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LncRNA SNHG8 promotes cell migration and invasion in breast cancer cell through miR-634/ZBTB20 axis

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Abstract. – OBJECTIVE: Small nucleolus RNA Host Gene 8 (SNHG8) belongs to a subgroup of long non-coding RNAs. SNHG8 is upregulated in many cancers, such as gastric cancer, liver cancer, and esophageal squamous cell cancer. However, whether SNHG8 is abnormally expressed in breast cancer and its biological functions remain unclear. Therefore, our research intended to determine the expression status of SNHG8 in breast cancer, explore the effects of SNHG8 on the development of breast cancer, and investigate the potential molecular mechanisms in cancer progression.

PATIENTS AND METHODS: The expression levels of SNHG8 were detected in tissue samples and cell lines via qRT-PCR. The effects of SNHG8 on viability of breast cancer cells were detected via CCK-8, EdU, transwell, and flow cytometry analyses.

RESULTS: qRT-PCR results showed that the expression level of SNHG8 was significantly upregulated in tumor tissues and cell lines. Gene functional studies showed that the downregulation of the expression level of SNHG8 significantly inhibited the breast cancer cells migration and invasion, and induced apoptosis. Meanwhile, we found that SNHG8 served as an inhibitor of miR-634 in tumor tissues. SNHG8 may participate in the malignancy of breast cancer by sponging the miR-634 to increase the expression level of ZBTB20.

CONCLUSIONS: The SNHG8-miR-634-ZBTB20 pathway may be a potential target for the treatment of breast cancers.

Key Words:
Small nucleolus RNA Host Gene 8, MicroRNA-634, Breast cancer, ZBTB20.

Introduction

Breast cancer is a malignant tumor that occurs in breast epithelial tissue[1]. In the past few decades, breast cancer has become the most commonly diagnosed cancer among women, especially in developed countries, breast cancer has become the second leading cause of cancer death in women after lung cancer[1-3]. However, the specific pathogenesis of breast cancer is still unclear[4,5]. Not only genetic and endocrine factors but also the improper expressions of Lnc/miRs and proteins were involved in the occurrence of breast cancer[6,7].

Long non-coding RNAs (lncRNA) have no function of coding proteins[8-10]. LncRNAs regulate gene expression through a variety of mechanisms, including genomic interactions, protein content, miRNA competition, and chromatin modification[9]. Moreover, they play a key roles in various biological and pathological behaviors, particularly in carcinogenesis and cancer progression[10]. LncRNAs have been reported abnormally expressed in breast cancer's progression. In particular, Lnc-UCA1, Lnc-Smad7, and Lnc-LSINCT5 were indicated overexpressed in various breast cancers and play oncogenes roles in the malignant phenotypes[11]. LncRNA SNHG8 has been observed overexpressed in various types of cancer, indicating that it may be participated in the progression of these tumors[12-14]. However, whether lncRNA SNHG8 is abnormal expressed in breast cancer and its underlying molecular mechanisms have not to be clarified. Our research may provide a new theoretical basis for

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understanding the molecular events that cause BC pathogenesis.

Patients and Methods

Collection of Tissue Specimens

All breast cancer tissues and paired peritoneal tissues were obtained from BC patients who underwent surgery at Tangdu Hospital, The Second Affiliated Hospital of Air Force Military Medical University from May 2017 to May 2019. The diagnostic criteria refer to the 2018 edition of the Chinese Breast Cancer Diagnostic and Treatment Guidelines. There were 16 patients in the study (all female) with an average age of 48.8 years old in the study. All protocols about the use of patient samples were approved by the Medical Ethics Committee of Tangdu Hospital, The Second Affiliated Hospital of Air Force Military Medical University. Informed consent was signed by all patients who participated in the study. The research was conducted in accordance with the World Medical Association Declaration of Helsinki.

Cell Lines and Culture Conditions

Human breast cancer cell lines (MCF7, Hs-578T, ZR-75-30, HCC1973) and human normal breast cell MCF-10A were purchased from the Chinese Academy of Sciences, Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). All tissue samples were fixed with 10% neutral formalin. ALL cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, South Logan, UT, USA), and grown in a humidified 37°C incubators supplied with 5% of CO_2. CO_2 cell incubator purchased from Thermo Fisher Scientific (Waltham, MA, USA) and FACS Calibri flow cytometer purchased from BD Biosciences (Franklin Lakes, NY, USA). ABI7300 fluorescence quantitative PCR instrument was purchased from Applied Biosystems Inc. (Foster City, CA, USA).

