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CircRNA ZNF609 promotes growth and metastasis of nasopharyngeal carcinoma by competing with microRNA-150-5p

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Abstract. – OBJECTIVE: This study aims to explore the biological function of circular RNA ZNF609 (circ-ZNF609) in regulating the occurrence and progression of nasopharyngeal carcinoma (NPC), and to investigate the possible underlying mechanism.

PATIENTS AND METHODS: The expression levels of circ-ZNF609, microRNA-150-5p and Sp1 in NPC tissues and normal nasopharyngeal epithelial tissues were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Circ-ZNF609 expression was silenced in NPC cell lines (5-8F and HONE-1). Cellular behaviors of NPC cells were determined using Cell Counting Kit-8 (CCK-8), wound healing and transwell assay. The binding relationship among microRNA-150-5p, circ-ZNF609 and Sp1 was detected by Dual-Luciferase reporter gene assay. In addition, the protein expression of Sp1 after altering expression of circ-ZNF609 or microRNA-150-5p was detected by Western blot.

RESULTS: The expression levels of circ-ZNF609 and Sp1 in NPC tissues were markedly higher than those of normal nasopharyngeal epithelial tissues. However, the expression of microRNA-150-5p was significantly lower in NPC tissues. The knockdown of circ-ZNF609 in NPC cells 5-8F and HONE-1 significantly inhibited the proliferative, migratory and invasive behaviors of NPC cells. Meanwhile, microRNA-150-5p knockdown in NPC cells showed the opposite effect on cellular behaviors of NPC cells. Dual-Luciferase reporter gene assay revealed that circ-ZNF609 could bind to microRNA-150-5p, and Sp1 was a target gene of microRNA-150-5p. Western blot results showed that circ-ZNF609 could stabilize the expression of Sp1, while microRNA-150-5p degraded Sp1 expression. Furthermore, the knockdown of Sp1 in NPC cells reversed the carcinogenic effect of circ-ZNF609.

CONCLUSIONS: Highly expressed circ-ZNF609 adsorbs microRNA-150-5p to upregulate Sp1 expression, thereby promoting the proliferation and metastatic ability of NPC cells.

Key Words:
Circ-ZNF609, MicroRNA-150-5p, Proliferation, Migration, Invasion.

Introduction

Nasopharyngeal carcinoma (NPC) derives from nasopharyngeal epithelium, presenting a high malignant level. NPC is characterized by a high rate of local metastasis and early distant metastasis[1]. The pathogenesis of NPC is related to various factors, such as environmental factors, genetic susceptibility and Epstein-Barr (EB) virus infection[2]. Currently, radiotherapy is widely applied in the treatment of NPC. Although the remission rate of NPC has been greatly enhanced, its 5-year survival rate remains about 70%. Quite a number of NPC patients experience tumor recurrence or distant metastasis within a few years after treatment[3]. Hence, it is of great significance to explore the crucial pathways and molecules in the pathogenesis of NPC, to improve the early diagnostic and effective therapeutic approaches of NPC.

In recent years, a large number of non-coding RNAs, especially microRNAs and lncRNAs, have exerted biological functions and diagnostic values in NPC[4-6]. The discovery of circular RNA (circRNA) further enriches the mechanism of post-transcriptional regulation[7]. Previously scholars[8] have shown that accumulating circRNAs interact and participate

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in the translation regulation through controlling modification and gene expressions.

The competing endogenous RNA (ceRNA) hypothesis was first proposed in 2011, which was then verified through bioinformatics, cell biology and animal models[9,10]. CeRNA molecules, such as lncRNAs, pseudogenes and circRNAs, are thought to compete with microRNA response elements (MREs) for binding to the same miRNAs, thus regulating their expression levels[11]. CeRNA hypothesis provides novel directions for elucidating the occurrence and development of tumors at the transcriptional level[12]. CircRNA differs from linear RNA, which does not contain a cap structure at 5' terminal end and a poly A at 3' terminal end. CircRNA presents a closed loop structure that is connected end to end by covalent bonds. Recent studies have found that circRNA is expressed in eukaryotic cell lines of different species[13]. Some circRNAs have been shown to contain multiple conserved miRNA binding sites that regulate gene expressions. The development and progression of tumors are regulated by these epigenetic changes[14]. Furthermore, multiple circRNAs are confirmed to participate in the regulation of tumor cell biological functions through ceRNA mechanism, including circ-BNDP, circ-HIPK3, and circ-MYLK[15-17].

Recently, the crucial regulatory role of circRNA HIPK3 in the progression of NPC has been confirmed[15]. However, the biological effects and specific mechanisms of most circRNAs in NPC development remain unclear. Circ-ZNF609 (ch: hsa_circ_0000615 in circBase) is located at chr15:64791491-64792365, exerting a crucial biological role in promoting the proliferation and metastasis of various diseases[18-21]. However, its function in NPC still needs to be fully elucidated.

Patients and Methods

Sample Collection

16 cases of NPC tissues were collected from NPC patients at the Affiliated Hospital of Southwest Medical University from July 2016 to July 2017. None of the patients were treated with radiothera-py, chemotherapy or other adjuvant therapy. Meanwhile, 16 cases of normal nasopharyngeal epithelial tissues were collected from patients with chronic nasopharyngeal inflammation confirmed by biopsy pathology. Collected tumor tissues or normal tissues were pathologically confirmed.

