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# Circ\_0000020 elevates the expression of PIK3CA and facilitates the malignant phenotypes of glioma cells via targeting miR-142-5p

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## Abstract

**Background:** Multiple circular RNAs (circRNAs) have been recently described as crucial oncogenic factors or tumor suppressors. This study aimed to investigate the role of circ\_0000020 in glioma progression.

**Methods:** Circ\_0000020 and miR-142-5p expressions in glioma samples were assessed through qRT-PCR, and then the association between pathological indexes and circ\_0000020 expressions was analyzed. Functional experiment was performed with human glioma cell lines U251 and U87. Gain-of-function and loss-of-function models were established. CCK-8 assay was used to detect glioma cell proliferation. Transwell assay was used to examine glioma cell migration and invasion. The regulatory relationships among circ\_0000020, miR-142-5p and phosphatidylinositol 3-kinase C (PIK3CA) were investigated by bioinformatics analysis, luciferase reporter assay, qRT-PCR and Western blot. In vivo tumorigenesis assay was performed with nude mice to further validate the demonstrations of in vitro experiments.

**Results:** Circ\_0000020 expression in glioma samples was remarkably increased compared with that in normal brain tissues and its high expression was associated with unfavorable pathological indexes. Circ\_0000020 overexpression remarkably accelerated proliferation, migration and invasion of glioma cells. Accordingly, circ\_0000020 knockdown suppressed the malignant phenotypes of glioma cells. Circ\_0000020 overexpression significantly reduced miR-142-5p expression by sponging it, and circ\_0000020 could enhance the expression of PIK3CA, which was a target gene of miR-142-5p.

**Conclusions:** Circ\_0000020 promotes glioma progression via miR-142-5p/PIK3CA axis.

**Keywords:** Glioma, circ\_0000020, miR-142-5p, PIK3CA

## Background

Glioma accounts for 40–60% in all intracranial tumors [1]. Despite the continuous improvement of treatment strategies such as surgery, radiotherapy, and chemotherapy, the median survival time of patients is far from

satisfactory [2, 3]. Clarifying the mechanism of glioma progression may provide clues for the diagnosis and treatment of this deadly disease.

Circular RNA (circRNA) is a type of RNA that has a closed-loop structure formed by reverse splicing and covalent bonding [4]. CircRNA has complex biological functions [5]. Accumulating studies authenticate that abnormal expression of circRNA is related to the occurrence and progression of human malignancies, gliomas

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included [6–8]. For example, circTTBK2 expression is increased in glioma tissues and cell lines, and its overexpression accelerates cancer cell proliferation, migration, and invasion [6]; circ\_0074362 expression is dramatically up-regulated in glioma tissues, and its high expression is markedly related to the unfavorable pathological characteristics and poor prognosis of patients [7].

MicroRNAs (miRNAs) are an endogenous small non-coding RNA (ncRNAs) containing 18–25 nucleotides [9]. MiRNAs can completely or incompletely pair with the 3'-UTR of target mRNA, therefore leading to mRNA degradation or post-transcriptional translational inhibition [10]. MiRNAs are important players in cancer biology [11, 12]. MiR-142-5p expression is increased in colorectal cancer, and miR-142-5p enhances the cancer progression via targeting SDHB [13]. Besides, miR-142-5p blocks the progression of pancreatic cancer by targeting RAP1A [14]. Moreover, reportedly, miR-142-3p expression is decreased in gliomas, and miR-142-3p overexpression can remarkably repress glioma cell migration and invasion [15]. Instead, the role of miR-142-5p in glioma still await more investigations. Interestingly, in our preliminary work, bioinformatics analysis suggests that circ\_0000020 can probably repress miR-142-5p, suggesting circ\_0000020 may exert oncogenic functions in glioma via inhibiting miR-142-5p.

Phosphatidylinositol kinase 3 catalytic subunit alpha (PIK3CA) gene is located on chromosome 3q26.3 and encodes a protein containing 1068 amino acid residues. PIK3CA encodes the p110 catalytic subunit of class I phosphatidylinositol 3-kinases (PI3Ks), namely PI3Kp110a [16]. PIK3CA mutations can not only strengthen the catalytic activity of PI3Ks, but also contribute to tumorigenesis [17, 18]. Importantly, previous studies report that PIK3CA expression is elevated in gliomas and it promotes glioma progression [19, 20].

In this work, we confirmed that circ\_0000020 expression was elevated in glioma tissues and cell lines. The high expression of circ\_0000020 was markedly associated with unfavorable clinicopathological indicators. Additionally, we demonstrated that circ\_0000020 promoted the proliferation, migration and invasion of glioma cells via modulating miR-142-5p/PI3KCA axis. These results implied that circ\_0000020 might be a biomarker and therapeutic target for gliomas.

## Materials and methods

### Tissue samples

Our study was ratified by the Research Ethics Committee of Xiangyang Central Hospital and obtained consent from all of the subjects involved. Tissue specimens (paired cancerous tissues and adjacent tissues) were from 50 randomly selected glioma patients, 29 males and

21 females included, who had undergone tumor resection in Xiangyang Central Hospital from 2015 to 2017. Adjacent tissues were the non-cancerous tissues of the same patient (about 3 cm away from the surgical margin). Before this study, the tissues were diagnosed and re-evaluated according to the World Health Organization (WHO) criteria by two experienced pathologists. All samples were stored in liquid nitrogen at  $-196^{\circ}\text{C}$  until RNA extraction.

### Cell culture

Cell Center of Chinese Academy of Sciences (Shanghai, China) was the provider of normal human astrocyte (NHA) and glioma cell line (U87, SW1008, T98G, U251 and U138). Cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, MA, USA) and 100 U/mL penicillin, 100  $\mu\text{g}/\text{mL}$  streptomycin (Hyclone, Logan, UT, USA) at  $37^{\circ}\text{C}$  in 5%  $\text{CO}_2$ .

### Cell transfection

pcDNA empty vector (Control), pcDNA-circ\_0000020 (circ\_0000020), siRNA normal control (si-NC), siRNA against circ\_0000020 (si-circ\_0000020), siRNA against PIK3CA (si-PIK3CA), miRNA control (miR NC, sense 5'-UUCUCCGAACGUGUCACGUTT-3' and antisense 5'-ACGUGACACGUUCGGAGAATT-3'), inhibitor control (Inhibitor NC) (5'-CAGUACUUUUGUGUAGUACAA-3'), miR-142-5p inhibitors (5'-AGUAGUCU UUCUACUUUAUG-3') and miR-142-5p mimics (sense 5'-CAUAAAGUAGAAAGCACUACU-3' and antisense 5'-UAGUGCUUUCUACUUUAUGUU-3') were available from GenePharma (Shanghai, China). For about  $5 \times 10^5$  cells, 500 ng of plasmids, siRNAs or miRNAs were transfected employing 1.5  $\mu\text{L}$  of Lipofectamine<sup>®</sup> 3000 (Invitrogen, Carlsbad, CA, USA). 48 h after the transfection, the transfection efficiency was determined by quantitative real-time polymerase chain reaction (qRT-PCR), and then the cells were harvested for other experiments.