RNA Extraction and RT-qPCR

We used TRIZOL reagent (Thermo Fisher Scientific, Waltham, MA, USA) to extract total RNA by in cells and tissues. TaqMan probes (Thermo Fisher Scientific, Waltham, MA , USA) were used to quantify miRNAs. Briefly, 1 μg of total RNA was transcribed to cDNA using AMV reverse transcriptase (TaKaRa, Otsu, Shiga, Japan) and a RT primer. The reaction conditions were: 16°C for 30 min, 42°C for 30 min and 85°C for 5 min. Real Time-PCR was performed using a TaqMan PCR kit on an Applied Biosystems 7300 sequence detection systems (Thermo Fisher Scientific, Waltham, MA, USA). The reactions were performed in a 96-well plate at 95 C for 10 min, followed by 40 cycles of 95 C for 10 sec and 60 C for 1 min. U6 was used as the internal control. Primers: LncRNA SNHG8 Forward 5'-AAGTTTACAAGCATGCGCGG-3', Reverse 5'-TCAAACTGACGGTTCTCGGG-3'. MiR-634 Forward 5'-CCCGCACGATTTCATTGAAC-3', Reverse 5'-AGGGCGGATTGGAAATGAAC-3'. ZBTB20 Forward 5'-GTCGGTGGTGTCCGTACAATC-3', Reverse 5'-GTGCCTCTTGGTGTGTCCTG-3'. β-actin Forward 5'-CTCCATCCTGGCCTCGCTGT-3', Reverse 5'-GCTGTCACCTTCACCGTTCC-3'.

Western Blotting Analysis

The samples were cracked in RIPA lysis buffer plus PMSF in low temperature, and BCA assay kit (Santa Cruz Biotechnology, Santa Cruz, CA, USA) detected total protein concentration. The prepared protein samples were separated in SDS-PAGE, and transferred into 0.22 μm polyvinylidene difluoride (PVDF) membranes and incubated with prepared antibodies. Finally, enhanced chemiluminescence (ECL; Thermo Fisher, Waltham, MA, USA) visualized this membrane. Antibodies against HIF1α and GAPDH were purchased from CST (Beverly, MA, USA). Antibodies against Bax, Bcl-2, MMP-2 and MMP-9 were purchased from Abcam (Shanghai, China).

Scratch Assay

When cells reached 90% confluence, a single wound was created and phase-contrast images were digitally photographed immediately and 24 hours after incubation. The original opening distances of the wound were set as 100%. The opening distance after 24 h were measured from three areas randomly selected per well, and the distances in three wells of each group were quantified and normalized by the original opening distance. The experiment was performed three times in triplicate, and the percentage of the migration rate was calculated by measuring the length of cell migration and expressed as a percentage compared to the control group. Migration rates =

(treatment group cell migration distance/control group migration distance) * 100%.

Cell Counting Kit-8 (CCK-8) Assay

24 hours after transfection, the cells were collected and resuspended in culture medium (Syngene, Nanjing, China). A total of 100 µl of a cell suspension containing 2×10^3 cells was seeded into 96-well plates. The cells were incubated in a humidified incubator at 37°C and 5% CO_2 to determine cell proliferation at specific time points (0, 1, 2, and 3 days after seeding). At each time point, 10 µl of CCK-8 solution (Syngene, Nanjing, China) was added to each well, and then, incubated at 37°C for another 2 hours. The absorbance of each well was measured at a wavelength of 450 nm on a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA)[15].

EdU Assay

Cell lines were cultured using EdU (Syngene, Nanjing, China) and fixed with 1 mL 4% paraformaldehyde (Syngene, Nanjing, China) at room temperature for 30 min, then, the cell lines were washed with 3 % BSA twice, 5 min each time, and permeated with 1 mL osmotic agent (0.5 % tritonx-100) to permeate the cytoplasm and nuclear membrane. After 20 min of permeation, each cell line was washed with 3% BSA twice for 5 min each time to remove the osmotic agent. 0.5 ml of the KeyFluor488 (Syngene, Nanjing, China) click-it reaction mixture was added to each culture dish and incubated at room temperature, for 30 min in darkness. Click-it reaction mixture was discarded, and the reaction mixture was rinsed with 3% BSA twice, 5 min each time. The reaction liquid was removed, add antifluorescence quenching agent and observed with a fluorescence microscope (Thermo Fisher Scientific, Waltham, MA, USA).

Transwell and Invasion Assay

Cell invasion was checked in a 24-well transwell chamber (8 µm). First, we covered the back of the upper chamber with Matrigel (10 µg/mL; BD Biosciences, Franklin Lakes, NJ, USA) and coated 70 µL Matrigel (1 mg/mL) on the surface. Breast cancer cells (5×10^4) in 200 µl of FBS-free medium were inoculated in the upper chamber, and 700 µL of complete medium (containing 10% FBS) was added to the bottom chamber. After incubating for 24 hours, cells entering the lower surface were gently washed and fixed with methanol for 10 minutes, and then, stained with 0.1% crystal violet for 10 minutes. Five random areas were observed from the membrane, and the number of migrated cells was counted and photographed under an inverted phase contrast microscope (Olympus, Tokyo, Japan). The migratory and invasive abilities were measured by counting respectively the migratory and invading cells[16].

