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Circular RNA hsa-circ-0012129 Promotes Cell Proliferation and Invasion in 30 Cases of Human Glioma and Human Glioma Cell Lines U373, A172, and SHG44, by Targeting MicroRNA-661 (miR-661)

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Circular RNA (circRNA) is a stable non-coding RNA without 5'-3' polarity and without a poly-A tail, that contains response elements for microRNAs (miRNAs) such as miR-661. There have previously been few reported studies on the role of circRNAs in glioma. The aim of this study was to investigate the effects of the expression of the circRNA, hsa-circ-0012129, and miR-661 in human glioma tissue and human glioma cell lines.

Material/Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression of hsa-circ-0012129 and miR-661 in glioma tissues from 31 patients (WHO grades I–IV), compared with adjacent normal tissue, and in human glioma cell lines, U373, A172, and SHG44, compared with the normal human astrocyte cell line, NHA. The MTT assay, colony formation assay, transwell and wound scratch assays were performed to analyze and compare cell viability, cell migration, and invasion.

Results: Expression of hsa-circ-0012129 was significantly increased in glioma tissues and cell lines; hsa-circ-0012129 knockdown significantly suppressed the proliferation, migration, and invasion abilities of U373 and SHG44 cells. A dual-luciferase reporter assay showed that hsa-circ-0012129 contained the complementary binding region with miR-661 and that hsa-circ-0012129 expression negatively regulated miR-661. Rescue experiments showed that miR-661 could reverse the effects of hsa-circ-0012129 on cell viability, cell migration and invasion of glioma cells *in vitro*.

Conclusions: The findings of this study indicated that, in human glioma cells, the circRNA, hsa-circ-0012129 might act as a natural miR-661 sponge, and that miR-661 could have suppressive effects on the expression of circ-0012129.

MeSH Keywords: **Astrocytoma • MicroRNAs • Neoplasm Invasiveness • RNA, Untranslated**

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Background

Worldwide, cerebral glioma is the most common malignant intracranial tumor, with high-grade glioma havening an infiltrative growth, and a high rate of recurrence following surgery [1,2]. The World Health Organization (WHO) has divided glioma into grades I-IV, with grade I (low-grade) glioma considered to be curable by surgery and having a better prognosis [3–5]. WHO grades II and III glioma have a more malignant progression, and WHO grade IV glioma, or glioblastoma, has the most rapid malignant clinical course and worst prognosis [3–5]. Primary glioblastoma (Grade IV glioma) occurs in the brain parenchyma and may arise from malignant transformation of grade II or III glioma [6,7].

Currently, the primary treatment of glioma is surgical resection with radiotherapy and chemotherapy. However, postoperative survival time in high-grade glioma patients is still only one year, or less [8,9]. One of the main reasons for the recurrence of glioma is that glioma stem cells can be resistant to radiotherapy and chemotherapy [10,11]. Therefore, it is still important to investigate the underlying molecular mechanisms and explore new potential diagnostic or therapeutic targets for the treatment of glioma.

Circular RNA (circRNA) is an endogenous non-coding RNA that is produced in the process of RNA shearing [12]. The RNA ring structure is formed by head and tail covalent bonds, is common in biological systems, and is mainly composed of exons and (or) introns [13]. CircRNA, as a relatively new member of non-coding RNA family, is characterized by a stable structure, is highly conserved within species, specialized tissues, and cells, and is now of interest in molecular medicine [14]. CircRNA mainly regulates the expression of target genes through microRNA (miRNA) and has recently been shown to play a role in the occurrence and development of several diseases, including human tumors [15,16]. Because of the stability of circRNAs and the available methods to detect circRNAs in body fluids and tissues, increasing knowledge of the specificity of circRNAs in human disease, including in specific types of malignancy, raise the possibility that circRNAs may become new molecular diagnostic, prognostic, or therapeutic biomarkers.

MicroRNAs (miRNAs) are a group of small (20-25 nucleotide) non-coding RNA fragments that can bind to target messenger RNAs (mRNAs) and result in translational repression or silencing of their target mRNAs [17]. miRNAs have been shown to be involved in the pathogenesis and progression of many types of human cancers [18,19]. Therefore, miRNAs have potential to transform cancer diagnosis and treatment. So far, more than 1,000 miRNAs have been identified, and have been shown to be either upregulated or downregulated in many different types of human cancer [20,21]. Previously published studies have shown that multiple miRNAs might have roles in the pathogenesis of glioma and have the potential to be used as prognostic biomarkers [22–25]. Previously published studies have also shown that miR-661 functions as a tumor suppressor in malignancy, and acts as a tumor suppressor in breast cancer where it inhibits cell proliferation, cell motility, and cell invasion [26]. Also, miR-661 expression has been shown to be downregulated in glioma cells and to inhibit glioma cell proliferation, migration, and invasion [27]. Accumulating published results have shown that circRNAs function as natural miRNA sponges and regulated miRNA activity in cancer [28,29]. However, the function of the hsa-circ-0012129 and miR-661 network in the human glioma remains unknown.

The aim of this study was to investigate the effects of the expression of the circRNA, hsa-circ-0012129, and miR-661 in human glioma tumor tissue and cell lines U373, A172, and SHG44, by targeting miR-661.

Material and Methods

Ethical statement

This study was approved by the Ethics Committee of the Third Affiliated Hospital of Bengbu Medical College, China. Written informed consent was obtained from all patients.

Glioma samples and cell lines

Thirty-one paired glioma tumor tissue samples, and adjacent non-tumor tissues were collected from patients undergoing treatment at the Third Affiliated Hospital of Bengbu Medical College, China. After washing with sterile phosphate-buffered saline (PBS), samples were immediately stored at –80°C until use.