All tissue samples were quickly frozen in liquid nitrogen af-ter resection and placed in a -80°C refrigerator. The study was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University. All patients signed an informed consent prior to the experiment.

Cell Culture

Human normal nasopharyngeal epithelial cell line (NP69) and NPC cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 IU/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA), and placed in a 37°C, 5% CO_2 incubator.

Cells in logarithmic growth phase were selected and transfected with circ-ZNF609 siRNA, circ-ZNF609 overexpression plasmid, microRNA-150-5p mimics, microRNA-150-5p inhibitor or Sp1 siRNA (GenePharma, Shanghai, China), respectively. Transfection was performed according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA in cells or tissues were first extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) reagent, centrifuged, and the aqueous phase was transferred to a new tube. RNA in the aqueous phase was precipitated using isopropanol and washed with 75% ethanol. Extracted RNA was air dried, diluted in diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China) and preserved at -20°C. Subsequently, extracted RNA was reversely transcribed into complementary Deoxyribose Nucleic Acid (cDNA) according to the instructions of Revert Aid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). QRT-PCR was performed in strict accordance with SYBR® Green Master Mix (TaKaRa, Otsu, Shiga, Japan). The reaction conditions were as follows: pre-denaturation at 95°C for 15 min, denaturation at 94°C for 15 s, 55°C for 30 s, and extension at 72°C for 30 s, for a total of 40 cycles. Fluorescence was collected at 75-80°C and finally analyzed the melting curve at 65-95°C. Primer sequences were as follows: Circ-ZNF609: F: 5′-CAGCGCTCAATCCTTTGGGA-3′, R: 5′-GACCTGCCACATTGGTCAGTA-3′; GAP-

DH: F: 5′-CACCCACTCCTCCACCTTTG-3′, R: 5′-CCACCACCCTGTTGCTGTAG-3′; MicroRNA-150-5p: F: 5'-ACACTCCAGCTGGGTCTCCCAACCCTTGTA-3', R: 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCACTGGTA-3'; U6: F: 5′-CTCGCTTCGGCAGCACA-3′, R: 5′-AACGCTTCACGAATTTGCGT-3′; Sp1: F: 5′-TGGTGGGCAGTATGTTGT-3′, R: 5′-GCTATTGGCATTGGTGAA-3′.

RNase R Digestion

Total RNA (5 μg) was incubated for 15 min at 37°C using 3 U/μg of RNase R (Epicentre Biotechnologies, Madison, WI, USA) twice, according to previously published procedures.

Cell Proliferation Assay

After 24 h of transfection, cells were digested and inoculated into 96-well plates at a density of 4000 cells/well. Each group had 6 replicates. After culturing for 24 h, 48 h, 72 h, and 96 h, respectively, the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan) assay was performed. Briefly, 10 μL of CCK-8 solution was added to each well, followed by incubation at 37°C for 1 h in the dark. The absorbance of each well at 450 nm was recorded by a microplate reader. Each experiment was repeated three times independently.

Cell Migration Assay

A total of 1×10^5 transfected NPC cells were seeded in the apical chamber. Meanwhile, the medium containing 10% FBS was added as a chemotactic agent to the basolateral chamber. Then the cells were maintained in a 37°C, 5% CO_2 incubator for 48 h. Cells invading to the basolateral chamber were fixed in 70% ethanol for 30 min and stained with 0.1% crystal violet for 10 min. The number of cells migrating to the lower chamber was counted under an inverted microscope. Five fields were randomly selected for each sample. This experiment was repeated three times.

Cell Invasion Assay

The fibronectin (FN) was diluted to a final concentration of 100 μg/mL. Matrigel was diluted at a ratio of 1:9 with serum-free medium. Cell density was adjusted to 1×10^5 cells/mL, and 100 μL of cell suspension was seeded to the apical transwell chamber pre-coated with 100 μL of Matrigel overnight. Meanwhile, 600 μL of medium with 10% FBS was added to the basolateral chamber pre-coated with 50 μL of FN. After 24-h of incubation, cells were fixed with methanol, stained with trypan blue and washed 3 times with PBS. Un-penetrating cells were wiped off with a cotton swab, and the number of invaded cells was photographed under a microscope. Each experiment was repeated three times independently.

Wound Healing Assay

Transfected 5-8F or HONE-1 cells were seeded into 6-well plates and subjected to serum starvation for 24 h in serum-free medium. On the other day, an artificial wound was created in the confluent cell monolayer using a 200 μL pipette tip. The images were taken at 0 and 24 h using an inverted microscope, respectively.

Chromatin Fractionation

Until cell grew to 1×10^6 cells, 200 μL of Lysis Buffer J was added to the culture bottle to fully lyse them. After centrifugation, the supernatant was transferred to a new tube. Subsequently, Buffer SK and absolute ethanol were added to cytoplasmic and nucleus RNA, respectively, followed by extraction with column centrifugation. Cytoplasmic or nuclear levels of circ-ZNF609 were detected by qRT-PCR.

