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Original Article

Circular RNA 001569 acts as an oncogene and correlates with aggressive characteristics in hepatocellular carcinoma

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Abstract: Circular RNAs (circRNAs), a type of noncoding RNAs, may serve as biomarkers and therapeutic targets for cancer. However, the role of circular RNA 001569 in hepatocellular carcinoma (HCC) remains unclear. We aimed to explore the potential role of hsa_circ_001569 in the development process of HCC. Quantitative real-time reverse transcription PCR (qRT-PCR) results indicated that hsa_circ_001569 was upregulated in HCC tissues, and was related to tumor differentiation and TNM stages. CCK-8 and colony formation experiment were performed to measure the proliferation ability of HCC cells. The results revealed that silence of hsa_circ_001569 inhibited the proliferation ability of HCC *in vitro*. Furthermore, tumorigenicity assay in nude mice showed that inhibition of hsa_circ_001569 by siRNAs suppressed the growth of HCC tumor *in vivo*. These results proved that hsa_circ_001569 acts as a potential therapeutic target and biomarker for HCC.

Keywords: Circular RNA 001569, hepatocellular carcinoma, oncogene, proliferation

Introduction

Hepatocellular carcinoma (HCC) is a major health problem worldwide and was caused by genetic factors, alcoholic injury, chronic infection such as hepatitis B virus and hepatitis C virus [1]. At present, there were about 745,000 patients died of HCC every year [2-4]. Because of a low detection rate of HCC at the curable stages and a high rate of recurrence and metastasis, its survival remains unsatisfactory in spite of recent developments of therapeutic strategies. Chemotherapy is one of the main treatment methods for terminal-stage HCC. However, most anti-cancer drugs have strong cytotoxic effect and the emergence of drug resistance which limits its widespread application and curative effect. Therefore, the underlying molecular mechanisms of HCC, especially the correlations between genes and their functions need be further studied. Here, we aimed to investigate the aggressive characteristics of hsa_circ_001569 in HCC.

Circular RNA (circRNA) is a class of non-coding RNAs (ncRNAs) and generated a closed continuous loop from thousands of genes by back-splicing and covalent binding in both human and mouse [5-7]. Although circular transcripts were found for at least 20 years, they were regarded as aberrant RNA splicing or specific pathogens [8-10]. However, recent studies have shown that circRNAs play important roles in gene expression regulation and biological processes [11-14]. Emerging researches suggest that large numbers of circRNAs are endogenous RNAs to mammalian cells, and are abundant and stable [15]. In addition, recent research indicated that both exonic and intronic circRNAs have important functions in the regulation of gene expression [16-18]. CircRNAs may have novel and potential biological functions compared with other RNAs, and may be ideal biomarkers in the diagnosis and therapy of cancers. At present, plentiful unknown circRNAs have been found in numerous human cell types [19-22]. However, the biological functions and molecular mechanisms of hsa_circ_001569 in HCC are not entirely clear.

Studies have shown that hsa_circ_001569 was served as a sponge of miR-145, and

increased the expression levels of miR-145 targeting genes such as E2F5, BAG4 and FMNL2; hsa_circ_001569 plays a major role in cell proliferation and invasion in colorectal cancer (CRC). This suggests that hsa_circ_001569 may be a novel biomarker in the progression of CRC [23]. In our study, for the first time, we revealed that hsa_circ_001569 was upregulated in HCC tissues, and was related to tumor differentiation and TNM stages. Silence of hsa_circ_001569 inhibited the proliferation ability of HCC *in vitro* and *in vivo*.

Materials and methods

Clinical specimens

A total of 30 HCC samples (tumor and adjacent normal tissues) were collected from the department of the First Affiliated Hospital of Bengbu Medical College between 2014 and 2016. This study has been approved by the Research Ethics Committee of the First Affiliated Hospital of Bengbu Medical College. Animal researches were performed according to the national guidelines for the care and use of laboratory animals. Written informed consent was obtained from each patient. We handled retrospective samples based on the ethical and legal standards. All tissue samples were stored at -80°C immediately.

Cell culture

Normal hepatocytes LO2 cell line and HCC cell lines (HepG2, Hep3B, QGY-7703, and SMMC-7721) were purchased from American Type Culture Collection (ATCC, Manassas, VA). LO2, HepG2, Hep3B, QGY-7703, and SMMC-7721 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM, High glucose, Invitrogen) including with 10% fetal bovine serum (FBS) (Sigma Aldrich), and 100 U/mL penicillin/streptomycin (Invitrogen, Carlsbad, CA) in an appropriate incubator containing 5% CO_2 at 37°C.

