**ORIGINAL RESEARCH ARTICLE**

**PCBP2 promotes the development of glioma by regulating FHL3/TGF-β/Smad signaling pathway**

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**Abstract**

The purpose of this study was to investigate the role of Poly (C)-binding protein 2 (PCBP2) and the related signaling pathway in glioma progression. Quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemistry (IHC) were performed to measure PCBP2 messenger RNA and protein expression in glioma tissues or cells. Cell transfection was completed using Lipofectamine 2000. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, Transwell assay and flow cytometry assay were used to explore the effects of PCBP2 expression on biological behaviors of glioma cells. Western blot assay was used for the detection of pathway related proteins. Expression of PCBP2 in glioma tissues and cells were higher than that in paracancerous tissues and normal cells (both \( p < .01 \)). Moreover, the elevated expression of PCBP2 was significantly correlated with tumor size \( (p = .001) \) and WHO stage \( (p = .010) \). Knockdown of PCBP2 could suppress proliferation, migration and invasion of glioma cells and promote apoptosis. Besides, the expression of transforming growth factor-β (TGF-β) pathway related proteins TGF-β1, p-Smad2 and p-Smad7 were decreased following the downregulation of PCBP2. PCBP2 also inhibited FHL3 expression by binding to FHL3-3′UTR. The inhibition of FHL3 could reverse the antitumor action caused by PCBP2 silencing. In vivo assay, PCBP2 was also found to inhibit the tumor growth of glioma. PCBP2 activates TGF-β/Smad signaling pathway by inhibiting FHL3 expression, thus promoting the development and progression of glioma.

**KEYWORDS**

FHL3, glioma, PCBP2, TGF-β/Smad pathway

1 | **INTRODUCTION**

Gliomas are the most frequently diagnosed intracranial malignancies with high morbidity and mortality in worldwide (de Robles et al., 2015). They are characterized by rapid metastasis and aggressive infiltration, leading to poor prognosis (Diksin, Smith, & Rahman, 2017; Ostrom et al., 2014). According to World Health Organization Classification of Tumors of the Central Nervous System proposed in 2016, glioblastoma (GBM) is the most common type of malignant gliomas, and the mean survival time of GBM patients is only approximately 15 months, even after systematic treatments (Lara-Velazquez et al., 2017; Louis et al., 2016). Growing evidence have demonstrated that the malignant degree of gliomas may be attributed to various genetic and epigenetic alterations in tumorigenesis (Masui, Kato, Sawada, Mischel, & Shibata, 2017; Tsankova & Canoll, 2014). Exploring...
the molecular mechanisms underlying the aggressive progression of glioma may provide the novel therapeutic targets for the fatal cancer.

Poly(C) binding protein 2 (PCBP2), a RNA-binding protein with 39 kDa, can regulate RNA stabilization, translational silencing and enhancement (Collier, Goobar-Larsson, Sokolowski, & Schwartz, 1998; Wan et al., 2016). It contains three K homology domains employed as the main RNA recognized regions (Chen et al., 2016; Leffers, Dejaegar, & Celis, 1995). Dysregulation of PCBP2 may damage multiple biological processes through RNA-binding pathways. For instance, PCBP2 could regulate the genome circularization and replication of various RNA viruses, like norovirus (Lopez-Manriquez et al., 2013), severe acute respiratory syndrome coronaviruses (SARS-CoV; Shi et al., 2014), poliovirus (Walter, Parsley, Ehrenfeld, & Semler, 2002), and hepatitis C virus (Li, Masaki, Shimakami, & Lemon, 2014). The upregulation of PCBP2 may contribute to the formation of ribosomal initiation complex, thus enhancing the replication of virus (Asnani, Pestova, & Hellen, 2016). Additionally, PCBP2 could regulate cell growth, migration and invasion (Chen et al., 2018; Mao et al., 2016). The abnormalities in PCBP2 expression have been observed in several cancers, such as gastric cancer (Chen et al., 2018) and hepatocellular carcinoma (Zhang et al., 2016). In glioma, it has been reported that the knockdown of PCBP2 may inhibit tumor cell proliferation and growth (Tang, Gao, & Chen, 2018) and hepatocellular carcinoma (Zhang et al., 2016). In glioma, it has been reported that the knockdown of PCBP2 may inhibit tumor cell proliferation and growth (Tang, Gao, & Chen, 2015). PCBP2 may act as an oncogene in etiology of glioma. However, the molecular mechanisms of PCBP2 in glioma progression were poorly known.

