



Original Article

## Effect of circFOXM1/miR-218-5p on the proliferation, apoptosis and migration of glioma cells



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### Article Info

### Abstract



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This study aimed to explore the influence of circFOXM1/miR-218-5p molecular axis in the proliferation, apoptosis and migration of glioma cells. The levels of circFOXM1 and miR-218-5p in glioma and adjacent tissues were tested by qRT-PCR. Cultured human glioma U251 cells were randomly split into groups: si-NC, si-circFOXM1, miR-NC, miR-218-5p, si-circFOXM1+anti-miR-NC, si-circFOXM1+anti-miR-218-5p. MTT method, plate clone formation, flow cytometry and Transwell experiments were utilized for detecting the proliferation, clone formation, apoptosis and migration of glioma cells. Dual-luciferase reporter experiment authenticated the targeted relation of circFOXM1 and miR-218-5p. Western blot tested the levels of E-cadherin and N-cadherin. CircFOXM1 was upregulated while miR-218-5p was low expressed in glioma tissues versus normal tissues. After circFOXM1 silence or miR-218-5p overexpression, miR-218-5p level was increased, and cell apoptosis rate and E-cadherin expression were enhanced, whereas cell proliferation, cell clone formation and migration abilities, and N-cadherin level were reduced. CircFOXM1 could affect miR-218-5p level by negative regulation. Furthermore, miR-218-5p silence could reverse the stimulative influence of si-circFOXM1 on apoptosis rate, and E-cadherin level, and the repressive effect on cell viability, cell number of colony formation and migration, and N-cadherin expression. Inhibition of circFOXM1 expression could block the proliferation, clone formation, and migration and induce apoptosis of glioma cells by upregulating miR-218-5p.

**Keywords:** Glioma; circFOXM1; miR-218-5p; Cell proliferation; Migration; Apoptosis

## 1. Introduction

Glioma is a common major pernicious tumor with an incidence of about 50% [1]. Currently, surgical resection and radiotherapy are mainly used for treatment, but the strong invasion ability of glioma cells leads to poor prognosis of patients [2, 3]. Circular RNA (circRNA) is a non-coding RNA molecule formed by connecting the 3' end to the 5' end, which can affect the biological behavior of glioma cells by sponging microRNA (miRNA) [4, 5]. CircFOXM1 expression was upregulated in thyroid papillary carcinoma tissues and cells, and downregulated circFOXM1 expression can inhibit the growth of thyroid papillary carcinoma [6]. However, the correlation between circFOXM1 and glioma has not been totally clear.

The circRNA/miRNA axis has been mentioned in multifarious diseases, and it can offer new ideas for remedying diseases and exploiting new drugs [7, 8]. Also, circRNA/miRNA axis is common in glioma research and can be used for identifying curative options for glioma [9, 10]. Starbase predicted that circFOXM1 had complementary sequence with miR-218-5p. Previous work has suggested

the reduction of miR-218-5p level in the tissues of glioma, which was relevant to poor prognosis of patients; and upregulation of miR-218-5p could impede cell proliferative and transferable abilities in glioma by targeting and regulating the expression of TCF12 [11]. However, the action theory of circFOXM1/miR-218-5p axis in glioma progress is indistinct. Here, this research focused on exploring if circFOXM1 could facilitate the growth of glioma cells by absorbing miR-218-5p.

## 2. Materials and methods

### 2.1. Clinical specimens

Tumor tissue and adjacent tissue specimens of 50 glioma patients admitted to our hospital from April 2020 to September 2020 were gathered and kept in an ultra-low temperature refrigerator at -80°C. All patients were diagnosed with glioma by pathological examination. 30 males and 20 females among 40 and 60 ages were involved, the mean age of them was about 52.32. The patients or near relations them were informed and agreed, that this project met the related demands of the Declaration of Helsinki of

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the World Medical Association.

## 2.2. Cell culture and experimental grouping

Human glioma cell line (U251) was obtained from Procell (Wuhan, China) and fostered at 37°C in DMEM complete medium (Beyotime, Shanghai, China) plus 10% FBS (Beyotime) and 1% P/S antibiotics in 5% CO<sub>2</sub> incubator.

For cell transfection, U251 cells were fostered and transfected in 6-well plates according to instructions of Lipofectamine2000 (Invitrogen, Carlsbad, CA, USA) when the growth and fusion of cells reached 70%. The experimental groups were divided according to different transfections: si-NC, si-circFOXM1, miR-NC, miR-218-5p mimics, si-circFOXM1+anti-miR-NC, si-circFOXM1+anti-miR-218-5p. Therein, above plasmids or oligonucleotides were purchased from RiboBio Biotechnology (Guangzhou, China).

