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ORIGINAL RESEARCH

CircCSNK1G1 Contributes to the Tumorigenesis of Gastric Cancer by Sponging miR-758 and Regulating ZNF217 Expression

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Background: Increasing evidence indicates that circular RNAs (circRNAs) act as vital regulators in various cancers. Nevertheless, the effect of circCSNK1G1 on gastric cancer (GC) is still unknown.

Methods: The mRNA levels of circCSNK1G1, miR-758, and ZNF217 were measured by RT-qPCR. The protein levels of ZNF217 were evaluated by Western blotting. Cell migration, invasion, proliferation, and apoptosis were detected by Transwell, CCK-8, and flow cytometry assays. The association between miR-758 and circCSNK1G1/ZNF217 was confirmed by RIP and luciferase reporter assays. Xenograft assay was employed for in vivo experiment.

Results: In the current study, it was demonstrated that the expression levels of circCSNK1G1 and ZNF217 were upregulated in GC tissues and cells, while the level of miR-758 was declined. Furthermore, functional assays indicated that circCSNK1G1 depletion suppressed GC progression in vitro and in vivo. In addition, circCSNK1G1 directly interacted with miR-758, and the supplementation of miR-758 suppressed the development of GC, which was abolished following pcDNA3.1-circCSNK1G1 transfection. Then, we explored the downstream mechanism of miR-758 and found that miR-758 could target the 3′ UTR of ZNF217 mRNA. The overexpression of miR-758 neutralized the ZNF217-mediated effects on facilitating the progression of GC. Finally, we revealed that circCSNK1G1 could upregulate ZNF217 expression by sponging miR-758 in GC cells.

Conclusion: Our study revealed that circCSNK1G1 accelerated GC progression via the miR-758/ZNF217 axis, suggesting that circCSNK1G1 might be a potential biomarker for GC diagnosis and treatment.

Keywords: gastric cancer, GC, circCSNK1G1, miR-758, ZNF217

Introduction

Gastric cancer (GC) is the third most frequent malignancy in the world[1] and nearly a million new cases of GC are reported annually, with two-thirds of all cases in developing countries.[2,3] Despite great progress has been achieved in the diagnosis and therapy of GC, the five-year survival rate remains unsatisfactory owing to the high metastasis and recurrence of GC.[4,5] Therefore, the identification of novel possible biomarkers for GC diagnosis and treatment is critical.

Circular RNAs (circRNAs) are a category of RNA with a covalent closed loop structure.[6–8] Increasing research has indicated that circRNAs are involved in the development of various cancers through the regulation of cellular processes, such as cell growth, migration, apoptosis, and cell cycle.[9] Liu et al disclosed that the

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upregulation of circRNA-5692 attenuated hepatocellular carcinoma cell proliferation, migration, and invasion in vitro and inhibited tumor growth in vivo.[10] Zeng et al revealed that c-Myb-induced circHIPK3 facilitated cell growth and metastasis by regulating miR-7 expression in colorectal cancer.[11] Moreover, many circRNAs have been identified as important regulators in GC. To cite an instance, Yu et al identified down-regulated expression and vital diagnostic value of circ_0067582 in GC.[12] A report from Sun et al uncovered that circMAN2B2 facilitated GC cell proliferation and metastasis by acting as a sponge for miR-145.[13] It was also revealed that circ_0023642 contributed to GC development via interaction with miRNA-223.[14] Ding et al reported that circCSNK1G1 was one of the markedly upregulated circRNAs in GC.[15] However, the biological role of circCSNK1G1 in GC is still unclear.

MicroRNAs (miRNAs), another type of ncRNAs, frequently participate in the regulation of the tumorigenesis of multiple human cancers, including GC.[16] For example, the supplementation of miR-129 suppressed the tumorigenesis of GC by targeting COL1A1.[17] MiR-4317 impeded GC cell proliferation by inhibiting ZNF322.[18] miR-197 repressed GC cell viability and invasion through mediating MTDH.[19] The dysregulation of miR-758 has been reported to participate in the development of various human cancers, including bladder,[20] ovarian,[21] and cervical cancers.[22] Moreover, Guo et al identified that miR-758 played an antitumor role in the GC progression.[23] Nonetheless, the exact function and molecular mechanism of miR-758 in GC need to be further explained.

In this research, we uncovered a new mechanism that circCSNK1G1 modulated the miR-758/ZNF217 axis to facilitate GC progression. Our findings suggest that circCSNK1G1 may be a novel biomarker for GC diagnosis and treatment.

Materials and Methods

Clinical Samples

From the First People's Hospital of Huzhou, 48 GC tissues and 48 corresponding normal tissues were collected. Besides, serum samples were also collected from 48 healthy volunteers as a healthy control grouThis study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the First People's Hospital of Huzhou, and each participant provided the informed consent. None of the patients received preoperative treatment or had been diagnosed with other cancers. The specimens were quickly frozen in liquid nitrogen and stored at −80°C for further study.

Cell Line Culture

Human GC cell lines (AGS, HGC-27, and MKN45) and normal gastric epithelial cell line (GES-1) were purchased from ATCC. Cells were incubated in RPMI 1640 (HyClone, UT, USA) containing 10% FBS (Gibco, Rockville, MD, USA) maintained in a 37°C, 5% CO_2 incubator.

Cell Transfection

siRNA targeting circCSNK1G1 (si-circCSNK1G1#1: 5′-GGUACCGACGACUAGUCUUGU-3′; si-circCSNK1G1#2: 5′- UGGCCCUCGUAACCCAGCUGAU-3′) with its control group (si-NC), miR-758 mimics (5′-UUUGUGACCUGGUCCACUAACC-3′) with its control (NC mimics, 5′-ACAUCUGCGUAAGAUUCGAGUCUA-3′), miR-758 inhibitor (5′-GGUUAGUGGACCAGGUCACAAA-3′) with its control (NC inhibitor, 5′-GCGUAACUAAUACAUCGGAUUCGU-3′) were obtained from GenePharma (Shanghai, China). circCSNK1G1 or ZNF217 overexpression plasmid (pcDNA3.1-circCSNK1G1 or pcDNA3.1-ZNF217) was established by inserting the full-length circCSNK1G1 or ZNF217 sequence into pcDNA3.1 vector. Lipofectamine 2000 (Invitrogen, CA, USA) was performed to transfect these oligonucleotides or vectors into GC cells.