### qRT-PCR

Total RNA was obtained from glioma tissue and cells with RNAiso Plus reagent (Takara, Dalian, China) according to the manufacturer's instruction. The complementary DNA (cDNA) was synthesized with 1  $\mu\text{g}$  of RNA as template using PrimeScript 1st Stand cDNA Synthesis Kit (Takara, Dalian, China). The total volume of PCR system was 30  $\mu\text{L}$ , and each sample contained 300 ng cDNA. qRT-PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Shanghai, China). The relative expressions of circ\_0000020, PI3KCA and miR-142-5p

were calculated through  $2^{-\Delta\Delta C_t}$  method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH; for circ\_0000020 and PI3KCA mRNA) or U6 (for miR-142-5p) as internal reference. The primer sequence information was listed in Table 1.

#### Cell counting kit-8 (CCK-8) assay

Glioma cells were inoculated into the 96-well plate ( $2 \times 10^3$  cells in each well). 6 wells were set for each group. After 12 h of culture, 10  $\mu$ L CCK-8 reagent (Beyotime, Shanghai, China) was loaded into each well. A blank well only contained the medium and CCK-8 reagent. The culture was continued in the incubator for 4 h, and then the absorbance of the cells at 450 nm was recorded by a microplate reader. With this method, the absorbance of U87 and U251 cells was measured at 12, 24, 48, 72 and 96 h to plot the proliferation curve.

#### Cell migration and invasion assay

Transwell assay was performed with Transwell chambers (Millipore, Billerica, USA). In migration assay, glioma cells were collected after the transfection, centrifuged at 100g for 3 min, and then resuspended in serum-free DMEM to a density of  $1 \times 10^5$  cells/mL. 200  $\mu$ L of the cell suspension and 700  $\mu$ L of complete medium with 10% FBS were dripped into the upper compartment and the lower compartment of each Transwell chamber (8  $\mu$ m pore size, Corning, NY, USA), respectively. Then the cells were incubated in at 37 °C in 5% CO<sub>2</sub> for 24 h. Then the cells on the upper surface of the membrane were gently wiped off with cotton swabs, and then the membrane was immersed in crystal violet solution, and stained for 30 min. After that, the cells were observed and photographed with a microscope (Olympus, Tokyo, Japan). For each chamber, 5 visual fields were selected randomly,

and the cells were counted, and then the average was taken. In invasion assay, Matrigel (Clontech, Madison, WI, USA) was diluted, and used to cover the membrane. Other experimental steps were the same as in the migration experiment.

#### Luciferase reporter assay

The sequence of circ\_0000020 or PIK3CA 3'-UTR containing the predicted wild-type (WT) or mutant (Mut) miR-142-5p binding sites was inserted into the luciferase reporter vector pmirGLO (Promega, Madison, WI, USA) to generate the reporter vectors, namely WT-pmirGLO-circ\_0000020, Mut-pmirGLO-circ\_0000020, WT-pmirGLO-PIK3CA 3' UTR, Mut1-pmirGLO-PIK3CA 3' UTR, Mut2-pmirGLO-PIK3CA 3' UTR, Mut3-pmirGLO-PIK3CA 3' UTR, and Mut1&2&3-pmirGLO-PIK3CA 3' UTR. U87 and U251 cells ( $4.5 \times 10^4$  cells/mL) were inoculated in 48-well plates and cultured to 70% confluence. Then the reporter vectors were co-transfected with miR-142-5p mimics or miR NC into the glioma cells, respectively, with Lipofectamine<sup>®</sup>3000 (Invitrogen, Carlsbad, CA, USA). 48 h after the transfection, luciferase activity was detected using dual-luciferase reporter assay system (Promega, Madison, WI, USA) according to the manufacturer's instruction. Firefly luciferase activity was normalized to renilla luciferase activity.

#### Western blot

After the cells were washed, RIPA lysis buffer (Beyotime, Shanghai, China) was used to extract the total protein. Then the total protein was separated by SDS-PAGE, followed by being electrically transferred to PVDF membrane (Millipore, Bedford, MA, USA). The PVDF membranes were blocked with 5% skim milk at room temperature for 1 h and washed with TBST 3 times, each time for 10 min. Then the membranes were incubated overnight with the primary antibodies Anti-PIK3CA (ab40776, Abcam, 1:1000) and anti-GAPDH (ab8245, Abcam, 1:1000) at 4 °C. After that, TBST was adopted to rinse the membrane 3 times, each time for 15 min, and then the membrane and the secondary antibody (Beyotime, Shanghai, China, 1:2000) were incubated at room temperature for 2 h before the membrane was rinsed with TBST three times again. After that, enhanced chemiluminescence kit (Millipore, Bedford, MA, USA) was utilized to develop the protein bands.

#### In vivo tumorigenesis assay

4-week old female BALB/c athymic nude mice were used for animal experiments. All animal experiments were endorsed by the Animal Experimental Committee of Xiangyang Central Hospital. U87 cells transfected with si-NC or si-circ\_0000020 were harvested, and

**Table 1** qRT-PCR primer sequences

| Name         | Primer sequences  |
|--------------|---|
| circ_0000020 | Forward: 5'-GAGAGGATGTACGCCAGAG-3'<br>Reverse: 5'-AAACTTTCGGAGCCTCTTC-3'    |
| miR-142-5p   | Forward: 5'-GGATCATAAAGTAGAAAA-3'<br>Reverse: 5'-CAGTGTGTCGTGGAGT-3'        |
| PIK3CA       | Forward: 5'-CCACGACCATCATCAGGTGAA-3'<br>Reverse: 5'-CCTCACGGAGATTCTAAAGT-3' |
| DDI2         | Forward: 5'-CTCCGAGGTGACCTTTTCCC-3'<br>Reverse: 5'-CTGTGAGAGGTCTTTCCGCA-3'  |
| GAPDH        | Forward: 5'-GAAGGTGAAGTCCGGAGTC-3'<br>Reverse: 5'-GAAGATGGTGATGGGATTTC-3'   |
| U6           | Forward: 5'-CTCGCTTCGGCAGCAC-3'<br>Reverse: 5'-AACGCTTCACGAATTTGCGT-3'      |

resuspended in 200  $\mu$ L of phosphate-buffered saline. Then the cells were subcutaneously injected into the left flank of the nude mice ( $n=5$  in each group,  $5 \times 10^6$  cells per mice). Tumor growth was examined every 5 d. 30 d later, the mice were euthanized and the tumors were resected and weighted. Lung metastasis model was established by injecting U87 cells into the caudal vein of the mice ( $n=5$  in each group,  $5 \times 10^6$  cells per mice). 14 d later, the mice were euthanized, and the lungs were harvested, and hematoxylin and eosin (H&E) staining was performed.

### Statistical analysis

All experiments were performed in triplicate (or more) and repeated for at least three times. The data were processed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). The results were expressed in the form of mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). One-Sample Kolmogorov–Smirnov test was used to determine whether the data are normally distributed or not. If the data were normal distributed, comparisons between the two groups were tested by paired or unpaired *t*-test. The comparisons among three or more groups were performed with one-way ANOVA and Tukey's post hoc test. For data that were skewed distributed, paired sample Wilcoxon signed-rank test was used to make the comparison. Survival analysis was determined by Kaplan–Meier method and log-rank test. The association between circ\_0000020 expression and pathological characteristics was assessed by Chi square test. The correlation between genes were analyzed by Pearson's correlation coefficient.  $P < 0.05$  was statistically meaningful.