In this study, we investigated the expression of PCBP2 in glioma tissues and cells and revealing its functional roles in glioma progression. Furthermore, cell experiments were constructed to explore the molecular mechanisms underlying the oncogenic function of PCBP2 in the pathogenesis of glioma.

## Materials and Methods

### Patients and tissue samples collection

The present study was carried out in Harrison international Peace Hospital. A total of 106 adult glioma patients who were diagnosed through pathological examinations were enrolled in our study. Glioma tissues and corresponding noncancerous tissues were collected from the patients who received surgical procedures and put into liquid nitrogen immediately. Then the specimens were stored at -80°C for usage in the next step. None of the patients had received any treatments before tissue collection, such as chemotherapy or/and radiotherapy. Clinical characteristics of glioma patients were also obtained from medical records. This study was approved by the Ethics Committee of Harrison international Peace Hospital. All patients had signed the written informed contents.

### Cell culture

Human glioma cell line U251 and normal glial cell HEB were purchased from Cell Bank of Shanghai Institutes for Biological Science (Shanghai, China). Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies) supplemented with 10% fetal bovine serum (FBS; Life Technologies). The cells were incubated at 37°C with 5% CO₂. Medium for cell culture was changed every 2–3 days.

### Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of glioma tissues and cell specimens was extracted employing TRIzol reagent (Invitrogen, Carlsbad, CA) following the instruction of the manufacturer. Quality and concentration of RNA samples were checked using NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). Complementary DNA (cDNA) was synthesized via reverse-transcription reaction which was completed using PrimerScript RT reagent kit (Takara, Chiga, Japan). Relative expression of PCBP2 mRNA was estimated using qRT-PCR. qRT-PCR reactions were performed by SYBR Green PCR Master Mix (Applied Biosystems) in the 7900 Real-time PCR System (Applied Biosystems). Specific primer sequences for genes were as follows: PCBP2 forward: 5'-AGGCAGGTT ACCATCAGCG-3', reverse: 5'-CATTGCATCGCTCCACCC-3'; FHL3 forward: 5'-CATGGTCTCTAGCTGC-3', reverse: 5'-GCACCAGGACGCTGC-3'; GAPDH was employed as the internal control, and its primer sequences were as the following, forward: 5'-TGCACAGCTGCTTCGACC-3', reverse: 5'-GGCATGAGACTGTGGTGTA CG-3'. The relative expression level of the detected genes was calculated using 2⁻ΔΔCT method. Each test was repeated three times.

### Immunohistochemistry (IHC)

Protein expression of PCBP2 in glioma tissue samples were estimated using IHC analysis staining with streptavidin-peroxidase (S-P). IHC kit (Boster, Wuhan, China) was used according to manufacturer’s instruction. The used antibody included anti-PCBP2 antibody (1:200, Abcam, UK). Staining results were recorded by two independent investigators, and they did not know the histopathologic features and patients’ information. Staining results were scored according to the following standards: 0: for staining < 5%; 1: staining 6–25%; 2: staining 26–50%; 3: staining 51–75%; 4: staining > 75%. Patients with score <1 were confirmed as negative staining, otherwise, the patients were confirmed as positive staining.

### Vector construction and cell transfection

To investigate the role of PCBP2 in glioma progression, siRNA-PCBP2 was designed to silence PCBP2 expression in glioma cells in vitro. si-PCBP2 and corresponding negative control (si-NC) sequences were synthesized by GenePharma (Shanghai, China). The obtained
sequences were cloned into pLV-shRNA plasmid, and then transfectected into glioma cells using Lipofectamine™ 2000 (Invitrogen, Thermo Fisher Scientific, Inc.). Transfection for 48 hr, qRT-PCR was conducted to measure the relative expression of PCBP2 so as to estimate the transfection effects.