RT-qPCR

Trizol reagent (Invitrogen) was used for the extraction of RNA. cDNA was then synthesized using PrimeScript RT Reagent kit (Takara, Otsu, Japan) qPCR was performed using the SYBRGreen PCR kit (Takara) and a 7500 Fast PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). GAPDH or U6 was used as reference control, and the relative gene expression was calculated by the $2^{-\Delta\Delta Cq}$ method. The primer sequences used were as follows: circCSNK1G1 forward, 5′-GCCATCACAACAGCAGCCT-3′ and reverse, 5′-AGGTCAAACAAGTCCTCCAAG-3′; miR-758 forward, 5′-ACACTCCAGCTGGGTTTGTGACCTGGTCCA-3′ and reverse, 5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGGTTAGTG-3′; ZNF217 forward, 5′-GAGAAGCGAATGGTGAAAGC-3′, and reverse, 5′-CAGCGCTCAAGTATGCAAAA-3′; GAPDH forward, 5′-GCACCGTCAAGGCTGAGAAC-3′ and

reverse, 5′-TGGTGAAGACGCCAGTGGA-3′; and U6 forward, 5′-CTCGCTTCGGCAGCACA-3′ and reverse, 5′-AACGCTTCACGAATTTGCGT-3′

Transwell Assay

The abilities of invasion and migration of GC cells were detected using transwell chambers (Corning, NY, USA). For detecting invasion ability, the transwell chambers were coated with Matrigel (BD Biosciences). The transfected cells ($1x10^5$ cells) were suspended in RPMI 1640 without serum and added to the upper chambers. Then, the lower chamber was filled with 600μL RPMI 1640 supplemented with 10% FBS. After 24 h, the invaded cells were stained with 0.5% crystal violet. The procedure for cell migration was the same as for cell invasion, except that the transwell chambers were not coated with Matrigel.

CCK-8 Assay

Transfected cells were seeded onto 96-well plates and then incubated for 0, 24, 48, and 72 h. The CCK-8 reagent was added to each well and then incubated for 4 h. The absorbance value of each well was detected at 490 nm.

Xenograft Assay in Nude Mice

5-week-old BALB/c nude mice bought from Shanghai Laboratory Animal Center (Shanghai, China) were used for the experiment. 1×10^7 AGS transfected with si-circCSNK1G1#1 or si-NC were subcutaneously injected into mice. Every 7 days, the tumor volume was monitored. 28 days after injection, mice were sacrificed and tumors were weighted. The animal experiment was performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Ethics Committee of the First People's Hospital of Huzhou.

Immunohistochemistry (IHC)

Tumor tissues collected from mice were processed by paraformaldehyde (4%) for fixation, and then the fixed tissues were dehydrated and embedded in paraffin. Subsequently, sections (4-mm-thick) were obtained via intersecting the paraffin-embedded tissues. Then, sections were deparaffinized and incubated at 4°C overnight with Ki67 antibody (Abcam). After incubation with a secondary antibody, sections were stained by hematoxylin, and photographed under a microscope.

RIP

RIP assay was applied using EZ-Magna RiP Kit (Millipore). Cells were lysed and incubated by anti-Ago2 antibody, with magnetic beads (Millipore, USA) or control antibody (Millipore, USA). The RT-qPCR was conducted after the immune precipitated RNA was purified.

Flow Cytometry

The transfected cells were digested with 0.25% trypsin, and then suspended in 100 μL binding buffer to produce 1×10^6 cells/mL suspension. Subsequently, the suspension was added with 10 μL AnnexinV-FITC and 10 μL PI for 10 min in darkness, and the apoptosis of cells was analyzed using FACScan flow cytometer (BD Biosciences).

Luciferase Reporter Assay

Wild-type circCSNK1G1 (circCSNK1G1-WT), mutant-type (circCSNK1G1-Mut), wild-type ZNF217 (ZNF217-WT), and mutant-type ZNF217 (ZNF217-Mut) were constructed by GenePharma. The reporter plasmids were co-transfection with miR-758 mimics or NC mimics via Lipofectamine 2000. The relative luciferase activity was detected by a dual-luciferase reporter assay system (Promega Corporation).

Western Blotting

Total protein was isolated from GC cells by using RIPA lysis buffer. The protein was separated with 10% SDS-PAGE and then moved to the PVDF membrane. After being blocked with 5% skimmed milk, the membrane was mixed with primary antibodies (anti-ZNF217: ab136678 and anti-GAPDH: ab181602; Abcam, USA) at 4°C all night. Then, the membrane was cultured with horseradish peroxidase-labeled secondary antibodies. Subsequently, the bands were evaluated with the enhanced chemiluminescence (ECL) Kit (Pierce, Thermo Fisher Scientific, IL, USA).

Statistical Analysis

The experiments were all conducted 3 times. Data were presented as mean ± standard deviation. SPSS 17.0 was performed for statistical analysis. Student's *t*-test and ANOVA were utilized to measure the differences. Kaplan-Meier method was used to calculate the overall survival curve. $P< 0.05$ was considered as statistical significance.

Results

CircCSNK1G1 Was Upregulated in GC and Associated with the Poor Prognosis of GC Patients

Firstly, the levels of circCSNK1G1 in GC tissues and adjacent normal tissues were detected by RT-qPCR and the data showed that circCSNK1G1 was considerably upregulated in GC tissues (Figure 1A). In the clinical sample, Kaplan–Meier survival analysis showed that the GC patients with high expression of circCSNK1G1 exhibited a lower survival rate than those with low expression of circCSNK1G1 (Figure 1B). Besides, the upregulation of circCSNK1G1 was related to tumor size, histological grades and TNM stage, but not with patients' ages, gender or lymph node metastasis (Table 1). In addition, we also assessed the diagnostic value of serum circCSNK1G1, and the results showed that area under curve (AUC) was 0.8840, implying that circCSNK1G1 might be used as an indicator for GC diagnosis (Figure 1C). Moreover, we further observed that GC cell lines (AGS, HGC-27, and MKN45) established higher circCSNK1G1 expression than GES-1 (Figure 1D). Hence, we speculated that circCSNK1G1 might be an oncogene of GC.

