Hsa_circ_0001017 inhibits proliferation and metastasis *via* regulating the let-7g-3p/NDST3 axis in glioma

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Abstract

Introduction: *Glioma is one of the common types of intracranial malignancies, which seriously threatens human health. Circular RNAs (circRNAs) play critical roles in various tumours. This study aims to observe the functions and mechanism of circ_0001017 in glioma progression.*

Material and methods: *Hsa_circ_0001017 expression was analysed in glioma tissue and cells using qRT-PCR assay. Flow cytometer, Cell Counting Kit-8 (CCK-8), colony formation, wound healing, and Transwell were used to analyse glioma cell apoptosis, viability, colony-forming ability, migration, and invasion. The expression of N-deacetylase and N-sulfotransferase 3 (NDST3) and epithelial-mesenchymal transition (EMT)-related proteins was measured using western blot analysis. Luciferase reporter and RNA immunoprecipitation (RIP) assay were performed to determine the target relationship.*

Results: *In glioma tissues and cells, hsa_circ_0001017 expression was decreased. The overexpression of hsa_circ_0001017 inhibited glioma cell proliferation, EMT, migration, and invasion and promoted glioma cell apoptosis, while the knockdown of hsa_circ_0001017 caused the opposite results. Mechanistically, hsa_circ_0001017 sponged hsa-let-7g-3p and NDST3 was the target gene of hsa-let-7g-3p. Moreover, the tumour-suppressive role of circ_0001017 was associated with hsa-let-7g-3p and NDST3.*

Conclusions: *Hsa_circ_0001017 suppressed the growth and metastasis and increased cell apoptosis of glioma, and this effect was associated with hsa-let-7g-3p/NDST3 axis.*

Key words: *hsa_circ_0001017, hsa-let-7g-3p, NDST3, glioma.*

Introduction

Glioma is a common intracranial malignancy in adults, which has a high mortality rate [4,31]. Currently, multimodal treatments, such as radical surgery and chemo-radiotherapy, have been improved, but the incidence rate of glioma continues to increase and the prognosis of glioma remains poor [22,40]. The poor therapeutic effect is mainly because many

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patients suffer from advanced and metastatic disease [38]. Thus, the potential mechanisms of glioma need to be explored, and novel and effective therapeutic targets for glioma need to be determined.

Circular RNAs (circRNAs), expressed in highly divergent eukaryotes, are endogenous noncoding RNAs (ncRNAs) [27,30]. CircRNAs have no 5' cap and 3' ploy (A) tail, but have a covalently closed loop structure; thus, they can maintain high cellular stability [12,21]. CircRNAs are considered to be novel plasma biomarkers [17]. Moreover, circRNAs play an important part in various types of cancer, and the abnormal function of circRNAs affects the biological process of tumour cells [8,9,19]. Interestingly, many circRNAs have been found to be upregulated or downregulated in glioma. For example, Wang *et al.* [33] found that hsa_circ_0001649 expression decreased in glioma tissues and cells, and cell growth was inhibited by the overexpression of hsa_circ_0001649. Therefore, the role of circRNAs as novel biomarkers or therapeutic targets in glioma needs to be examined.

Emerging evidence shows that circRNA can serve as a competing endogenous RNA (ceRNA) in binding to microRNAs (miRNAs) to modulate gene expression [25,36]. MiRNAs are 22-nucleotide-long ncRNAs identified to take part in tumour progression [39]. A previous paper showed that circ_0034642 could increase BATF3 expression through miR 1205 to facilitate glioma cell proliferation and invasion [37]. Another paper [16] reported that hsa_circ_0046701 could promote carcinogenesis of glioma, and this effect was associated with the miR-142-3p/ITGB8 axis. However, the role of circRNA in glioma is under investigation.

Hsa_circ_0001017 was downregulated in dysplasia tissues and cancer, and the hsa_circ_0001017 expression levels were significantly related to distant metastasis [18]. However, the function of hsa_circ_0001017 in glioma is not fully clarified. In the present study, hsa_circ_0001017 expression in glioma tissues and cells was analysed. Moreover, the function of hsa_circ_0001017 and its potential molecular pathway in glioma were explored. The findings might offer a new insight into circRNA-directed treatment in glioma.

Material and methods

Patients and tissue samples

In this study, tumour tissue samples were obtained from 40 patients diagnosed with glioma in our hospital between January 2019 and June 2020. Normal brain tissues were obtained from 20 patients without craniocerebral injuries (cerebral haemorrhage, cerebral contusion and laceration). All registered patients did not receive preoperative radiotherapy and chemotherapy before surgery, and they signed informed consent. The research was authorised by the Ethics Committee of our hospital.

Cell culture

Human normal astrocytes (NHA) and glioma cells (U118, U251, U87MG and LN229) were purchased from the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in DMEM medium containing 10% foetal bovine serum (FBS; Gibco, CA, USA) and penicillin/streptomycin at 37°C and 5% CO_2.

Cell transfection

U87MG cells were grouped into control (without treatment), si-NC (cells transfected with siRNA negative control), si1-circ (cells transfected with siRNA1-circ-0001017) and si2-circ group (cells transfected with siRNA2-circ-0001017) groups. U251 cells were divided into control (without treatment), vector (cells transfected with pcDNA3.1-NC), over-circ (cells transfected with pcDNA3.1-circ-0001017), let-7g-3p mimic (cells transfected with let-7g-3p mimic), over-circ + let-7g-3p mimic (cells transfected with let-7g-3p mimic and pcDNA3.1-circ-0001017) and let-7g-3p + N-deacetylase and N-sulfotransferase (NDST3) (cells transfected with let-7g-3p mimic and pcDNA3.1-NDST3) groups. Negative control, circ-0001017 siRNA, pcDNA3.1-circ-0001017, pcDNA3.1-NDST3, and let-7g-3p mimic were purchased from GenePharma (Shanghai, China). Cell transfection was performed using Lipofectamine 3000 transfection reagent (Invitrogen, CA, USA).

Quantitative reverse transcription polymerase chain reaction

Total RNA was extracted using TRIzol reagent (Invitrogen) from tissue and cells. Complementary deoxyribonucleic acid (cDNA) was synthesized using a PrimeScript Reverse Transcription (RT) Reagent Kit with a gDNA Eraser (Takara, Shiga, Japan). In line with the instructions of SYBR® Premix Ex Taq™ II (Takara), mRNA expression was measured by quantitative RT-polymerase chain reaction (qRT-PCR) and

Table I. The sequences of primers

Primers	Sequences (5'-3')
circ-0001017-F	AACCAGTGCGAAGTAATCTATGC
circ-0001017-R	TCTTTGCTGGGCTCCTTCT
let-7g-3p-F	TGGTACTGATGTGATGGACT
let-7g-3p-R	TCATATCACACAGCACCGAT
NDST3-F	GCCCCTCCCAGGAGTAAGTA
NDST3-R	AGGTGGTGGAGAGAACAGGT
GAPDH-F	GGTCGGAGTCAACGGATTT
GAPDH-R	TACCAGAGTTAAAAGCAGCC

normalized to GAPDH. The $2^{-\Delta\Delta Ct}$ method was used to analyse the data. The primer sequences were shown in Table I.

