).

Protein stability assay

Lentivirus-infected U251 cells and corresponding control group cells were cultured and cycloheximide (CHX) at a concentration of 0.2 mg/mL was introduced into the medium to assess protein stability. Cell lysates were prepared at specified time points (0, 2, 4, and 8 hours). U251 cells were harvested and lysed with IP lysis buffer on ice for 10 min. The lysate was then centrifuged at 13,000×g for 10 min, and the protein concentration was determined using the BCA Protein Assay Kit (Cat. P0009, Beyotime Biotechnology). Subsequently, 20 µg of protein was used for WB to assess the levels of FOXC1 protein.

Co-IP assay and in vivo ubiquitination assays

For the Co-IP (Co-immunoprecipitation) assay, 1.0 mg of proteins (3.2 µg/µL, 312.5 µL) was immunoprecipitated with anti-FOXC1 (6 µg, Cat. ab227977, Abcam) or IgG (1 mg/ml, 6 µl, Cat. A7016, Beyotime) at 4°C overnight. Then, 40 µL of Protein A+G Magnetic Beads (Cat. P2108, Beyotime) were added and incubated with the protein complex at 4°C for 2 h. The protein complex was collected and subjected to WB using antibodies against FOXC1 (1:1000, Cat. ab227977, Abcam) and USP34 (1:1000, Cat. 18827-1-AP, Proteintech), followed by the incubation of HRP-conjugated goat anti-rabbit IgG polyclonal secondary antibody (1:3000, Cat. A0208, Beyotime). The proteins of interest were visualized using a Chemiluminescence imaging system (GE, USA).

For the *in vivo* ubiquitination assays, U251 cells were treated with 20 µM MG132 for 8h before harvesting. Ubiquitylated proteins were detected using an antibody against ubiquitin (1:2000, Cat. sc-8017, Santa Cruz) followed by the incubation of HRP-conjugated goat anti-mouse IgG polyclonal secondary antibody (1:3000, Cat. A0216, Beyotime).

Statistics analysis

Each experiment was repeated in triplicate, and data were represented as means ± SD (standard deviation). A *P*-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using SPSS v22.0 or GraphPad Prism 7. The data were analyzed using the Student's t-test for comparisons between two groups. The relationship and correlation between FOXC1 expression and clinical characteristics in patients with glioma cancer were analyzed using the Mann-Whitney U test and Spearman's rank correlation test, respectively. Survival analysis was conducted using the Kaplan-Meier method, and the log-rank test was used for comparison.

Results

BPTF regulates cell proliferation and apoptosis of glioma through FOXC1

To investigate the regulatory role of BPTF on the expression level of FOXC1 and determine if FOXC1 is necessary for BPTF-mediated regulation of glioblastoma cell phenotypes, a BPTF knockdown cell model was first constructed, and the knockdown efficiency was validated