Table 1 The Relationship Between circCSNK1G1 Expression Level and the Clinicopathologic Features of GC

Parameters	Number	circCSNK1G1 Expression		P-value
		Low (<Median)	High (≥Median)	
Age (years)				
< 65	30	16	14	0.561
≥ 65	18	8	10	
Gender				
Male	26	12	14	0.75
Female	22	12	10	
Tumor size				
< 3cm	21	15	6	0.002
≥ 3cm	27	9	18	
Histological grade				
Well	26	17	9	0.004
Moderately and poorly	22	7	15	
TNM stage				
I + II	28	18	10	0.003
III + IV	20	6	14	
Lymph node metastasis				
Absent	36	20	16	0.158
Present	12	4	8	

Interference of circCSNK1G1 Inhibited the Progression of GC in vitro

To determine the function of circCSNK1G1 in GC, AGS and HGC-27 cells were transfected with si-circCSNK1G1#1, si-circCSNK1G1#2, or si-NC, and a remarkable downregulation of circCSNK1G1 level in cells transfected with si-circCSNK1G1#1 and si-circCSNK1G1#2 was detected by RT-qPCR (Figure 2A). CCK-8 assay revealed that circCSNK1G1 depletion restrained the proliferation of GC cells (Figure 2B). Furthermore, transwell assays indicated that the interference of circCSNK1G1 attenuated the migration and invasion of GC cells (Figure 2C and D). In addition, flow cytometry assay indicated that circCSNK1G1 knockdown promoted the cell apoptosis of GC (Figure 2E). These results demonstrated that the knockdown of circCSNK1G1 could inhibit GC development.

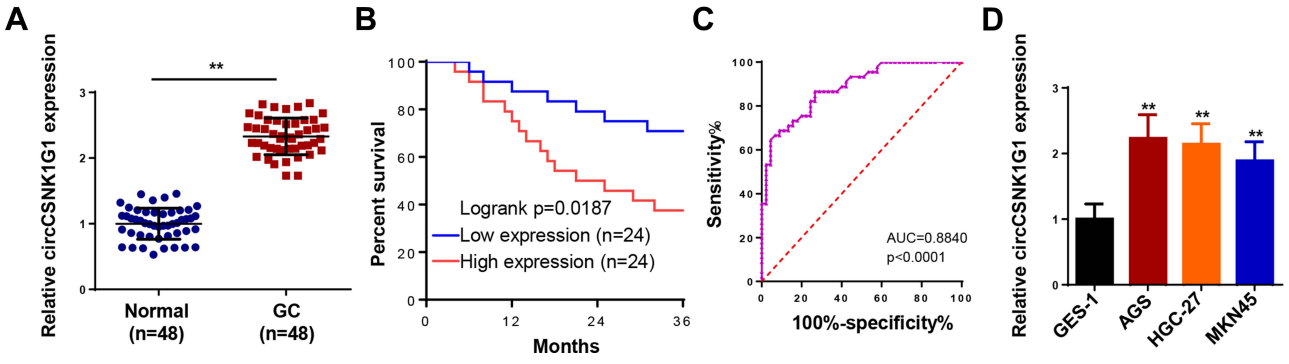

Figure 1 CircCSNK1G1 was upregulated in GC and associated the prognosis of GC patients. (**A**) The level of circCSNK1G1 in GC tissues and paired normal tissues was measured using RT-qPCR. (**B**) Kaplan-Meier analysis reveals the association of high crcCSNK1G1 expression with poor overall survival in patients with GC. (**C**) ROC curve analysis was used to assess the diagnostic values of the circCSNK1G1. (**D**) The expression of circCSNK1G1 in GC cell lines and normal gastric epithelial cell line was detected using RT-qPCR. **P < 0.01.