Luciferase Reporter Gene Assay

Bioinformatics methods were used to predict the potential targets of microRNA-150-5p, circ-ZNF609 and Sp1. Wild-type sequences of Sp1 3'UTR and circ-ZNF609 3'UTR, mutant-type sequences of Sp1 3'UTR and circ-ZNF609 3'UTR were inserted into pGL3 promoter vector (Realgene, Nanjing, China). One day prior to co-transfection, NPC cells were seeded into 24-well plates at a density of 5×10^5 cells/well. Then, the cells were co-transfected with Luciferase reporter vector (0.12 μg) and 50 nM microRNA-150-5p or negative control for 48 h. The Luciferase activity was determined using the Dual-Luciferase reporter gene assay kit (Promega, Madison, WI, USA). The experiment was repeated three times.

Western Blot

Cells were collected for extracting total protein with lysis buffer on ice. After centrifugation, the supernatant was harvested for determining the protein concentration using the bicinchoninic acid (BCA) protein quantification kit (Beyotime, Shanghai, China). After heating at 100°C, the protein was denatured and loaded for gel electrophoresis using sodium dodecyl

sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein was then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membrane was cut according to the molecular weight, blocked in 5% skim milk and incubated with primary and secondary antibodies. Finally, the image of the protein band was captured by the Tanon detection system using the enhanced electrochemiluminescence (ECL) reagent (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (SPSS Inc. Chicago, IL, USA) was used for all statistical analysis, and GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) was introduced for figure editing. All experiments were repeated three times. All measurement data were expressed as mean ± SD. Kaplan-Meier method was introduced to calculate the survival curve. Student's *t*-test was used to compare the difference between the two groups. $p<0.05$ indicated that the difference was statistically significant.

Results

Circ-ZNF609 Was Highly Expressed in NPC

We first detected the expression of circ-ZNF609 in 16 cases of NPC and corresponding normal nasopharyngeal epithelial tissues by qRT-PCR. The results showed that circ-ZNF609 was highly expressed in NPC tissues (Figure 1A). Compared with the normal nasopharyngeal epithelial cell line NP69, the expression of circ-ZNF609 in NPC cell lines (SUNE-1, CNE-1, HONE-1 and 5-8F) was identically higher than controls (Figure 1B). Particularly, 5-8F and HONE-1 cells showed the most pronounced expression of circ-ZNF609 among the four NPC cell lines, which were chosen for subsequent experiments. To verify whether circ-ZNF609 exerted the stabilization function as a typical circRNA, total RNA in NPC cells was treated with RNase R. RNase R can degrade linear RNA characterized by free 3' terminal end, but has no impact on circRNA. It is suggested that circ-ZNF609 was resistant to RNase R digestion, while the internal control GAPDH did not has such ability (Figure 1C).

Knockdown of Circ-ZNF609 Inhibited Proliferation and Metastasis of NPC Cells

Three circ-ZNF609 siRNAs were constructed and their transfection efficacy in 5-8F and HONE-1 cells were verified. It was found that si-circ-ZNF609-1 was the most effective siRNA to inhibit circ-ZNF609 expression in NPC cells (Figure 1D). We tested the proliferative change in 5-8F and HONE-1 cells after circ-ZNF609 knockdown. The CCK-8 results showed that circ-ZNF609 knockdown remarkably inhibited the proliferative potential of NPC cells (Figure 1E). Subsequently, we examined the effects of circ-ZNF609 on migratory and invasive changes by wound healing and transwell assays. The knockdown of circ-ZNF609 expression in NPC cells significantly attenuated migratory and invasive behaviors (Figure 1F-1H).

Circ-ZNF609 Regulated Proliferative and Migratory Behaviors of NPC Cells by Binding to MicroRNA-150-5p

It is well known that the subcellular localization of circRNA determines its biological role. Therefore, we examined the cytoplasmic and nuclear distribution of circ-ZNF609 in 5-8F and HONE-1 cells by chromatin fractionation assay. Circ-ZNF609 was mostly distributed in the cytoplasm of 5-8F and HONE-1 cells, suggesting that circ-ZNF609 possibly plays a regulatory role at the post-transcriptional level (Figure 2A). Bioinformatics was used to predict the potential miRNAs that bound to circ-ZNF609, and verified by functional analysis. MicroRNA-150-5p was finally screened out (Figure 2B). Subsequently, microRNA-150-5p expression in NPC tissues and corresponding normal nasopharyngeal epithelial tissues was determined by qRT-PCR. The results showed a significantly higher expression of microRNA-150-5p in NPC tissues (Figure 2C). The Luciferase reporter gene assay demonstrated that, after transfection of microRNA-150-5p mimics in 5-8F and HONE-1 cells, the Luciferase activity was remarkably reduced in the circ-ZNF609-WT 3'UTR group. However, no significant changes in the Luciferase activity were found in the circ-ZNF609-MUT 3'UTR group, confirming that microRNA-150-5p could bind to circ-ZNF609 (Figure 2D). After silencing circ-ZNF609 in 5-8F and HONE-1 cells, qRT-PCR assay revealed an increase in microRNA-150-5p expression (Figure 2E). This suggested that circ-ZNF609 was capable of binding to microRNA-150-5p and inhibiting its expression. Besides, we found a negative cor-

relation between the expressions of circ-ZNF609 and microRNA-150-5p in NPC cells (Figure 2E). Subsequently, we examined the regulatory effects of microRNA-150-5p on the proliferative, migratory and invasive behaviors of NPC cells. The results indicated that circ-ZNF609 knockdown in 5-8F and HONE-1 cells markedly inhibited the proliferative potentials of NPC cells. However, interfering with microRNA-150-5p could markedly reverse the inhibitory effect (Figure 2F). Similarly, the inhibitory effects of circ-ZNF609 on migratory and invasive potentials of NPC cells were reversed by microRNA-150-5p interference as well (Figure 2G-I). These data indicated that circ-