In addition, to explore the molecular mechanisms underlying the function of PCBP2 in the progression of glioma, si-FHL3 and FHL3-overexpression (pLV-FHL3) recombinant plasmids and corresponding controls were also constructed.

2.6 | 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay

The effect of PCBP2 on the proliferation of glioma cells was tested via MTT assay. After the cultivation of 48 hr, the transfected cells were seeded to 96-well plate (5 × 10^3 cells/well). The cells were incubated at the specific condition (37°C, 5% CO2). Each well was added 20 µl MTT (5 mg/ml; Sigma, St. Louis, MO) when transfected cells were incubated for 0, 24, 48, and 72 hr, respectively, and an additional 4 hr was needed to maintain the incubation. Then, 150 µl dimethyl sulfoxide (DMSO, Sigma-Aldrich) was added to dissolve the purple formazan for 15 min. And then the absorbance at 490 nm was read by a Microplate Reader (TECAN, Salzburg, Austria).

2.7 | Transwell assay

Cell migration and invasion abilities were tested using Transwell chambers coated or not by Matrigel (Corning Glass Works, Corning, NY) on the upper chamber, respectively. The upper chamber of Transwell (Corning Glass Works, Corning, NY) was added 200 µl serum-free medium, the bottom chamber was filled with the medium containing 10% FBS. Transfected cells with the density of 4 × 10^5/ml were seeded to the upper chamber. Incubation for 48 hr, the number of cells at the bottom chamber were determined by crystal violet staining and quantified based on gray value. Each test was repeated three times.

2.8 | Flow cytometry

Flow cytometry with Annexin V/PI double staining method was applied to detect cell apoptosis rate. After transfection for 48 hr, the cells were collected and adjusted to the density of 1 × 10^5/ml. Then they were stained by Annexin V FITC/PI (fluorescein isothiocyanate/propidium iodide) Apoptosis Detection Kit (Beyotime) according to the manufacturer’s specification. Apoptosis rate was assessed by flow cytometry (BD Biosciences, San Jose, CA). Each test was repeated in triplicate.

2.9 | Luciferase reporter assay

3′-Untranslated region (3′-UTR) of FHL3 gene contained the putative binding site of PCBP2. PCR method was used to amplify 3′-UTR of FHL3, and then it was cloned to p-GL3 vector (Promega, Madison, WI) containing firefly luciferase reporter gene (wild-type [WT]). In addition, 3′UTR of FHL3 with a mutant sequence of PCBP2 binding site was also amplified and cloned to the vector (mutate type [MT]). Then the vectors were transfected into U251 cells with or without si-PCBP2 vector. Transfection for 48 hr, the cells were harvested and the luciferase activity was detected using a dual-luciferase reporter assay system (Promega) and normalized to Renilla activity.

2.10 | Western blot analysis

Expression of proteins was detected using western blot analysis. Precold radioimmunoprecipitation assay buffer (RIPA buffer) (Beyotime, Shanghai, China) with protease inhibitor was used to isolate protein samples from the cells and protein samples were extracted and quantified by a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, San Jose, CA). The equal protein was separated in 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis, and transferred to the polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA) at 48 V for 3.5 hr. Then 5% bovine serum albumin (BSA) was applied to incubate the membranes at room temperature for 2 hr. After washing by 1 × Tris-buffer saline with Tween 20 (TBST), the membranes were blocked for 60 min and incubated for 1 hr at room temperature after adding the primary antibodies: mouse anti-human TGF-β1 (1:1,000), p-Smad2 (1:1,000), p-Smad7 (1:1,000), and Smad2 (1:1,000), and Smad7 (1:1,000) (Cell Signaling Technologies, Beverly, MA). Next, the membranes were incubated with the secondary goat anti-rabbit antibody (1:4,000; Cell Signaling Technologies, Beverly, MA), and visualized under UV transilluminator (Uvitec Ltd., Avebury House, Cambridge, UK). β-Actin was acted as an internal control. Protein levels were quantified based on gray value. Each test was repeated three times.