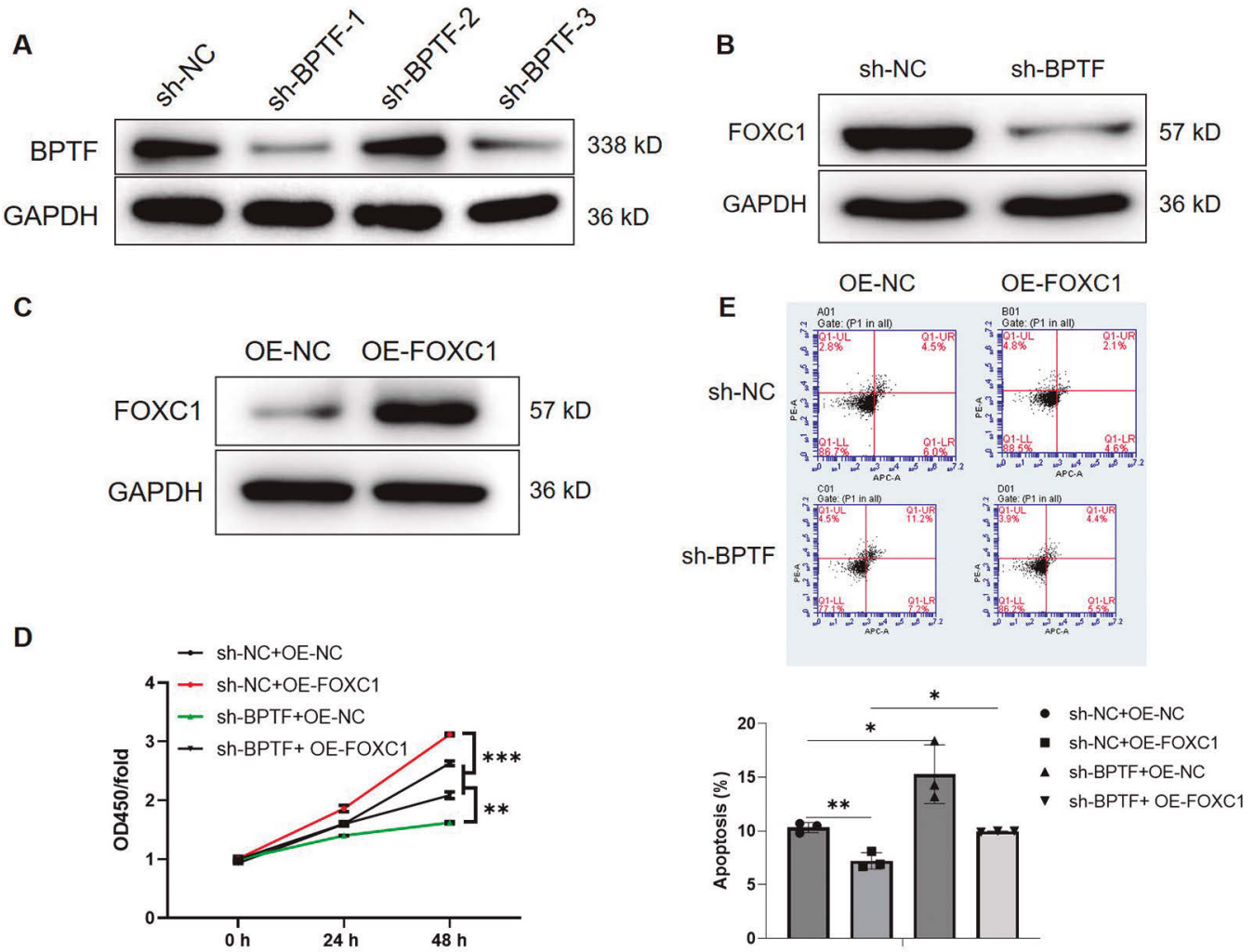

Fig. 1. BPTF regulates glioma cell proliferation and apoptosis through FOXC1. **A.** Western blot was performed to detect the protein level of BPTF in U251 cells transfected with the indicated lentivirus for evaluating knockdown efficiencies. **B.** The protein levels of BPTF and FOXC1 in U251 cells transfected with sh-NC or sh-BPTF were detected by western blot. **C.** Western blot was performed to detect the protein level of FOXC1 in U251 cells transfected with the indicated lentivirus for evaluating the overexpression efficiency. **D.** The CCK8 assay was performed to examine cell viability and draw cell a proliferation curve for U251 cells transfected with the indicated lentiviruses. **E.** Flow cytometry was used to examine cell apoptosis for U251 cells transfected with the indicated lentiviruses. Sh-NC, negative control for gene knockdown; sh-BPTF, lentivirus for BPTF knockdown; OE-NC, negative control for gene overexpression; OE-FOXC1, lentivirus for FOXC1 overexpression. Data are presented as mean ± SD (n≥3); **P*<0.05; ***P*<0.01; ****P*<0.001.