The human glioma cell lines U373, A172, and SHG44, and normal human astrocytes (NHA) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Cat. No: 11960-044) (Invitrogen) (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 100 U/mL penicillin (Cat. No: 10378016) (Gibco, Grand Island, NY, USA), and 100 ng/mL streptomycin (Cat. No: 15140-122) (Gibco, Grand Island, NY, USA). All cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Cell transfection with small interfering RNA (siRNA)

For transfection with small interfering RNA (siRNA), negative control (NC) siRNAs, and siRNAs for hsa-circ-0012129 were

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obtained from GenePharma Co., Ltd (Shanghai, China). One day before transfection, U373 and SHG44 cells (2×10^4 cells/well) in the logarithmic phase were seeded into six-well plates in 2 mL of medium and incubated overnight. The next day, cells were transfected with 50 μM of scramble siRNA as the negative control (NC), and hsa-circ-0012129 siRNAs using Lipofectamine 3000 reagent (Invitrogen) (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. After 48 hrs, the cells were harvested for further experiments.

For miRNA transfection, the miR-661 inhibitor (anti-miR-661) and miRNA controls (miR-NC) were purchased from Shanghai GeneChem Co., Ltd. (Shanghai, China). U373 cells were seeded in six-well plates (1×10^5 cells/well) and were transfected with anti-miR-661 and miR-NC using Lipofectamine 3000 reagent (Invitrogen) (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from glioma tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Then the RNA was reversely transcribed to cDNA by using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The reactions were performed on ABI 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) using the SYBR Green Master Mixture (Roche, USA). The relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [30].

The involved primers were as follows:
hsa-circ-0012129, forward 5'-GAT GAC CGC AAC CTA TAC CG-3',
reverse 5'-AAG TAG AGG ATG ACG GCC AC-3';
GAPDH, forward 5'-GCC ATC ACA GCA ACA CAG AA-3',
reverse 5'-GCC ATA CCA GTA AGC TTG CC-3';
miR-661, forward 5'-ACA CTC CAG CTG GGT GCC TGG GTC TCT GGC CT-3',
reverse 5'-CTC AAC TGG TGT CGT GGA-3';
U6, forward 5'-CTC GCT TCG GCA GCA CA-3',
reverse 5'-AAC GCT TCA CGA ATT TGC GT-3'.

Colony formation assay

The same densities of U373 and SHG44 cells were seeded into six-well plate after being transfected with different vectors. After two weeks, the cells were fixed with 4% paraformaldehyde (PFA) (Cat. No: 16005) (Sigma-Aldrich, St. Louis, MO, USA) and stained with 0.5% crystal violet (Cat. No: C-3886) (Sigma-Aldrich, St. Louis, MO, USA). After washing with phosphate buffered saline (PBS) (Cat. No: D8537) (Sigma-Aldrich, St. Louis, MO, USA), the total number of colonies from three independent transfections was counted, and the average value was used to evaluate the colony formation ability.

Cell viability assay

The treated U373 and SHG44 cells were seeded into a 96-well plate (3×10^3 cells/well) with complete medium for different points in time. Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. When the cells were adherent, 10 μl of MTT (0.5 mg/ml) (Sigma–Aldrich, St. Louis, MO, USA) was added to each well and incubated for 4 hours. Then, the MTT solution was removed, and 100 μl of dimethyl sulfoxide (DMSO) (Cat. No: 2225) (Ajax Finechem, Australia) was added. The absorbance was measured at 490 nm.

Wound healing assay

The treated U373 and SHG44 cells were plated in six-well plates with serum-free medium. The constant-diameter stripes were scratched by removing a line of cells with a 10 μl disinfected Eppendorf tip. The wound areas were photographed at 0 and 48 hours using a light microscope. The results of the measured migration distance were recorded.

In vitro cell invasion assays

Cell invasion assays were performed using the transwell assay according to the manufacturer's instructions. The treated U373 and SHG44 cells (5×10^4) were seeded in the upper chambers of 24-well transwell plates with serum-free media. The complete medium, supplemented with 10% FBS, was added to the lower chambers. After 24 hours of incubation, invasive cells on the bottom surface of the filters were fixed by 4% paraformaldehyde (PFA) (Cat. No: 16005) (Sigma-Aldrich, St. Louis, MO, USA), and stained with 0.1% crystal violet solution (Cat. No: C-3886) (Sigma-Aldrich, St. Louis, MO, USA). Four random fields were examined by light microscopy and cells were counted for each group.

Luciferase reporter assay

The sequences of the wild-type (WT) and mutant-type (Mut) hsa-circ-0012129-3'UTR were amplified by polymerase chain reaction (PCR) using human genomic DNA of the HT29 cell line and cloned into the pGL3-promoter vector (Promega Corporation, Madison, WI, USA) (Cat. No: E1751). U373 and SHG44 cells (5×10^4 cells/well) were seeded in 24-well plates and co-transfected with miR-661 or miR-NC and WT or Mut 3'-UTR of hsa-circ-0012129 by using Lipofectamine 3000 reagent (Invitrogen) (Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol. The Renilla plasmid (RL-SV40) was used as the reference. At 48 hours

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after transfection, the relative intensities of luciferase were measured with the Dual-Luciferase Reporter Assay System (Promega, Wisconsin, WI, USA).