2.11 | Animal assay

Twenty Balb/c nude mice (6–8 weeks) were routinely cultured, U251 cells (3 × 10^6/200 µl) were inoculated on the upper part of the groin, mice were performed the subcutaneous injection. They were equally divided into two groups: experimental group (si-PCBP2) and the control group (si-NC), every group was 10 cases. The major axis (a) and minor axis (b) of the subcutaneous tumor were measured once 3 days, tumor volume: V = 1/2 × a × b^2. After 4 weeks, nude mice were killed for the measurement of tumor weight.

2.12 | Statistical analysis

All the data analysis and figure drawing were performed using SPSS 18.0 software (SPSS Inc., Chicago, IL), and GraphPad Prism version 5.0 (GraphPad, San Diego, CA), respectively. Continuous variables were shown as mean ± standard deviation (SD). Comparison between case and control groups via student’s t test. χ^2 test was used to explore the association of PCBP2 expression
with clinical parameters of glioma patients. \( p < .05 \) were considered as statistical significance.

3 | RESULTS

3.1 | Characteristics of study subjects

A total of 106 glioma patients were enrolled in our study, including 55 males and 51 females. The average age of glioma patients was 54.28 ± 11.025 years. Forty-four patients had family history of cancer, and the tumor size of 49 patients were larger than 5 cm. According to World Health Organization (WHO) classification, 65 patients were confirmed as Stage I-II, and 41 patients were Stage III-IV. The clinical characteristics of the patients were summarized in Table 1.

3.2 | Expression patterns of PCBP2 in glioma tissues and cells

mRNA levels of PCBP2 were detected using qRT-PCR in glioma tissues and cells. Analysis results demonstrated that PCBP2 mRNA level was significantly higher in glioma tissues and cells than that in noncancerous tissues and normal cells (\( p < .01 \) for both; Figure 1a,b). Then the protein levels of PCBP2 in glioma tissues were also examined by western blot assay. The data showed that PCBP2 protein level in glioma tissues was significantly increased compared with noncancerous tissues (\( p < 0.01 \); Figure 1b). Additionally, IHC was applied to investigate the expression of PCBP2 protein in glioma tissues. As shown in Figure 1d, PCBP2 exhibited the strong positive expression in glioma tissues, but weak positive expression in noncancerous tissues. The positive rate was 87.73% (93/106) in glioma tissues, while the positive rate was only 16.98% (18/106) in adjacent normal tissues.

3.3 | Association of PCBP2 mRNA levels with clinical characteristics of glioma patients

In this study, the relationship between PCBP2 expression and clinical parameters of glioma patients was evaluated. The included patients were divided into high expression group (\( n = 48 \)) and low expression group (\( n = 58 \)) according to the mean level of PCBP2 mRNA in glioma tissues. The results indicated that the high expression of PCBP2 was positively associated with tumor size (\( p = .001 \)), and advanced WHO stage (\( p = .010 \)). However, the expression of PCBP2 was not correlated with age, gender, family history, and KPS score of glioma patients (\( p > .05 \) for all; Table 1).

3.4 | Effects of PCBP2 expression on biological behaviors of glioma cells in vitro

To investigate the role of PCBP2 in etiology of glioma, si-PCBP2 vector was constructed in our study. After transfection, qRT-PCR and western blot analysis were used to detect the transfection efficiency of si-PCBP2, the result indicated that the expression of PCBP2 was remarkably downregulated in glioma cells which were transfected by si-PCBP2 (\( p < .001 \); Figure 2a,b). Effects of PCBP2 on biological behaviors of glioma cells were estimated in our study. MTT assay demonstrated that the knockdown of PCBP2 could distinctly suppress the proliferation of glioma cells (\( p < .05 \) and Figure 3a). Migration and invasion abilities of glioma cells were also significantly inhibited after