siRNA transfection

Hsa_circ_001569 siRNAs (si-Circ) and negative control (si-NC) were purchased from GenePharma (GenePharma Co., Ltd., Shanghai, China). The sequences of hsa_circ_001569 siRNAs were 5'-GCA UCG UGC AGG ACU GGA AdT dT-3' (sense), and 3'-dTdT CG UAG CAC GUC CUG ACC UU-5' (antisense). The sequences of NC siRNAs were 5'-UUC UCC GAA CGU GUC ACG UTT-3' (sense), and 5'-ACG UGA CAC GUU CGG AGA ATT-3'. The QGY-7703 and SMMC-7721 cells were cultured in 6-well plates, and were transfected with hsa_circ_001569 siRNAs and negative control (NC) using Lipofectamine™ 3000 (Invitrogen, Carlsbad, CA, USA) respectively according to the manufacturer's protocols. At the indicated time, cells were harvested for further experiments.

Quantitative real-time reverse transcription PCR (qRT-PCR)

Total RNA was extracted from HCC tissues and the transfected QGY-7703 and SMMC-7721 cells using TRIzol reagent (Invitrogen, CA, USA). Then the cDNA was synthezed by using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher) with random primers and corresponding total RNA. The reaction conditions were 95°C for 30 seconds, followed by 40 cycles of 95°C for 5 seconds, 60°C for 34 seconds using the standard SYBR-Green PCR Master Mix kit (Takara) on ABI 7500 system (Applied Biosystems, Foster City, CA, USA). The primer sequences for hsa_circ_001569 are 5'-TCC CCT GAA CAT TCT CCC CAT-3' (the forward primer) and 5'-GAA AGC ACT TGG TGA AGT CGG-3' (the reverse primer). GAPDH was used as an internal control. The primer sequences for GAPDH are 5'-TGT TCG TCA TGG GTG TGA AC-3' (the forward primer) and 5'-ATG GCA TGG ACT GTG GTC AT-3' (the reverse primer) [23]. The expression level of hsa_circ_001569 was calculated using $\Delta\Delta$Ct ($2^{-\Delta\Delta Ct}$) levels.

Nucleic acid electrophoresis

Total RNA was extracted from SMMC-7721 cells, and cDNA was synthezed. Genomic DNA was extracted using a genome DNA extraction kit (Tiangen) according to the manufacturer's protocol. The cDNA and gDNA PCR products were amplified using a Takara RNA polymerase chain reaction (PCR) kit (alfalfa mosaic virus) according to the manufacturer's protocol, and then separated by 2% agarose gels with TE buffer at 110 V for 30 min. The DNA marker was DL600 (KeyGen, Nanjing, cat 37061). The images were obtained by UV irradiation. The divergent primer sequences for hsa_circ_001569 are shown in qRT-PCR assay. The convergent primer sequences for hsa_circ_001569

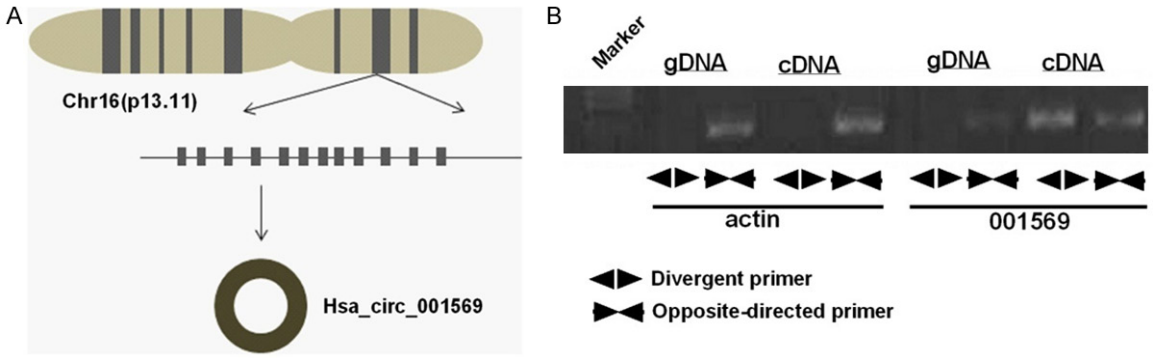

Figure 1. Identification and characterization of hsa_circ_001569. A. The location of hsa_circ_001569 is shown in human chromosome 16p13.11. B. Hsa_circ_001569 is confirmed by PCR using divergent and convergent primers. β-actin mRNA serves as a negative control; gDNA, genomic DNA; cDNA, complementary DNA.

are 5'-ATC AAC CGG GGA AGC TGT TA-3' (the forward primer) and 5'-CGC AGA GAC ACA TGC CTT AC-3' (the reverse primer). The primer sequences for β-actin are 5'-CGC TCT CTG CTC CTC CTG TTC-3' (the forward primer) and 5'-ATC CGT TGA CTC CGA CCT TCA C-3' (the reverse primer).