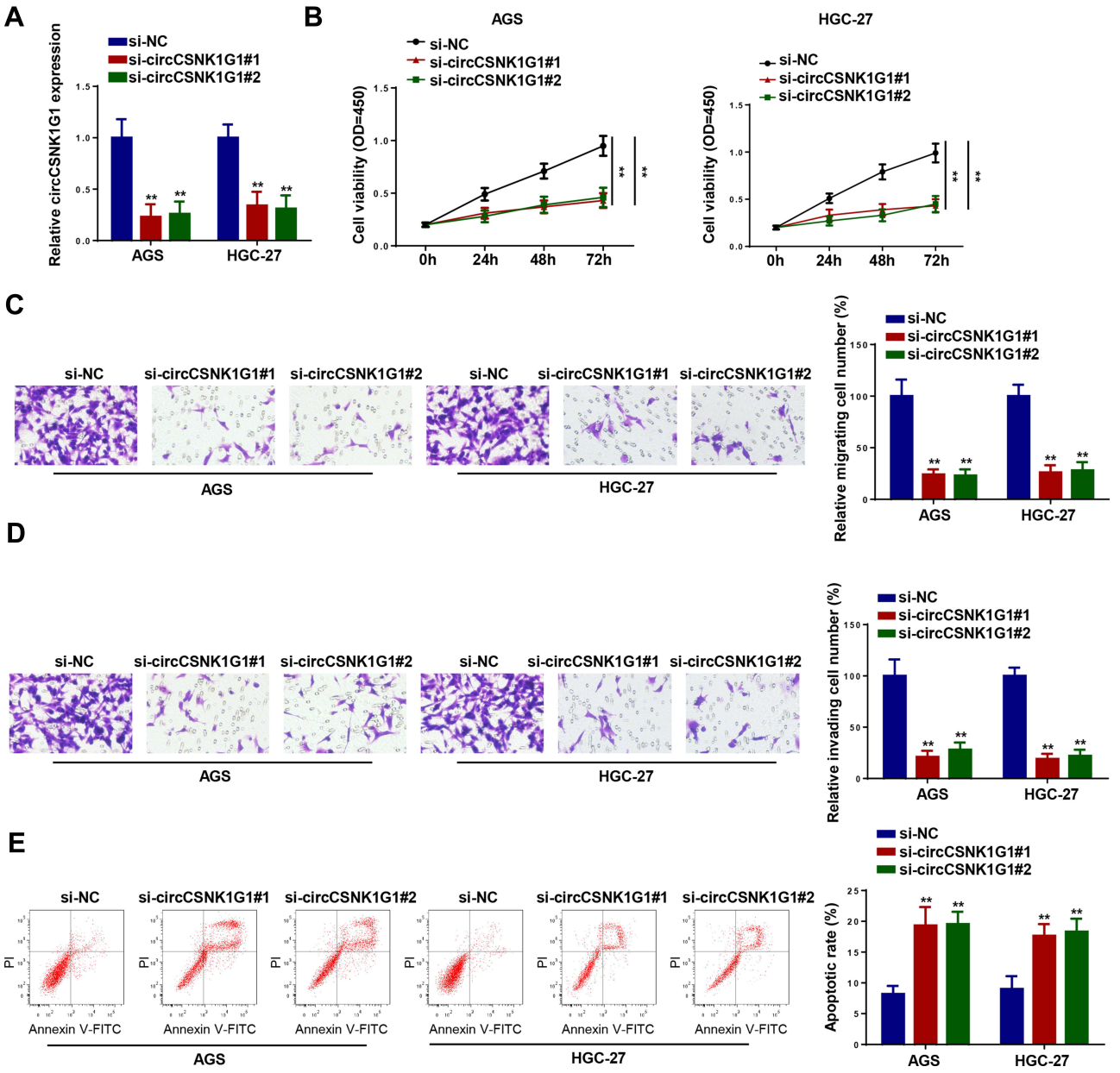

Figure 2 Interference of CircCSNK1G1 inhibited the progression of GC. (**A**) The expression of circCSNK1G1 was detected by RT-qPCR in AGS and HGC-27 cells transfected with si-circCSNK1G1#1, si-circCSNK1G1#2, or si-NC. (**B**) The proliferation of AGS and HGC-27 cells was measured by CCK-8 assay. (**C** and **D**) Transwell was used to detect the migration and invasion of AGS and HGC-27 cells transfected with si-circCSNK1G1#1, si-circCSNK1G1#2, or si-NC. (**E**) Flow cytometry assay showed the apoptosis of AGS and HGC-27 cells transfected with si-circCSNK1G1#1, si-circCSNK1G1#2, and si-NC. **P < 0.01.

CircCSNK1G1 Knockdown Represses GC Tumorigenesis in vivo

To further elaborate the biological role of circCSNK1G1 in GC in vivo, AGS cells transfected with si-NC or si-circCSNK1G1#1 were subcutaneously injected into nude mice. As presented in Figure 3A and B, circCSNK1 depletion significantly restrained tumor growth in mice. Moreover, IHC staining showed that circCSNK1 knockdown decreased Ki67 expression with comparison of the control group (Figure 3C). Altogether, these results implied that circCSNK1G1 knockdown suppressed GC tumorigenesis in vivo.

CircCSNK1G1 Was a Sponge of miR-758

The downstream target genes were screened via the StarBase website to further explore the molecular mechanism of circCSNK1G1 in GC, and the results indicated that miR-758 might be a candidate downstream target of

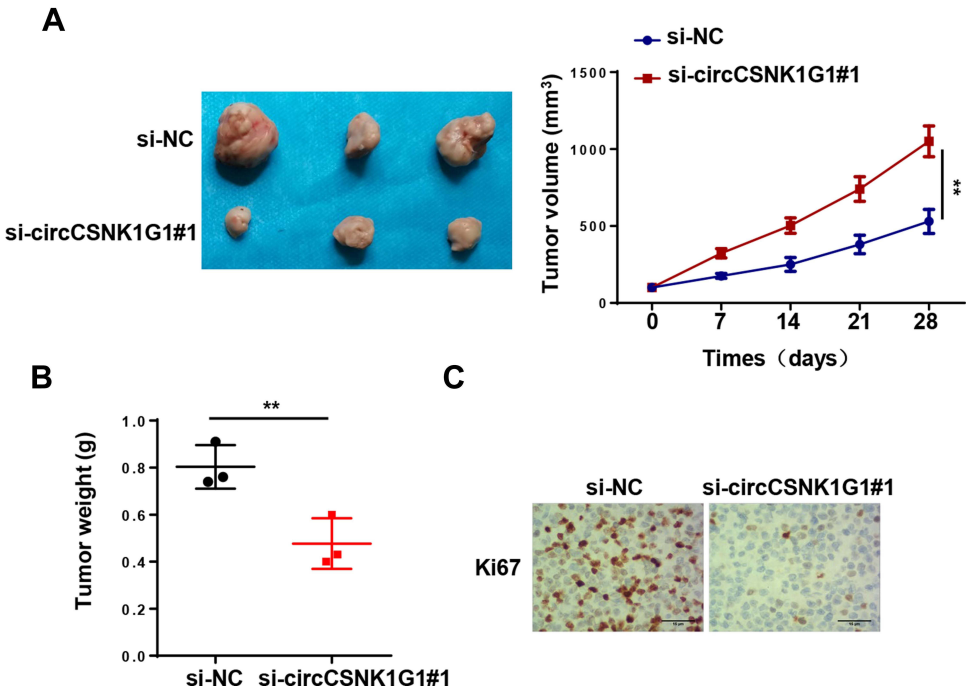

Figure 3 CircCSNK1G1 knockdown represses GC tumorigenesis in vivo. (**A**) AGS cells stably transfected with si-circCSNK1G1#1 or si-NC were subcutaneously injected into nude mice. Tumor growth curves indicated that circCSNK1G1 knockdown led to suppression on GC growth in mice. (**B**) The subcutaneous tumors were harvested and weighted. (**C**) IHC staining showed that Ki67 expression in AGS cells transfected with si-circCSNK1G1 and si-NC. **P < 0.01.

circCSNK1G1 (Figure 4A). Moreover, it was found that miR-758 mimics transfection remarkably diminished the luciferase activity of circCSNK1G1-WT reporter, but not the activity of circCSNK1G1-Mut in AGS and HGC-27 cells (Figure 4B). RIP assay showed that circCSNK1G1 and miR-758 were significantly enriched in the Ago2 group compared with the IgG group (Figure 4C). Furthermore, miR-758 expression was distinctly highly expressed in normal tissues and cells compared to GC tissues and cells (Figure 4D and E). Besides that, the deletion of circCSNK1G1 upregulated miR-758 expression (Figure 4F). These data showed that circCSNK1G1 could directly interact with miR-758.