Fluorescence *in situ* hybridization (FISH) assay

FISH was performed using a probe to hsa-circ-0012129. PCR products were obtained with the specific primers for the back-splice region of hsa-circ-0012129. PCR products were labeled with biotin-labeled RNA probes by using biotin RNA labeling mix (Roche Applied Science, Mannheim, Germany) (Cat. No: 11685597910) and T7 RNA polymerase (Cat. No: M0251L) according to the manufacturer's instructions. Cells were seeded in six-well plates and were hybridized in Ambion ULTRAhyb hybridization buffer (Cat. No: AM8670), with biotin-labeled RNA probes to hsa-circ-0012129, at 60°C overnight. Cells were treated with the DyLight 549-conjugated antibody (Cat. No: 711-506-152) (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA). Cell nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) (Invitrogen). The results were evaluated and quantified using a fluorescence microscope (Leica Microsystems, Mannheim, Germany).

Statistical analysis

The data were presented as the mean ± standard deviation (SD) and analyzed by the Student's t-test and one-way analysis of variance (ANOVA) using the SPSS version 19.0 software. Each experiment was performed in triplicate. $P<0.05$ was considered to be statistically significant.

Results

The circular RNA (circRNA) hsa-circ-0012129 was highly expressed in glioma tissues and glioma cell lines

Glioma tumor tissues and matched normal tissues were obtained from 31 patients. The relative expression levels of the circular RNA (circRNA) hsa-circ-0012129 were determined by the quantitative real-time polymerase chain reaction (qRT-PCR) assay. As shown in Figure 1, hsa-circ-0012129 expression was significantly increased in tumor tissues compared with the matched normal tissues ($P<0.05$). The expression levels of hsa-circ-0012129 were also significantly increased in three glioma cell lines (U373, A172, and SHG44) compared with normal human astrocytes (NHA) ($P<0.05$). The results also showed that the expression levels of hsa-circ-0012129 were significantly increased in gliomas of WHO grades III–IV compared with grades I–II. ($P<0.05$) (Figure 1C). The hsa-circ-0012129 expression levels were significantly related to the clinical grade of the gliomas ($P=0.014$) (Table 1).

Also, fluorescence *in situ* hybridization (FISH) showed that the expression of hsa-circ-0012129 was predominately located in the cytoplasm of U373 and SHG44 cells ($P<0.05$) (Figure 1D, 1E). This finding was in accordance with the previously published findings that most circRNAs were located in the cell cytoplasm [31].

hsa-circ-0012129 knockdown inhibited the proliferative ability of glioma cells

To evaluate the possible roles of hsa-circ-0012129 in glioma, the effects of transfection with small interfering RNA (siRNA) was compared with the negative control (NC). The siRNA was used to analyze whether hsa-circ-0012129 knockdown affected glioma cell viability and proliferation. U373 and SHG44 cells were transfected with siRNA or NC, and showed that the gene silencing efficacies of hsa-circ-0012129 were significant ($P<0.05$) (Figure 2A, 2B). Also, silence of hsa-circ-0012129 inhibited cell proliferation (Figure 2C–2F). These findings supported the view that hsa-circ-0012129 functioned as an oncogene in glioma.

hsa-circ-0012129 knockdown inhibited glioma cell migration and invasion

The wound healing assay was performed to explore the effects of siRNA on the migration of U373 and SHG44 cells. The results showed that hsa-circ-0012129 knockdown significantly decreased the migration abilities of U373 and SHG44 cells at 48 hours ($P<0.05$) (Figure 3A, 3B). Transwell chamber inserts were then used to explore the effects of siRNA on the invasion of U373 and SHG44 cells. The results supported the view that hsa-circ-0012129 knockdown significantly inhibited the invasive abilities of U373 and SHG44 cells ($P<0.05$) (Figure 3C, 3D).

hsa-circ-0012129 was a target gene of miR-661 in glioma cells

U373 and SHG44 cells were co-transfected with miR-661 and a luciferase reporter containing the full length of hsa-circ-0012129 3'-UTR (wild-type) or a mutant. Luciferase intensity was measured after 48 hours for transfection. miR-661 markedly decreased the luciferase intensity in Luciferase wild-type reporter construct ($P<0.05$) (Figure 4A). However, the luciferase intensity was not changed markedly in the mutant type reporter construct ($P>0.05$) (Figure 4B). Also, overexpression of hsa-circ-0012129 decreased miR-661 expression in U373 and SHG44 cells ($P<0.05$) (Figure 4C). However, hsa-circ-0012129 knockdown significantly increased miR-661 expression in U373 and SHG44 cells ($P<0.05$) (Figure 4D). Consequently, hsa-circ-0012129 was identified as a target gene of miR-661 in glioma cells, and miR-661 expression was negatively correlated with hsa-circ-0012129 expression.