| TABLE 1 | The influences of PCBP2 expression on clinical characteristics of glioma patients |
|-----------------------------|-------------------------------------------------|--------------------------|--------------------------|--------------------------|
| Characteristics            | N (\( n = 106 \))                              | PCBP2 low expression (\( n = 58 \)) | PCBP2 high expression (\( n = 48 \)) | \( p \) values |
| Age (years)                |                                                |                          |                          | .072                     |
| ≥60                        | 43                                             | 19                       | 24                       |                          |
| <60                        | 63                                             | 39                       | 24                       |                          |
| Gender                     |                                                |                          |                          | .669                     |
| Male                       | 55                                             | 29                       | 26                       |                          |
| Female                     | 51                                             | 29                       | 22                       |                          |
| Family history             |                                                |                          |                          | .107                     |
| Yes                        | 44                                             | 20                       | 24                       |                          |
| No                         | 62                                             | 38                       | 24                       |                          |
| Tumor size (cm)            |                                                |                          |                          | .001                     |
| <5                         | 57                                             | 40                       | 17                       |                          |
| ≥5                         | 49                                             | 18                       | 31                       |                          |
| KPS scores                 |                                                |                          |                          | .588                     |
| ≥70                        | 39                                             | 20                       | 19                       |                          |
| <70                        | 67                                             | 38                       | 29                       |                          |
| WHO stage                  |                                                |                          |                          | .010                     |
| I-II                       | 65                                             | 42                       | 23                       |                          |
| III-IV                     | 41                                             | 16                       | 25                       |                          |

Abbreviations: KPS, Karnofsky performance score; PCBP2, poly (C)-binding protein 2; WHO, World health organization.
si-PCBP2 transfection, compare with si-NC group \((p < .05, \text{Figure 3b,c}).\) Furthermore, the apoptotic rate of si-PCBP2 transfected cells was obviously increased, compared with si-NC group \((p < .001, \text{Figure 3d}).\) Therefore, the knockdown of PCBP2 could obviously inhibit the progression of glioma cells.

### 3.5 PCBP2 knockdown could promote FHL3 expression and suppress TGF-β/Smad signaling pathway

In our study, qRT-PCR and western blot were carried out to investigate the expression of FHL3 in si-PCBP2 transfected cells.
FIGURE 3  Effects of poly (C)-binding protein 2 (PCBP2) expression on biological behaviors of glioma cells in vitro. The knockdown of PCBP2 expression could significantly inhibit proliferation (a), migration (b) and invasion (c) of glioma cells. Furthermore, inhibition of PCBP2 expression could promote cell apoptosis (d). Data are presented as mean ± SD (n = 3). *p < .05, **p < .01, and ***p < .001. SD, standard deviation.
The results suggested that the expression of FHL3 was obviously increased in glioma cells with the knockdown of PCBP2 (p < .05 and Figure 4a,b). PCBP2 could regulate the expression of FHL3 in glioma.

Moreover, western blot method was used to investigate the expressions of TGF-β/Smad signaling pathway proteins in glioma cells transfected by si-PCBP2 and the results showed that the expression of TGF-β1, p-Smad2, and p-Smad7 proteins were downregulated in glioma cells after PCBP2 knockdown. However, the expression of Smad2 and Smad7 did not show obvious changes (Figure 4c).

3.6 | FHL3 was a potential target of PCBP2 in glioma

Luciferase reporter assay was used to investigate the link between PCBP2 and FHL3. The glioma cell U251 was cotransfected by wt or mt and si-PCBP2 or si-NC plasmids. The relative luciferase activity was significantly reduced after PCBP2 knockdown in cells transfected by FHL3-wt (p < .01, Figure 5a). However, the luciferase activity in cells transfected by FHL3-mt did not show obvious changes after PCBP2 silencing (NS: nonsignificant). All the data revealed the target relationship between PCBP2 and FHL3.

Nucleotide sequence of FHL3 target binding site and the core recognition sequence are red (Figure 5a and Han et al., 2013). pLV-FHL3 vector was designed to enhance the expression of FHL3 in glioma cells. The glioma cell line U251 was transfected by pLV-FHL3 and corresponding control (pLV-NC) in vitro. qRT-PCR method was used to investigate the relative expression of PCBP2 and FHL3 in transfected cells. Expression of FHL3 mRNA was significantly increased after pLV-FHL3 transfection (p < .01, Figure 5b), while PCBP2 expression did not show obvious changes (p > .05, Figure 5d). The results of western blot analysis was also showed after pLV-FHL3 transfection, the expression of FHL3 protein was increased significantly (p < 0.01 and Figure 5c), while the expression of PCBP2 did not change significantly (p > .05 and Figure 5e). Therefore, PCBP2 could inhibit FHL3 expression, while FHL3 did not influence PCBP2 expression. The data indicated that FHL3 was located on the downstream of PCBP2. FHL3 might be a potential target gene of PCBP2 in glioma.