CircCSNK1G1 Overexpression Counteracted miR-758-Mediated Effects on the Progression of GC

In order to deeply explore the interaction between circCSNK1G1 and miR-758 in GC, the level of miR-758 was assessed in AGS and HGC-27 cells transfected with NC mimics, miR-758 mimics, miR-758 mimics+pcDNA3.1, and miR-758 mimics+pcDNA3.1-circCSNK1G1. RT-qPCR assay indicated that the addition of circCSNK1G1 counteracted the promotive effects of miR-758 mimics on miR-758 expression (Figure 5A). Moreover, the supplementation of miR-758 hindered the progression of GC, which was neutralized following pcDNA3.1-circCSNK1G1 transfection (Figure 5B–E). The above data indicated that the opposite functions of circCSNK1G1 and miR-758 in GC progression.

ZNF217 Was Directly Targeted by miR-758

According to the prediction of TargetScan, ZNF217 might be a target of miR-758 (Figure 6A). Though GEPIA database, ZNF217 expression was higher expressed in stomach adenocarcinoma (STAD) tissues than that in normal tissues (Figure 6B). To validate the binding ability between miR-758 and ZNF217, luciferase reporter and RIP assays were conducted. The results revealed that miR-758 mimics resulted in an obvious reduction in relative luciferase activity of ZNF217-WT but not in ZNF217-Mut (Figure 6C). Meanwhile, miR-758 and ZNF217 were both enriched in the Ago2 group (Figure 6D). Moreover, RT-qPCR and Western blotting revealed that the upregulation of miR-758 decreased ZNF217 expression, and the downregulation of miR-758 increased ZNF217 expression (Figure 6E and F). After that, an increased ZNF217 expression was observed in GC tissues and cells (Figure 6G and H). To summarize, miR-758 negatively regulated ZNF217 level by directly targeting 3′-UTR of ZNF217 mRNA.

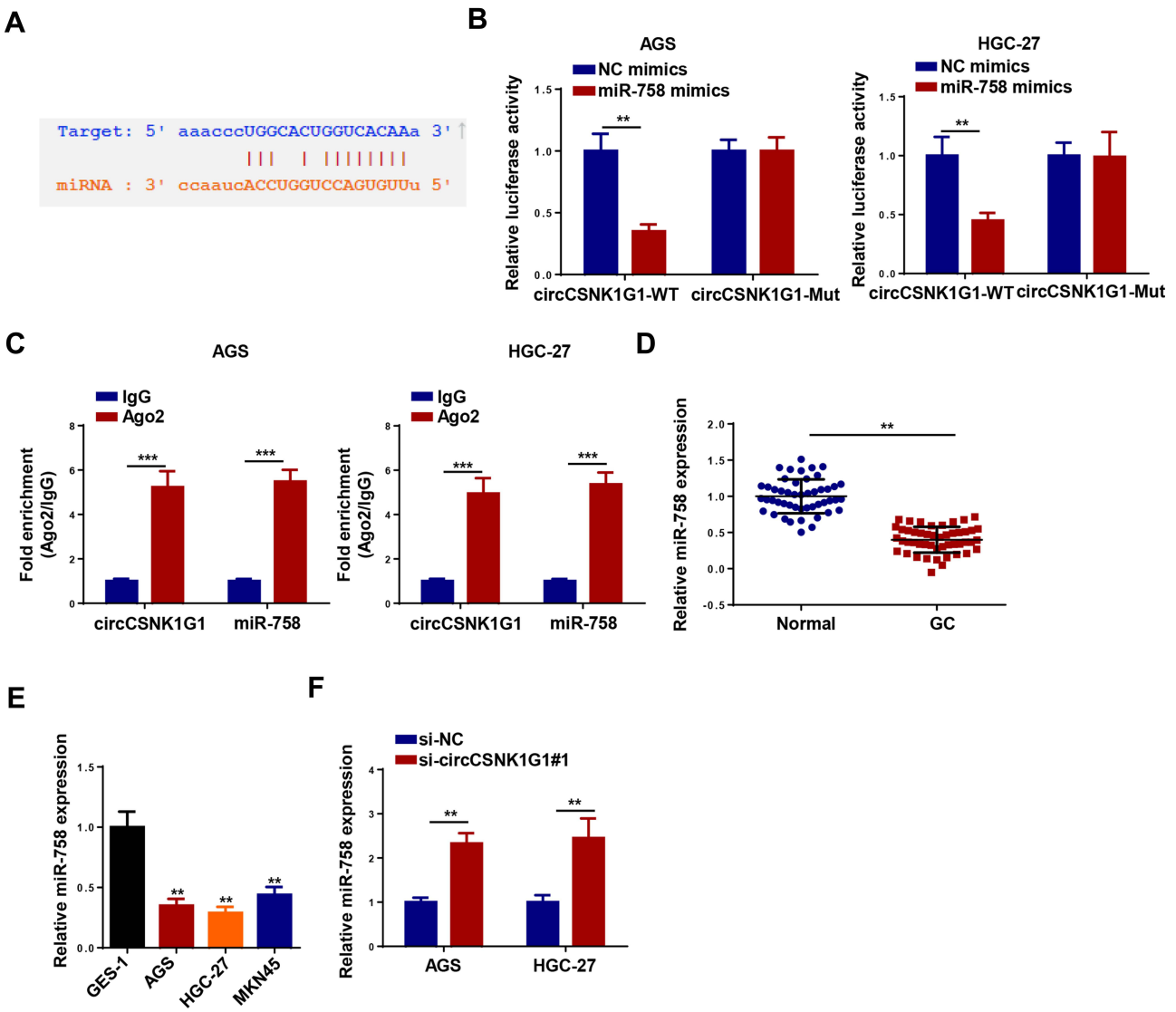

Figure 4 CircCSNK1G1 was a sponge of miR-758. (**A**) Putative binding regions of circCSNK1G1 in miR-758 predicted with biological software. (**B**) Luciferase activity of wild-type or mutant circCSNK1G1 in AGS and HGC-27 cells following NC mimics, or miR-758 mimics transfection determined by luciferase reporter assay. (**C**) Correlations between circCSNK1G1 and miR-758 detected by RIP assay. (**D**) The level of miR-758 in GC tissues and paired normal tissues was measured using RT-qPCR. (**E**) miR-758 in GC cell lines and normal gastric epithelial cell line was detected using RT-qPCR. (**F**) The expression of miR-758 was detected by RT-qPCR in AGS and HGC-27 cells transfected with si-circCSNK1G1 and si-NC. **P < 0.01 and ***P < 0.001.