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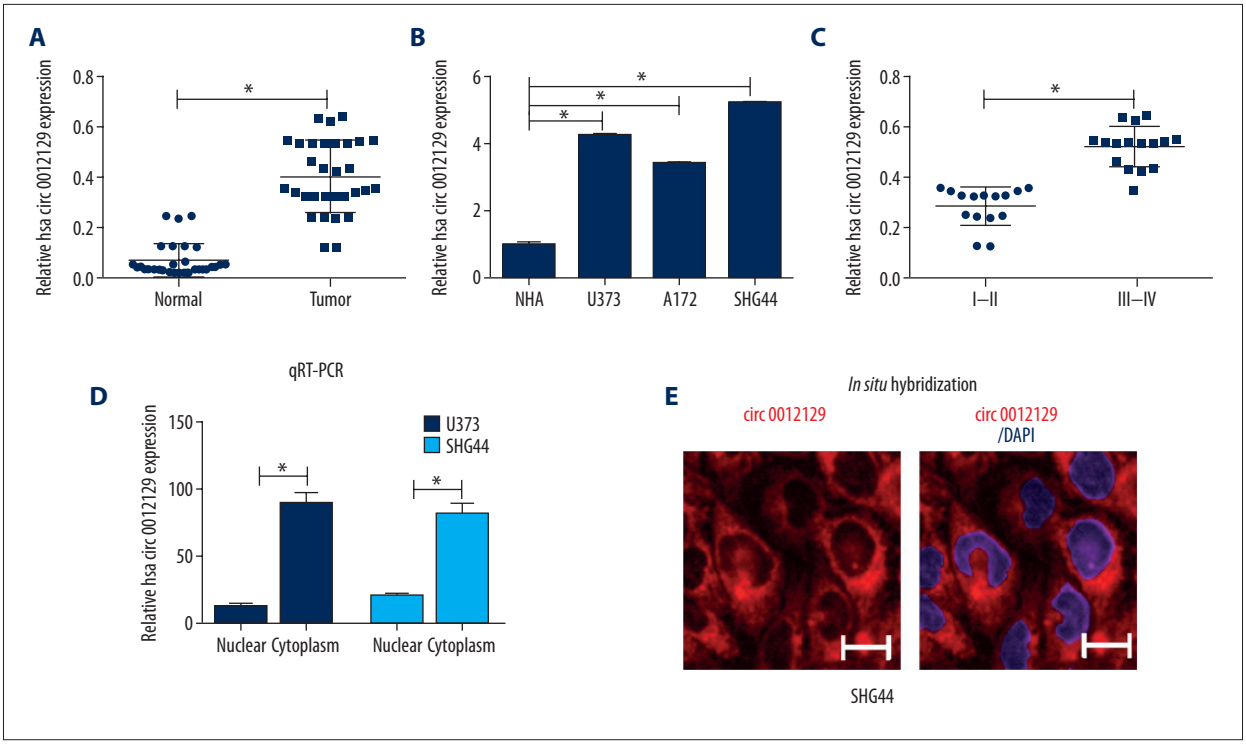

Figure 1. Circular RNA (circRNA), hsa-circ-0012129 is highly expressed in glioma tumor tissues and the U373 and SHG44 human glioma cells. (**A**) Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression of hsa-circ-0012129 and miR-661 in glioma tissues from 31 patients, compared with adjacent normal tissue, and showed a significant increase in expression in human glioma tissue (* P<0.05). (**B**) Hsa-circ-0012129 expression, measured by qRT-PCR in a normal human astrocyte cell line (NHA) and three human glioma cell lines (U373, A172, and SHG44) lines showed significantly increased expression in the glioma cell lines (* P<0.05). (**C**) Hsa-circ-0012129 expression, measured by qRT-PCR, was significantly increased in WHO grades III–IV glioma compared with grades I–II glioma, respectively (* P<0.05). (**D**) Hsa-circ-0012129 expression, measured by fluorescence *in situ* hybridization (FISH) assay, was compared between the cytoplasm and the nucleus of U373 and SHG44 human glioma cells, respectively (* P<0.05). (**E**) The FISH assay showed the localization of hsa-circ-0012129, with 4,6-diamidino-2-phenylindole (DAPI), used to stain the cell nuclei.

Table 1. The association between hsa_circ_0012129 expression and clinic pathological parameters in 31 cases of glioma tissues.

	All cases	hsa_circ_0012129		*P* value
		High expression	Low expression	
Age (year)				
>60	14	6 (42.9%)	8 (57.1%)	0.551
≤60	17	8 (47.1%)	9 (52.9%)	
Gender				
Male	16	9 (56.3%)	7 (43.8%)	0.578
Female	15	8 (53.3%)	7 (46.7%)	
Clinical stage				
I & II	23	8 (34.8%)	15 (65.2%)	**0.014***
III & IV	8	7 (87.5%)	1 (12.5%)	

* Probability (P) values <0.05 was considered statistically significant.