Proliferation assay

Cell proliferation assay was performed using the Cell Counting Kit-8 (CCK8) Kit (Promega) according to the manufacturer's protocol. The transfected QGY-7703 and SMMC-7721 cells (3000 cells/well) were plated in 96-well plates and were incubated at 37°C. Cholecystokinin octapeptide (10 μL, CCK-8; Dojindo Laboratories, Kumamoto, Japan) was added to each well at 0, 12, 24, 48, and 72 hrs respectively. Cells then were incubated for 3 h at 37°C. The absorbance value was measured at 450 nm with a micro-plate reader (Bio Tek Instruments, Inc., Winooski, VT, USA).

Cell proliferation assay was also performed by colony formation experiment. The transfected QGY-7703 and SMMC-7721 cells (100 cells/well) were plated in 6-well plates and were incubated with 2 ml complete media at 37°C for 14 days. The complete medium was replaced every 3 days. Then cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Visible colonies were photographed and counted.

Tumorigenicity assay in nude mice

This study was approved by the Animal Care and Use Committee. Six-week-old female Balb/c athymic nude mice (Vitalriver Laboratory Animals, Beijing, China) were purchased from Beijing Vital River Laboratories (Beijing, China). QGY-7703 and SMMC-7721 cells were transfected with hsa_circ_001569 siRNAs and NC respectively. Nude mice were injected subcutaneously with the transfected QGY-7703 and SMMC-7721 cells (3×10^6 cells/well). The tumor volume (mm^3) and weight (g) were measured at 0, 7, 14, 21, 28, and 35 days.

Statistical analysis

Statistical analyses were performed using SPSS version 21.0 (SPSS, Chicago, IL, USA). Student's *t*-test was used to analyze the differences between the groups. All data were shown as the mean ± SD. All experiments were performed in triplicate. $P < 0.05$ was considered to be statistically significant.

Results

Identification and characterization of hsa_circ_001569

Previous study indicates hsa_circ_001569 accelerates the proliferation and invasion abilities of CRC, and directly inhibits miR-145 [23]. However, hsa_circ_001569 in HCC progress in not clear. Hsa_circ_001569 is located in chromosomal 16 region p13.11 (**Figure 1A**). We designed pairs of primers, one is a divergent primer that amplifies circular RNA, and the other is an opposite-directed primer that amplifies linear RNA. RT-PCR assay was performed to verify the expression level of hsa_circ_001569 in HCC cells. Our results showed

