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Long noncoding RNA SNHG14 promotes breast cancer cell proliferation and invasion via sponging miR-193a-3p

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Abstract. – OBJECTIVE: Breast cancer (BC) is one of the most ordinary fatal cancers. Recent studies have identified the vital role of long noncoding RNAs (lncRNAs) in the development and progression of BC. In this research, lncRNA SNHG14 was studied to identify how it functioned in the development and metastasis of BC.

PATIENTS AND METHODS: SNHG14 expression of tissues was detected by Real Time Quantitative Polymerase Chain Reaction (RT-qPCR) in 50 paired patients with BC. And cell proliferation assay, colony formation assay, and transwell assay were enrolled to observe the biological behavior changes of BC cells with gain or loss of SNHG14. In addition, luciferase assays and RNA immunoprecipitation assay (RIP) were performed to discover the potential targets of SNHG14 in BC cells.

RESULTS: SNHG14 expression level of BC samples was higher than that in adjacent ones. Besides, cell growth ability and cell invaded ability of BC cells were suppressed after SNHG14 was silenced, while cell growth ability and cell invaded ability of BC cells were promoted after SNHG14 was overexpressed. In addition, miR-193a-3p was upregulated after silence of SNHG14 in BC cells, while miR-193a-3p was downregulated after overexpression of SNHG14 in BC cells. Furthermore, luciferase assays and RNA immunoprecipitation assay (RIP) showed that miR-193a-3p was a direct target of SNHG14 in BC.

CONCLUSIONS: Our study uncovers a new oncogene in BC and suggests that SNHG14 could enhance BC cell proliferation and invasion by sponging miR-193a-3p, which provided a novel therapeutic target for BC patients.

Key words
Long noncoding RNA, SNHG14, Breast cancer, MiR-193a-3p.

Introduction

Breast cancer (BC), accounting for 29% of all cancer diagnoses in women, is the most frequently diagnosed malignancy in female both in China and worldwide. It is estimated that 246,660 new cases were diagnosed of BC and 40,450 cases died due to BC in America in 2016[1]. Despite great advances that have been made in the diagnosis and therapeutic treatment in BC, the 5-year survival rate of BC patients remains below 25% because of the high rate of metastasis[2]. However, the mechanism of pathogenesis in BC has not been completely explained. Therefore, it is urgent to have a better understanding of the molecular characteristics of BC.

Long non-coding RNAs (lncRNAs), more than 200 nt in length, have been reported to participate in a diversity of biological progression. For example, by targeting miR-130a-5p/HMGB2, the lncRNA HOXA11-AS promotes glioma cell growth and metastasis[3]. Cell proliferation and cell migration of ovarian cancer are inhibited after knockdown of lncRNA MNX1-AS1 in ovarian cancer[4]. LncRNA PCAT-1 modulates TP53-miR-215-PCAT-1-CRKL axis and exerts a vital function in tumorigenesis of hepatocellular carcinoma[5]. LncRNA AC132217.4 promotes cell metastasis in oral squamous cell carcinoma by regulation of IGF2 expression[6]. In addition, lncRNA PlncRNA-1 accelerates the progression of colorectal cancer cell *via* PI3K/Akt signaling pathway[7]. However, the clinical role and underlying mechanisms of SNHG14 in the development of BC remain unexplored. In the present study, we performed function and mechanism assays to explore whether SNHG14 functioned in the proliferation and metastasis of BC.

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Patients and Methods

Patients and Clinical Samples

50 BC patients who received surgery at our hospital from 2015 to 2018 were enrolled for human tissues. Written informed consent was achieved before the operation. No radiotherapy or chemotherapy was performed before the surgery. All tissues were saved immediately at –80°C. The protocol of the study was approved by the Ethics Committee of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine.

Cell Culture

Human BC cell lines (MCF-7, LCC9, T-47D, SKBR3) and MCF-10A (normal human breast cell line) were got from the Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China). Culture medium consisted of 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA), Dulbecco's modified Eagle's medium (DMEM) (Gibco, Rockville, MD, USA). Besides, cells were cultured in an incubator containing 5% CO_2 at 37°C.