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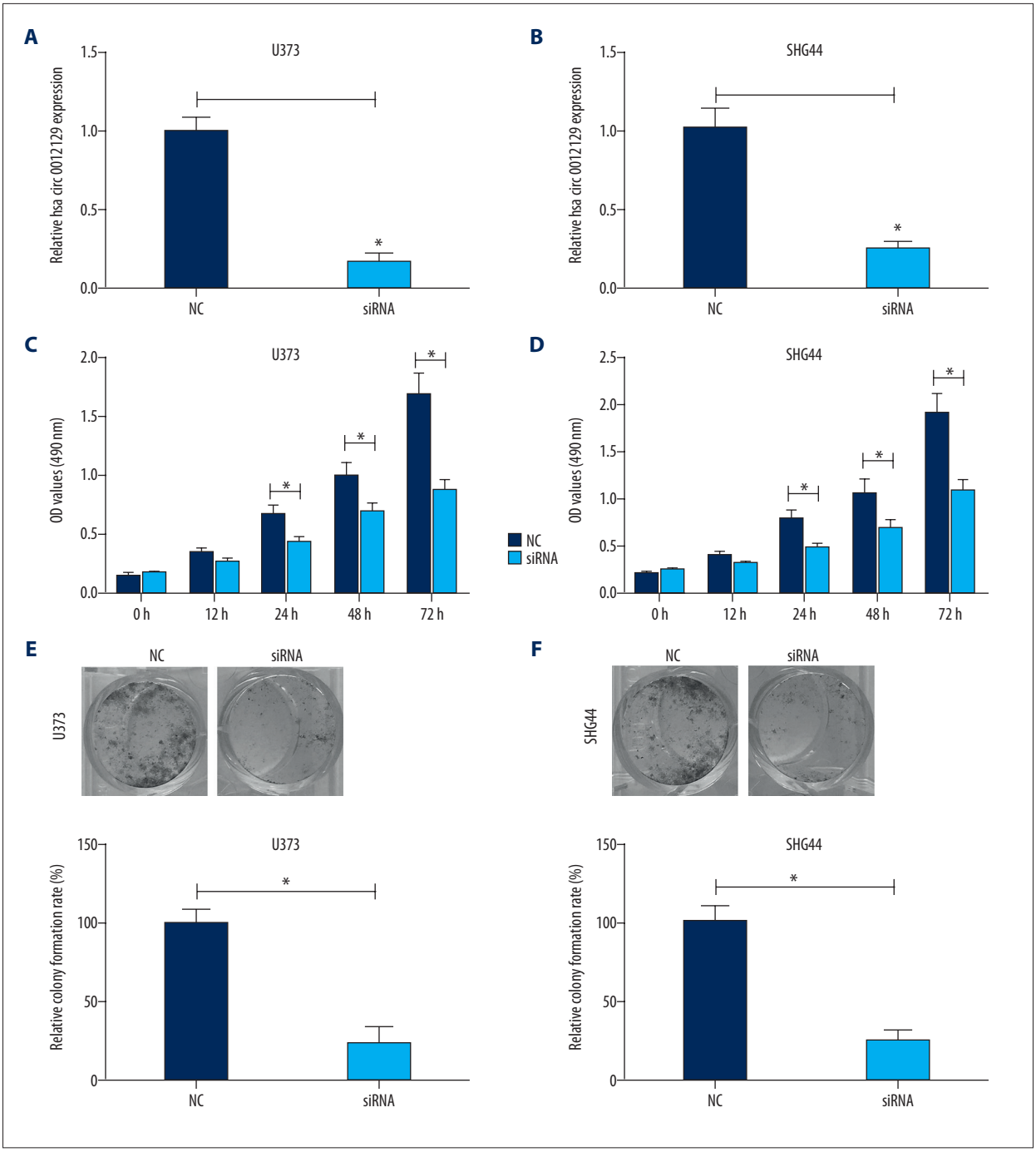

Figure 2. Silence of circular RNA (circRNA), hsa-circ-0012129 by transfection of the U373 and SHG44 human glioma cells with small interfering RNA (siRNA) suppresses glioma cell proliferation. (**A, B**) Circular RNA (circRNA), hsa-circ-0012129 expression was assessed by quantitative real-time polymerase chain reaction (qRT-PCR) in the transfected U373 and SHG44 human glioma cells, respectively (*P <0.05). (**C, D**) Cell viability of the U373 and SHG44 human glioma cells were measured by the MTT assay at 0, 12, 24, 48, and 72 hrs after transfection (* P <0.05). (**E, F**) The cell proliferation abilities were then detected using a colony formation assay in the treated U373 and SHG44 human glioma cells (* P<0.05). NC – negative control.

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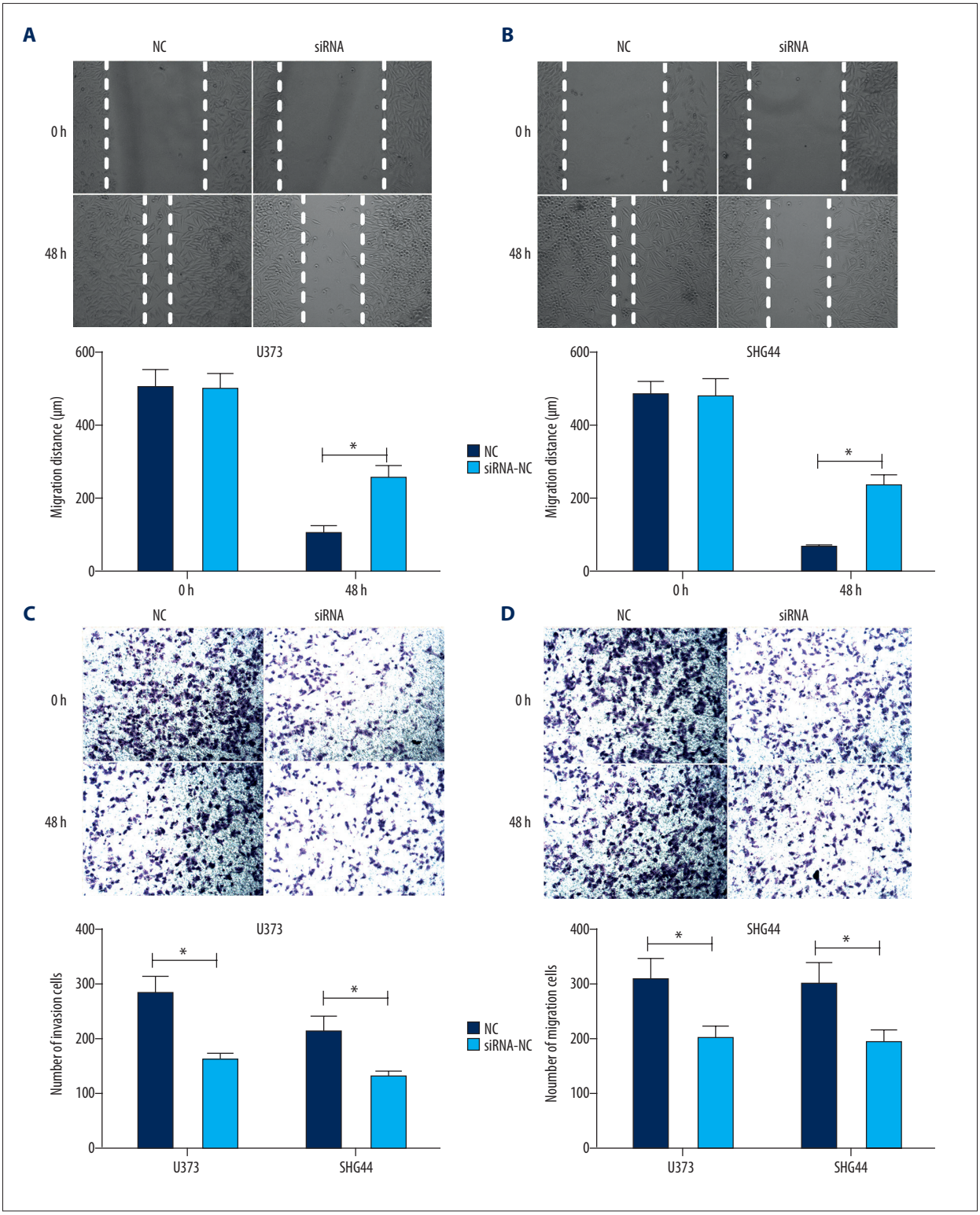