Cell viability assay

After culturing for 0, 24, 48, and 72 h, the transfected cells were incubated with Cell Counting Kit-8 (CCK-8) solution (Beyotime Institute of Biotechnology, China). After incubation at 37°C for 2 h, the optical density (OD) value was measured using a microplate reader (MultiScan FC, Thermo Scientific, NY, USA) at 450 nm.

Colony formation assay

The transfected cells (1000 cells/well) were seeded into a 6-well plate. After 12 days of incubation, the cells were fixed with methanol (Sigma-Aldrich, Merck, Darmstadt, Germany) for 20 min and stained with crystal violet solution (Sigma-Aldrich) for 15 min. Subsequently, the colonies were imaged and counted.

Cell apoptosis assay

The cells were collected after transfection for 48 h and re-suspended in 400 µl of 1 × binding buffer. Next, the cells were incubated with Annexin V-FITC (5 µl) and propidium iodide (PI, 5 µl; BD Biosciences, NJ, USA) in the dark for 15 min. Finally, flow cytometer analysis (BD Biosciences) was used to detect the cell apoptosis.

Wound healing assay

The transfected cells (5×10^5 cells/well) were seeded into a 6-well plate and maintained in serum-free medium. A 200-µl pipette tip was used to create a straight scratch (time 0 h) when the cell confluence reached about 90% confluence. After scratch for 0 and 48 h, the images were captured under a microscope (Olympus, Japan).

Transwell assay

The 24-well Transwell chamber (BD Biosciences) with membranes having 8-mm pores was used to detect cell invasion. In short, 5×10^4 cells/well transfected cells were added into the Matrigel-coated upper chamber containing 500 µl of serum-free medium. DMEM medium with 10% FBS was added to the lower chamber. The cells in the lower chamber were fixed with methanol for 10 min and stained with 0.1% crystal violet after incubation for 48 h. Finally, under a microscope, the invasive cells were counted and imaged.

Western blot analysis

RIPA lysis buffer (CWBio, Beijing, China) with protease inhibitors was performed to extract total proteins. Equal amounts of protein sample was loaded into SDS-PAGE to fractionate the extracted proteins, and then transferred to PVDF membranes (Merck Millipore, Burlington, MA). Blocking with 5% non-fat milk for 1 h at 37°C, the membranes were incubated with primary antibodies (Vimentin, 5741; N-cadherin, 13116; E-cadherin, 3195; GAPDH, 5174, 1 : 1000; Cell Signaling Technologies, Danvers, MA, USA; NDST3, SAB2101559, 1 : 1000; Sigma-Aldrich) at 4°C overnight. Next, the membranes were incubated with HRP-labelled secondary antibody (1 : 5000, Cell Signaling Technologies) at room temperature for 1 h. Finally, the enhanced chemiluminescence (ECL) system (Thermo Scientific, USA) was used to visualize the bands.

RNA immunoprecipitation assay

U251 cells were lysed in complete RNA immunoprecipitation (RIP) lysis buffer (Merck Millipore). Next, the cell lysates were hatched with RIP buffer containing magnetic beads bound to a human anti-Argonaute2 (Ago2) antibody (Merck Millipore) or immunoglobulin G (IgG, Merck Millipore). Proteinase K was incubated with the samples, and then immunoprecipitated RNA was isolated for detecting the levels of hsa_circ_0001017, hsa-let-7g-3p, and NDST3.

Luciferase reporter assay

GeneChem Co., Ltd (Shanghai, China) constructed the luciferase reporter plasmid (hsa_circ_0001017-WT, hsa_circ_0001017-MUT, NDST3-WT and NDST3-MUT). U251 cells were co-transfected with corresponding plasmids and let-7g-3p mimics or its negative control using Lipofectamine 3000 transfection reagent. The luciferase activity was measured using Dual-Luciferase Reporter Assay System (Promega, WI, USA) after transfection for 48 h.

Statistical analysis

Statistical analysis was performed by GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). Data were shown as mean ± standard deviation (SD) from three independent experiments. The Student t test and one-way ANOVA were used to analyse the difference. $P < 0.05$ was considered to be statistically significant.

Results

Hsa_circ_0001017 expression decreases in glioma tissues and cells

The hsa_circ_0001017 expression in glioma tissue and cells was detected using a qRT-PCR assay to assess the role of hsa_circ_0001017 in glioma. The hsa_circ_0001017 expression was decreased in glioma tissues compared with non-neoplastic brain tissues (Fig. 1A, $p < 0.01$). Furthermore, compared with the NHA cells, the hsa_circ_0001017 expression was decreased in glioma cells (U251, U118, LN229, and U87MG) (Fig. 1B, $p < 0.01$).
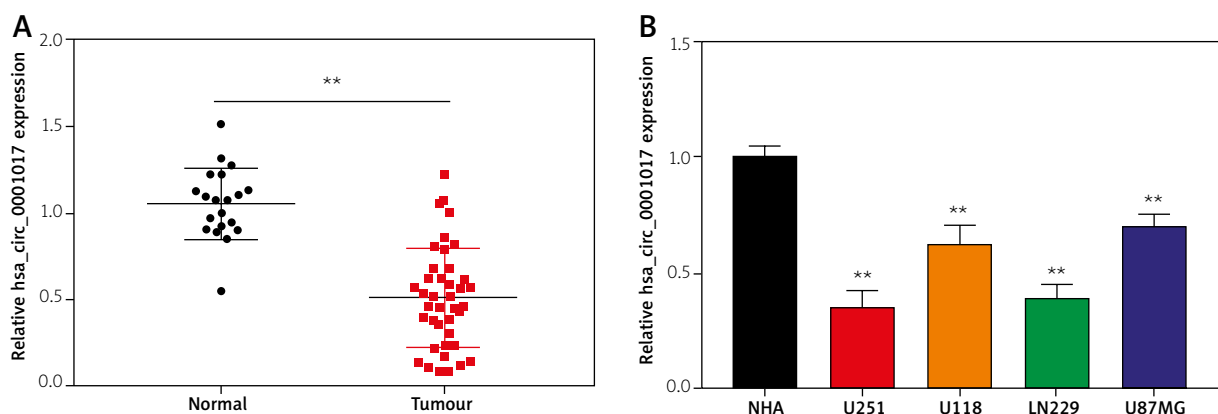
Hsa_circ_0001017 restrains glioma cell proliferation and induces glioma cell apoptosis

U251 cells with the lowest hsa_circ_0001017 expression and U87MG cells with the highest hsa_circ_0001017 expression were used for gain or loss-of-function experiments to illustrate the function of hsa_circ_0001017 in glioma. Hsa_circ_0001017 was stably overexpressed or knocked down in glioma cells (Fig. 2A). According to Figure 2B-D, the overexpressed hsa_circ_0001017 restrained the proliferation ability and increased the apoptosis rate of U251 cells ($p < 0.05$), while the knockdown of hsa_circ_0001017 increased the proliferation ability and decreased the apoptosis rate of U87MG cells ($p < 0.05$).