using WB experiments. Based on the results shown in Figure 1A, sh-BPTF-1, which exhibited the highest knockdown efficiency, was selected for subsequent experiments without further elaboration. The results in Figure 1B demonstrated a significant downregulation of FOXC1 protein levels in BPTF knockdown cells, indicating the regulatory effect of BPTF on FOXC1 expression levels. Subsequently, to explore the synergistic regulatory role of BPTF and FOXC1 in glioma cell phenotypes, a FOXC1-overexpression vector was first prepared and verified (Fig. 1C). Then, functional rescue experiments were conducted based on BPTF knockdown and FOXC1 overexpression. CCK-8 assays demonstrated that BPTF knockdown inhibited the proliferation of U251 cells, while FOXC1 overexpression promoted cell proliferation (Fig. 1D). Importantly, we observed that simultaneous overexpression of FOXC1 partially restored the suppressed cell proliferation induced by BPTF knockdown (Fig. 1D). Similarly, in apoptosis assays, opposite regulatory effects with the same trend were observed, whereby FOXC1 overexpression attenuated the alterations caused by BPTF knockdown (Fig. 1E). Moreover, according to the outcomes of wound-healing (Fig. 2A) and Transwell assays (Fig. 2B), both of which were performed to detect cell migration, BPTF and FOXC1 also co-operated in the regulation of cell migration.

FOXC1 is upregulated in glioma and negatively correlated with prognosis

Although there have been a few reports on the function of FOXC1 in glioma (Cao et al., 2019), its clinical significance remains unclear. A tissue microarray containing 144 tumor tissues and 29 normal tissues was utilized for IHC staining to evaluate the expression of FOXC1. Based on median IHC scores, the expression levels of FOXC1 in the tissues were categorized as "high" or "low". According to the statistical analysis presented in Table 1, it was observed that, generally, the expression level of FOXC1 in tumor tissues was significantly higher than that in normal tissues. Furthermore, after incorporating the pathological parameters of the 144 tumor tissues into the analysis, a significant correlation was observed between FOXC1 expression and tumor grade, as well as recurrence (Table 2-3). These findings suggested a clear positive correlation between FOXC1 expression and glioma malignancy, which is also evident in the representative image provided in Figure 3A. Moreover, the corresponding Kaplan-Meier survival analysis indicated that patients with high FOXC1 expression tended to have shorter disease-free survival (Fig. 3B) and overall survival (Fig. 3C). Collectively, these results revealed the critical role of FOXC1 in the progression of glioma.