Figure 3. Silence of circular RNA (circRNA) hsa-circ-0012129 inhibits glioma cell migration and invasion. (**A, B**) Wound-healing assay, performed to detect the migration ability of the treated U373 and SHG44 human glioma cells at 0 and 48 hours, respectively (* P<0.05). (**C, D**) A transwell assay was used to measure the invasion ability of the transfected U373 and SHG44 human glioma cells. The representative images were shown on the upper panel, and the quantification results are shown in the lower panel (* P<0.05). NC – negative control.

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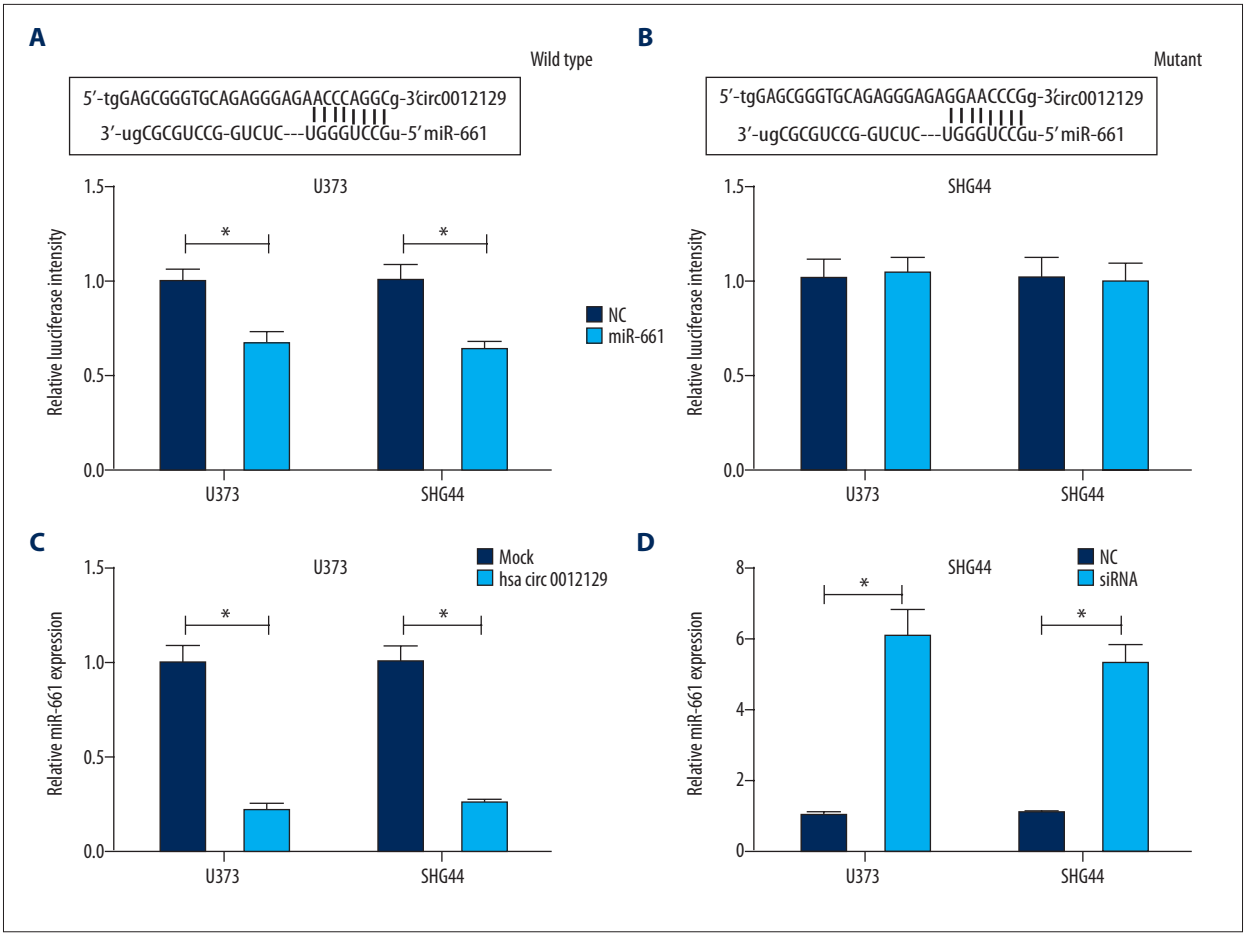