Cell Transfection

Lentivirus expressing short-hairpin RNA (shRNA; Biosettia Inc., San Diego, CA, USA) against SNHG14 was synthesized. SNHG14 shRNA (SNHG14/shRNA) and negative control (control) were then used for transfection of T-47D BC cells. 48 h later, Real Time Quantitative Polymerase Chain Reaction (RT-PCR) was used to detect transfection efficiency in these cells. Besides, lentivirus (Biosettia Inc., San Diego, CA, USA) against SNHG14 (SNHG14/shRNA) was synthesized and then used for transfection of SKBR3 BC cells. Empty vector was used as control. 48 h later, RT-PCR was used to detect transfection efficiency in these cells.

RNA Extraction and RT-qPCR

Trizol reagent (Invitrogen, Carlsbad, CA, USA) was utilized to separate the total RNA. High fidelity Transcription Kit (TaKaRa Biotechnology Co., Dalian, China), complementary deoxyribose nucleic acid (cDNA) was then reverse transcribed from total RNA. Thermocycling conditions were as follows: pre-denaturation at 95°C for 5 min, denaturation at 95°C for 10 s, annealing at 60°C for 30 s, a total of 35 cycles. Following are the primers using for RT-qPCR: SNHG14, forward: 5′-GGGTGTTTACGTAGACCAGAACC-3′ and reverse: 5′-CTTCCAAAAGCCTTCTGCCTTAG-3′; β-actin, forward: 5'-CCAACCGCGAGAAGATGA-3' and reverse: 5'-CCAGAGGCGTACAGGGATAG-3'.

Cell Proliferation Assay

Cell proliferation of BC cells in 96-well plates was monitored every 24 h by cell counting kit-8 (CCK-8) assay (Dojindo, Kumamoto, Japan). Spectrophotometer (Thermo Scientific, Waltham, IL, USA) was applied for measurement of the absorbance at 450 nm.

Transwell Assay

8-μm pore size insert with Matrigel by Corning (Corning, NY, USA). 4×10⁴ cells in 150 μL serum-free medium were transferred to the top chamber of the inserts coated with 50 μg Matrigel (BD, Bedford, MA, USA). The bottom chamber was filled with DMEM containing 10% FBS. 48 h later, the top surface of chambers was immersed for 10 min with precooled methanol and was stained in crystal violet for 30 min.

Luciferase Assay and RNA Immunoprecipitation Assay (RIP)

The 3′-UTR of SNHG14 was cloned into the pGL3 vector (Promega, Madison, WI, USA) as wild-type (WT) 3′-UTR. Site-direction mutagenesis of the miR-193a-3p binding site in SNHG14 3′-UTR as mutant (MUT) 3′-UTR was conducted through quick-change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). Then, they were used for transfection of BC cells. The luciferase assay was conducted on the dual Luciferase reporter assay system (Promega, Madison, WI, USA). For RIP assay, Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, Billerica, MA, USA) was performed according to the protocol. Then, the RT-qPCR was used to detect Co-precipitated RNAs. Treated BC cells were collected and lysed using RIP lysis buffer containing protease inhibitor and RNase inhibitor. Cells were incubated with the RIP buffer containing magnetic beads coated with Ago2 antibodies (Millipore, Billerica, MA, USA). IgG acted as a negative control (input group). After incubation for 2 h at 4°C, coprecipitated RNAs were isolated and measured by RT-qPCR analysis.

Statistical Analysis

All statistical analyses were carried out using GraphPad Prism 5.0 (La Jolla, CA, USA). The difference between the two groups was compared by the Student *t*-test. The statistical significance was defined as $p<0.05$.

Results

SNHG14 Expression Level in BC Tissues and Cells

Firstly, SNHG14 expression was detected *via* RT-qPCR in 50 patients' tissues and 4 BC cell lines. As a result, SNHG14 was significantly upregulated in BC tissue samples (Figure 1A). The SNHG14 expression level of BC cells was higher than that of MCF-10A (Figure 1B).