CircCSNK1G1 Regulated ZNF217 by Sponging miR-758 in GC Cells

Subsequently, we examined whether ZNF217 was a downstream regulator of circCSNK1G1/miR-758 axis. RT-qPCR and Western blotting indicated that ZNF217 was notably downregulated by miR-758 mimics transfection, which could be abolished by pcDNA3.1-ZNF217 in AGS cells (Figure 7A and B). Moreover, the effect of miR-758 mimics on proliferation, migration, invasion, and apoptosis of AGS cells was abolished by upregulating ZNF217 expression (Figure 7C–F). Furthermore, we investigated whether circCSNK1G1 could regulate ZNF217 expression via miR-758, and the results indicated that interference of circCSNK1G1 downregulated the expression of ZNF217, which was abolished following miR-758 inhibitor transfection (Figure 7G and H). To sum up, these results demonstrated that circCSNK1G1 regulated GC progression via miR-758/ZNF217 axis.

Discussion

With the continuous development of next-generation sequencing technology, increasing circRNAs have been identified, as well as their function in human cancers, including GC. For instance, circ-HuR was downregulated

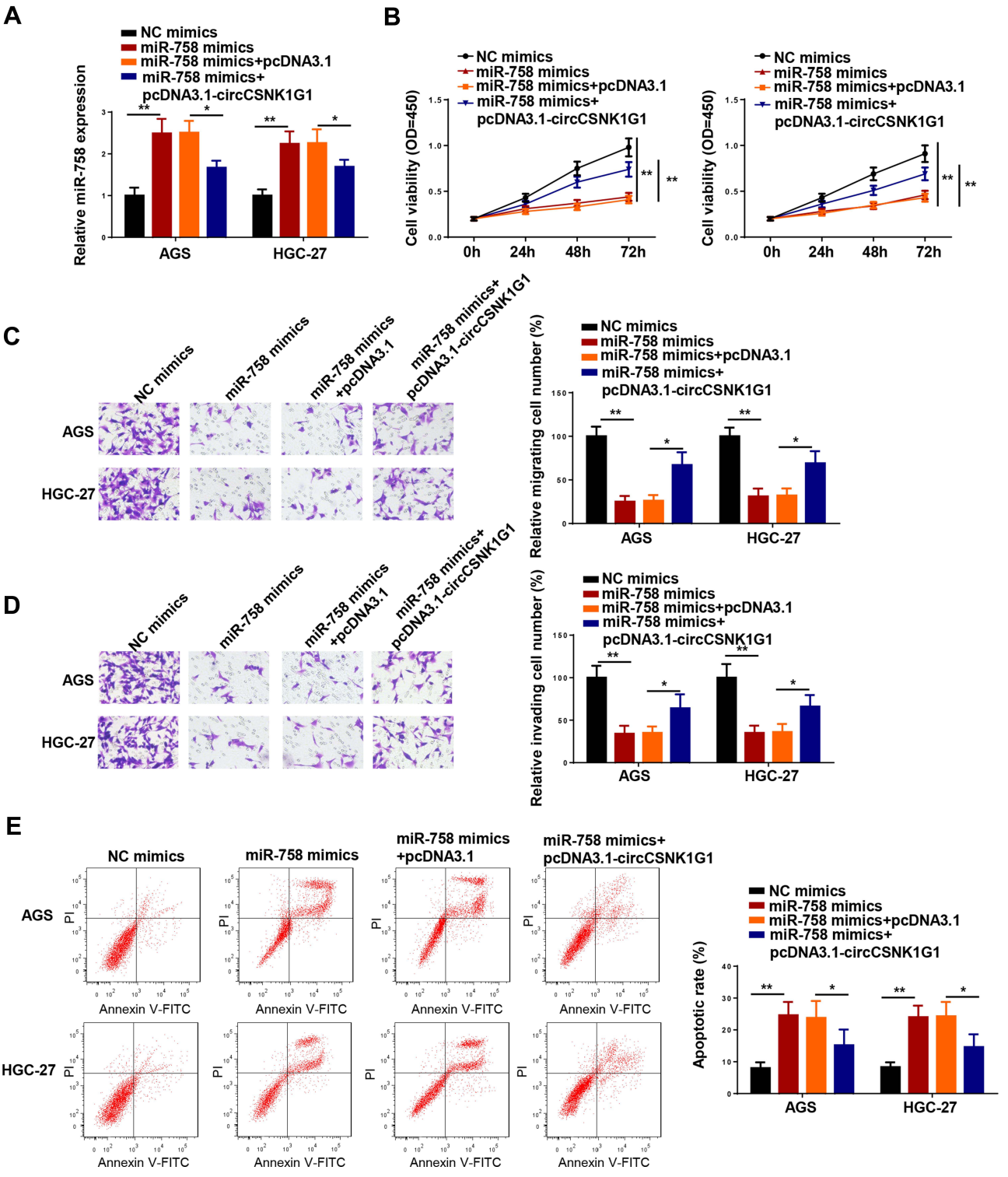

Figure 5 CircCSNK1G1 overexpression counteracted miR-758-mediated effects on the progression of GC. (**A**) The expression of miR-758 was measured by RT-qPCR in AGS and HGC-27 cells transfected with NC mimics, miR-758 mimics, miR-758 mimics+pcDNA3.1, and miR-758 mimics+pcDNA3.1-circCSNK1G1. (**B**) The proliferation of AGS and HGC-27 cells was measured by CCK-8 assay. (**C** and **D**) Transwell was used to detect the migration and invasion of AGS and HGC-27 cells transfected with NC mimics, miR-758 mimics, miR-758 mimics+pcDNA3.1, and miR-758 mimics+pcDNA3.1-circCSNK1G1. (**E**) Flow cytometry assay showed the apoptosis of AGS and HGC-27 cells transfected with NC mimics, miR-758 mimics, miR-758 mimics+pcDNA3.1, and miR-758 mimics+pcDNA3.1-circCSNK1G1. *P < 0.05 and **P < 0.01.