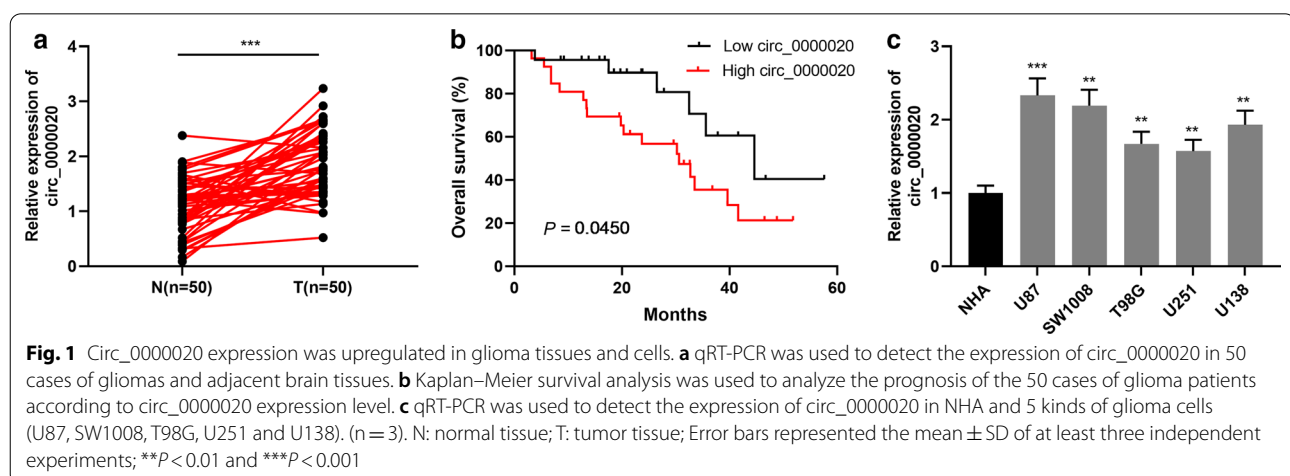
## Results

### Circ\_0000020 expressions in glioma and its correlation with clinicopathological characteristics

To investigate the expression of circ\_0000020 in gliomas, qRT-PCR was employed to measure circ\_0000020 expressions in cancer tissues and adjacent normal tissues from 50 glioma patients, the results of which manifested that, in most cases, circ\_0000020 expression in glioma tissues was dramatically elevated as against in normal brain tissues, independent of histologic glioma subtype (Fig. 1a). Kaplan–Meier analysis and log-rank test showed that patients with higher circ\_0000020 ( $n=27$ ) expression had a significantly poorer overall survival rate compared to patients with lower circ\_0000020 expression ( $n=23$ ) ( $P=0.0450$ ) (Fig. 1b). Additionally, Chi square test was adopted to analyze the relationship between the expression of circ\_0000020 and clinical pathological indicators, and the results implied that circ\_0000020 high expression was remarkably correlated with advanced WHO grade (III–IV) and larger tumor size (Table 2,  $P < 0.05$ ). Besides, qRT-PCR was adopted to detect circ\_0000020 expressions in NHA and 5 glioma cell lines. As shown, compared with in NHA cells, circ\_0000020 expression in five glioma cells was observably up-regulated (Fig. 1c, Additional file 1: Figure S1). These data hinted that circ\_0000020 exerted an oncogenic role in gliomas.

### Effects of circ\_0000020 on cell proliferation, migration and invasion of glioma cells

Next, we investigated the role of circ\_0000020 in modulating the malignant phenotypes of glioma cells. We selected U87 and U251 cell lines to construct the circ\_0000020 knockdown models and overexpression models (Fig. 2a). Notably, the siRNA targeting circ\_0000020 effectively down-regulated the expression of circ\_0000020 in U87 and U251 cells, and didn't



**Table 2 Correlation between clinicopathological characteristics and hsa\_circ\_0000020 expression levels in glioma patients**

| Characteristics              | Number of patients | hsa_circ_0000020 expression |          | $\chi^2$ | P value       |
|------------------------------|--------------------|-----------------------------|----------|----------|---------------|
|                              |                    | Low (n)                     | High (n) |          |               |
| Gender                       |                    |                             |          |          |               |
| Male                         | 29                 | 11                          | 18       | 1.8098   | 0.1785        |
| Female                       | 21                 | 12                          | 9        |          |               |
| Age (years)                  |                    |                             |          |          |               |
| < 45                         | 22                 | 12                          | 10       | 1.1549   | 0.2825        |
| ≥ 45                         | 28                 | 11                          | 17       |          |               |
| Family history of cancer     |                    |                             |          |          |               |
| Yes                          | 18                 | 10                          | 8        | 1.0338   | 0.3093        |
| No                           | 32                 | 13                          | 19       |          |               |
| Tumor size (cm)              |                    |                             |          |          |               |
| < 5                          | 19                 | 13                          | 6        | 6.2019   | <i>0.0128</i> |
| ≥ 5                          | 31                 | 10                          | 21       |          |               |
| WHO grade                    |                    |                             |          |          |               |
| I-II                         | 17                 | 13                          | 4        | 9.6275   | <i>0.0019</i> |
| III-IV                       | 33                 | 10                          | 23       |          |               |
| Tumor location               |                    |                             |          |          |               |
| Supratentorial               | 27                 | 10                          | 17       | 1.8983   | 0.1683        |
| Infratentorial               | 23                 | 13                          | 10       |          |               |
| Peritumoral Brain Edema (cm) |                    |                             |          |          |               |
| ≥ 1                          | 24                 | 14                          | 10       | 2.8263   | <i>0.0927</i> |
| < 1                          | 26                 | 9                           | 17       |          |               |
| Histological type            |                    |                             |          |          |               |
| OT                           | 8                  | 5                           | 3        | 1.7340   | 0.4202        |
| AT                           | 35                 | 16                          | 19       |          |               |
| Other                        | 7                  | 2                           | 5        |          |               |

OT: Oligoastrocytic type; AT: Astrocytic type; Data in italics indicates statistical significance at  $P < 0.05$

repress DDI2 (whose pre-mRNA probably gives rise to circ\_0000020) expression (Additional file 1: Figure S2). CCK-8 assay was performed to detect glioma cell proliferation. As shown, compared with the control group, knocking down circ\_0000020 dramatically inhibited glioma cell proliferation, while overexpression of circ\_0000020 remarkably promoted glioma cell proliferation (Fig. 2b). In addition, compared with si-circ\_0000020#2, si-circ\_0000020#1 not only had a higher knockdown efficiency, but also more significantly inhibited the viability of glioma cells. Therefore, si-circ\_0000020#1 has been used in subsequent experiments. Subsequently, through Transwell experiments, it was revealed that knockdown of circ\_0000020 remarkably blocked cell migration and invasion, and overexpression of circ\_0000020 exerted the opposite effects (Fig. 2c). The above data indicated that circ\_0000020