Silence of SNHG14 Inhibited Cell Proliferation and Invasion in T-47D BC Cells

In this study, we chose the T-47D BC cell line for the silence of SNHG14. Then, the SNHG14 expression was detected by RT-qPCR (Figure 2A). Moreover, results of CCK-8 assay and colony formation assay showed that the silence of SNHG14 significantly inhibited the ability of cell growth in BC cells (Figure 2B and 2C). The outcome of the transwell assay also revealed that the number of invaded cells was remarkably decreased after SNHG14 was silenced in BC cells (Figure 2D).

Overexpression of SNHG14 Promoted Cell Proliferation nd Invas in SKBR3 BC Cells

In this study, we chose the SKBR3 BC line for the overexpression of SNHG14. The the SNHG14 expression was RT-qPCR (Figure3A). Moreover lts of K-8 assay and colony formatio ay sho the overexpression of SNHG14 significantly promoted the ability of cell growth in BC cells (Figure 3B and 3C). The outcome of the transwell assay also revealed that the number of invaded remarkably increased after SNHG14 overe pressed in BC cells (Figure 3D).

The Interaction Betwee MiR-193a-3p and SNH in BC

DIANA LncBASE cted v.2 (htt olina.imis.athena-in ana_to web/index.php?r=l ndex-p edicted) was used to f the s that ined complementary e with G14. selected miR-193a-3p h contained g area of SNHG14 ( ). RT-qPCR ults showed that upr lated 193a-3p was observed in sh-SNHG14 grou mpared with control gr ure 4B). R R results showed t downregulated miR-193a-3p was observed NHG14 gro compared with empty vector p (Figure 4 urthermore, results of lucif- assay sho that luciferase activity was sig ced through co-transfection of SNHG1 and miR-193a-3p, while no signifi- nt changes of luciferase activity were observed co-transfection of SNHG14-MUT and a-3p (Figure 4D). Meanwhile, RIP assay identified that SNHG14 and miR-193a-3p were significantly enriched in Ago2-containing beads compared to input group (Figure 4E). All these data revealed that miR-193a-3p was a direct target of SNHG14.

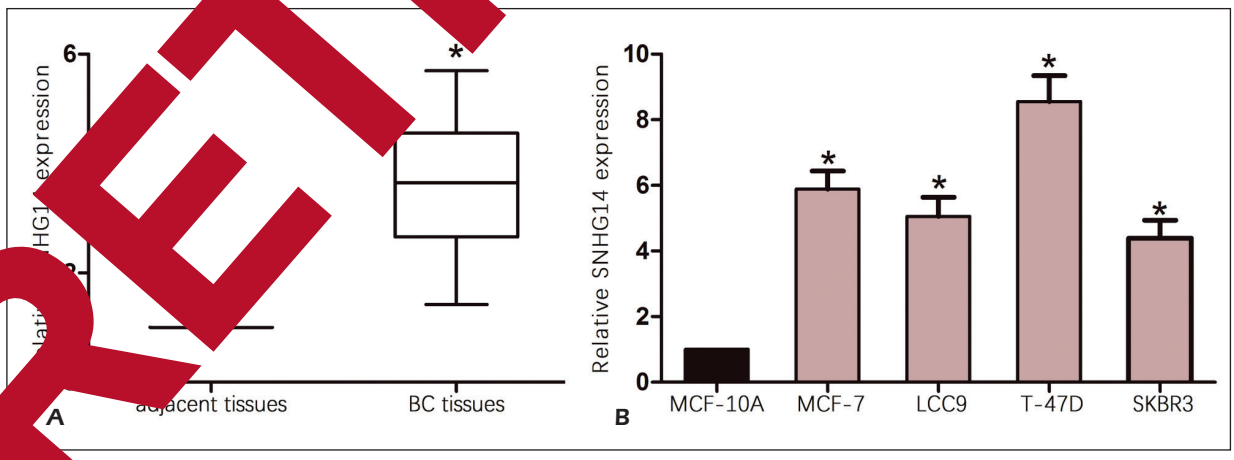

Figure 1. Expression level of SNHG14 was increased in BC tissues and cell lines. **A**, SNHG14 expression was significantly increased in the BC tissues compared with adjacent tissues. **B**, Expression levels of SNHG14 relative to β-actin were determined in the human BC cell lines and MCF-10A (normal human breast cell line) by RT-qPCR. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