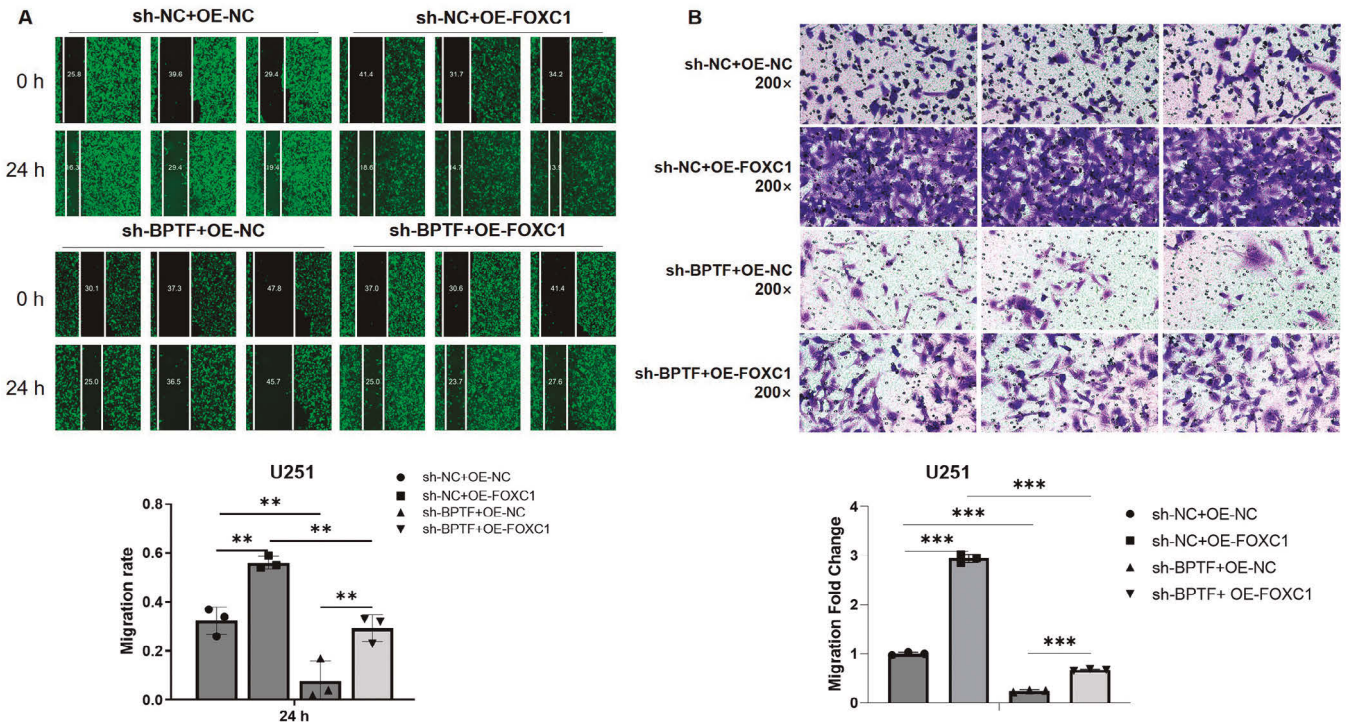

Fig. 2. BPTF regulates glioma cell migration through FOXC1. **A.** A wound-healing assay was performed to detect cell migration of U251 cells transfected with the indicated lentiviruses. **B.** The Transwell assay was used to examine cell migration of U251 cells transfected with the indicated lentiviruses. Sh-NC, negative control for gene knockdown; sh-BPTF, lentivirus for BPTF knockdown; OE-NC, negative control for gene overexpression; OE-FOXC1, lentivirus for FOXC1 overexpression. Data are presented as mean ± SD (n≥3); ***P*<0.01; ****P*<0.001.

BPTF regulates FOXC1 through USP34-mediated de-ubiquitination

Considering the regulatory effects of BPTF on FOXC1 at the protein level described in the previous results, as well as the critical role of post-translational modifications in protein degradation, the regulatory ability of BPTF on FOXC1 protein stability was investigated. The results in Figure 4A revealed a significant acceleration in FOXC1 protein degradation in BPTF knockdown cells, which may explain the

Table 1. Expression patterns of FOXC1 in glioma and normal tissues revealed by immunohistochemistry analysis.

FOXC1 expression	Tumor tissue		Normal tissue	
	Cases	Percentage	Cases	Percentage
Low	74	51.4%	29	100%
High	70	48.6%	0	0%

P<0.001.

Table 2. Relationship between FOXC1 expression and tumor characteristics in patients with glioma.

Features	No. of patients	FOXC1 expression		*P* value
		low	high	
All patients	144	74	70	
Age (years)				0.500
<42	70	38	32	
≥42	74	36	38	
Gender				0.002
Female	51	35	16	
Male	93	39	54	
Tumor recurrence				<0.001
No	65	51	14	
Yes	79	23	56	
Grade				<0.001
I	16	16	0	
II	65	42	23	
III	44	14	30	
IV	19	2	17	