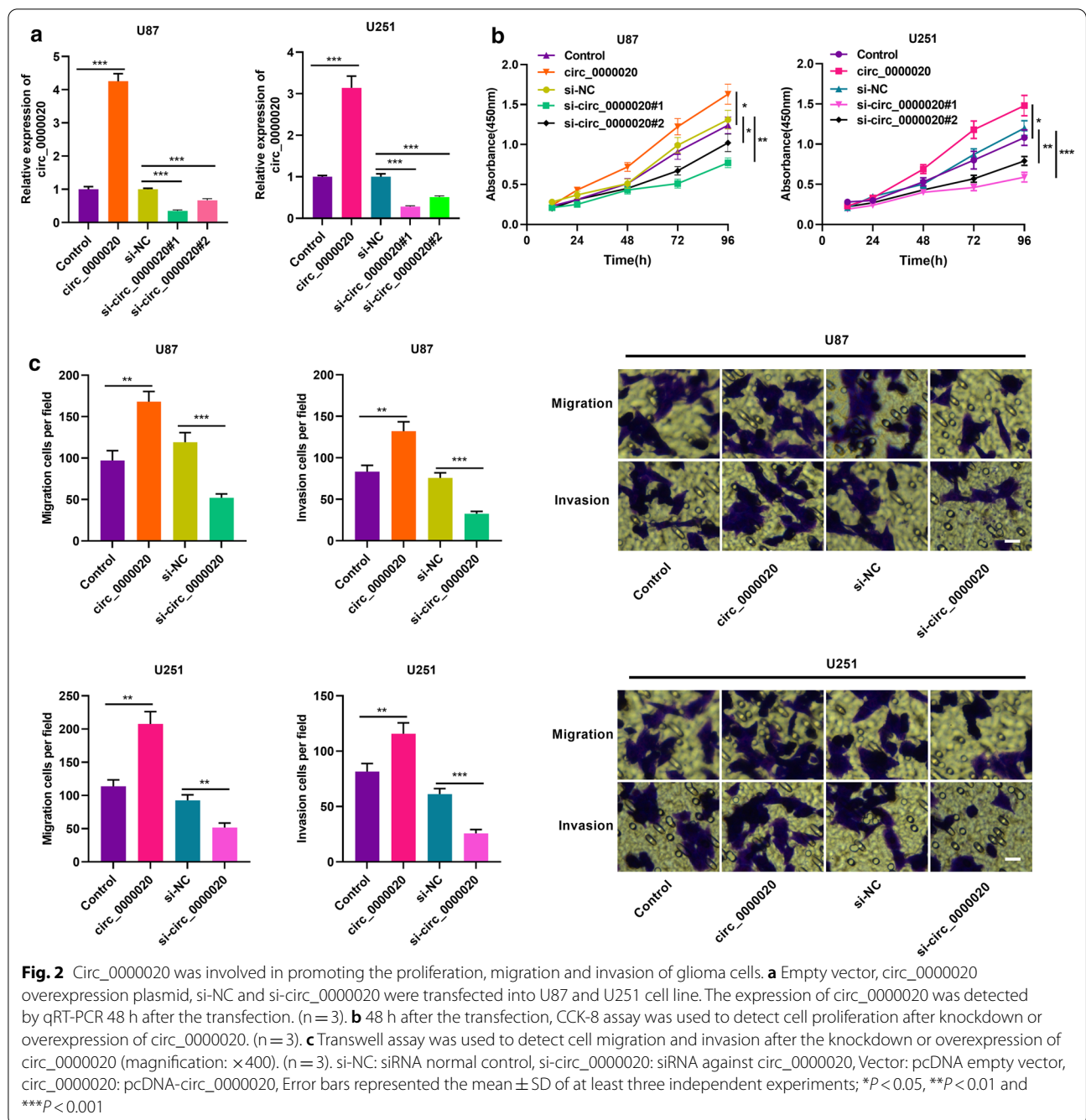
participated in promoting the proliferation, migration and invasion of glioma cells.

#### Circ\_0000020 could target miR-142-5p

To elucidate the downstream mechanism by which circ\_0000020 regulates the phenotypes of glioma cells, bioinformatics analysis was performed using the CircInteractome database to predict the miRNA that could pair with circ\_0000020 (Additional file 1: Figure S3). As shown, the potential binding site between circ\_0000020 and miR-142-5p was predicted (Fig. 3a). Next, we detected miR-142-5p expression in glioma tissues and cell lines by qRT-PCR. As shown, miR-142-5p expression in glioma tissues was markedly lowered (Fig. 3b). Consistently, miR-142-5p expression in five glioma cell lines was also dramatically reduced compared with that in NHA cells (Fig. 3c). Then we analyzed the correlation between circ\_0000020 and miR-142-5p expressions in 50 glioma tissues and Person's correlation coefficient indicated that there was a negative correlation between circ\_0000020 and miR-142-5p in glioma tissues (Fig. 3d). Then dual-luciferase reporter assay was utilized to verify the predicted binding site. The results demonstrated that miR-142-4p mimics could repress the luciferase activity of circ\_0000020-WT reporter, but had no obvious effect on circ\_0000020-MUT reporter (Fig. 3e). Moreover, qRT-PCR was utilized to detect the expression level of miR-142-5p after knockdown or overexpression of circ\_0000020 in glioma cell lines. It was observed that miR-142-5p expression was elevated in U87 and U251 cells with circ\_0000020 knockdown, but restrained in U87 and U251 cells with circ\_0000020 overexpression (Fig. 3f). These data indicated that circ\_0000020 targeted miR-142-5p and negatively modulated its expressions in glioma cells.

#### MiR-142-5p could participate in inhibiting the proliferation and metastasis of glioma cells

Then we transfected miR-142-5p inhibitors, mimics and their negative controls into U87 and U251 cells, and the successful transfection was ascertained by qRT-PCR, and the results showed that miR-142-5p expression was inhibited by the transfection of miR-142-5p inhibitors in U87 and U251 cells, but it was increase after the transfection of miR-142-5p mimics in U87 and U251 cells (Fig. 4a). Afterwards, we used CCK-8 and Transwell assays to detect cell proliferation, migration, and invasion. The results indicated that these malignant biological behaviors of glioma cells were dramatically facilitated after the transfection of miR-142-5p inhibitors, while the transfection of miR-142-5p mimics worked oppositely (Fig. 4b, c). These results implied that miR-142-5p