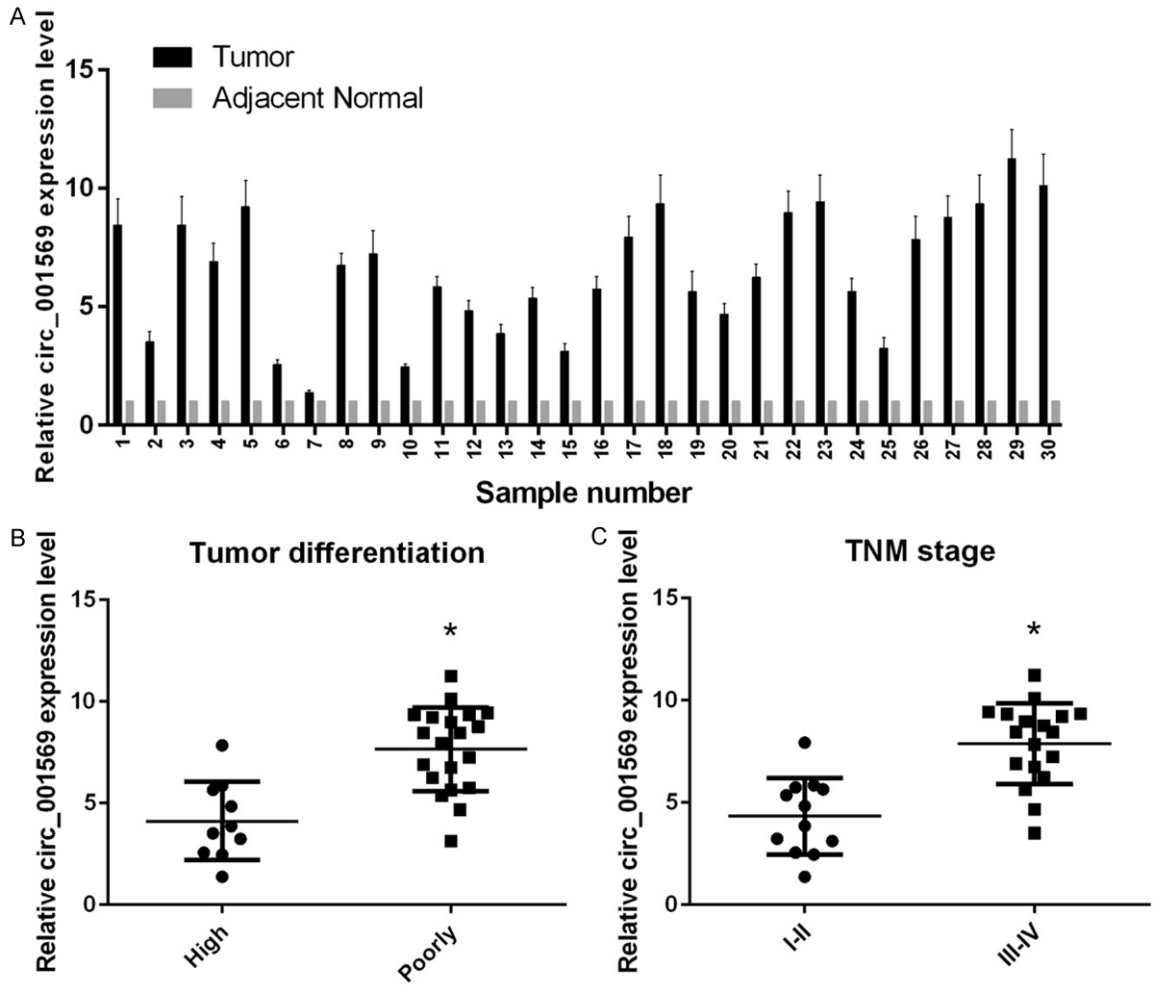

Figure 2. Hsa_circ_001569 is high-expressed in human HCC tissues. A. The expression level of hsa_circ_001569 was analyzed by qRT-PCR in 30 pairs of HCC tissues and paired adjacent normal tissues. B. The mRNA expression level of hsa_circ_001569 was measured by qRT-PCR in high differentiated tumor and low differentiated tumor (*P < 0.05). C. Hsa_circ_001569 expression level was detected by qRT-PCR in TNM stage (I & II) and TNM stage (III & IV) (*P < 0.05).

that hsa_circ_001569 was amplified using cDNA and divergent primers. However, genomic DNA was not amplified with the divergent primers. Actin was served as a linear RNA control (**Figure 1B**). Our results indicated that hsa_circ_001569 can be expressed in HCC, and has_circ_001569 may be a potential regulator in HCC.

Hsa_circ_001569 is high-expressed in human HCC tissues

To study the expression level of hsa_circ_001569 in human HCC tissues, qRT-PCR was performed to analyze the expression of hsa_circ_001569 in 30 pairs of HCC tissues and paired adjacent normal tissues. The results revealed that hsa_circ_001569 was significantly higher in HCC tissues than adjacent noncancerous tissues (**Figure 2A**). Then we found that the expression level of hsa_circ_001569 was increased in low differentiated HCC tissues compared to high differentiated HCC tissues (P < 0.05) (**Figure 2B**). In addition, hsa_circ_001569 was highly expressed in HCC tissues with TNM stage (III & IV) relative to HCC tissues with TNM stage (I & II) (P < 0.05) (**Figure 2C**). In conclusion, hsa_circ_001569 was upregulated in HCC tissue, and is related to tumor differentiation and TNM stages.

Silence of hsa_circ_001569 suppresses the proliferation ability of HCC

We detected the expression of hsa_circ_001569 in HCC cancer cell lines by qRT-PCR.