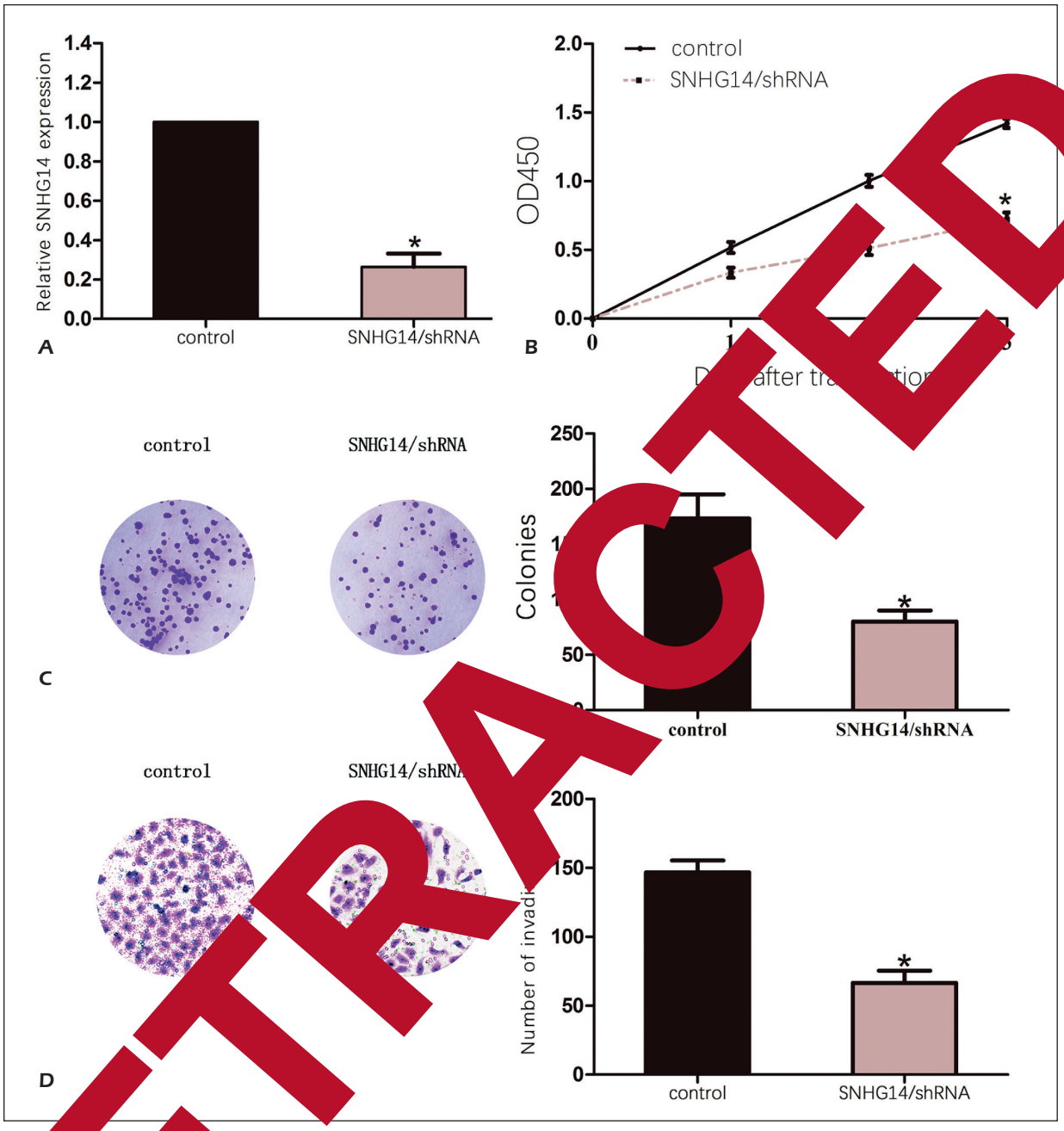

Fig. . Silen SNHG14 inhibited T-47D BC cell proliferation and invasion. **A**, SNHG14 expression in BC cells transduc SN shRNA HG14/shRNA) and the negative control (control) was detected by RT-qPCR. β-actin was used as an in trol. **B**, 8 assay showed that silence of SNHG14 significantly repressed cell growth ability of BC cells. lony on as howed that silence of SNHG14 significantly repressed cell growth ability of BC cells (magnifica-). **D**, say showed that number of invaded cells was significantly decreased via silence of SNHG14 in BC cell gnificati ×). The results represent the average of three independent experiments (mean ± standard error of the mea $p \leq 0.05$, as compared with the control cells.