Figure 4. Circular RNA (circRNA), hsa-circ-0012129 might act as a natural 'sponge' that directly targets the microRNA, miR-661. (**A, B**) Wild-type and mutated 3'-UTR sequences of hsa-circ-0012129 are shown. The U373 and SHG44 human glioma cells were co-transfected with hsa-circ-0012129 wild-type or mutated 3'UTRs, and miR-661, or negative control. Relative luciferase intensity of hsa-circ-0012129 3'UTR (wild-type and mutant constructs) was detected by a dual-luciferase reporter assay after transfection (* P<0.05). (**C, D**) The U373 and SHG44 human glioma cells were transfected with hsa-circ-0012129, small interfering RNA (siRNA), or negative control. The microRNA, miR-661 expression was detected by quantitative real-time polymerase chain reaction (qRT-PCR) in transfected U373 and SHG44 human glioma cells (* P<0.05).

hsa-circ-0012129 knockdown mediated the glioma tumorigenesis promotion through miR-661

Matched tumor tissues and normal tissues were obtained from 31 patients, and the relative expression level of miR-661 was determined by qRT-PCR. The results showed that the expression level of miR-661 was decreased in glioma tumor tissues (P<0.05) (Figure 5A). Pearson's correlation indicated that hsa-circ-0012129 was negatively correlated to miR-661 expression in glioma (r=–0.4834; P<0.01) (Figure 5B). Because previous experiments had verified that hsa-circ-0012129 knockdown promoted the tumorigenicity of glioma cell lines and hsa-circ-0012129 acted as an miR-661 sponge, we performed the rescue experiments to assess whether hsa-circ-0012129 knockdown mediated the promotion of tumorigenesis in glioma by targeting miR-661. The results showed that hsa-circ-0012129 knockdown suppressed colony formation ability, which was rescued by miR-661 inhibition (P<0.05) (Figure 5C). Also, miR-661 inhibitor co-transfection significantly reversed the cell invasion induced by has-circ-0012129 siRNAs (P<0.05) (Figure 5D). Rescue experiments showed that the oncogenesis effect of hsa-circ-0012129 was carried out by targeting miR-661, which also illustrated the important role of the competing endogenous mechanism of hsa-circ-0012129 and miR-661 (Figure 5E).

Discussion

Gliomas are the most common primary brain tumors in adults and have a high morbidity and mortality rate [32,33]. The patients with glioma suffer poor prognoses because of the aggressive and invasive characteristics of high-grade gliomas.

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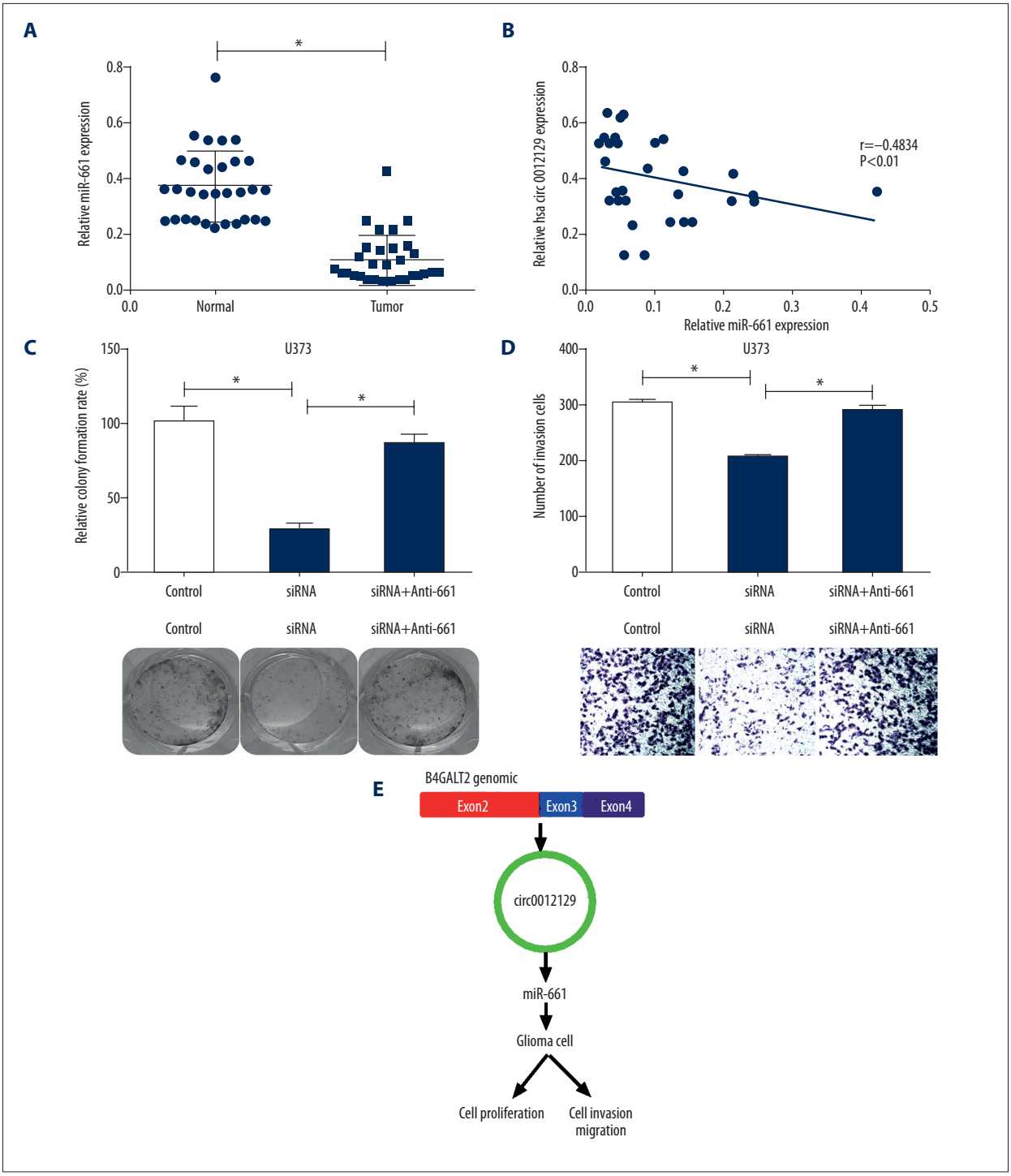