Hsa_circ_0001017 restrains glioma cell migration, invasion, and epithelial-mesenchymal transition

Wound healing and Transwell assays were performed to assess the influence of hsa_circ_0001017 on cell metastatic property. The over-expressed hsa_circ_0001017 restrained the migration capacity of U251 cells, but the knockdown of hsa_circ_0001017 promoted the migration capacity of U87MG cells ($p < 0.01$, Fig. 3A). Simultaneously, the Transwell invasion assay also showed a similar result (Fig. 3B). The epithelial-mesenchymal transition (EMT)-related proteins expression was examined using western blot analysis to clarify further the mechanism of action of hsa_circ_0001017 in cell metastatic
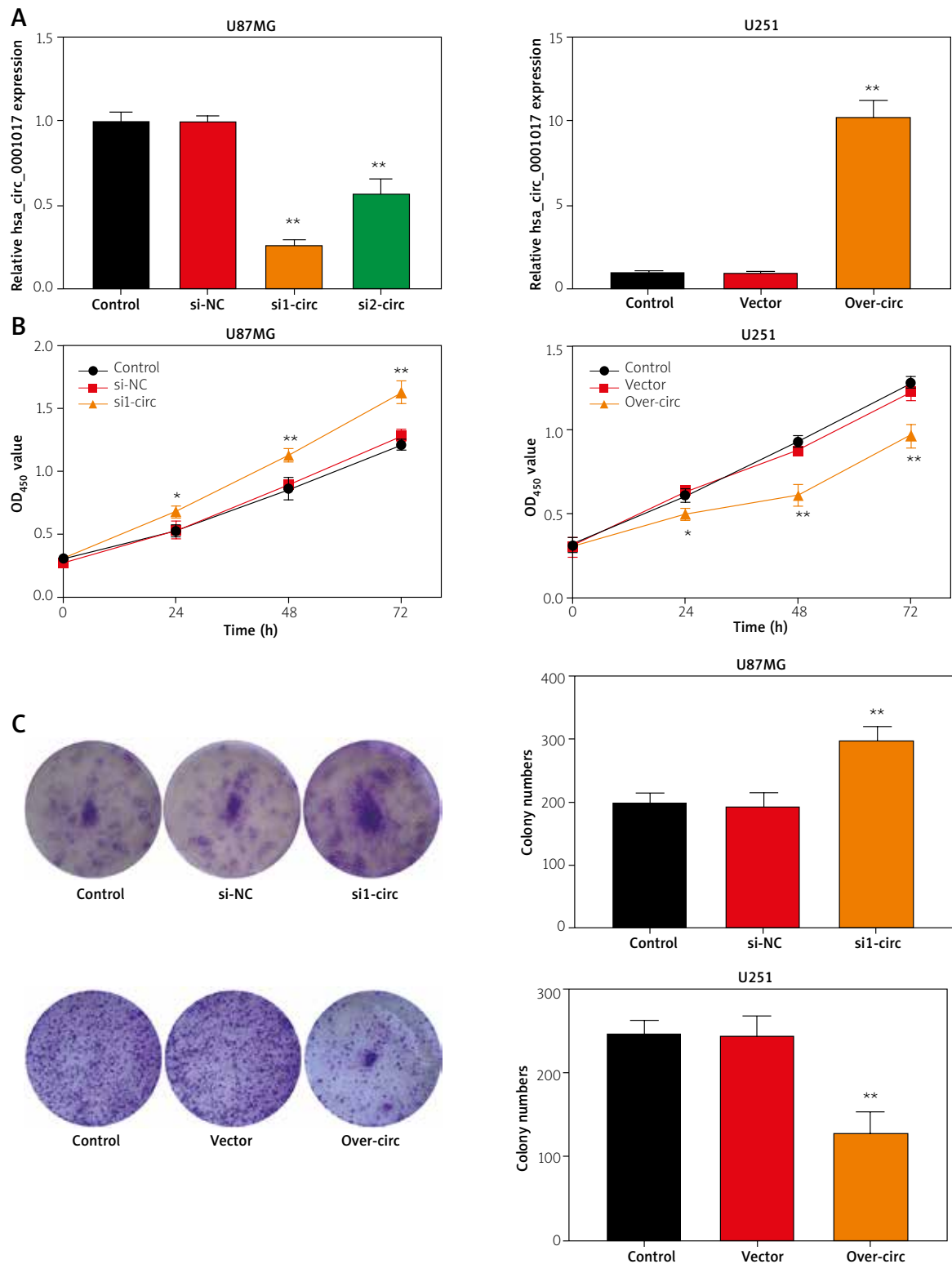

Fig. 1. Hsa_circ_0001017 expression was decreased in glioma tissues and cells. **A)** The hsa_circ_0001017 expression in glioma tissue ($n = 40$) and non-neoplastic brain tissues (normal, $n = 20$) was measured using qRT-PCR assay. **B)** The hsa_circ_0001017 expression in human normal astrocytes (NHA) and glioma cells (U118, U251, U87MG and LN229) was detected using qRT-PCR assay. **$p < 0.01$ vs. normal group or NHA cells.

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Fig. 2. Hsa_circ_0001017 inhibited glioma cell proliferation and promoted glioma cell apoptosis. **A)** The hsa_circ_0001017 expression in U87MG and U251 cells was analysed through qRT-PCR assay. **B)** The cell viability of U87MG and U251 cells was tested by CCK-8 assay. **C)** The cell colony-forming ability of U87MG and U251 cells was detected using colony formation assay. *$p < 0.05$, **$p < 0.01$ vs. Control, si-NC or Vector groups.
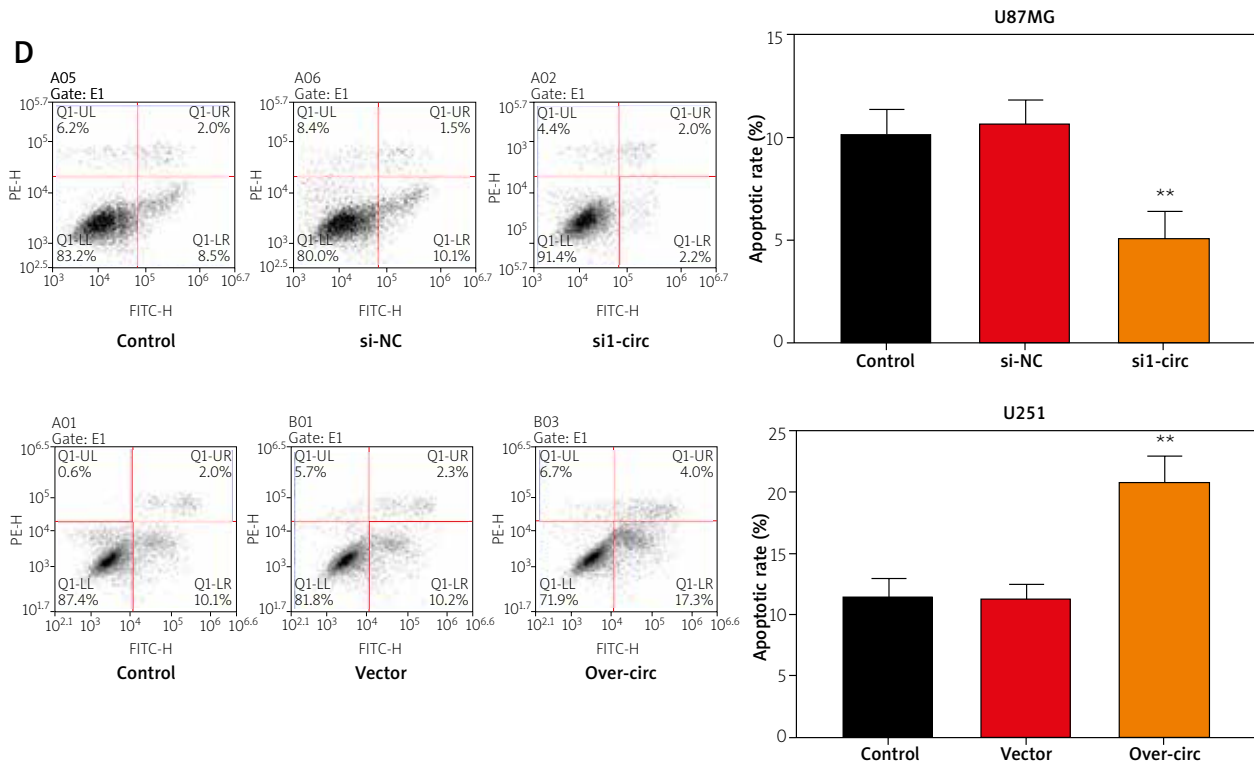
Fig. 2. Cont. **D**) Cell apoptosis of U87MG and U251 cells was detected using flow cytometer analysis. ******$p < 0.01$ vs. Control, si-NC or Vector groups.