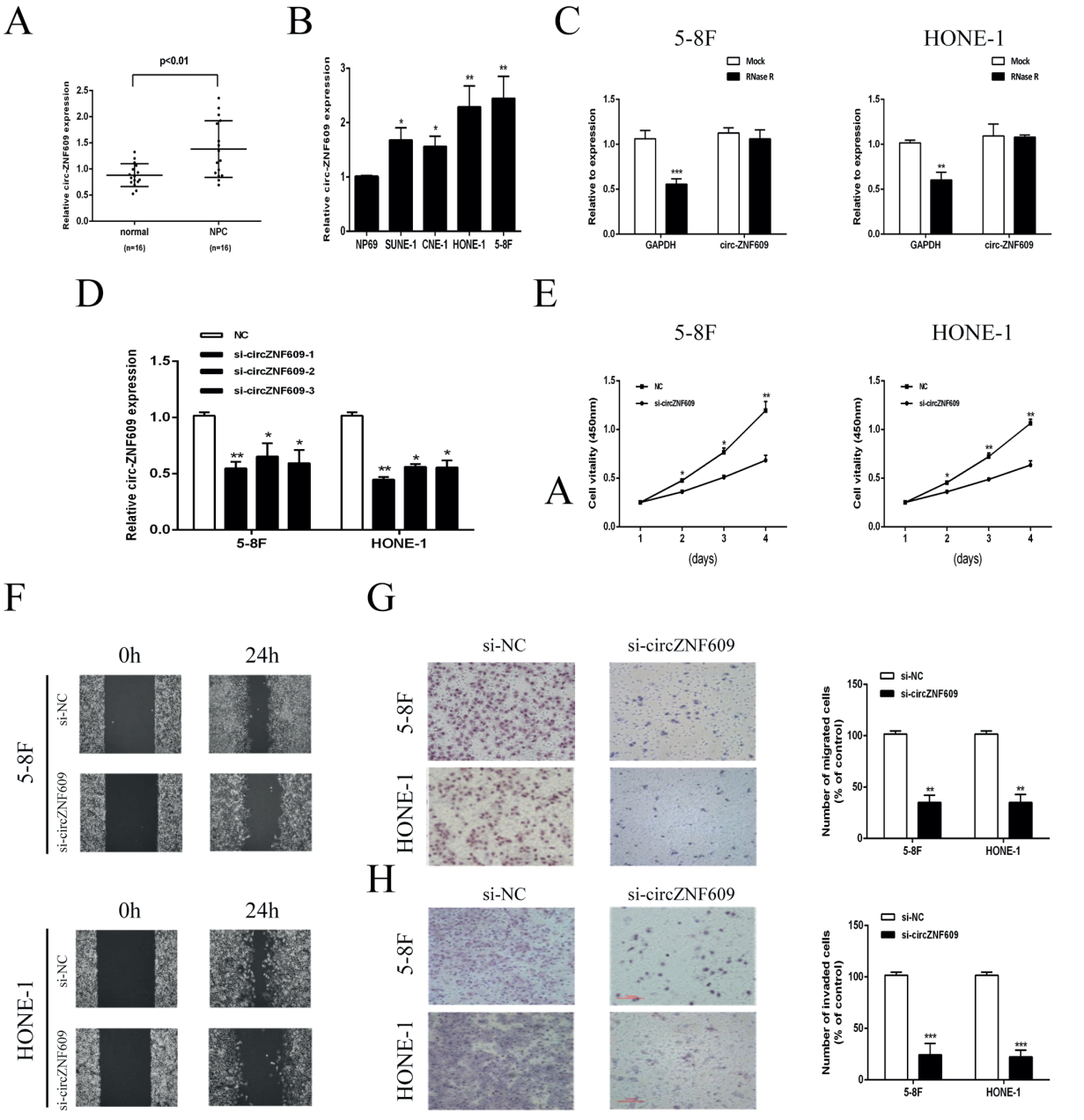

Figure 1. The knockdown of circ-ZNF609 inhibited the proliferation and metastasis of NPC cells. ***A,*** Circ-ZNF609 was highly expressed in NPC tissues. ***B,*** Circ-ZNF609 was highly expressed in NPC cell lines. ***C,*** Expression of circ-ZNF609 in 5-8F and HONE-1 cells after RNase R treatment. ***D,*** Circ-ZNF609 expression after interference with 3 lines of siRNA circ-ZNF609 in 5-8F and HONE-1 cells. ***E-G,*** Changes in cell proliferation, migration and invasion after interfering with circ-ZNF609 in 5-8F and HONE-1 cells. CCK-8 assay (***E***), wound healing assay (***F***), cell migration assay (***G***) (Magnification x 40) and cell invasion assay (***H***) (Magnification x 40). Data were presented as mean ± SD; *p<0.05, **p<0.01, ***p<0.001.