Figure 5. Circular RNA (circRNA), hsa-circ-0012129 knockdown inhibits glioma cell proliferation and invasion by targeting miR-661. (**A**) Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the expression level of the microRNA, miR-661 in glioma tissues and normal tissues (* P<0.05). (**B**) The correlation between circular RNA (circRNA), hsa-circ-0012129 and miR-661 expression was analyzed by Pearson's correlation (P<0.01; r=0.4834) in glioma tissue samples from 31 patients with adjacent normal tissues. (**C**) The human glioma U373 cells were transfected with control, hsa-circ-0012129 siRNAs or miR-661 inhibitor and hsa-circ-0012129 small interfering RNA (siRNA). Cell proliferation was measured by a colony formation assay (* P<0.05). (**D**) A transwell assay was used to measure the invasion ability of the treated human glioma U373 cells (* P<0.05). (**E**) Schematic representation of the oncogenesis effect of the circular RNA (circRNA), hsa-circ-0012129.

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Recently, despite ongoing research on advanced chemotherapy, radiotherapy, and surgery for glioma, there are still no effective treatments to improve the long-term survival in patients with glioma [34–36]. Because of the poor five-year survival rate for patients with cerebral glioma, it is important to investigate the molecular mechanisms of glioma.

The role of circular RNA (circRNA), an endogenous non-coding RNA, has recently received attention in tumorigenesis [37]. An increasing number of studies have now shown that the expression levels of circRNAs undergo dysregulation in several human tumors [38,39]. Previous studies have shown that circRNAs are also involved in the development and progression of glioma, including a recent study by Yang et al., which showed that circ-FBXW7 inhibited glioma tumorigenesis [40]. Zheng et al. showed that circ-TTBK2 inhibition inhibited the progression of glioma cells by upregulating miR-217 [41]. Yang et al. found that circ-ZNF292 silencing inhibited glioma cell proliferation and the cell cycle [42]. Despite the increasing number of studies on the role of circRNA functions, their role in human cerebral glioma remains poorly understood.

In the present study, a novel circRNA, hsa-circ-0012129, was shown to be aberrantly expressed in glioma, and that hsa-circ-0012129 was upregulated in glioma tissues and cell lines. A series of experiments were performed to explore the potential regulatory mechanism of hsa-circ-0012129 in glioma. The results indicated that hsa-circ-0012129 knockdown could effectively suppress the cell proliferation, migration and invasion of glioma cells U373 and SHG44 *in vitro*, which suggested that hsa-circ-0012129 functioned as an oncogene in glioma.

The miRNAs are involved in the regulation of approximately 30% of human genes at the posttranscriptional level by either degrading or inhibiting translation of mRNA [43, 44]. Therefore, miRNAs are capable of regulating a variety of cellular events including cell proliferation, differentiation, cell death, and cell metabolism [45]. Dysfunction of miRNAs could lead to the initiation and progression of malignant tumors [46]. miR-661 has been shown to be dysregulated in various tumors, and has been shown to inhibit breast cancer cell proliferation, colony formation, and invasion via targeting MTA1 [26]. Also, miR-661 has previously been shown to promote tumor cell growth, invasion and metastasis in non small-cell lung cancer (NSCLC) [47]. A recently published study also showed that miR-661 could inhibit glioma cell proliferation, migration, and invasion by targeting hTERT [27]. Recently published studies have begun to provide results that support a role for circRNAs as natural miRNA sponges that have a role in modulating the function of miRNA networks [48]. In support of this role, recently published studies have shown that circ-005169 functions as an oncogene by acting as a sponge for miR-145 in colorectal cancer cells [49], and the circular RNA CiRS-7 modulates miR-7 expression in pancreatic islet cells to regulate insulin transcription [50].

These previously published studies support the findings of the present study, which showed that miR-661 was downregulated in glioma tissues and hsa-circ-0012129 was upregulated in glioma cells, with the Pearson's correlation indicating that hsa-circ-0012129 was negatively correlated with miR-611 expression in glioma. The findings of the present study also showed that there was a complementary binding region between hsa-circ-0012129 and miR-661, which supports a role for hsa-circ-0012129 as a sponge for miR-661 that modulates biological processes in human glioma.

Conclusions

The findings of this study showed that human glioma cells in tumor tissue and in human glioma cells *in vitro*, the circular RNA (circRNA), hsa-circ-0012129, might act as a natural sponge for the microRNA, miR-661 and that miR-661 could have a suppressive role on the expression of hsa-circ-0012129. Further large-scale controlled studies are required to confirm these findings and to investigate whether the circRNA, hsa-circ-0012129, has the potential to be a diagnostic or prognostic biomarker, or provide a potential therapeutic target for human glioma.

Conflict of interest.

None.

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