Discussion

Numerous reports have indicated that lncRNAs are important regulators in the development of BC. For instance, lncRNA SNAR facilitates cell proliferation and metastasis of BC cells and may be a novel therapeutic target[8]. LncRNA UCA1 functioned as an oncogene in BC and modulated cell proliferation and apoptosis by targeting miR-143[9]. LncRNA LINP1 promotes repair of DNA

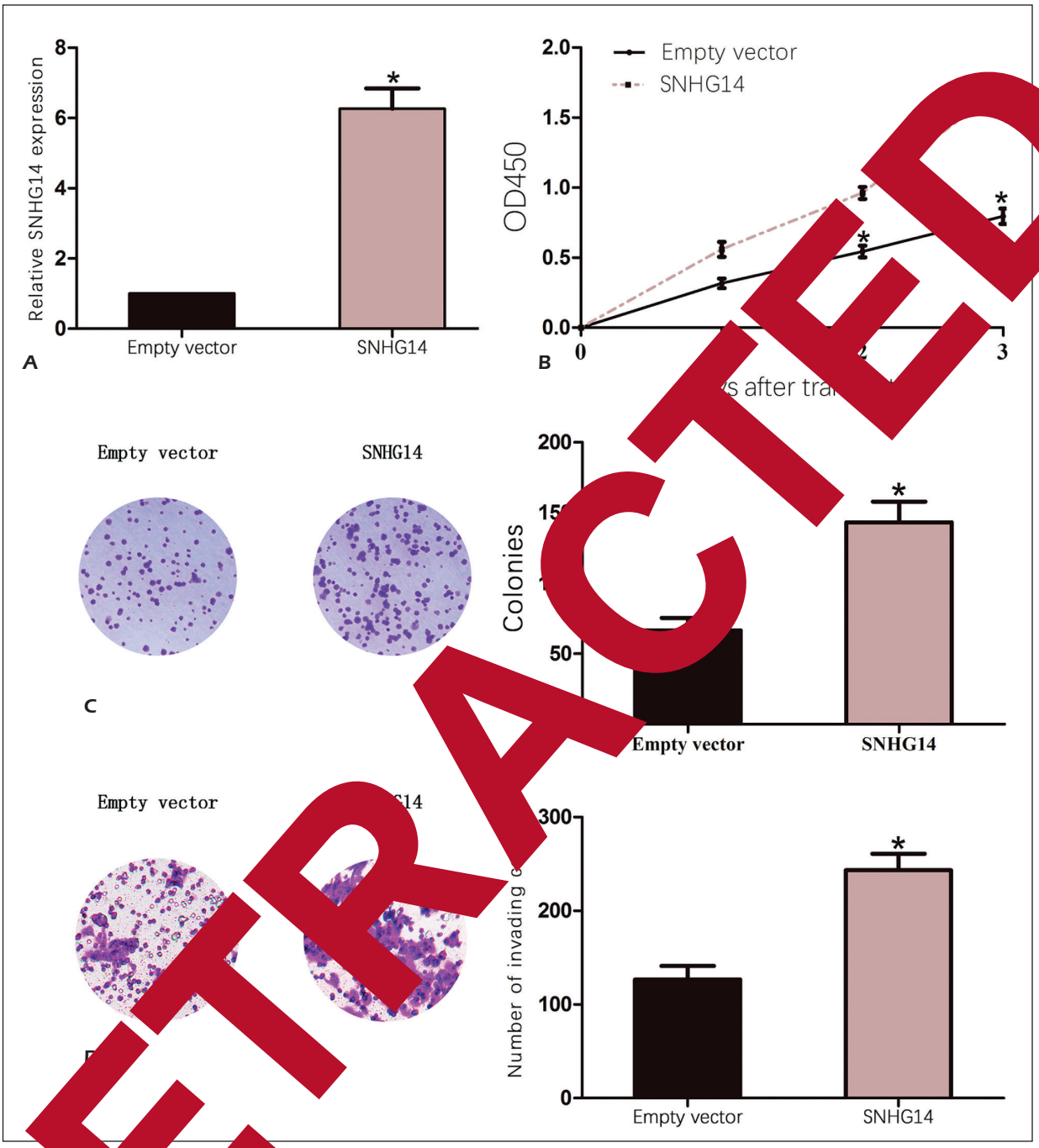

Figure … expressi… SNHG14 promoted SKBR3 BC cell proliferation and invasion. **A**, SNHG14 expression in BC …ransd… …ith S… …14 lentivirus (SNHG14) and the empty vector was detected by RT-qPCR. β-actin was used as an … control… …8 assay showed that overexpression of SNHG14 significantly promoted cell growth ability of BC cells… Colony fo… …on assay showed that overexpression of SNHG14 significantly promoted cell growth ability of BC cells (ma… …ation: 40×). **D**, Transwell assay showed that number of invaded cells was significantly increased via overexpression o… cells (magnification: 40×). The results represent the average of three independent experiments (mean ± …dard erro… the mean). *p<0.05.

dou…-strand breaks and raises the sensitivity of cancer cell to radiotherapy in BC[10]. Moreover, lncRNA MEG3 inhibits angiogenesis and invasion in BC through AKT signal pathway[11]. Downregulated lncRNA linc-ITGB1 suppresses metastasis and aggressiveness of BC[12].