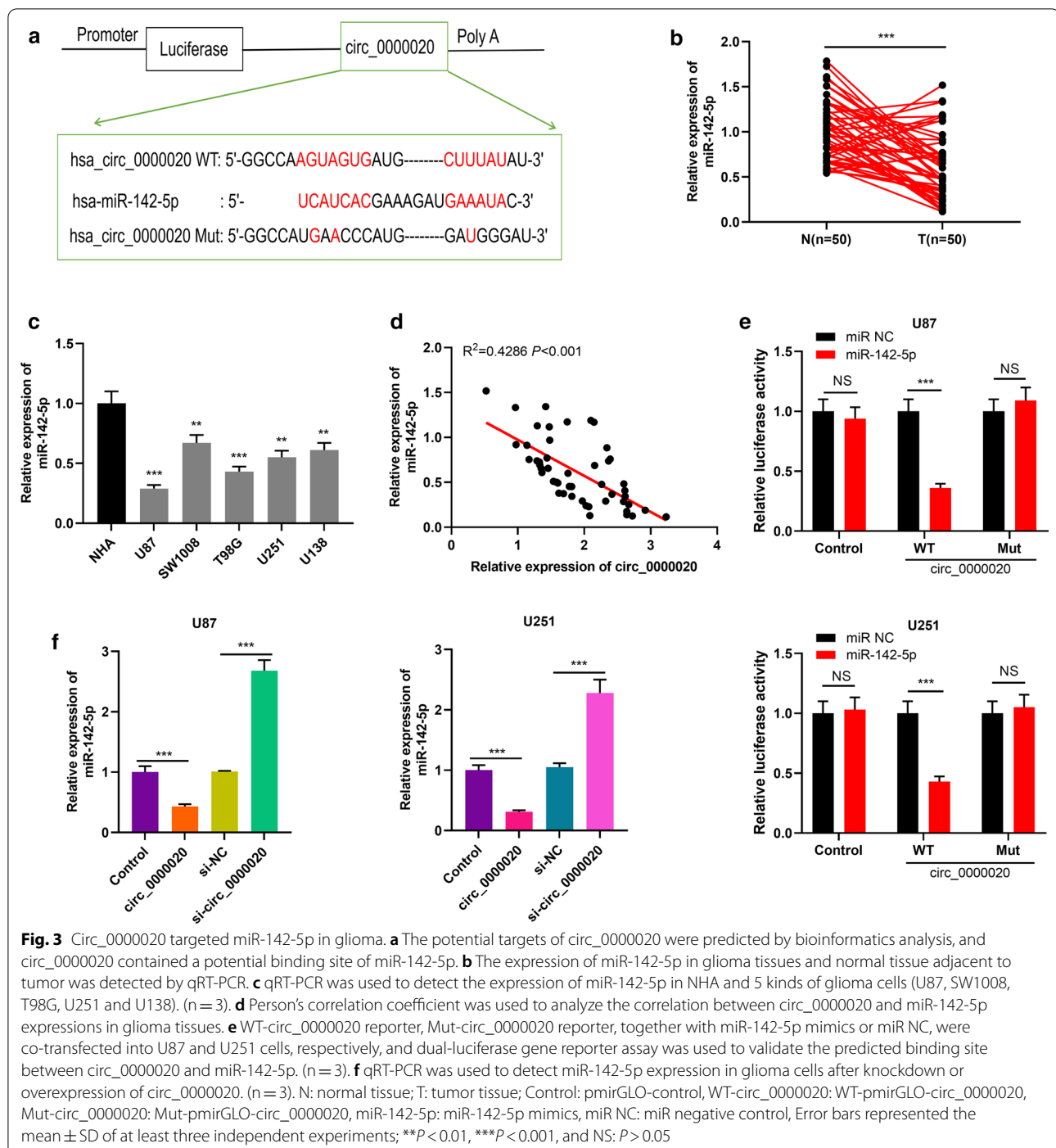


inhibited the proliferation, migration and invasion of glioma cells.

**Circ\_0000020 participated in regulating proliferation, migration, and invasion of glioma cells by targeting miR-142-5p**

To further clarify the function of the circ\_0000020/miR-142-5p axis in glioma progression, we then carried out “rescue experiments”. We found that inhibiting

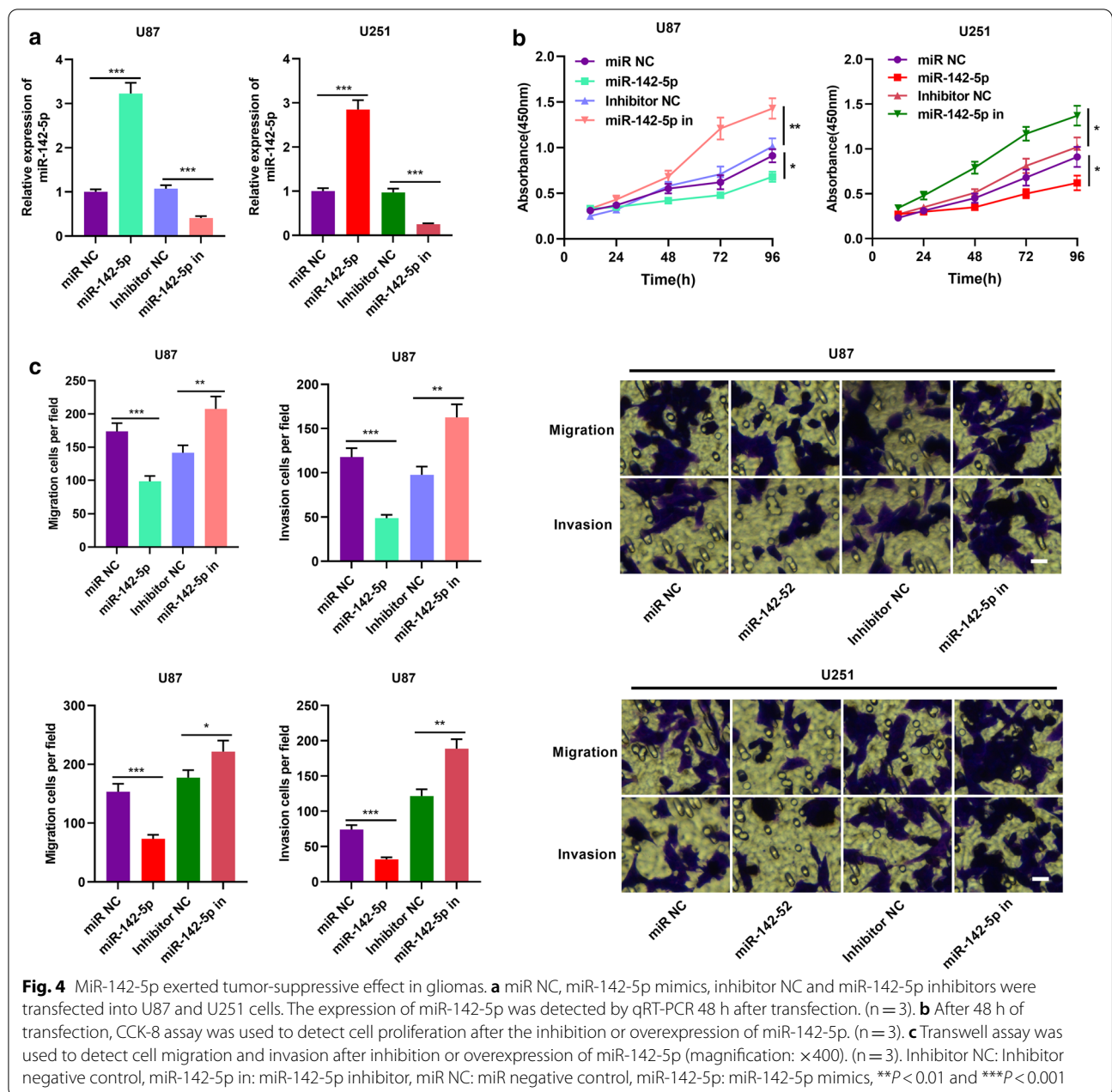
miR-142-5p partially reversed the inhibitory effect of knocking down circ\_0000020 on the proliferation, migration, and invasion of U87 cells. The effect of overexpression of circ\_0000020 on the proliferation, migration, and invasion of U251 cells was partly weakened by miR-142-5p mimics (Fig. 5a, b). These data indicated that circ\_0000020 was involved in regulating the proliferation, migration, and invasion of glioma cells via targeting miR-142-5p.