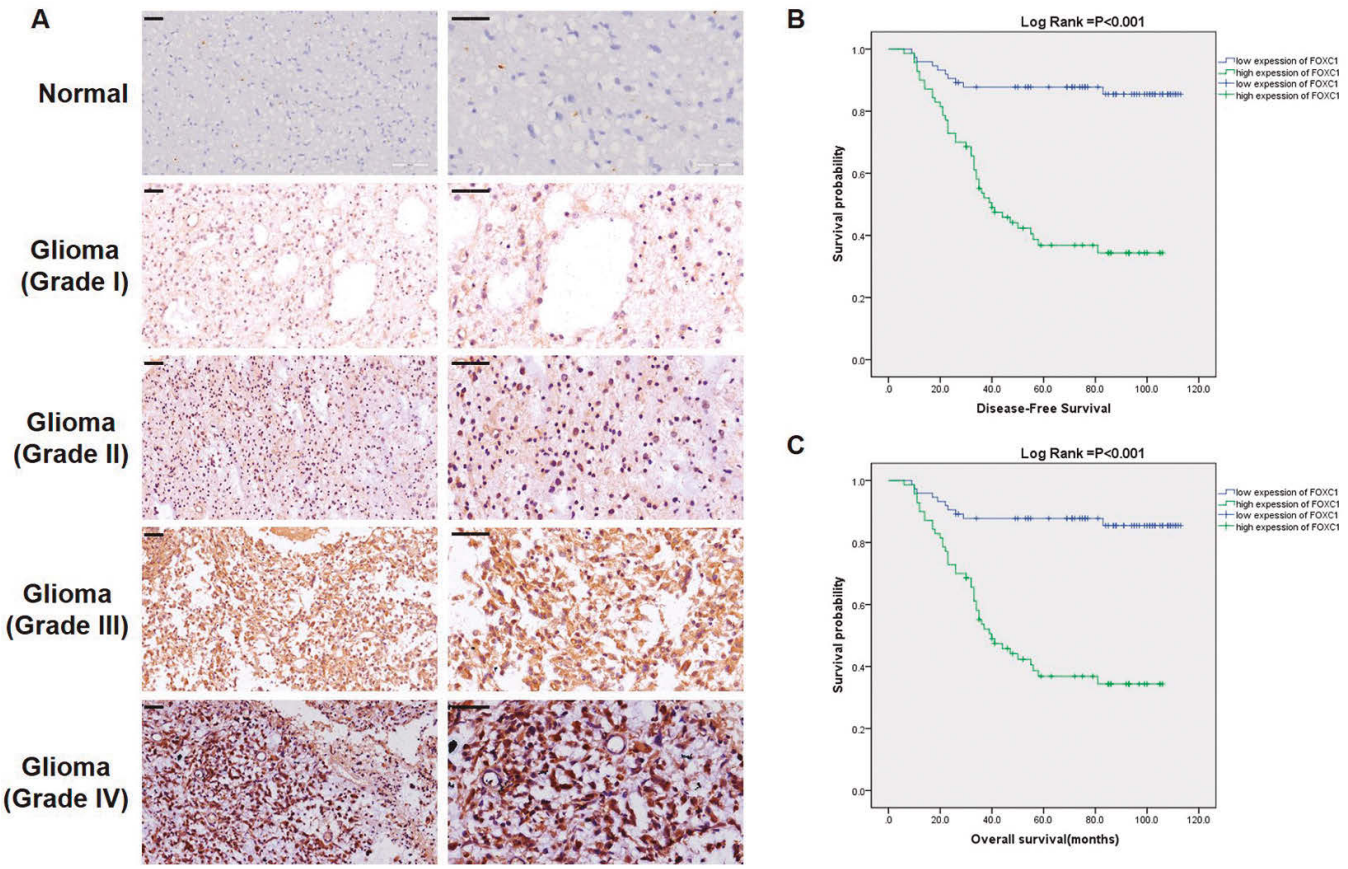

Fig. 3. FOXC1 is upregulated in glioma tissues and correlated with poor prognosis. **A.** Immunohistochemical staining was used to indicate the protein levels of FOXC1 in normal brain and glioma tissues with different pathological tumor grades (I-IV). **B, C.** Kaplan-Meier survival analysis was carried out to establish the correlation between FOXC1 expression and disease-free survival or overall survival of glioma patients.