## 2.3. Quantitative real-time PCR (qRT-PCR)

Trizol (Beyotime) was added into tissues and cells for the acquisition of total RNA, and the synthesis of cDNA from 2 µg RNA was proceeded by Reverse transcription kit (Tiangen, Beijing, China). SYBR Green kit (Tiangen) was used for qRT-PCR amplification. CircFOXM1 and miR-218-5p expression were comparatively computed using 2<sup>-ΔΔCt</sup> tactic, GAPDH or U6 severally utilized as the inner reference.

## 2.4. MTT assay

U251 cells were gathered and put into 96-well plates, and 20 µL MTT (5 mg/mL; Solarbio, Beijing, China) was put in each aperture. 4 h later, 150 µL DMSO was incorporated into each aperture for 5 min-incubation after the abandonment of supernatant. The absorbance value of each aperture (A 490 nm) was detected by plate reader.

## 2.5. Colony formation assay

U251 cells were collected and appended in 6-well plate (500 cells/well), and fostered for 14 days, then DMEM was discarded. The cells were precooled by PBS, followed by the 20-minute fixation of methanol and 15-minute staining of 1% crystal violet. The formed cell clones were photographed and observed.

## 2.6. Flow cytometry

U251 cells were gathered and digested, and supernatant was abandoned. The pre-cooled PBS was used for the washing of cell pellet, and 500 µL binding buffer suspended cells, and later 5 µL Annexin V-FITC followed by PI (all from apoptosis detection kit, Solarbio) were respectively added. FACS Calibur flow cytometer was utilized for detecting apoptosis after 10 min-incubation.

## 2.7. Transwell assay

U251 cells (2×10<sup>5</sup> cells/well) from each group were inoculated into top Transwell chamber (Solarbio), and the underneath was added with 600 µL DMEM containing 10% FBS, which was continued and fostered for 24 h. Then, cells were subjected to 20 min-fixation of paraformaldehyde and 10 min-tinting of 0.1% crystal violet. A microscope was utilized for observing cell number of migration.

## 2.8. Dual-luciferase reporter assay

There is a complementary site between circFOXM1 and miR-218-5p, to detect the targeting relation between them, the mutation of the complementary site was conducted, and the complementary and mutational sites were cloned into pmirGLO vector to obtain wild-type (WT)-circFOXM1 and mutant (MUT)-circFOXM1 vectors. Luciferase reporter gene vectors WT-circFOXM1 and MUT-circFOXM1 were purchased from (Promega, Madison, WI, USA) and Double luciferase activity detection kit was obtained from Solarbio. The experimental groups were as follows: miR-NC and WT-circFOXM1 group, miR-218-5p and WT-circFOXM1 group, miR-NC and MUT-circFOXM1 group, miR-218-5p and MUT-circFOXM1 group. The transfection process was conducted to refer to Lipofectamine2000 transfection reagent.

## 2.9. Western blot

Collected U251 cells were added with 500 µL RIPA to extract total protein. Protein samples of 40 µg were taken for SDS-PAGE reaction, and PVDF membrane was utilized for the transfer of protein. After 1 h-sealing of 5% nonfat-dried milk, PVDF membrane interacted with dilution solution of E-cadherin (1:1000, Abcam, Cambridge, UK), N-cadherin (1:1000, Abcam) and inner standard GAPDH (1:2000, Abcam) antibodies for 24 h at 4°C. The second antibody (1:3000, Abcam) was added for 2h incubation at room temperature and developed after ECL luminescence. The grayscale of strips received the analysis of ImageJ software.

## 2.10. Statistical analysis

SPSS 21.0 software was utilized for analyzing the data, which were expressed as (x±s). Comparing the differences of multiple groups or between two groups severally proceeded using ANOVA or *t*-test. *P* < 0.05 was deemed to be statistically significant.