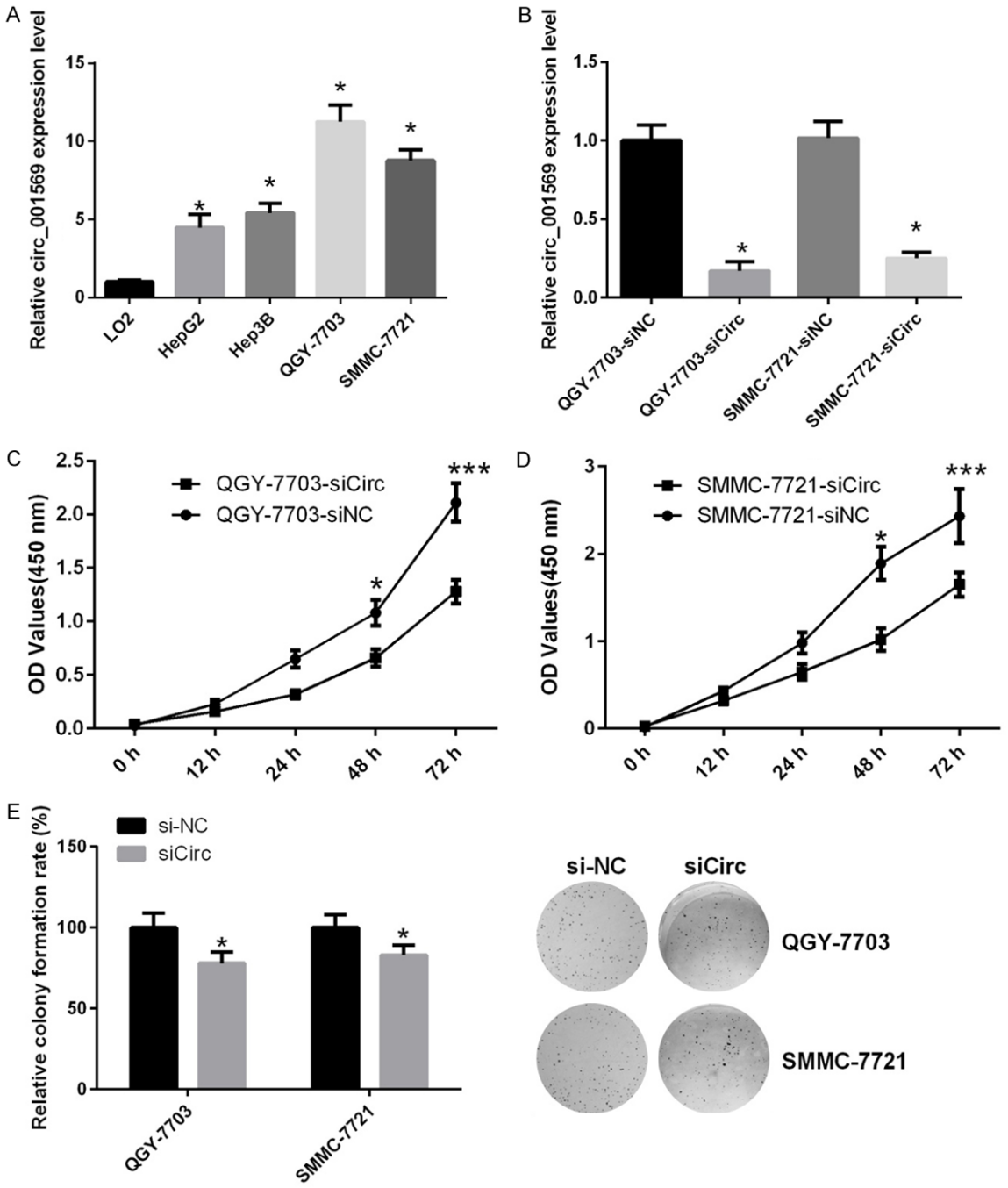

Figure 3. Silence of hsa_circ_001569 suppresses the proliferation ability of HCC. A. The mRNA expression level of hsa_circ_001569 was analyzed by qRT-PCR in normal hepatocytes LO2 cells and HCC cell lines (HepG2, Hep3B, QGY-7703, and SMMC-7721) (*P < 0.05). B. QGY-7703 and SMMC-7721 cells were transfected with hsa_circ_001569 siRNAs and negative control (NC) using Lipofectamine 3000, and the expression level of hsa_circ_001569 was detected by qRT-PCR in the treated QGY-7703 and SMMC-7721 cells (*P < 0.05). C. The proliferation ability was detected by CCK-8 in the treated QGY-7703 cells (*P < 0.05, ***P < 0.001). D. The proliferation ability was detected by CCK-8 in the treated SMMC-7721 cells (*P < 0.05, ***P < 0.001). E. The proliferation ability was detected by colony formation in QGY-7703 and SMMC-7721 cells treated as B (*P < 0.05).

Our results demonstrated that hsa_circ_001569 was highly expressed in HCC cell lines (HepG2, Hep3B, QGY-7703, and SMMC-7721) when compared with normal hepatocytes LO2