property. According to Figure 3C, the overexpressed hsa_circ_0001017 reduced N-cadherin and Vimentin expression and raised E-cadherin expression in U251 cells ($p < 0.01$). Meanwhile the opposite results were observed due to the knockdown of hsa_circ_0001017 of U87MG cells.

Hsa_circ_0001017 sponges hsa-let-7g-3p in glioma

Many studies showed that circRNAs functioned as miRNA sponges. Next, DGEs and RegRNA2.0 were used to predict miRNAs, and then the intersection of DGEs and RegRNA2.0 identified 12 miRNAs likely to be sponged by hsa_circ_0001017 (Fig. 4A). After overexpressing hsa_circ_0001017, hsa-let-7g-3p expression presented the most down-regulated miRNA ($p < 0.01$, Fig. 4B). Therefore, hsa-let-7g-3p was selected for further analysis. The Cancer Genome Atlas (TCGA) database showed that hsa-let-7g-3p expression was increased in patients with recurrent glioma compared with glioma patients with non-recurrent glioma, but the difference was not statistically significant ($p = 0.052$, Fig. 4C). Nota-

bly, the survival analysis using the TCGA database showed that high hsa-let-7g-3p expression in low-grade glioma predicted poor survival ($p = 0.017$, Fig. 4D). A series of experiments were conducted to explore whether hsa_circ_0001017 could sponge hsa-let-7g-3p. First, the qRT-PCR assay showed that hsa-let-7g-3p expression in glioma (tumour) tissues was increased ($p < 0.01$, Fig. 4E), which was opposite to hsa_circ_0001017 expression. As shown in Figure 4F, hsa_circ_0001017 and hsa-let-7g-3p could be efficiently pulled down by anti-Ago2 in U251 cells ($p < 0.01$). The potential binding sites of hsa-let-7g-3p within the sequence of hsa_circ_0001017 were shown in Figure 4G. The luciferase activity was decreased in U251 cells co-transfected with hsa-let-7g-3p mimics and circRNA-0001017-WT, but with no statistically significant differences in other groups (Fig. 4H).

NDST3 is the target gene of hsa-let-7g-3p in glioma

Three miRNA target gene databases (miRDB, TargetScan, and microRNA.org) were used to seek
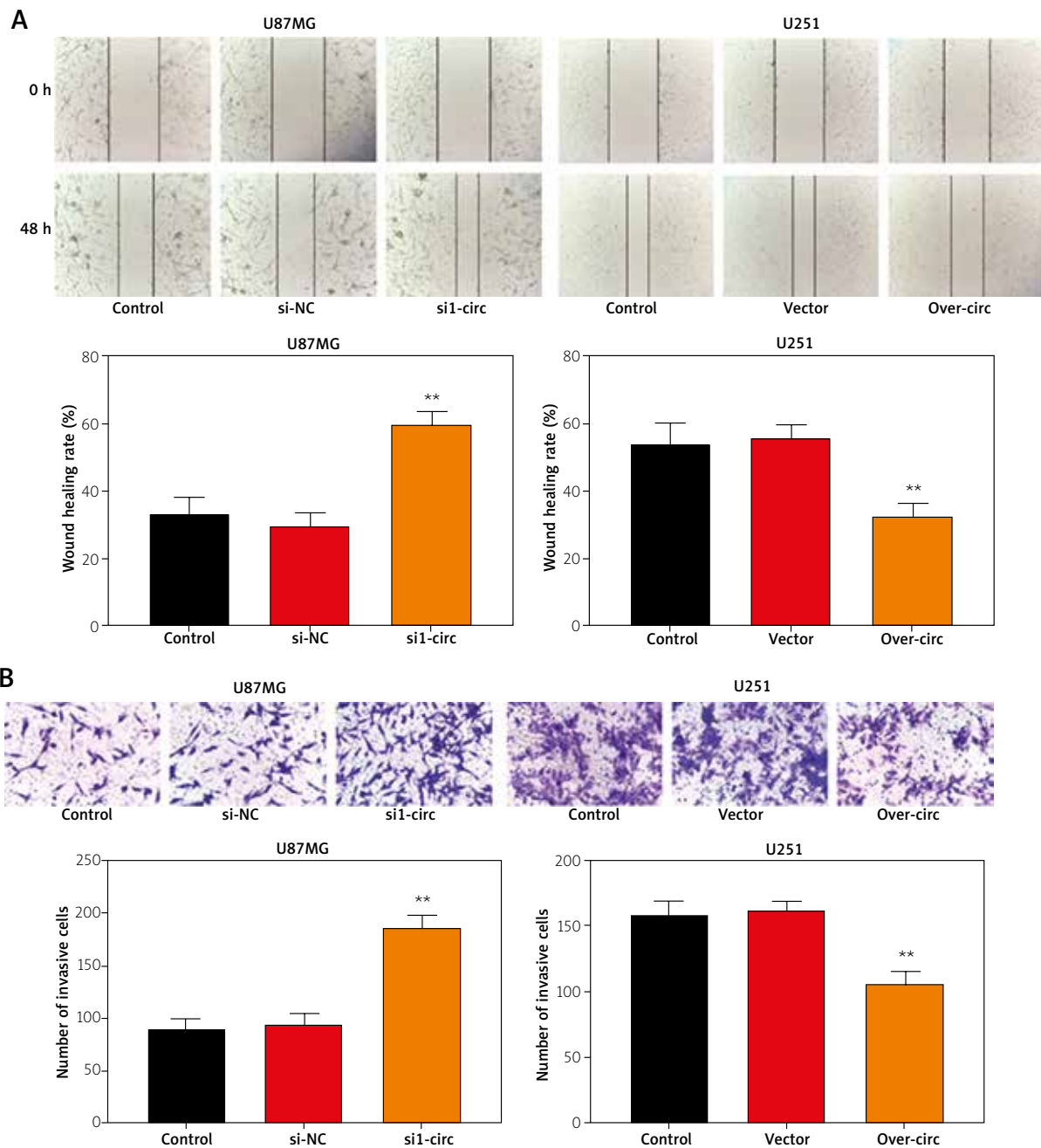
Fig. 3. Glioma cell migration, invasion, and EMT were inhibited by hsa_circ_0001017. **A**) The cell migration of U87MG and U251 cells was detected using wound healing assay. **B**) Transwell assay was performed to detect cell invasion of U87MG and U251 cells. **p < 0.01 vs. Control, si-NC or Vector groups.