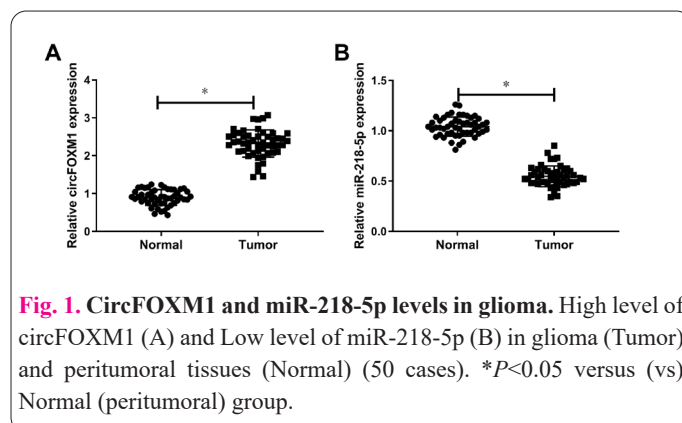
## 3. Results

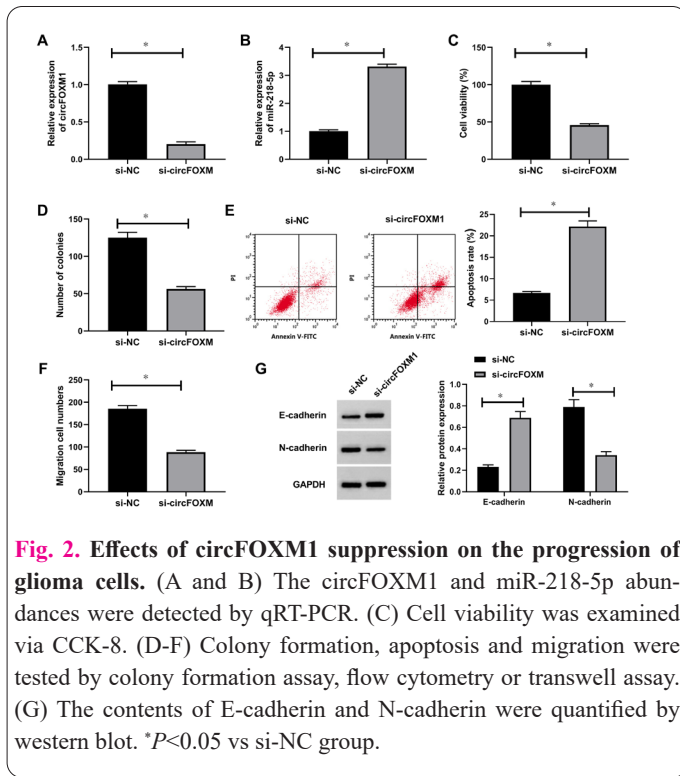
### 3.1. Change of circFOXM1 and miR-218-5p in glioma

In comparison with peritumoral tissues, circFOXM1 level in glioma tissues of 50 cases of patients diagnosed as glioma was elevated, whereas miR-218-5p level declined, as illustrated in Figure 1A-1B.

### 3.2. Inhibition of circFOXM1 on growth, apoptosis and metastasis of U251 cells

After circFOXM1 silence, miR-218-5p level had tripled (Figure 2A-2B). Meanwhile, cell viability and cell colony formation ability declined, whereas cell apoptosis rate was elevated in circFOXM1-silenced U251 cells





**Fig. 2.** Effects of circFOXM1 suppression on the progression of glioma cells. (A and B) The circFOXM1 and miR-218-5p abundances were detected by qRT-PCR. (C) Cell viability was examined via CCK-8. (D-F) Colony formation, apoptosis and migration were tested by colony formation assay, flow cytometry or transwell assay. (G) The contents of E-cadherin and N-cadherin were quantified by western blot. \* $P < 0.05$  vs si-NC group.

(Figure 2C-2E). After circFOXM1 inhibition, U251 cell migration and N-cadherin protein level declined, while E-cadherin expression was upregulated (Figure 2F-2G).

### 3.3. circFOXM1 targeted miR-218-5p

The combinative site of circFOXM1 and miR-218-5p was existed and listed (Figure 3A). Moreover, elevation of miR-218-5p lessened the luciferase activity of WT-circFOXM1 vector, but had no impact on the luciferase activity of MUT-circFOXM1 vector, as shown in Figure 3B.

### 3.4. Influences of miR-218-5p on the vicious progress of U251 cells

After the introduction of miR-218-5p mimics, cell viability, cell number of colony formation, migration, and N-cadherin protein level were lessened, while apoptosis and E-cadherin expression in U251 cells were upregulated (Figure 4A-4F).