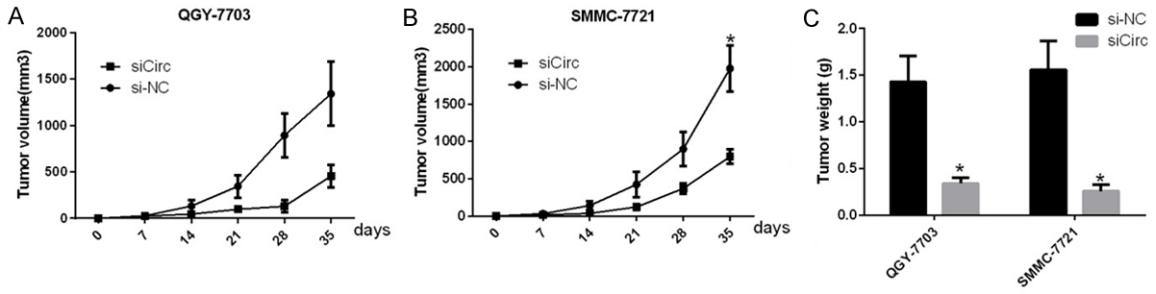

Figure 4. Silence of hsa_circ_001569 inhibits the growth of HCC tumor *in vivo*. A. Nude mice were subcutaneously injected with 3 × 10^6 QGY-7703 cells were transfected with hsa_circ_001569 siRNAs and NC respectively. The tumor volume (mm^3) was measured at 0, 7, 14, 21, 28, and 35 days. B. Nude mice were subcutaneously injected with 3 × 10^6 SMMC-7721 cells were transfected with hsa_circ_001569 siRNAs and NC respectively. The tumor volume (mm^3) was measured at 0, 7, 14, 21, 28, and 35 days (*P < 0.05). C. The tumor weight (g) in nude mice was measured at particular points in time (*P < 0.05).

cells, and the expression of hsa_circ_001569 was highest in QGY-7703 and SMMC-7721 cells (P < 0.05) (**Figure 3A**). Then QGY-7703 and SMMC-7721 cells were transfected with hsa_circ_001569 siRNAs and negative control (NC) using Lipofectamine 3000. qRT-PCR assay was performed to analyze the transfection effects of hsa_circ_001569 siRNAs. The results showed that the expression level of hsa_circ_001569 was dramatically down-regulated in QGY-7703 and SMMC-7721 cells which were transfected with hsa_circ_001569 siRNAs compared to NC groups (P < 0.05). Therefore, our result indicated that hsa_circ_001569 was effectively decreased by siRNAs (**Figure 3B**). Furthermore, compared to the NC groups, silence of hsa_circ_001569 by siRNAs notably inhibited the cellular growth of QGY-7703 cells (P < 0.001) (**Figure 3C**). Similarly, silence of hsa_circ_001569 significantly suppressed the proliferation ability of SMMC-7721 cells (P < 0.001) (**Figure 3D**). Consistently, the colony formation experiment results revealed that silence of hsa_circ_001569 significantly inhibited the proliferation of both QGY-7703 and SMMC-7721 cells (P < 0.05) (**Figure 3E**).

Silence of hsa_circ_001569 inhibits the growth of HCC tumor in vivo

We further detected the effects of hsa_circ_001569 inhibition on tumor growth *in vivo*. Nude mice were subcutaneously injected with 3 × 10^6 QGY-7703 and SMMC-7721 cells which were transfected with hsa_circ_001569 siRNAs and NC respectively. Then we measured the tumor volume at 0, 7, 14, 21, 28, and 35 days. The results indicated that the group with QGY-7703 cells transfected with hsa_circ_001569 siRNAs formed substantially smaller tumors than the NC group (**Figure 4A**). Likewise, the group with SMMC-7721 cells transfected with hsa_circ_001569 siRNAs formed substantially smaller tumors than the NC group (P < 0.05) (**Figure 4B**). In addition, the tumor weight in nude mice was also detected. We found that silence of hsa_circ_001569 inhibited the weight of HCC tumor (P < 0.05) (**Figure 4C**). In conclusion, we demonstrated that silence of hsa_circ_001569 inhibits the growth of HCC tumor *in vivo.*

Discussion

Noncoding RNAs (ncRNAs) are s kind of RNAs that are not encoded protein and mainly include microRNA (miRNA), long noncoding RNA (lncRNA), circRNAs, ribosomal RNA, transfer RNA, and small nucleolar RNA [24]. They regulate the process of gene expression such as transcription, mRNA stability, and translation [25, 26]. Studies found that ncRNAs have the great significance in tumorigenesis [27, 28]. Among them, miRNA, lncRNA and circRNA have important functions in the regulation of gene expression [29].

CircRNAs were ever discovered in RNA viruses as early as the 1970s [30, 31]. The phenomenon of RNA presenting a circular format was first reported by Coca-Prados and Hsu in eukaryotic cytoplasm [32]. CircRNAs are characterized by the presence of a covalent bond linking the 3' and 5' ends [33]. CircRNAs may

be generated from exons or introns through gene rearrangement or non-linear reverse splicing [34, 35]. For the past few years, as RNA sequencing and large scale application technology developing, more and more circRNAs are screened out gradually. An increasing number of studies have found that circRNAs act as gene regulators and participate in the biological process including tumorigenesis and tumor progression [17, 36]. A large number of studies at home and abroad have also found that circRNAs could act as miRNA sponge and regulate miRNAs expression. For example, ciRS-7 was found to specifically bind miR-7, and inhibit its biological function of regulating epidermal growth factor receptor (EGFR) signaling [18, 37]. Sex-determining region Y (SRY) regulates miR-138 expression and suppresses its biological effects such as enhancement of the proliferation, migration and invasion [37].