![Figure 4](image-url) Poly (C)-binding protein 2 (PCBP2) knockdown could promote FHL3 expression and suppress TGF-β/Smad signaling pathway. The knockdown of PCBP2 could significantly enhance the expression of FHL3 in glioma cells (a and b). Knockdown of PCBP2 might inhibit the activity of TGF-β/Smad signaling pathway (c). Data are presented as mean ± SD (n = 3). *p < .05, **p < .01, ***p < .001. SD, standard deviation.
3.7 | Oncogenic function of PCBP2 in glioma was mediated by FHL3

To explore the molecular mechanisms of PCBP2 in the progression of glioma, U251 was cotransfected by si-PCBP2 and si-FHL3. Western blot analysis suggested that compared with glioma cells only transfected by si-PCBP2, the cotransfection of si-PCBP2 and si-FHL3 could significantly enhance the expression of TGF-β1, p-Smad2, and p-Smad7 proteins, suggesting the activation of TGF-β/Smad signaling pathway (Figure 6).

FIGURE 5  FHL3 was a potential target of poly (C)-binding protein 2 (PCBP2) in glioma. The nucleotide sequence of FHL3 target binding site and the core recognition sequence are red (a). The loss of PCBP2 expression could reduce the luciferase activity of cell transfected by FHL3-wt, but it did not influence the cells transfected by FHL3-mt (b). The transfection of pLV-FHL3 vector could distinctly enhance the expression of FHL3 (c and d), but had no significant influence on PCBP2 expression (e and f). Data are presented as mean ± SD (n = 3). **p < .01, (NS, nonsignificant, p > .05)
In addition, the proliferation (p < .05, Figure 7a), migration (p < .01 and Figure 7b) and invasion (p < .01 and Figure 7c) abilities of cotransfected cells were distinctly enhanced, and the cell apoptosis (p < .001 Figure 7d) was decreased. All the data revealed that si-FHL3 transfection could reverse the antitumor action caused by the knockdown of PCBP2 in glioma.

3.8 | Knockout of PCBP2 inhibited the progression of glioma in vivo

In this study, we also carried out the animal assay to verify the role of PCBP2 in glioma progression. The results showed that tumor volume and weight in nude mice with glioma cells transfected by si-PCBP2 were significantly decreased, compared with si-NC transfection group (Figure 8a-c and p < .05). Meanwhile, in vivo experiment, FHL3 expression level was significantly upregulated in si-PCBP2 group (p < .001 and Figure 8d). Therefore, PCBP2 could regulate the progression of glioma through targeting FHL3.

4 | DISCUSSION

Glioma is a deadly malignancy, accounting for about 80% primary brain cancer (Omuro & DeAngelis, 2013). To improve the outcome of glioma, it is crucial to explore the molecular mechanisms underlying the tumor progression of glioma. In the current study, we found that PCBP2 silencing could inhibit glioma cell proliferation, migration and invasion, and promote cell apoptosis in vitro. PCBP2 might participate in glioma progression through regulating FHL3/TGF-β/Smad signaling pathway.

PCBP2 is a RNA-binding protein that can regulate RNA processing (Collier et al., 1998; Wan et al., 2016). Abnormal expression of PCBP2 may lead to genetic alterations, thus leading to human diseases, like cancer. In our study, we found that the expression of PCBP2 was significantly upregulated in glioma tissues and cells, compared with noncancerous tissues and normal cells. Furthermore, the elevated expression of PCBP2 showed the positive association with tumor size and WHO stages of glioma. The conclusion was consistent with the previous studies. Luo et al. reported that the high expression of PCBP2 was closely correlated with advanced tumor stage and poor prognosis of GBM patients and it might be a potential prognostic biomarker for GBM patients (Luo & Zhuang, 2017). Increased expression of PCBP2 may be a risk hallmark for glioma patients, predicting aggressive cancer progression.