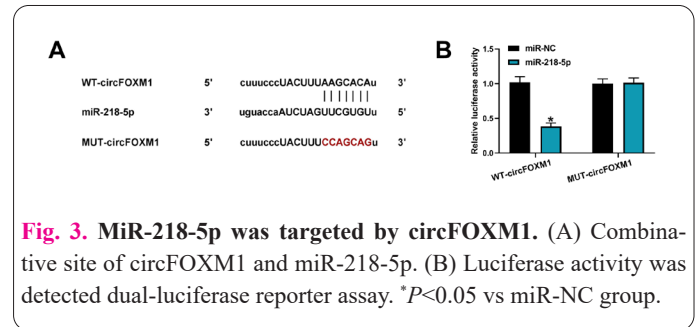
### 3.5. Influence of miR-218-5p inhibition on progression of circFOXM1 silence-treated U251 cells

Compared with si-circFOXM1+anti-miR-NC group, miR-218-5p silence in si-circFOXM1-treated cells had increased cell viability, number of cell clone formation, and also had reductive apoptosis rates (Figure 5A-5D). In the U251 cells with circFOXM1 silence, miR-218-5p downregulation enhanced the number of migrating cells, and lessened the level of E-cadherin protein, while improving N-cadherin protein expression (Figure 5E-5F).

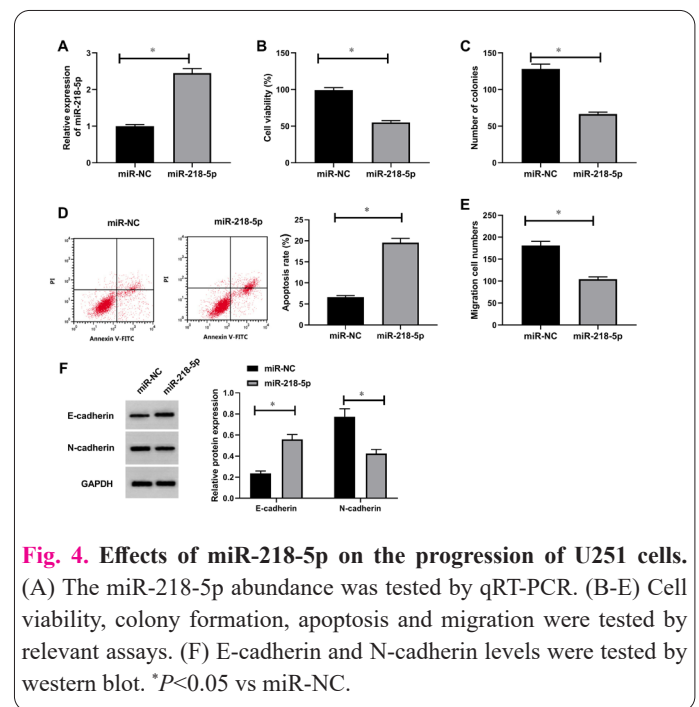
## 4. Discussion

There are a large number of miRNA binding sites on circRNA, which can bind to miRNA to inhibit miRNA expression, thus antagonizing the regulatory effect of miRNA on target genes. Research findings have attested that circRNA is unconventionally expressed in glioma and can work as potential biological indicator for early diagnosis of glioma [12, 13]. Upregulation or downregulation of circRNA expression can exert a corresponding function in the

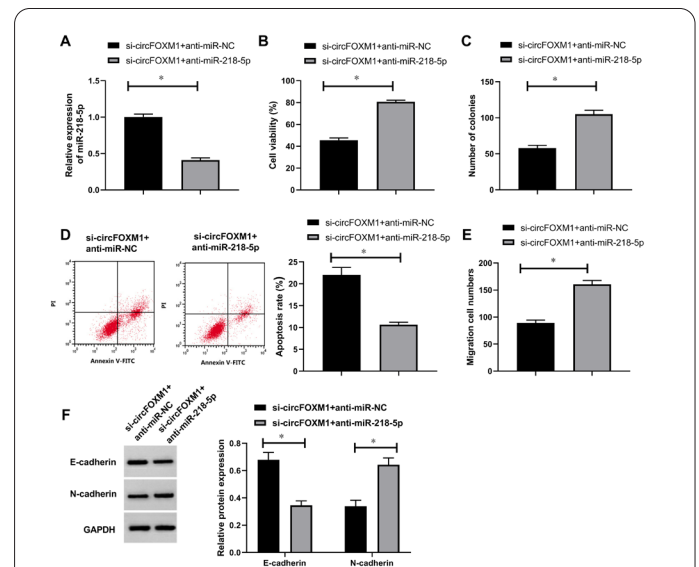
occurrence and advancement of glioma, that is, upregulation of circRNA expression can play the role of oncogene, and vice versa can play the role of tumor suppressor gene. CircRNA has been verified to be a potential target for molecular targeted therapy of glioma [14, 15]. CircFOXM1



**Fig. 3.** MiR-218-5p was targeted by circFOXM1. (A) Combinative site of circFOXM1 and miR-218-5p. (B) Luciferase activity was detected dual-luciferase reporter assay. \* $P < 0.05$  vs miR-NC group.