Cancer is a complicated biological processes, and there is no exception in HCC [38]. Various genes are involved in the occurrence and development of HCC. For example, the lncRNAs including UCA1 and GAS5 [39], microRNAs such as microRNA-1 and microRNA-122 [40], and circRANs including ciRS-7 [41] and hsa_circ_0001649 are associated with HCC [42]. Study indicated that hsa_circ_001569 promotes proliferation and invasion of CRC cells. Simultaneously, hsa_circ_001569 serves as a miRNA sponge to directly inhibit miR-145, and then increases the expression levels of miR-145 targets E2F5, BAG4 and FMNL2 in CRC cells [23]. As we all known, circRNAs are involved in several types of diseases, we focused on hsa_circ_001569 in our study (ID: hsa_circ_0000677 in CircBase: http://circbase.org/cgi-bin/simplesearch.cgi). Its gene is located at chr16:16101672-16162159, its Best transcript is NM_019899, and its associated-gene symbol is ABCC1. In this study, we proved the expression level of hsa_circ_001569 in HCC and the correlation with clinical characteristics. We then detected the potential roles of hsa_circ_001569 by siRNA mediated silencing in HCC.

In summary, our results found that hsa_circ_001569 is upregulated in HCC tumor tissues, and its expression is associated with tumor differentiation and TNM stages. Silence of hsa_circ_001569 inhibits the proliferation ability of HCC cells *in vitro*. Meanwhile, silence of hsa_circ_001569 suppresses the growth of HCC tumor *in vivo*. Therefore, we suggest that hsa_circ_001569 may be a potential target for HCC therapy. And further study will be performed to indicate hsa_circ_001569 and its downstream in HCC.

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Disclosure of conflict of interest

None.

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References

[1] Baffy G. Hepatocellular carcinoma in non-alcoholic fatty liver disease: epidemiology, pathogenesis, and prevention. J Clin Transl Hepatol 2013; 1: 131-137.

[2] Li Z, Zhang C, Lou C, Yan F, Mao Y, Hong X and Zhang Y. Comparison of percutaneous cryosurgery and surgical resection for the treatment of small hepatocellular carcinoma. Oncol Lett 2013; 6: 239-245.

[3] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[4] Asia-Pacific Working Party on Prevention of Hepatocellular Carcinoma. Prevention of hepatocellular carcinoma in the Asia-Pacific region: consensus statements. J Gastroenterol Hepatol 2010; 25: 657-663.

[5] Salzman J, Gawad C, Wang PL, Lacayo N and Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. PLoS One 2012; 7: 231-241.

[6] Hentze MW and Preiss T. Circular RNAs: splicing's enigma variations. EMBO J 2013; 32: 923-925.

[7] Chen I, Chen CY and Chuang TJ. Biogenesis, identification, and function of exonic circular RNAs. Wiley Interdiscip Rev RNA 2015; 6: 563-579.

[8] Sanger HL, Klotz G, Riesner D, Gross HJ and Kleinschmidt AK. Viroids are single-stranded covalently closed circular RNA molecules exist-