downregulation of FOXC1 protein expression upon BPTF knockdown. Interestingly, treatment of cells with MG132 (for 8h) almost completely abolished the regulatory effects of BPTF on FOXC1 protein expression, indicating that this effect is mediated through the ubiquitin-proteasome system (Fig. 4B). To explore the de-ubiquitinating enzymes acting on FOXC1, the ubibrowser (Wang et al., 2022) (http://ubibrowser.bio-it.cn/ubibrowser_v3/home/index) was utilized (Fig. 4C), and we discovered that USP34 interacts with FOXC1 through protein-protein interactions (Fig. 5A). Additionally, knocking down the expression of USP34 in U251 cells not only significantly increased the ubiquitination level of FOXC1 (Fig. 5B) but also decreased its protein level (Fig. 5C). In summary, BPTF regulates the protein stability of FOXC1 through the ubiquitin-proteasome system, thereby influencing its expression, and this process may be associated with the de-ubiquitination of FOXC1 mediated by USP34.

Table 3. Relationship between FOXC1 expression and tumor characteristics in patients with glioma analyzed by Spearman's rank correlation analysis.

Tumor characteristics	Index	Value
Grade	Spearman's correlation	0.519
	Significance (two-tailed)	P<0.001
	n	144
Tumor recurrence	Spearman's correlation	0.491
	Significance (two-tailed)	P<0.001
	n	144

Discussion

BPTF, the largest member of the nucleosome remodeling complex (NURF), remains largely uncharacterized in terms of the functions of most of its structural domains. However, BPTF has increasingly been recognized as an oncogenic factor, which has drawn considerable attention to its regulatory role in tumor progression. In recent years, several studies have demonstrated the aberrant overexpression of BPTF in tumors, contributing to the promotion of various malignant phenotypes (Dar et al., 2015; Zhang et al., 2021). For instance, in breast cancer samples collected by Bezrookove et al. copy number gain and amplification were observed in 34.1% and 8.2% of cases, respectively, showing a significant correlation with increased tumor grading. Inhibition of BPTF has been shown to significantly suppress the *in vitro* and *in vivo* growth of triple-negative breast cancer cells and induce apoptosis, leading to notable anti-cancer effects