**Fig. 4.** Effects of miR-218-5p on the progression of U251 cells. (A) The miR-218-5p abundance was tested by qRT-PCR. (B-E) Cell viability, colony formation, apoptosis and migration were tested by relevant assays. (F) E-cadherin and N-cadherin levels were tested by western blot. \* $P < 0.05$  vs miR-NC.



**Fig. 5.** Impact of miR-218-5p inhibition on U251 cell apoptosis after circFOXM1 silence. (A) The miR-218-5p level was tested by qRT-PCR. (B-E) Cell viability, colony formation, apoptosis and migration were examined by relevant assays. (F) The expression of E-cadherin and N-cadherin was examined by western blot. \* $P < 0.05$  vs si-circFOXM1+anti-miR-NC.

was highly expressed in hepatocellular carcinoma (HCC) tissues with sorafenib resistance, and downregulation of circFOXM1 level can block the growth of HCC cells [16]. CircFOXM1 was upregulated in lung cancer cells, and downregulation of it could restrain lung cancer cell metastasis and inhibit the tumor growth of transplants [17]. However, the role of circFOXM1 in glioma is not totally reported. This study verified that circFOXM1 level was elevated in glioma tissues, and circFOXM1 silence could reduce the proliferation and clonogenesis of glioma cells, implying that high expression of circFOXM1 might promote the pernicious progression of glioma. E-cadherin is used as an epithelial cell marker of EMT, and N-cadherin is used as a mesenchymal cell marker of EMT. When the expression of both is dysregulated, EMT transformation can be promoted to promote cell metastasis [18, 19]. Here, we certified that inhibition of circFOXM1 expression reduced the migration ability and elevated the apoptosis rate of glioma cells, suggesting that inhibition of circFOXM1 expression could facilitate apoptosis and impede migration of glioma cells.

The circFOXM1/miRNA axis has been mentioned in the progress of multifold diseases [20, 21]. Moreover, circFOXM1 could regulate miR-432/Gα12 and miR-577/E2F5 axis in glioma to accelerate cell malicious development [22, 23]. For exploring the action mechanism of circFOXM1 in the growth of glioma, this study confirmed that circFOXM1 has a targeted regulatory effect on miR-218-5p, and there is a binding site of miR-218-5p on the circFOXM1 gene sequence. Research has certified that miR-218-5p had low expression in NSCLC and accelerated the transferability of NSCLC cells [24]. Downregulation of miR-218-5p was found in osteosarcoma, and circ\_0028171 promoted the expression of IKBKB as a sponge molecule of miR-218-5p, thus promoting the proliferation and metastasis of osteosarcoma cells [25]. MiR-218-5p upregulation can block cell growth and metastasis and accelerate cell apoptosis in bladder cancer [26-29]. In the results of this study, miR-218-5p level declined in the tissues of glioma, and enhancement of miR-218-5p blocked cell growth, clone formation and migration and facilitated apoptosis of glioma cells. Downregulation of miR-218-5p could weaken the inhibitory impact of circFOXM1 downregulation on the detrimental progress of glioma cells, and boost cell apoptosis. This suggested that circFOXM1 could facilitate the progress of glioma cells through negatively adjusting miR-218-5p level.

In summary, the level of circFOXM1 is upregulated in glioma tissues, while miR-218-5p is downregulated. CircFOXM1 can target and adsorb miR-218-5p, thus inhibiting miR-218-5p level. Silence of circFOXM1 in glioma cells can hinder the proliferation, clone formation and migration and promote apoptosis through upregulating miR-218-5p. CircFOXM1 may be a latent target for molecular targeted therapy of glioma, and it can also furnish an experimental basis for further elucidating the pathogeny of glioma. However, whether there is other miRNA in the circFOXM1 gene sequence and whether they can partake in the occurrence and development of glioma through absorbing and regulating other miRNA remains to be further investigated.

### Conflict of Interests

The author has no conflicts with any step of the article

preparation.

### Consent for publications

The author read and approved the final manuscript for publication.

### Ethics approval and consent to participate

No human or animals were used in the present research.

### Informed Consent

The authors declare not used any patients in this research.

### Availability of data and material

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

### Authors' contributions

DR and CJ conducted the experiments and wrote the paper; QJ,WL,ZD analyzed and organized the data; SX conceived, designed the study and revised the manuscript.

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