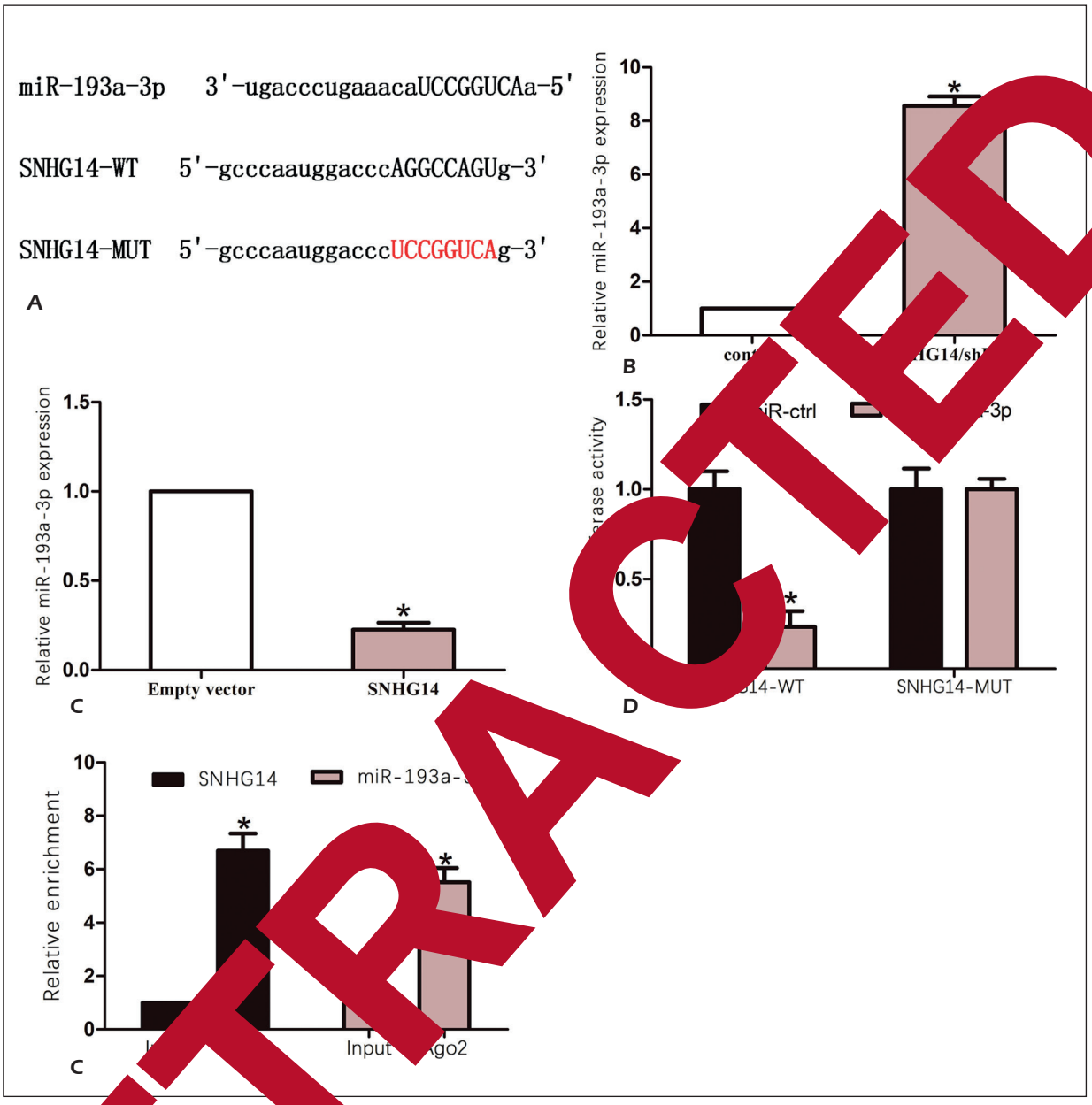

Figure sociation between S 4 and miR-193a-3p in BC cells and tissues. **A**, The binding sites of miR-193a-3p on SNHG , The mi 93a-3p expression was increased in sh-SNHG14 group compared with control group. **C**, The miR-193a-3p e sion wa reased in SNHG14 group compared with empty vector group. **D**, Co-transfection of miR-193a-3p and SNH T gly decr d the luciferase activity, while co-transfection of mir-ctrl and SNHG14-WT did not change the luci vity. **E**, ssay showed the enrichment of SNHG14 and miR-193a-3p Ago2-containing beads. The results sent t ge of independent experiments. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

LncRNA small nucleolar RNA host gene (SNHG14), as a novel lncRNA, is located q11.2. Recent researches[13-15] have indicat that SNHG14 plays an important role in the progression of tumorigenesis. In our study, SNHG14 was upregulated in BC tissues. Besides, the silence of SNHG14 inhibited cell proliferation and invasion in BC cells, while the overexpression of SNHG14 promoted cell proliferation and invasion in BC cells. Above results indicated that SNHG14 promoted tumorigenesis of BC and might act as an oncogene.