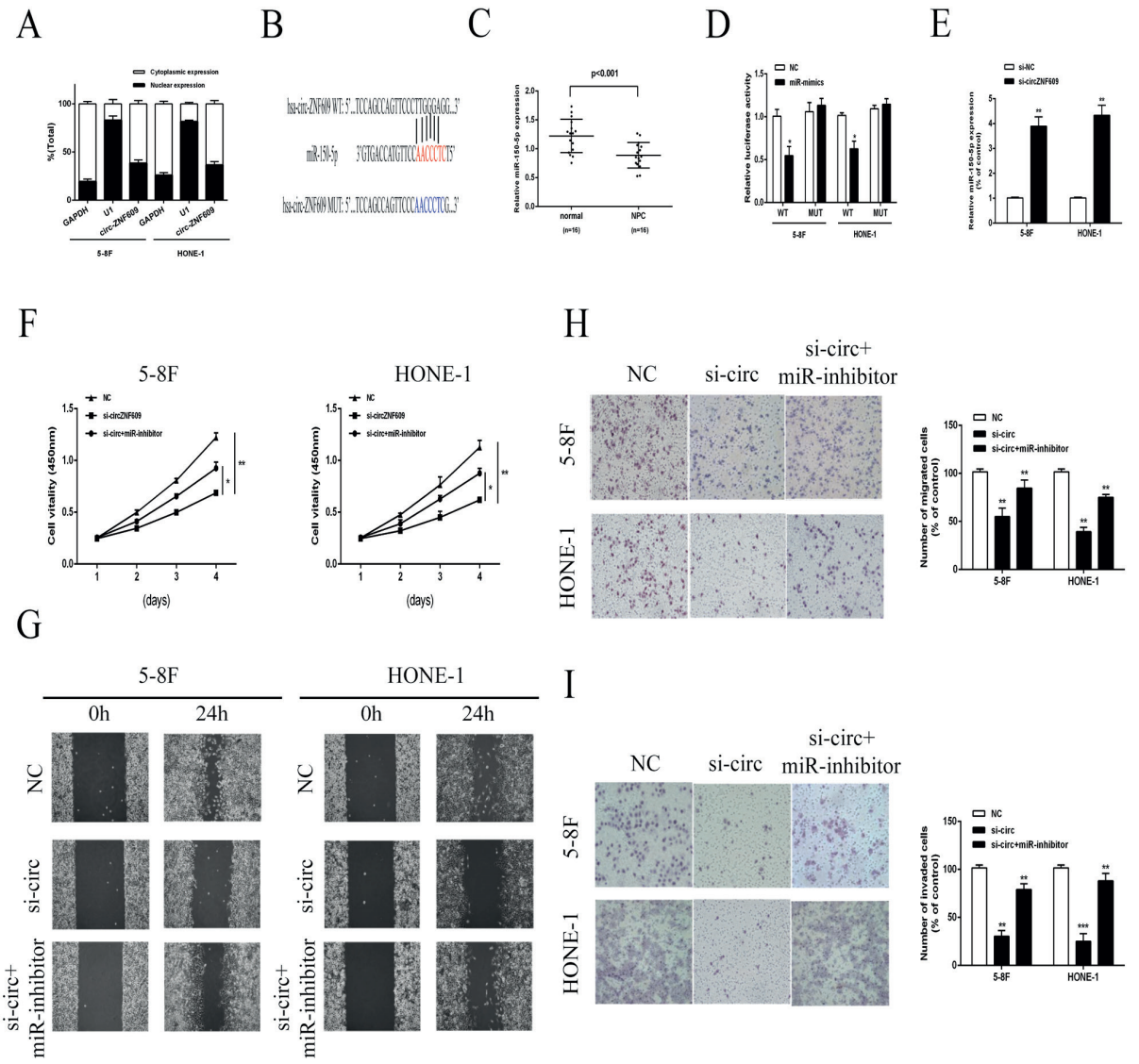

Figure 2. Circ-ZNF609 regulated proliferative and migratory behaviors of NPC cells by binding to microRNA-150-5p. ***A,*** Subcellular distribution of circ-ZNF609 in the nucleus and cytoplasm of 5-8F and HONE-1 cells. ***B,*** Bioinformatics predicted the binding sites of circ-ZNF609 and microRNA-150-5p. ***C,*** MicroRNA-150-5p was lowly expressed in NPC tissues. ***D,*** Dual-Luciferase reporter gene assay showed that microRNA-150-5p could bind to the 3'-UTR of circ-ZNF609. ***E,*** Interfering with circ-ZNF609 in 5-8F and HONE-1 cells upregulated the expression of microRNA-150-5p. ***F-I,*** Interfering with circ-ZNF609 in 5-8F and HONE-1 cells attenuated the proliferation, migration, and invasion ability of NPC cells. Meanwhile, the inhibitory effects were reversed after the inhibition of microRNA-150-5p. CCK-8 assay (***F***), would healing assay (***G***), cell migration assay (Magnification x 40) (***H***) and cell invasion assay (Magnification x 40) (***I***). Data were presented as mean ± SD; *p<0.05, **p<0.01, ***p<0.001.