### Circ\_0000020/miR-142-5p axis regulated PIK3CA expression

After confirming that circ\_0000020 could regulate miR-142-5p expression, we then explored the downstream targets of miR-142-5p. We searched for the candidate targets of miR-142-5p with StarBase, miRDB and TargetScan database, the findings of which depicted that

miR-142-5p could probably target the PIK3CA (Fig. 6a, Additional file 2). Then we verified the targeting relationship between miR-142-5p and PIK3CA through dual-luciferase assay (Fig. 6b). Additionally, after the transfection of miR-142-5p inhibitors and mimics, the expression levels of PIK3CA mRNA and protein in glioma cells were up-regulated and down-regulated,



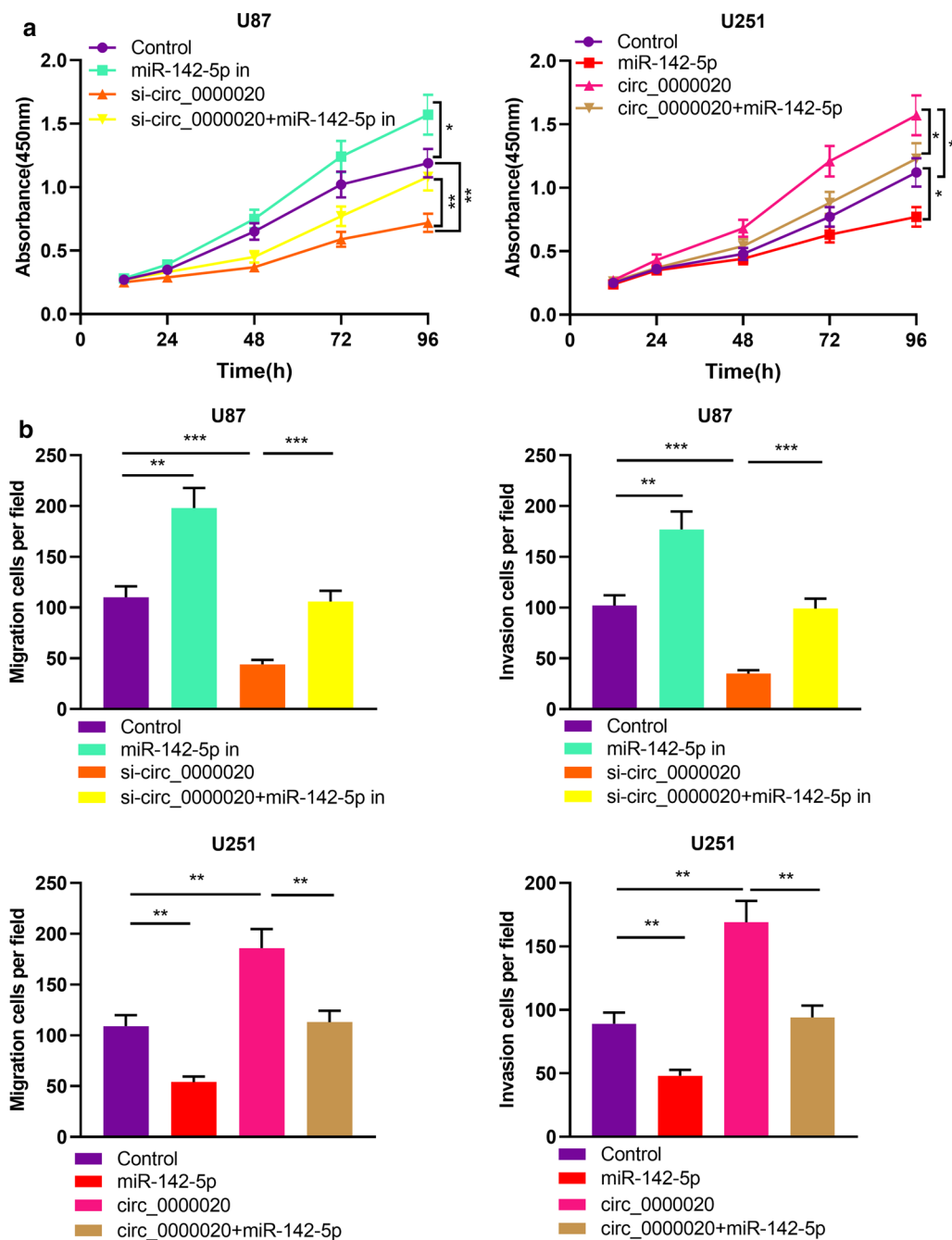
respectively, and the opposite effects could be observed after circ\_0000020 knockdown and overexpression; moreover, we also found that miR-142-5p inhibition partially reversed the down-regulation of PIK3CA mRNA and protein expressions induced by knocking down circ\_0000020; miR-142-5p overexpression partly attenuated the up-regulation of PIK3CA mRNA and protein expressions due to overexpression of circ\_0000020 (Fig. 6c, d). Besides, we also verified with qRT-PCR that PIK3CA mRNA expression was markedly elevated and positively correlated with circ\_0000020 expression in

glioma tissues and negatively correlated with miR-142-5p expression (Fig. 6e–g). The above results suggested that PIK3CA was a target of miR-142-5p in glioma and was positively regulated by circ\_0000020.

#### Overexpression of PIK3CA reversed the effect of circ\_0000020 knockdown on glioma cells

In order to further verify the role of PIK3CA in the process of circ\_0000020 promoting the development of glioma, siRNA against PIK3CA was transfected into U251 cell line with circ\_0000020 overexpression. As shown,