To further identify the underlying mechanism of how SNHG14 affects BC cell proliferation and invasion, we predicted and picked miR-193a-3p as the potential binding miRNA of SNHG14 by using bioinformatic analysis and experimental verification. MiR-193a-3p is widely known as a tumor suppressor in many carcinomas which regulates diverse biological processes. For example, miR-193a-3p promotes the development of colorectal cancer *via* targeting KRAS[16]. MiR-193a-3p suppresses metastasis of renal cell carcinoma through regulating PTEN[17]. MiR-193a-3p inhibits the aggressive ability of osteosarcoma cells *via* targeting Rab27B[18]. Yu et al[19] showed that miR-193a-3p modulates the development of BC. In the present work, the miR-193a-3p expression could be upregulated after knockdown of SNHG14, while the miR-193a-3p expression could be downregulated after overexpression of SNHG14. Moreover, miR-193a-3p could directly bind to SNHG14 through a luciferase assay, and miR-193a-3p was significantly enriched by SNHG14 RIP assay. All the results above suggested that SNHG14 might promote tumorigenesis of BC *via* sponging miR-193a-3p.

Conclusions

We found that SNHG14 was significantly upregulated in BC patients. Besides, SNHG14 could facilitate cell proliferation and invasion of BC through sponging miR-193a-3p. These findings suggest that SNHG14 may contribute to the therapy of BC as a candidate target.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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