ZNF609 might promote cell growth and metastasis by inhibiting microRNA-150-5p expression.

MicroRNA-150-5p Targeted and Degraded Sp1

The target gene binding to microRNA-150-5p was predicted through bioinformatics and verified by functional analysis. Finally, Sp1 was screened out (Figure 3A). QRT-PCR demonstrated that Sp1 was highly expressed in NPC tissues (Figure 3B). Subsequently, Luciferase reporter gene assay confirmed the binding of microRNA-150-5p and Sp1 (Figure 3C). Further, we assessed the association among circ-ZNF609, microRNA-150-5p and Sp1 in NPC by bivariate correlation analysis. As indicated, circ-ZNF609 was positively correlated with Sp1. However, microRNA-150-5p was negatively correlated with circ-ZNF609 and Sp1 (Figure 3D-3F). After overexpression of microRNA-150-5p in 5-8F and HONE-1 cells, both the mRNA and protein expressions of Sp1 significantly

decreased, while the overexpression of circ-ZNF609 reversed the inhibitory effect of microRNA-150-5p on Sp1 expression (Figure 3G-3H). The above results indicated that circ-ZNF609 might increase the expression of Sp1 by binding to microRNA-150-5p.

Circ-ZNF609 Promoted Growth and Metastasis of NPC Cells by Upregulating Sp1

The expression of Sp1 in 5-8F and HONE-1 cells was significantly upregulated after circ-ZNF609 overexpression, which was markedly downregulated by Sp1 interference (Figure 4A). Subsequently, the proliferative, migratory and invasive behaviors of NPC cells co-transfecting with circ-ZNF609 overexpression plasmid and si-Sp1 were evaluated. The results indicated that the promotive effects of circ-ZNF609 overexpression on proliferative, migratory and invasive behaviors of NPC cells were partially reversed by Sp1 knockdown. This suggested that the carcinogenic