Given their function in RNA processing process, PCBP2 is involved in several cellular processes, such as cell proliferation, migration and invasion, as well as apoptosis. In the present study, in vitro experiments were performed to investigate the effects of PCBP2 on biological behaviors of glioma cells. The results demonstrated that cell growth and motility (migration and invasion) were significantly inhibited, and cell apoptosis was enhanced in transfected glioma cells when PCBP2 expression was decreased. Tang et al. (2015) indicated that the upregulation of PCBP2 could remarkably promote the cell proliferation and growth of glioma in vitro. Lin et al. (2016) suggested that PCBP2 could facilitate the migration and invasion of glioma cells. These previous studies all supported the conclusions obtained in our study. PCBP2 may be an oncogene and therapeutic target in glioma.

In addition, we also investigated the molecular mechanisms of PCBP2 in glioma progression. We found that the knockdown of PCBP2 might lead to high expression of FHL3 and low activity of TGF-β/Smad signaling pathway in glioma cells. Besides, the expression of FHL3 did not obviously influence the expression of PCBP2. Therefore, FHL3 might be a downstream target gene of PCBP2 in glioma. Moreover, the vivo mice experiment also verified the results in our study. A gene chip assay performed by Han et al. also indicated the link between PCBP2 and FHL3 in glioma progression (Han et al., 2013). PCBP2 may bind to 3′ UTR of FHL3 gene (Han, Xia, Yin, & Peng, 2014). It has been reported to bind several target genes in diseases. PCBP2 could regulate CDK2 via direct 3′UTR binding in gastric cancer patients, which might be used as a potential biomarker for the therapy of gastric cancer (Chen et al., 2018). Xin et al. also reported that PCBP2 could promote the antiviral activity of IFN-α against HCV by stabilizing the mRNA of STAT1 and STAT2 (Xin et al., 2011). In addition, we found that the inhibition of FHL3 expression might enhance TGF-β/Smad signaling pathway activity in glioma cells which were transfected by si-PCBP2. FHL3 protein belongs to LIM-only proteins family and can enhance phosphorylation and unclear accumulation of Smad proteins, thereby leading to the activation of

![FIGURE 6](image-url) The knockdown of FHL3 expression could enhance the expression patterns of TGF-β/Smad signaling pathway proteins in glioma cells transfected by si-PCBP2 vector. *p < .05, **p < .01
tumor suppressor p21 and repression of oncogene c-myc (Ding et al., 2009). In the current study, we also found that knockdown of FHL3 could reverse the tumor-suppressive effects caused by PCBP2 silencing expression in glioma. Therefore, PCBP2 may activate TGF-β/Smad signaling pathway through suppressing FHL3, thus contributing to the progression of glioma.

Certainly, several limitations in the current study should be stated. First, the sample size was relatively small that might reduce the statistical power of the current study. Second, the interaction between PCBP2 and other genes, as well as relative signaling pathways which might play potential roles in cancer development were not investigated in our study. The progression
of glioma is a complex process regulated by a netweb consisting with various genes, noncoding RNAs, pathways and proteins. To explain the development and progression of glioma, further research are required to explore the genetic regulatory netweb in glioma.

In conclusion, the upregulated expression of PCBP2 can promote glioma progression including enhancing cell proliferation, migration and invasion, and inhibiting cell apoptosis. In glioma, PCBP2 activates TGF-β/Smad signaling pathway through targeting FHL3 to participate in glioma progression.

**AUTHOR CONTRIBUTIONS**

J. M., Z. S., and Y. C. conceived and designed the experiments; N. D. and H. G. conceived and performed the experiments; J. W., L. Z., and Z. H. prepared figures. J. W. and Z. H. wrote the main manuscript text. All authors reviewed the manuscript.

**DATA ACCESSIBILITY**

Data available on request from the authors.

**ETHICS STATEMENT**

With the approval of Central Laboratory, Harrison International Peace Hospital Ethics Committee, written informed consent was obtaining from every subject.

| FIGURE 8 | Tumor volume was significantly decreased in nude mice with PCBP2 knockdown (a and b); Tumor weight was obviously lower in si-PCBP2 group than that in si-NC group (c); The expression level of FHL3 was also upregulated in si-PCBP2 group (d). *p < .05, **p < .01, ***p < .001 |

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