ing as highly base-paired rod-like structures. Proc Natl Acad Sci U S A 1976; 73: 3852-3856.
[9] Kos A, Dijkema R, Arnberg AC, van der Meide PH and Schellekens H. The hepatitis delta (delta) virus possesses a circular RNA. Nature 1986; 323: 558-560.
[10] Cocquerelle C, Mascrez B, Hetuin D and Bailleul B. Mis-splicing yields circular RNA molecules. FASEB J 1993; 7: 155-160.
[11] Guttman M and Rinn JL. Modular regulatory principles of large non-coding RNAs. Nature 2012; 482: 339-346.
[12] Batista PJ and Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell 2013; 152: 1298-1307.
[13] Sabin LR, Delás MJ and Hannon GJ. Dogma derailed: the many influences of RNA on the genome. Molecular Cell 2013; 49: 783-794.
[14] Ulitsky I and Bartel DP. lincRNAs: genomics, evolution, and mechanisms. Cell 2013; 154: 26-46.
[15] Conn SJ, Pillman KA, Toubia J, Conn VM, Salmanidis M, Phillips CA, Roslan S, Schreiber AW, Gregory PA and Goodall GJ. The RNA Binding protein quaking regulates formation of circRNAs. Cell 2015; 160: 1125-1134.
[16] Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, Zhu S, Yang L and Chen LL. Circular intronic long noncoding RNAs. Mol Cell 2013; 51: 792-806.
[17] Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F and Rajewsky N. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 2013; 495: 333-338.
[18] Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK and Kjems J. Natural RNA circles function as efficient microRNA sponges. Nature 2013; 495: 384-388.
[19] Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 2013; 495: 333-338.
[20] Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF and Sharpless NE. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA 2013; 19: 141-157.
[21] Salzman J, Chen RE, Olsen MN, Wang PL and Brown PO. Cell-type specific features of circular RNA expression. PLoS Genet 2013; 9: e1003777.
[22] Danan M, Schwartz S, Edelheit S and Sorek R. Transcriptome-wide discovery of circular RNAs in Archaea. Nucleic Acids Res 2012; 40: 3131-3142.
[23] Xie HJ, Ren XL, Xin SN, Lan XL, Lu GF, Lin Y, Yang SS, Zeng ZC, Liao WT, Ding YQ and Liang L. Emerging roles of circRNA_001569 targeting miR-145 in the proliferation and invasion of colorectal cancer. Oncotarget 2016; 7: 26680-26691.
[24] Erhard F and Zimmer R. Classification of ncRNAs using position and size information in deep sequencing data. Bioinformatics 2010; 26: i426-i432.
[25] Qu ZP and Adelson DL. Identification and comparative analysis of ncRNAs in human, mouse and zebrafish indicate a conserved role in regulation of genes expressed in brain. PLoS One 2012; 7: e34121.
[26] Qu ZP and Adelson DL. Bovine ncRNAs are abundant, primarily intergenic, conserved and associated with regulatory genes. PLoS One 2012; 7: e35166.
[27] Zhao ZJ, Li S, Song EW and Liu SL. The roles of ncRNAs and histone-modifiers in regulating breast cancer stem cells. Protein Cell 2016; 7: 89-99.
[28] Irizar H, Munoz-Culla M, Saenz-Cuesta M, Osorio-Querejeta I, Sepulveda L, Castillo-Trivino T, Prada A, de Munain AL, Olascoaga J and Otaegui D. Identification of ncRNAs as potential therapeutic targets in multiple sclerosis through differential ncRNA-mRNA network analysis. BMC Genomics 2015; 16: 250-258.
[29] Hayes EL and Lewis-Wambi JS. Mechanisms of endocrine resistance in breast cancer: an overview of the proposed roles of noncoding RNA. Breast Cancer Res 2015; 17: 40.
[30] Wu Q, Wang Y, Cao M, Pantaleo V, Burgyan J, Li WX and Ding SW. Homology-independent discovery of replicating pathogenic circular RNAs by deep sequencing and a new computational algorithm. Proc Natl Acad Sci U S A 2012; 109: 3938-3943.
[31] Wilusz JE and Sharp PA. Molecular biology. A circuitous route to noncoding RNA. Science 2013; 340: 440-1.
[32] Hsu MT and Coca-Prados M. Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells. Nature 1979; 280: 339-340.
[33] Ebbesen KK, Kjems J and Hansen TB. Circular RNAs: identification, biogenesis and function. Biochim Biophys Acta 2016; 1859: 163-168.
[34] Salzman J, Gawad C, Wang PL, Lacayo N and Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. PLoS One 2012; 7: e30733.
[35] Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, Zhu S, Yang L and Chen LL. Circular

intronic long noncoding RNAs. Mol Cell 2013; 51: 792-806.
[36] Li J, Yang J, Zhou P, Le Y, Zhou C, Wang S, Xu D, Lin HK and Gong Z. Circular RNAs in cancer: novel insights into origins, properties, functions and implications. Am J Cancer Res 2015; 5: 472-480.
[37] Zhao ZJ and Shen J. Circular RNA participates in the carcinogenesis and the malignant behavior of cancer. RNA Biol 2015; 1-8.
[38] Hashimoto K, Mori N, Tamesa T, Okada T, Kawauchi S, Oga A, Furuya T, Tangoku A, Oka M and Sasaki K. Analysis of DNA copy number aberrations in hepatitis C virus-associated hepatocellular carcinomas by conventional CGH and array CGH. Mod Pathol 2004; 17: 617-622.
[39] Tu ZQ, Li RJ, Mei JZ and Li XH. Down-regulation of long non-coding RNA GAS5 is associated with the prognosis of hepatocellular carcinoma. Int J Clin Exp Pathol 2014; 7: 4303-4309.
[40] Koberle V, Kronenberger B, Pleli T, Trojan J, Imelmann E, Peveling-Oberhag J, Welker MW, Elhendawy M, Zeuzem S, Piiper A and Waidmann O. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. Eur J Cancer 2013; 49: 3442-3449.
[41] Xu L, Zhang M, Zheng X, Yi P, Lan C and Xu M. The circular RNA ciRS-7 (Cdr1as) acts as a risk factor of hepatic microvascular invasion in hepatocellular carcinoma. J Cancer Res Clin Oncol 2017; 143: 17-27.
[42] Qin M, Liu G, Huo X, Tao X, Sun X, Ge Z, Yang J, Fan J, Liu L and Qin W. Hsa_circ_0001649: a circular RNA and potential novel biomarker for hepatocellular carcinoma. Cancer Biomark 2016; 16: 161-169.