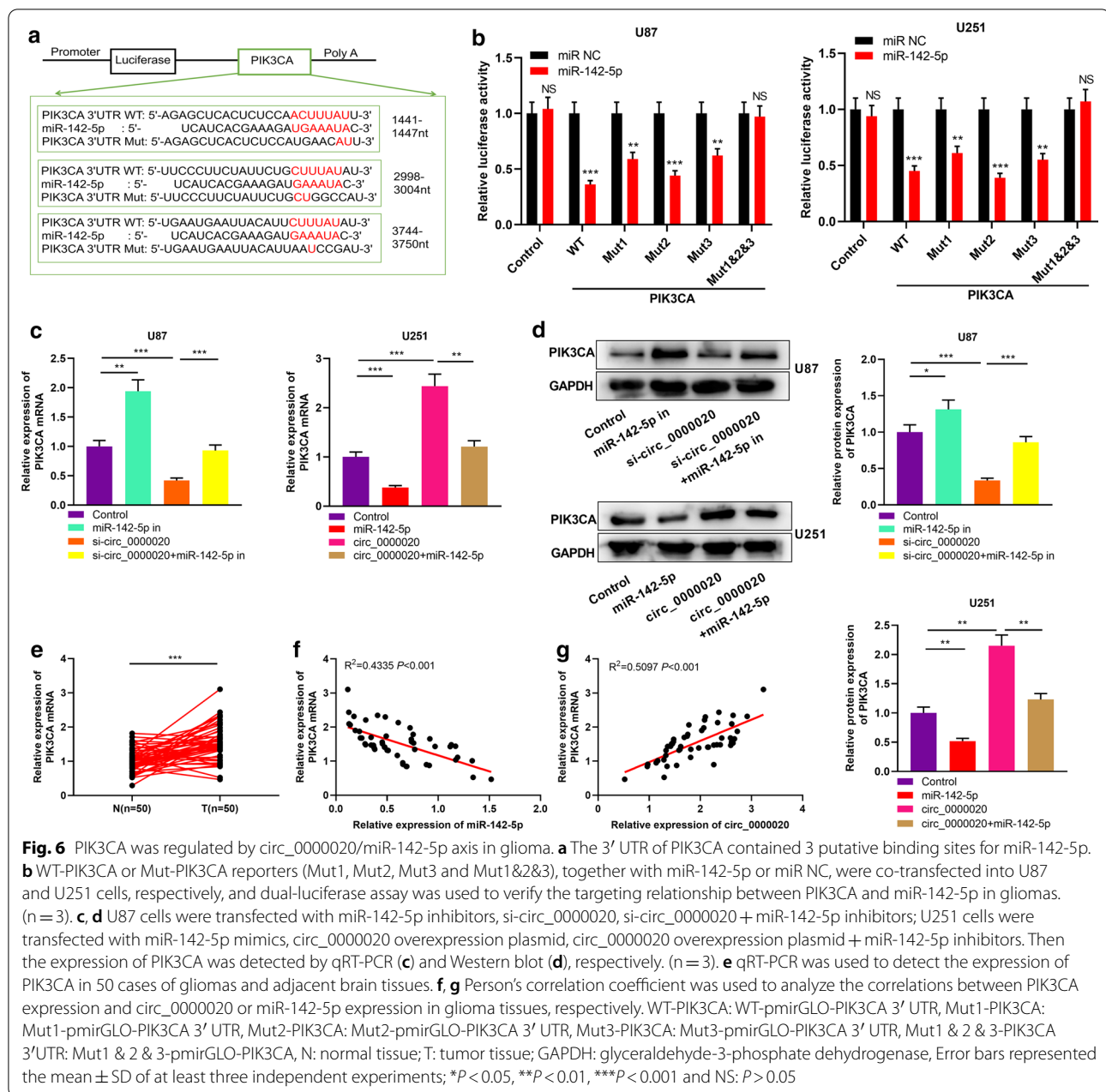




**Fig. 5** The effects of circ\_0000020 on glioma cells was partly dependent on miR-142-5p. U87 cells were transfected with si-circ\_0000020, miR-142-5p inhibitors, or si-circ\_0000020 + miR-142-5p inhibitors; U251 cells were transfected with circ\_0000020 overexpression plasmid, miR-142-5p mimics, or circ\_0000020 overexpression plasmid + miR-142-5p mimics. **a, b** The proliferation, migration and invasion of were detected by CCK-8 assay (**a**) and Transwell assay (**b**), respectively. (n = 3). Control: negative control, miR-142-5p in: miR-142-5p inhibitor, si-circ\_0000020: siRNA against circ\_0000020, miR-142-5p: miR-142-5p mimics, circ\_0000020: pcDNA-circ\_0000020, Error bars represented the mean ± SD of at least three independent experiments; \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001

the promoting effects of circ\_0000020 overexpression on PIK3CA expression in U251 cells were blocked by the co-transfection with si-PIK3CA (Additional

file 1: Figure S4A, B). Consistently, the enhanced proliferation, migration and invasion of U251 cells due to circ\_0000020 overexpression were also reversed by

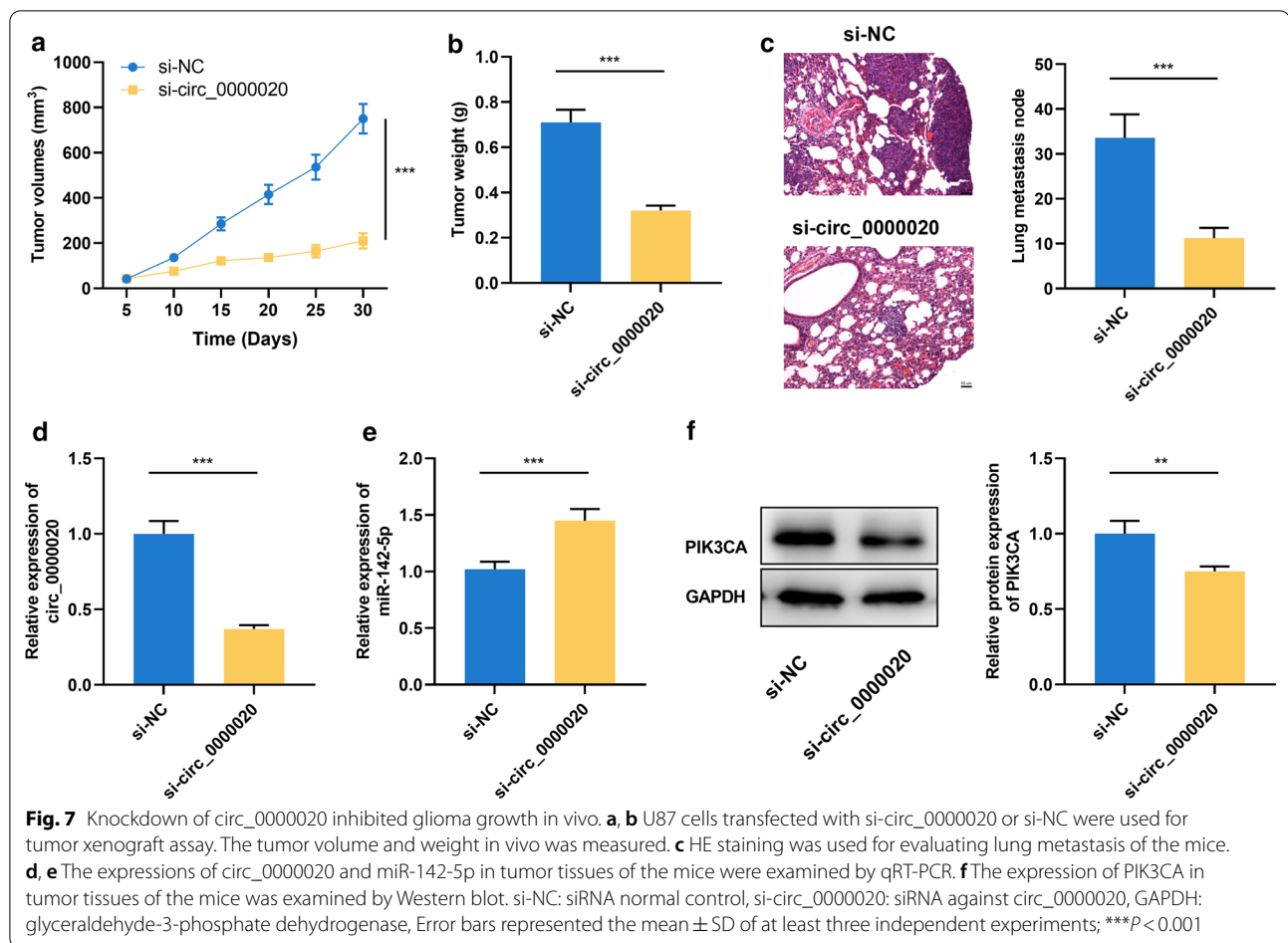


PIK3CA knockdown (Additional file 1: Figure S4C, D). These results suggested that the oncogenic properties of circ\_0000020 were dependent on PIK3CA.

#### Knockdown of circ\_0000020 inhibited glioma growth and metastasis in vivo

In addition to in vitro assays, xenograft tumor models were established in vivo in order to confirm the role of circ\_0000020 in glioma growth and metastasis. U87 cells with circ\_0000020 knockdown was subcutaneously injected into the nude mice. The result showed

that compared with in the si-NC group, the size of tumors in mice in si-circ\_0000020 group was significantly smaller (Fig. 7a). Moreover, the tumor weight in the si-circ\_0000020 group were also markedly reduced compared with the si-NC groups (Fig. 7a, b). Additionally, after the U87 cells were injected into the mice via caudal vein, 2 weeks later, HE staining of lung sections revealed that knockdown of circ\_0000020 in U87 cells markedly inhibited lung metastasis in mice (Fig. 7c). Furthermore, qRT-PCR was used to examine the expression of circ\_0000020 in excised tumor tissues, and the



result showed that the expression level of circ\_0000020 was remarkably decreased, and miR-142-5p expression was significantly increased in si-circ\_0000020 group compared with the si-NC group (Fig. 7d, e). Western blot showed that the protein expression of PIK3CA was remarkably decreased in si-circ\_0000020 group compared with the si-NC group (Fig. 7f). These data further validated that circ\_0000020 regulated glioma progression via regulating miR-142-5p and PIK3CA.