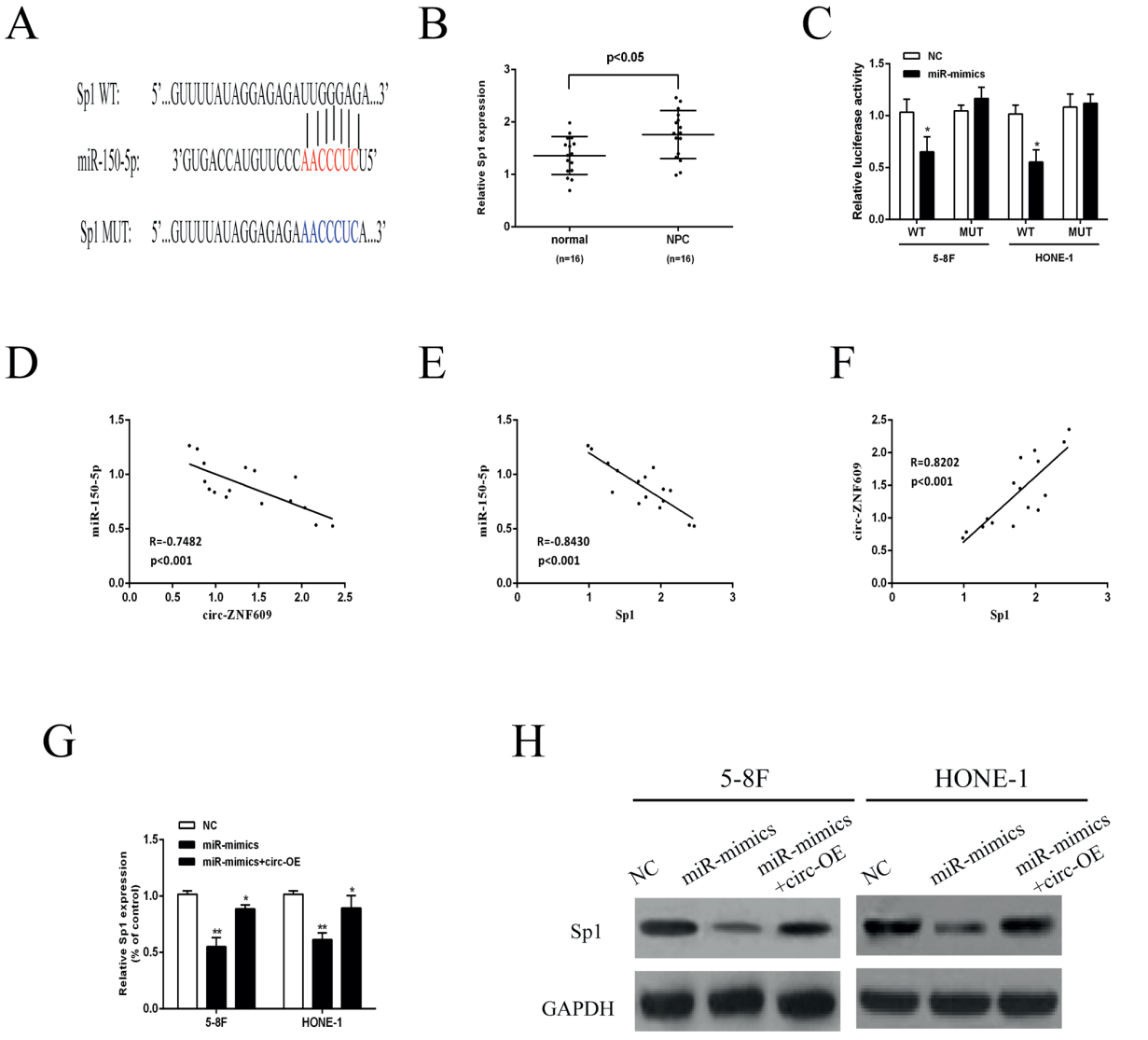

Figure 3. MicroRNA-150-5p targeted and degraded Sp1. ***A,*** Bioinformatics predicted the binding site of microRNA-150-5p to Sp1. ***B,*** Sp1 was highly expressed in NPC tissues. ***C,*** Dual-Luciferase reporter gene assay showed that microRNA-150-5p could bind to Sp1 and degrade Sp1. ***D,*** The expression of circ-ZNF609 in NPC was negatively correlated with the expression of microRNA-150-5p. ***E,*** The expression of Sp1 in NPC was negatively correlated with the expression of microRNA-150-5p. ***F,*** The expression of circ-ZNF609 in NPC was positively correlated with the expression of Sp1. ***G,*** Overexpresses microRNA-150-5p in 5-8F and HONE-1 cells inhibited the mRNA expression of Sp1, which was reversed by overexpression of circ-ZNF609. ***H,*** Overexpressed microRNA-150-5p in 5-8F and HONE-1 cells inhibited the protein expression of Sp1, which was reversed by overexpression of circ-ZNF609. Data were presented as mean ± SD; *p<0.05, **p<0.01, ***p<0.001.