the potential targets of hsa-let-7g-3p so as to illustrate the mechanism of hsa_circ_0001017 as a ceRNA. A total of four miRNAs (MAP4K3, NDST3, LIN28B, and FNDC3A) were identified in both databases (Fig. 5A). Next, the expression of MAP4K3, NDST3, LIN28B, and FNDC3A in glioma tissues was analysed

using Gene Expression Profiling Interactive Analysis 2 (GEPIA2). As shown in Figure 5B, NDST3 and LIN28B expression in glioma tissues was decreased, and the difference in NDST3 expression was statistically significant ($p < 0.05$). Therefore, NDST3 was selected for further analysis. Moreover, the TCGA
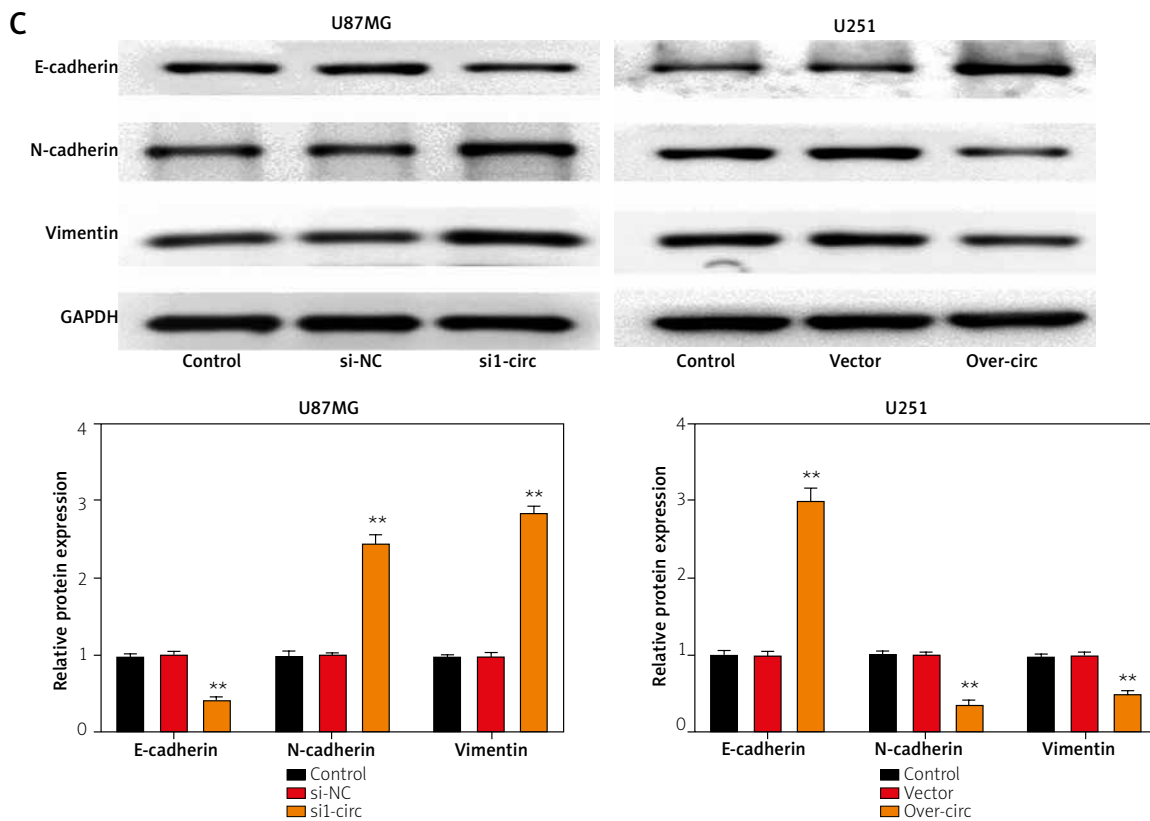
Fig. 3. Cont. **C)** Western blot analysis was performed to measure EMT-related proteins expression. **p < 0.01 vs. Control, si-NC or Vector groups.

database indicated that NDST3 expression was decreased in glioma (Fig. 5C) and high-grade glioma (Fig. 5D), and low NDST3 expression predicted poor survival ($p = 0.0076$, Fig. 5E). As shown in Figure 5F, NDST3 mRNA expression in glioma (tumour) tissues was decreased ($p < 0.01$). The RIP assay found that NDST3 and hsa-let-7g-3p could be efficiently pulled down by anti-Ago2 in U251 cells ($p < 0.01$, Fig. 5G). Figure 5H displayed the potential binding site of hsa-let-7g-3p within NDST3. The luciferase activity was decreased in U251 cells co-transfected with hsa-let-7g-3p mimic and NDST3-WT, but with no statistically significant differences in other groups (Fig. 5I).

Hsa_circ_0001017 restrains the growth and metastasis and induces glioma cell apoptosis *via* targeting the hsa-let-7g-3p/NDST3 axis

U251 cells were transfected with pcDNA 3.1-circ-0001017, let-7g-3p mimic, pcDNA 3.1-circ-0001017 + let-7g-3p mimic or let-7g-3p mimic + pcDNA3.1-NDST3 to conduct rescue assays so as to understand the interplay between hsa_circ_0001017, hsa-let-7g-3p, and NDST3. As indicated in Figure 6A-C, the overexpression of hsa_circ_0001017 reduced hsa-let-7g-3p expression and increased NDST3 expression ($p < 0.01$), and the effect of pcDNA3.1-circ-0001017 on NDST3 was restored by let-7g-3p mimic. According to Figure 6D-F, the tumour-suppressive role of circ_0001017 was reversed after co-transfection with let-7g-3p mimic. Moreover, the overexpression of let-7g-3p increased cell proliferation and invasion and decreased cell apoptosis ($p < 0.01$), while overexpression of let-7g-3p and NDST3 decreased cell proliferation and invasion, and increased cell apoptosis (Fig. 6D-F).