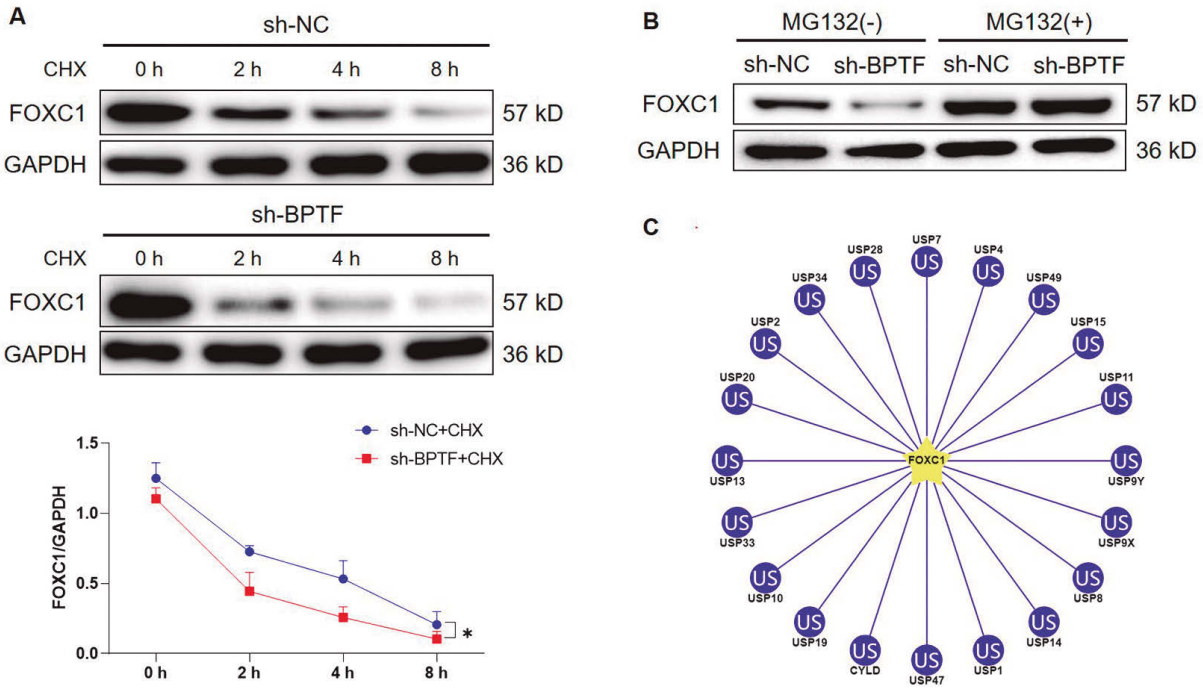

Fig. 4. BPTF regulates FOXC1 protein stability. **A.** A CHX chase assay was performed in sh-NC and sh-BPTF groups of U251 cells to evaluate the effects of BPTF knockdown on the protein stability of FOXC1. A protein degradation curve was drawn accordingly. The experiments were performed in triplicate. We have uploaded the original data for the other two independent experiments. **B.** Western blot was performed to detect the change in FOXC1 protein levels in U251 cells treated with sh-NC/sh-BPTF or sh-NC/sh-BPTF plus MG132 (10 μM). **C.** A series of de-ubiquitination enzymes for FOXC1 were predicted by the ubiquitination browser.

(Bezrookove et al., 2022). The study by Bai et al. revealed the regulatory role of BPTF in T-cell lymphoma. BPTF was overexpressed in T-cell lymphoma tissues compared with normal tissues and was associated with advanced clinical staging. Silencing of BPTF expression has been shown to inhibit tumor progression *in vitro* and *in vivo* by suppressing the activation of the MAPK pathway (Bai et al., 2022). BPTF has also been found to promote tumor progression in lung cancer, and its targeted regulation of tumor-related markers such as VEGF suggests its potential for predicting the efficacy of related therapies (Dai et al., 2019, 2022). In hepatocellular carcinoma, BPTF is also characterized by high expression. Knockdown of BPTF significantly inhibits the proliferation, clonogenicity, and stemness of cancer cells while increasing their sensitivity to chemotherapy drugs (Zhao et al., 2019). In our previous research, the high expression of BPTF in glioma tissues was found to be significantly associated with higher tumor grade and larger tumor size, and it was identified as an independent prognostic factor for glioma patients (Pan et al., 2019).

It is noteworthy that numerous studies have indicated that BPTF serves as an interacting factor of the oncogene MYC, playing a crucial role in MYC chromatin recruitment and transcriptional activity (Richart et al., 2016a). Correspondingly, it is essential for MYC-driven proliferation, G1-S progression, and replication stress (Richart, et al., 2016b). Research has shown that the expression of BPTF in colorectal cancer cells is transcriptionally regulated by MYC, thereby making significant contributions to the malignant progression of colorectal cancer (Guo et al., 2022). Lin et al. discovered that the circPDK1/miR-628-3p axis activates the BPTF/MYC axis in pancreatic cancer cells, leading to the induction of metabolic reprogramming of pancreatic cancer cells and tumor progression (Lin et al., 2022). Furthermore, targeted inhibition of the BPTF/MYC axis has been found to suppress the expression of ABC-transporter proteins in pancreatic ductal adenocarcinoma cells, thereby eliminating multidrug resistance (Munoz Velasco et al., 2022). Importantly, both the work of our group and that of Green et al. have elucidated the interconnected relationship between BPTF and the MYC pathway in regulating the malignant phenotype of glioma (Green et al., 2020).