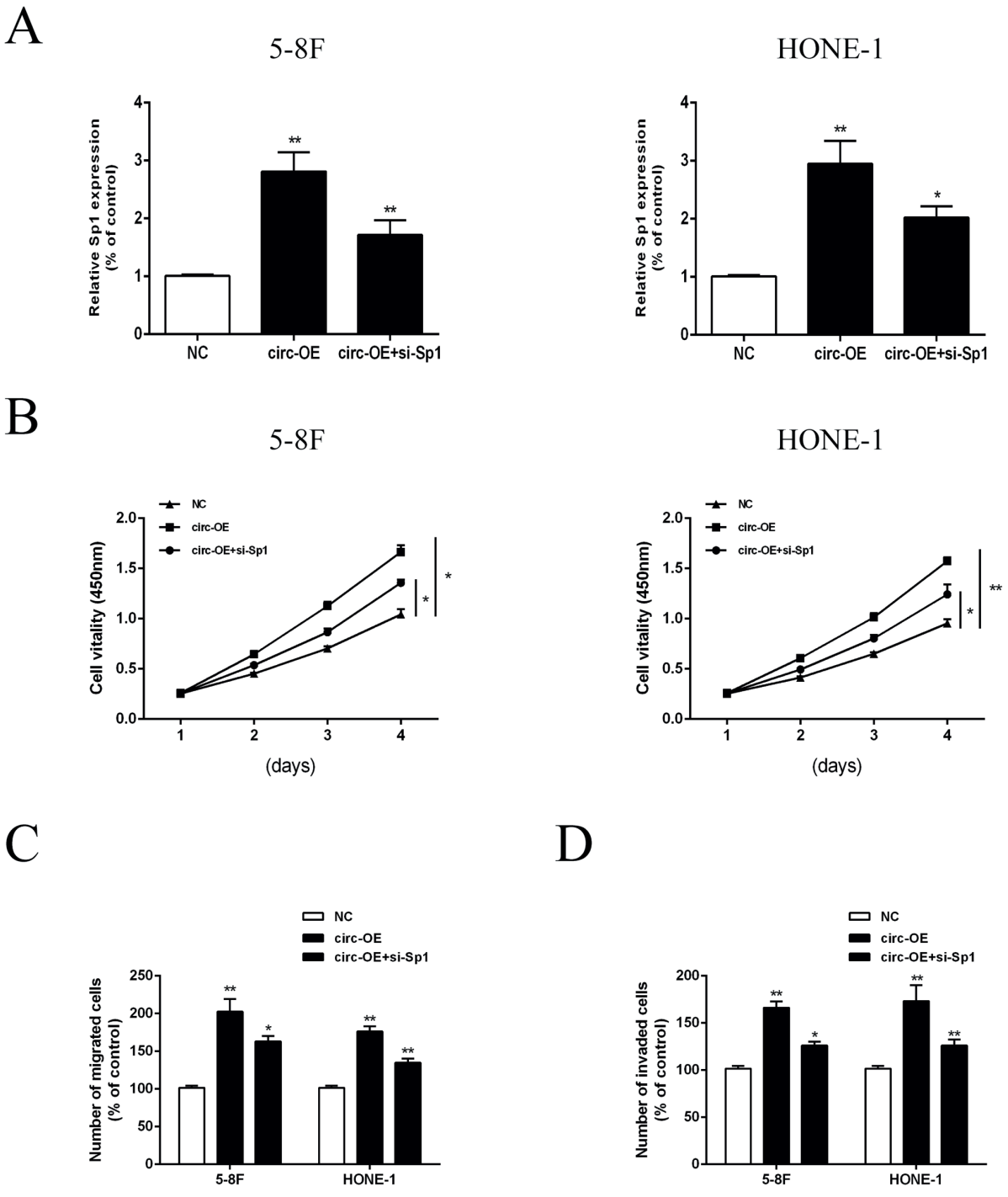

Figure 4. Circ-ZNF609 promoted growth and metastasis of NPC cells by upregulating Sp1. ***A,*** Sp1 expression was upregulated after circ-ZNF609 overexpression in 5-8F and HONE-1 cells, which was downregulated by Sp1 interference. ***B-D,*** The promotive effects of circ-ZNF609 overexpression on proliferative, migratory and invasive behaviors of NPC cells were partially reversed by Sp1 knockdown. CCK-8 assay (***B***), cell migration assay (***C***) and cell invasion assay (***D***). Data were presented as mean ± SD; **p*<0.05, ***p*<0.01, ****p*<0.001.

effect of circ-ZNF609 was attenuated after inhibition of Sp1 (Figure 4B-4D). We indicated that circ-ZNF609 might promote the progression of NPC by upregulating the expression of Sp1.

Discussion

NPC is characterized by low differentiation and high metastasis. Most NPC patients have already progressed into the advanced stage and suffered cervical lymph node metastasis at the first time of diagnosis[22]. Although the comprehensive treatment of radiotherapy and chemotherapy has greatly improved the local control rate of NPC, approaches for early detection and effective intervention are still lacking[23]. Therefore, the exploration of the potential pathogenesis of NPC contributes to developing novel diagnostic and therapeutic targets.

CircRNAs are structurally stable, tissue-specific and evolutionarily conserved. Meanwhile, they cannot be degraded by RNase. Many circRNAs are derived from protein-encoding genes, that is, exon sequences of genes. Only a few circRNAs are derived from introns. A great number of circRNAs do not display protein-encoding functions in cells, which are considered as non-coding RNAs. CircRNAs are abundantly present in eukaryotic cells and participate in the regulation of disease process. Our study found that circ-ZNF609 was highly expressed in NPC tissues and cell lines. Circ-ZNF609 could affect the proliferative, migratory and invasive behaviors of NPC cells. *In vitro* experiments showed that circ-ZNF609 knockdown significantly inhibited the growth and metastasis of NPC cells 5-8F and HONE-1. To investigate the mechanism of circ-ZNF609 in affecting the development and progression of NPC, we screened out that Sp1 was the target gene of circ-ZNF609 and subsequently elucidated its potential function.

Sp1 locates at 12q13.1, which is capable of translating Zinger-finger proteins to bind to the GC rich of the downstream gene promoter. This may eventually initiate the transcription of downstream genes[24]. Sp1 is considered to be a ubiquitous transcription factor involved in multiple cellular biological activities, including growth differentiation, aging, apoptosis, angiogenesis, DNA damage, etc. Recent studies[25] have shown that Sp1 is highly expressed in many tumors, which is closely related to tumor stage, metastasis and prognosis. Patients with high expression of Sp1 *in vivo* tended to have a low survival rate and poor prognosis. Therefore, Sp1 is recognized as an oncogenic gene. Meanwhile, it can be used as a downstream target for many genes, including some microRNAs and oncogenes. Up-regulation or phosphorylation of Sp1 promotes tumorigenesis, while Sp1 itself can also influence the proliferation and apoptosis of tumors by regulating downstream genes[24,26]. Our work found that Sp1 was highly expressed in NPC tissues and cells. Furthermore, Sp1 knockdown remarkably attenuated proliferative, migratory and invasive behaviors of NPC cells.

To investigate the mechanism by which circ-ZNF609 regulated Sp1, we searched for a bridge connecting circ-ZNF609 and Sp1. Previous studies have shown that circ-ZNF609 can bind to miRNAs and inhibit their degradative effects on downstream target genes[21]. We therefore postulated that circ-ZNF609 might regulate Sp1 by targeting some certain miRNAs. MicroRNA-150-5p complementary pairs to the base of circ-ZNF609, as well as Sp1 3'UTR. It is speculated that circ-ZNF609 may indirectly promote the expression of Sp1 by binding to microRNA-150-5p. Subsequently, our results found that circ-ZNF609 and Sp1 could competitively bind to microRNA-150-5p. Highly expressed circ-ZNF609 could bind to and inhibit the expression level of microRNA-150-5p, thereby abolishing the degradation of Sp1 by microRNA-150-5p. As a result, the expression level of Sp1 was indirectly upregulated. Both circ-ZNF609 and Sp1 could promote the growth and metastasis of NPC cells. However, microRNA-150-5p attenuated the proliferative, migratory and invasive behaviors of NPC cells. We believed that circ-ZNF609 indirectly promoted the expression of oncogene Sp1 by inhibiting microRNA-150-5p expression, thereby promoting the occurrence and development of NPC.

Conclusions

We found that high expression of circ-ZNF609 promotes the proliferation and enhances the metastatic ability of NPC cells by absorbing microRNA-150-5p and upregulating Sp1. In addition, circ-ZNF609 may serve as a diagnostic hallmark and therapeutic target for NPC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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