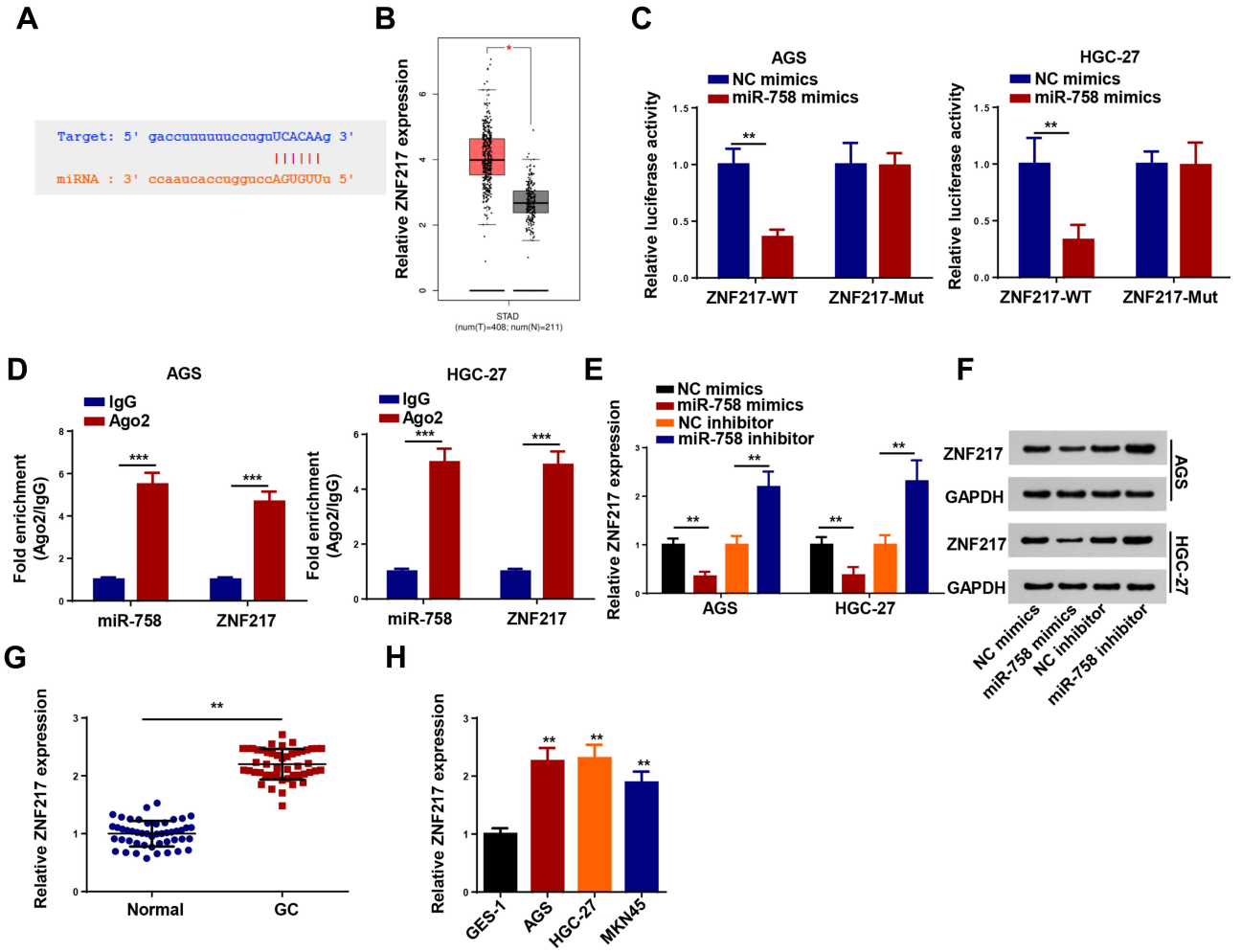

Figure 6 ZNF217 was directly targeted by miR-758. (**A**) Putative binding regions of miR-758 in ZNF217 3′UTR. (**B**) GEPIA database showed ZNF217 expression in stomach adenocarcinoma (STAD) tissues compared with that in normal tissues. (**C**) Luciferase activity of wild-type or mutant ZNF217 in AGS and HGC-27 cells following NC mimics, or miR-758 mimics transfection determined by luciferase reporter assay. (**D**) Correlations between ZNF217 and miR-758 detected by RIP assay. (**E** and **F**) The expression of ZNF217 was measured by RT-qPCR and Western blotting in AGS and HGC-27 cells transfected with miR-758 mimics or miR-758 inhibitor. (**G** and **H**) The expression of ZNF217 in GC tissues and cells detected by RT-qPCR. **P < 0.01 and ***P < 0.001.

in GC, and overexpression of circ-HuR inhibited the growth and metastasis of GC cells.[24] circRNA_001569 increased proliferation and decreased apoptosis of GC cells via inhibiting miR-145 expression.[25] CircCSNK1G1, a novel circRNA, was confirmed to be involved in the development of tumors. For example, Yao et al revealed that circCSNK1G1 facilitated hepatocellular carcinoma development by upregulating TRAF6 and MAPK11.[26] Huang et al reported that circCSNK1G1 accelerated colorectal cancer progression by elevating MYO6 level via interaction with miR-455-3p.[27] In this research, we focused on the role of circCSNK1G1 in GC. The results showed that circCSNK1G1 was upregulated in GC and deletion of circCSNK1G1 restrained GC progression in vitro and in vivo.