Discussion

Previous studies reported that rapid proliferation and infiltrative growth were the main causes of death in patients with glioma [5,23]. However, the mechanism underlying glioma growth and progression is not clear. Thus, it is particularly important to reveal the potential mechanisms of molecular-targeted therapy in glioma. Accumulating evidence has suggested that circRNAs are closely related to tumour progres-
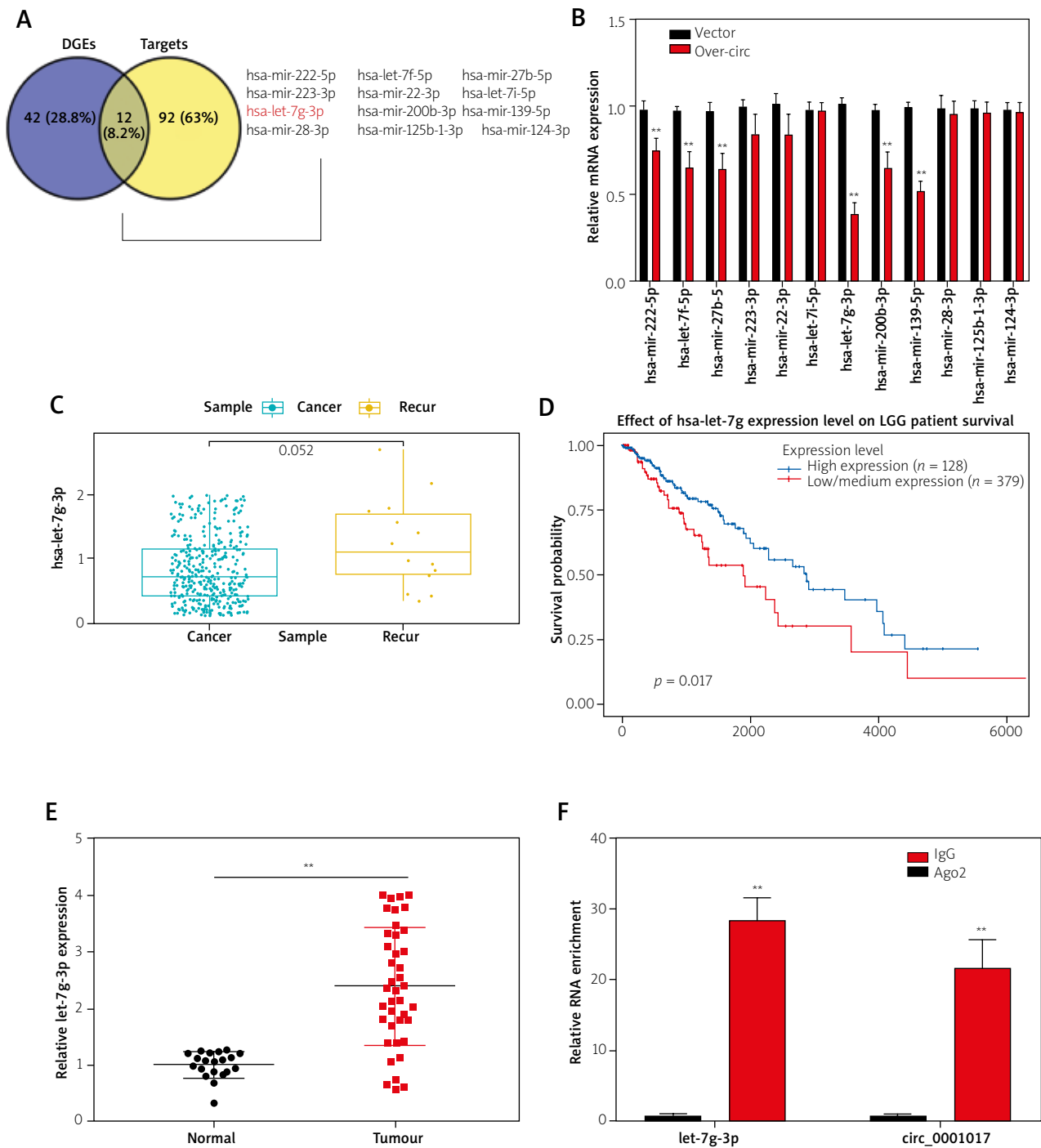
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Fig. 4. Hsa_circ_0001017 sponged hsa-let-7g-3p in glioma. **A**) DGEs and RegRNA2.0 were used to predict miRNAs sponged by hsa_circ_0001017. **B**) The candidate miRNAs expression in U251 cells with overexpressed hsa_circ_0001017 was measured using qRT-PCR assay; **p < 0.01 vs. Vector group. **C**) Hsa-let-7g-3p expression in recurrent patients with glioma and glioma patients from the Cancer Genome Atlas (TCGA) database. **D**) Kaplan-Meyer survival curves for two groups of low grade glioma (LGG) patients from TCGA database. **E**) The hsa-let-7g-3p expression in glioma (tumour) tissues (*n* = 40) and normal tissues (*n* = 20) was measured using qRT-PCR assay; **p < 0.01 vs. Normal group. **F**) After pulling down of Ago2 by RIP assay, hsa_circ_0001017 and hsa-let-7g-3p expression in U251 cells was measured using qRT-PCR assay; **p < 0.01 vs. IgG group.

G

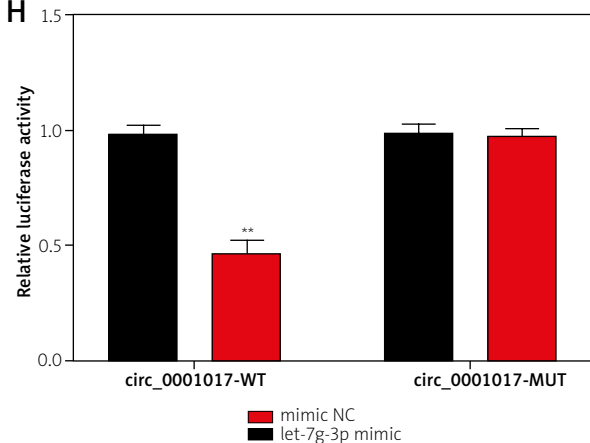|	
hsa_circ_0001017-WT	5'-TGGTGGCGTGTGCCTGTAATC-3'
hsa-let-7g-3p	3'-CGUUCCGUCACCCGAGGACAUGUC-5'
hsa_circ_0001017-MUT	5'-TGGTGGCGTGTGGGACAUATC-3'

H

Fig. 4. Cont. **G**) Potential binding sites of hsa-let-7g-3p within the sequence of hsa_circ_0001017. **H**) Luciferase reporter assay was performed to test the relative luciferase activity; **$p < 0.01$ vs. mimic NC group.

sion, such as cell proliferation, invasion, and apoptosis [2,3,13]. Specifically, many circRNAs have been identified to play a vital role in glioma. Chen *et al.* [1] found that circPTN promoted proliferation and stemness in glioma. Peng *et al.* [26] reported that circCPA4 affected the proliferation and metastasis, and served as a prognostic factor for glioma. The present study showed that hsa_circ_0001017 expression decreased in glioma tissues and cells. These findings indicated that hsa_circ_0001017 might participate in the initiation and development of glioma.

The basic biological feature of glioma includes glioma cell proliferation, migration, invasion, and apoptosis [11,33,35]. Therefore, better understanding of the biological features of gliomas may be advantageous to promoting the glioma diagnosis and treatment. The loss/gain of function studies were conducted by transfection with silencing or overexpression plasmid of hsa_circ_0001017 to examine the effects of hsa_circ_0001017 in glioma cells. The overexpressed hsa_circ_0001017 restrained glioma cell proliferation, migration, and invasion, and induced glioma cell apoptosis. However, the knockdown of hsa_circ_0001017 caused the opposite results. Invasion, dissemination, and metastasis are major steps in malignant cancer progression, which are related to EMT [10,32]. Thus, it is important to study the EMT process for glioma progression. The expression of EMT-related proteins was examined using western blot analysis to determine whether the influence of hsa_circ_0001017 on glioma migration and invasion resulted from EMT. Experimental evidence indicated that the overex-

pressed hsa_circ_0001017 decreased N-cadherin and Vimentin expression and increased E-cadherin expression, whereas the knockdown of hsa_circ_0001017 showed contrasting effects. Our findings pointed out that hsa_circ_0001017 might serve as an anti-oncogene in glioma.