FOXC1 is a member of the FOX transcription factor family, which can bind to target genes through its unique forkhead domain (FHD), thereby initiating the transcription process. In recent years, numerous studies have demonstrated the involvement of FOXC1 in tumor development, suggesting its potential clinical value for prognostic assessment of cancer patients (Han et al., 2017). In this study, IHC staining of clinical tissues and subsequent statistical analysis revealed that FOXC1 is highly expressed in glioma and indicates a higher degree of malignancy and worse prognosis. Of particular interest, multiple studies have indicated that FOXC1 is one of the upstream activating factors of MYC, playing a crucial role in activating its oncogenic functions (Tang et al., 2020, Wu et al., 2022). In this work, we investigated the regulatory role of BPTF, another known activator of MYC, on FOXC1 and found that BPTF positively regulates the protein expression of FOXC1, and both factors synergistically contribute to the regulation of glioma cell proliferation and migration. Mechanistically, inhibiting the expression of BPTF may promote the degradation of FOXC1 by reducing its protein stability, thereby modulating its expression. Studies have shown that the protein stability of FOXC1 is regulated by the de-ubiquitinating activity of the USP family (Liu et al., 2021). We also discovered that USP34 interacts with FOXC1 and decreases its protein ubiquitination levels.

In conclusion, it was demonstrated that BPTF may promote malignant cell phenotypes of glioma cells

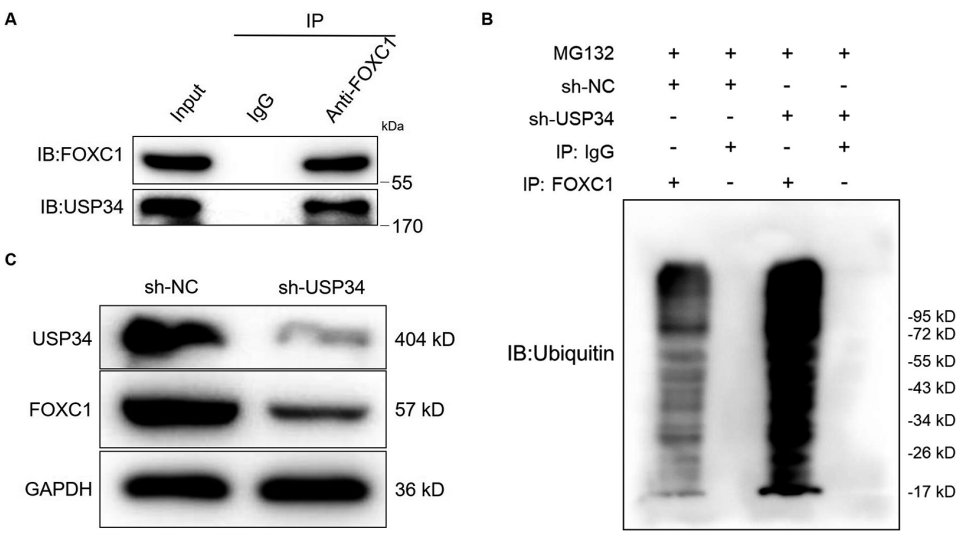

Fig. 5. BPTF regulates FOXC1 through USP34-mediated de-ubiquitination. **A.** A co-immunoprecipitation assay was performed using anti-FOXC1 to precipitate protein complex, followed by western blot analysis of FOXC1 and USP34 expression, to verify the protein-protein interaction between FOXC1 and USP34. **B.** A western blot using anti-ubiquitin was performed to detect the ubiquitination level of FOXC1 after the immunoprecipitation of the protein complex by anti-FOXC1 from the sh-NC or sh-USP34 groups of U251 cells. **C.** The protein levels of USP34 and FOXC1 in the sh-NC and sh-USP34 groups of U251 cells were detected by western blot analysis.

through enhancing USP34-mediated de-ubiquitination of FOXC1 and upregulating its expression. Therefore, the BPTF/FOXC1 axis may be a potential target for the inhibition of glioma development.

Ethics Statement. This research obtained ethical approval from the Ethical Committee of Haikou Affiliated Hospital of Central South University Xiangya School of Medicine (No. SC20180231).

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Author contributions. YL Pan designed this program. YL Pan and ZR Lin operated the cell and animal experiments. YL Pan, F Yuan, and YJ Li conducted the data collection and analysis. YL Pan produced the manuscript, which was checked by F Yuan and YJ Li. All authors have confirmed the submission of this manuscript.

Data availability statement. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest. The authors declare no conflict of interest.

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