Previous researches have indicated that circRNAs could act as a competing endogenous RNA (ceRNA) of miRNA to exert its effect in cellular physiology.[28] For example, circCACTIN served as a ceRNA of miR-331 to regulate TGFBR1 level and accelerated GC development.[29] CircMAT2B facilitated glycolysis and growth of GC through increasing HIF-1α expression by restraining miR-515-5p.[30] CircLDLRAD3 promoted GC progression and could function as ceRNA to target NRP2 by sponging miR-224.[31] In our study, miR-758 was identified as a downstream gene of circCSNK1G1. miR-758 has been verified as a downstream regulator of circRNAs in the tumorigenesis of human cancers. For instance, circRNA RBM33 contributed to the occurrence and development of cervical cancer by sponging miR-758.[32]

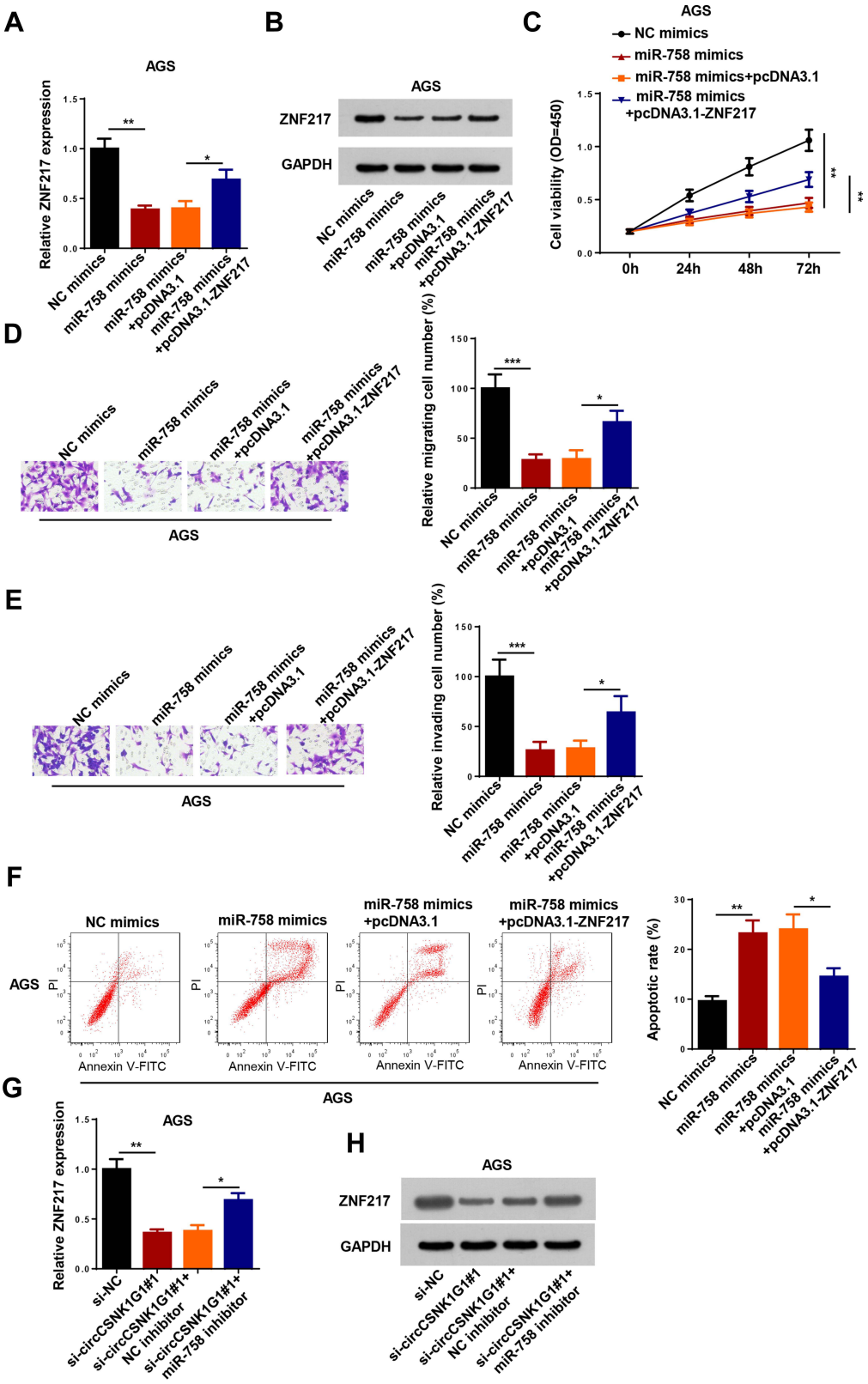

Figure 7 CircCSNK1G1 regulated ZNF217 by sponging miR-758 in GC cells. (**A** and **B**) The expression of ZNF217 was measured by RT-qPCR and Western blotting in AGS cells transfected with NC mimics, miR-758 mimics, miR-758 mimics+pcDNA3.1, and miR-758 mimics+pcDNA3.1-ZNF217. (**C**–**F**) CCK-8, Transwell and flow cytometry assays were used to detect the proliferation, migration and invasion and apoptosis of AGS cells transfected with NC mimics, miR-758 mimics, miR-758 mimics+pcDNA3.1, and miR-758 mimics+pcDNA3.1-ZNF217. (**G** and **H**) The expression of ZNF217 was measured by RT-qPCR and Western blotting in AGS cells transfected with si-NC, si-circCSNK1G1#1, si-circCSNK1G1#1+NC inhibitor, and si-circCSNK1G1+miR-758 inhibitor. *P < 0.05, **P < 0.01 and ***P < 0.001.

Hsa_circ_0002483 facilitates acute myeloid leukemia progression by downregulating miR-758 expression.[33] Here, we observed that supplementation of miR-758 restrained cell proliferation, migration and invasion, and promoted the apoptosis of GC cells, while circCSNK1G1 overexpression neutralized the effects on GC progression induced by miR-758.

ZNF217 is a potential oncogene located at chromosome 20q13.2, a region that is frequently involved in tumorigenesis of many cancers.[34] For example, the upregulation of ZNF217 predicted poor prognosis in breast cancer and promoted breast cancer progression.[35] LncRNA CTBP1-AS2 enhanced cervical cancer cell proliferation and migration via upregulation of ZNF217.[36] Moreover, Shida et al indicated high expression of ZNF217 contributed a worse relapse-free survival and overall survival of patients with GC.[37] In this study, we confirmed that ZNF217 was a target gene of miR-758. Functional assays indicated that the suppressive effect of miR-758 overexpression on GC progression was counteracted by ZNF217 overexpression. In addition, the absence of circCSNK1G1 resulted in a downregulation ZNF217, and miR-758 inhibition abolished the repressive effect of circCSNK1G1 inhibition on ZNF217 expression.

In summary, we demonstrated that circCSNK1G1 stimulated the progression of GC via regulating the miR-758/ZNF217 axis. These findings indicated that circCSNK1G1 might be a potential biomarker for GC diagnosis and therapy.

Disclosure

The authors report no conflicts of interest in this work.

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