CircRNAs regulate tumour progression through sponging miRNA [6]. CiRS-7 was the first circRNA approved as a miRNA sponge. Many studies showed that ciRS-7 sponged miR-7 to regulate gene expression [7,29,34]. In addition to ciRS-7, a lot of other circRNAs have been described to serve as miRNA sponges. CircMMP9 can sponge miR-124 and facilitate glioblastoma multiforme cell tumorigenesis [28]. Circ_001350 regulates glioma cell proliferation, apoptosis, and metastatic properties through sponging miR-1236 [20]. In our study, the bioinformatics analysis was performed to anticipate the target miRNAs of hsa_circ_0001017, revealing the binding sites for hsa-let-7g-3p. López-Aguilar *et al.* [14] have shown that miR-1303 is upregulated in paediatric astrocytoma, and let-7g-3p has a similar expression pattern to that of miR-1303. We found that hsa-let-7g-3p expression in glioma tissues was higher. In addition, hsa_circ_0001017 could directly interact with hsa-let-7g-3p and function by sponging hsa-let-7g-3p. These findings suggested that hsa-let-7g-3p was a functional target of hsa_circ_0001017 in glioma.

MiRNAs exert their biological function by binding to 3' UTRs of mRNA. Hence, the online miRNA target bioinformatics analysis was performed to explore possible target genes of hsa-let-7g-3p. NDST3 is expressed in both foetal and adult brains,
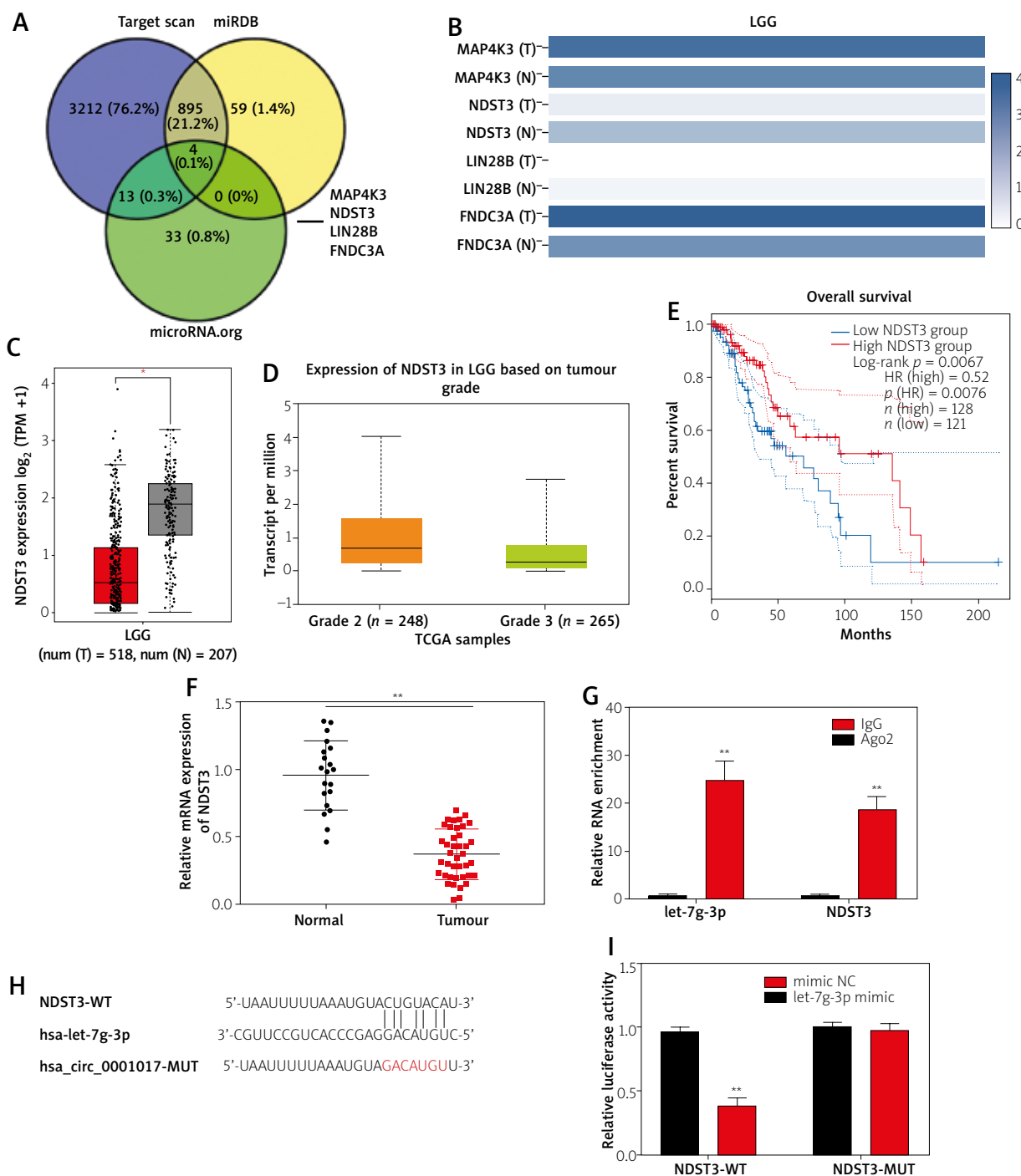
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Fig. 5. NDST3 was the target gene of hsa-let-7g-3p in glioma. **A**) Venn diagram for miRNA target gene data-bases (miRDB, TargetScan and microRNA.org) of predicting the potential targets of hsa-let-7g-3p. **B**) The expression of MAP4K3, NDST3, LIN28B and FNDC3A in glioma tissues was analysed using Gene Expression Profiling Interactive Analysis 2 (GEPIA2). **C**) NDST3 expression in glioma tissues and normal tissues from the Cancer Genome Atlas (TCGA) database. **D**) NDST3 expression in different grade glioma from TCGA data-base. **E**) Kaplan-Meyer survival curves for two groups glioma (LGG patients from TCGA database). **F**) NDST3 mRNA expression in glioma (tumour) tissues (*n* = 40) and normal tissues (*n* = 40) was tested using qRT-PCR assay; ***p < 0.01 vs. normal group. **G**) After pulling down of Ago2 by RIP assay, NDST3 and hsa-let-7g-3p expression was tested in U251 cells using qRT-PCR assay; ***p < 0.01 vs. IgG group. **H**) The potential binding site of hsa-let-7g-3p within NDST3. **I**) Luciferase reporter assay was performed to test the relative luciferase activity; ***p < 0.01 vs. mimic NC group.
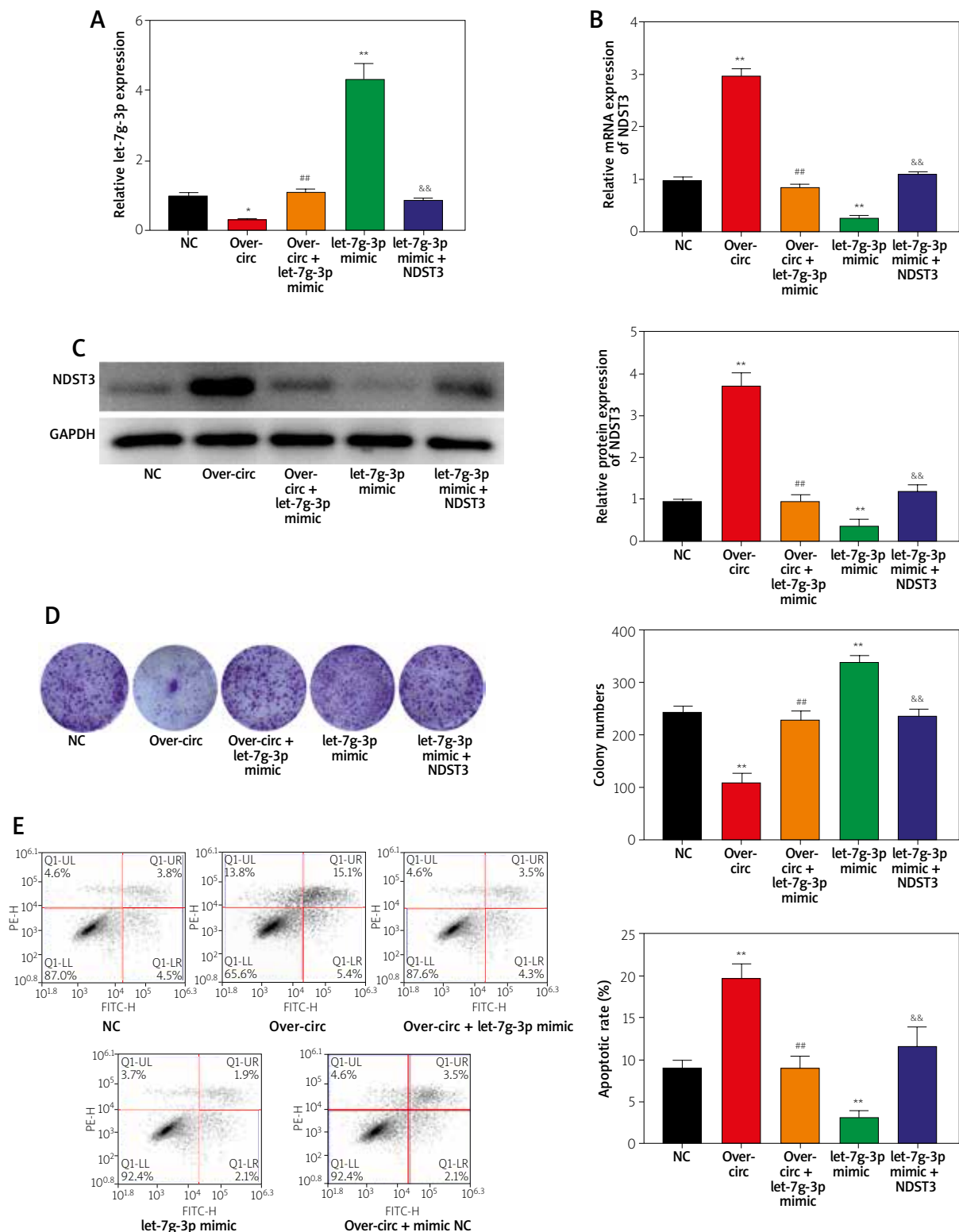
Fig. 6. Hsa_circ_0001017 inhibited the growth and metastasis and promoted cell apoptosis of glioma *via* targeting the hsa-let-7g-3p/NDST3 axis. **A)** The hsa-let-7g-3p expression in U251 cells was measured using qRT-PCR assay. **B)** qRT-PCR assay was performed to detect NDST3 mRNA expression in U251 cells. **C)** The protein expression of NDST3 was analysed using western blot analysis. **D)** Cell colony-forming ability of U251 cells was assessed through colony formation assay. **E)** Apoptosis of U251 cells was detected using flow cytometer analysis. *$p < 0.05$ and **$p < 0.01$ vs. NC group, ##$p < 0.01$ vs. Over-circ group, &&$p < 0.01$ vs. let-7g-3p mimic group.
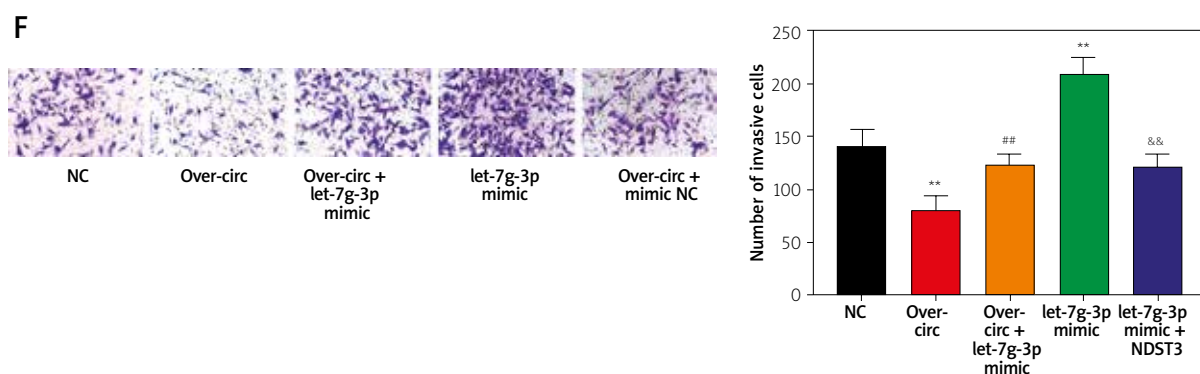
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F