## Discussion

In recent years, a large amount of endogenous circRNA has been detected in a variety of cells, which becomes a research hotspot in the field of non-coding RNA after miRNA and lncRNA [21–23]. CircRNA is abnormally expressed in many tumors and is involved in tumorigenesis and cancer progression [24–26]. Accumulating studies reveal that circRNA is abnormally expressed in gliomas and regulates the biological behaviors of glioma cells. For instance, circ\_0001649 expression is reduced in gliomas and it exerts tumor-suppressive effects [8]. CircNFIX expression is upregulated in gliomas and it promotes

cancer progression [27]. Circ\_000173 facilitates the proliferation and invasion of glioblastoma cells via the miR-326/Wnt7B axis [28]. In this work, we observed that circ\_0000020 expression in glioma tissues and cell lines was significantly up-regulated. It is reported that DDI2 is an oncogene [29], and circRNA's enrichment is related to the expression level of precursor mRNA [30]. Therefore, the increase of circ\_0000020 expression in glioma tissues and cell lines may be related to the expression of oncogene DDI2, which remains to be validated in the future. Furthermore, we demonstrated that circ\_0000020 high expression was interrelated with higher WHO grade and larger tumor size. These results imply that circ\_0000020 is promising to be an indicator to evaluate the prognosis of glioma patients. In addition, our functional experiments affirmed that overexpression of circ\_0000020 could markedly expedite the proliferation and metastasis of glioma cells, while its knockdown could inhibit the malignant phenotypes of glioma cells, suggesting it is a potential therapy target for gliomas.

MiRNA negatively modulates the expressions of target genes via targeting the 3'-UTR of mRNA, thereby

regulating the functions of various cells [31, 32]. MiR-142-5p expression is elevated and exerts an oncogenic role in colorectal cancer and renal cell carcinoma [13, 33]. Besides, miR-142-5p is low-expressed in pancreatic cancer, gastric cancer, and hepatocellular carcinoma, and functions as a tumor-suppressor [14, 34–36]. Also, previous studies have reported that miR-142-3p can remarkably inhibit glioma cell migration and invasion [15]. In this work, we confirmed that miR-142-5p expression was also reduced in glioma tissues and cells, and overexpression of miR-142-5p remarkably impeded the proliferation, migration, and invasion of glioma cells. After miR-142-5p expression was inhibited, the malignancy of glioma cells was significantly enhanced. These data verified that miR-142-5p suppressed the progression of glioma.

CircRNAs can function as competitive endogenous RNA (ceRNA) or miRNA molecular sponge, and adsorb miRNA to exert biological functions. For instance, circ\_0007534 regulates the proliferation and migration of glioma cells via adsorbing miR-761 [37]; circ\_0014359 is highly expressed in glioma tissues and functions as a miR-153 sponge to facilitate cancer progression [38]. In CircInteractome database, we found that multiple miRNAs could probably be targeted by circ\_0000020, among which many miRNAs have been reported to be involved in the development of glioma. This implies that circ\_0000020 plays an important role in glioma progression. In this work, we identified a binding site between circ\_0000020 and miR-142-5p. We also proved that miR-142-5p mimics partly abolished the promotion of glioma cell proliferation and metastasis due to overexpression of circ\_0000020. The inhibitory effect of knocking down circ\_0000020 on the proliferation and metastasis of glioma cells was partially alleviated by miR-142-5p inhibitors. These results indicated that circ\_0000020 could participate in regulating the proliferation, migration and invasion of glioma cells by adsorbing miR-142-5p.

PIK3CA, the activator of PI3K/AKT signaling, is expressed in normal human brain, lung, mammary gland, gastrointestinal tract, cervix, ovary and other tissues. PIK3CA is an oncogene that regulates somatic cell proliferation, differentiation, survival and many other important physiological functions [18]. Previous studies indicate that PIK3CA is frequently mutated in human solid tumors like head and neck squamous cell carcinoma, esophageal cancer, squamous cell lung cancer and NSCLC, which suggests its importance in the tumorigenesis of cancers [39–42]. PI3K/AKT signaling promotes cell growth and survival, and studies demonstrate that the expression of PI3K/AKT is up-regulated in glioma, which is consistent with circ\_0000020 expression. In addition, dysregulation of PIK3CA is also a usual event during cancer progression, and it is

regulated by multiple miRNAs. For example, miR-375 inhibits the development of osteosarcoma via targeting PIK3CA [43]; In liver cancer, miR-124 impedes the proliferation of cancer cells via targeting PIK3CA [44]. It is reported that PIK3CA is a target of miR-142-5p in non-small cell lung cancer, gastric cancer, and pancreatic cancer [45–47]. In this work, it was demonstrated that miR-142-5p could negatively regulate PIK3CA mRNA and protein expressions in glioma cells. What's more, circ\_0000020 could positively regulate PIK3CA mRNA and protein expressions. Intriguingly, miR-142-5p overexpression could partially reverse the promoting effect of circ\_0000020 on the expression of PIK3CA. Also, PIK3CA knockdown could partially reverse the promoting effect of circ\_0000020 overexpression on proliferation, migration and invasion. It is worth noting that miR-142-5p can inhibit the development of glioma by regulating other genes. For example, miR-142-5p inhibits the proliferation and invasion of glioma cells by targeting Sema3C [48]. Furthermore, miR-142-5p inactivates Wnt/ $\beta$ -catenin signaling via targeting Wnt3a, thus regulating the stem cell-like traits of glioma cells [49]. These studies suggest that circ\_0000020/miR-142-5p can probably regulate glioma progression via other downstream mechanisms, besides PIK3CA.

## Conclusion

This study confirms that circ\_0000020 is significantly overexpressed in glioma tissues and cell lines. The high expression level of circ\_0000020 is closely related to advanced WHO grade and larger tumor size. In addition, we find that circ\_0000020 promotes the proliferation and metastasis of glioma cells by regulating miR-142-5p/PI3KCA axis. These findings reveal the function and underlying mechanism of circ\_0000020 in gliomas, suggesting that circ\_0000020 may be a diagnostic marker and therapeutic target for gliomas.

## Supplementary information

The online version contains supplementary material available at <https://doi.org/10.1186/s12935-021-01767-5>.

**Additional file 1:** Additional figures.

**Additional file 2.** The targets of miR-142-5p in StarBase, miRDB and TargetScan databases.

**Additional file 3.** The original data of qRT-PCR, CCK-8 and luciferase reporter assay.

## Abbreviations

CircRNAs: Circular RNAs; miRNAs: MicroRNAs; PIK3CA: Phosphatidylinositol kinase 3 catalytic subunit alpha; PI3Ks: Phosphatidylinositol 3-kinases; FBS: Fetal bovine serum; qRT-PCR: Quantitative reverse transcriptase PCR; ceRNA: Competitive endogenous RNA; NSCLC: Non-small cell lung cancer.

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**Authors' contributions**

Conceived and designed the experiments: YZ; Performed the experiments: XW, YZ; Analyze statistic: XW; Wrote the paper: YZ. Both authors read and approved the final manuscript.

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**Data availability**

Data used to support the results of this study can be obtained from the corresponding authors as required (Additional file 3).

**Ethics and consent to participate**

Our study was endorsed by the Ethics Review Board of Xiangyang Central Hospital. All animal experiments were approved by the Animal Experimental Committee of Xiangyang Central Hospital and conducted in accordance with the institutional guidelines for the use of laboratory animals.

**Consent for publication**

All authors agreed on the manuscript.

**Competing interests**

The authors declare that they have no competing interests.

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