Fig. 6. Cont. **F)** Transwell assay was performed to detect the invasive ability of U251 cells, **$p < 0.01$ vs. NC group, ##$p < 0.01$ vs. Over-circ group, &&$p < 0.01$ vs. let-7g-3p mimic group.

and it plays a key role in maintaining heparan sulfate metabolism [15,24]. However, NDST3 expression has not yet been reported in glioma. In the present research, NDST3 mRNA expression was decreased in glioma tissues. RIP and luciferase reporter assays showed that NDST3 was the target gene of hsa-let-7g-3p in glioma. In addition, further functional studies demonstrated that the overexpression of hsa-let-7g-3p increased proliferation and invasion and decreased apoptosis of glioma cells, while the promotion effect of let-7g-3p overexpression was reversed by the overexpression of NDST3. In addition, the tumour-suppressive effects of circ_0001017 was reversed by overexpression of hsa-let-7g-3p.

Conclusions

In glioma tissues and cells, hsa_circ_0001017 expression was downregulated. Hsa_circ_0001017 inhibited glioma cell proliferation, migration, and invasion and induced glioma cell apoptosis. Additionally, the tumour-suppressive role of hsa_circ_0001017 was related to the hsa-let-7g-3p/NDST3 axis. Our findings may be conducive to the development of treatment methods for glioma.

Availability of data and material

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

All enrolled patients did not receive preoperative radiotherapy and chemotherapy before surgery, and they signed written informed consent. The study was approved by the Ethics Committee of the First Affiliated Hospital of Shandong First Medical University.

Disclosure

The authors report no conflict of interest.

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