Luciferase Reporter Assay

PMIR-SNHG8-3'-UTR-WT, pMIR-SNHG8-3'-UTR-Mut, as well as pMIR-ZBTB20-3'-UTR-WT, pMIR-ZBTB20-3'-UTR-Mut Luciferase reporter plasmids, were constructed by Synthgene Biotechnology (Nanjing, China). The sequences that could be binding to miR-634 were partly mutated and inserted into the reporter plasmid in order to identify the binding specificity. The implementation method refers to a previous study. Briefly, MCF7 and ZR-75-30 cells were seeded in 24 well plates until reaching 60% confluence. Each well was co-transfected with luciferase reporter plasmids (0.5 µg) and RNA mimics (100 mol) using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. The Luciferase activity was measured after 48 h of transfection, by using the Dual-Luciferase Reporter Assay (Promega, Madison, WI, China) according to the manufacturer's instructions and normalized to Renilla signals.

Overexpression Plasmid Construction

SNHG8 and ZBTB20 knockdown were accomplished by transforming cells with shRNA. SNHG8, ZBTB20 and control shRNA were synthesized by Synthgene (Nanjing, China). Control plasmid (pCMV6) and over expression plasmid (pCMV6-SNHG8/ZBTB20) was purchased from Synthgene (Nanjing, China). All cells were transferred with them using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instruction. Total protein was extracted after transfection for 24 h and 48 h, respectively.

Statistical Analysis

All experiments were repeated three times and the data are presented as the mean standard deviation using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA and post-hoc Dunnett's T3 test were performed in order to compare the differences among and between groups, respectively. $p<0.05$ was considered to indicate a statistically significant result.

Results

The Expression Level of SNHG8 is Upregulated both in Breast Cancer Tissues and its Cell Lines

In order to evaluate the potential expression of SNHG8 in breast cancer, we first measured its expression level in 16 pairs of breast cancer and normal tissues *via* qRT-PCR. The data indicate that the expression level of SNHG8 is expressed in tumor tissues at a higher level (Figure 1A). On the other hand, we also detected the SNHG8 expression in breast cancer cell lines, including MCF7, Hs-578T, ZR-75-30 and HCC1937 cells, and normal breast cell MCF-10A served as the control. The qRT-PCR data indicated that SNHG8 expression was upregulated in all tumor cell lines compared to MCF-10A cells, especially in MCF-7 and ZR-75-30 cell lines (Figure 1B). All these outcomes suggest that the upregulation of SNHG8 is correlated to the progression of breast cancer.

Knockdown of SNHG8 Inhibited Breast Cancer Cells Proliferation, Invasion, Migration and Promoted Apoptosis

In order to study the function of SNHG8 in breast cancer progression and its role in tumor development, we transformed MCF-7 and ZR-75-30 cell lines with SNHG8 transient knockdown shRNA (Figure 2A). The qRT-PCR data showed that compared with the NC group, the expression of SNHG8 in MCF-7 and ZR-75-30 cells was significantly downregulated after transfection. Then, we used EdU, CCK-8, scratch healing, transit, and flow cytometry tests to evaluate tumor cells proliferation, invasion, migration and apoptosis, respectively. As shown in Figure 2B-C and Figure 3A-B, compared with the NC group, MCF-7/sh-SNHG8 and ZR-75-30/sh-SNHG8 cells' cytoactive and invasive abilities were significantly inhibited. In addition, after transacting with sh-SNHG8, tumor cell apoptosis significantly increased.

Meanwhile, the expression level of apoptosis related proteins were evaluated *via* Western blotting test. The data showed that Bax expression was downregulated in both cell lines, but the expression level of Bcl-2 is opposite (Figure 2D). Similarly, the expression levels of MMP-2 and MMP-9 proteins related with cells migration and invasion were detected (Figure 3C). The data indicated that MMP-2 and MMP-9 expressions were downregulated at the same time.

SNHG8 is Directly Attached to MiR-634 in Breast Cancer Cells

As we all know, lncRNAs have many important functions in the body, and suppressing the expression of RNA is one of them. Therefore, we want to know whether SNHG8 can bind to miR-634 and inhibit miR-634 expression. By searching StarBase (http://starbase.sysu.edu.cn) we found that miR-634 has a binding site for SNHG8 (Figure 4A-B). We first detected the expression level of miR-634 in breast cancer

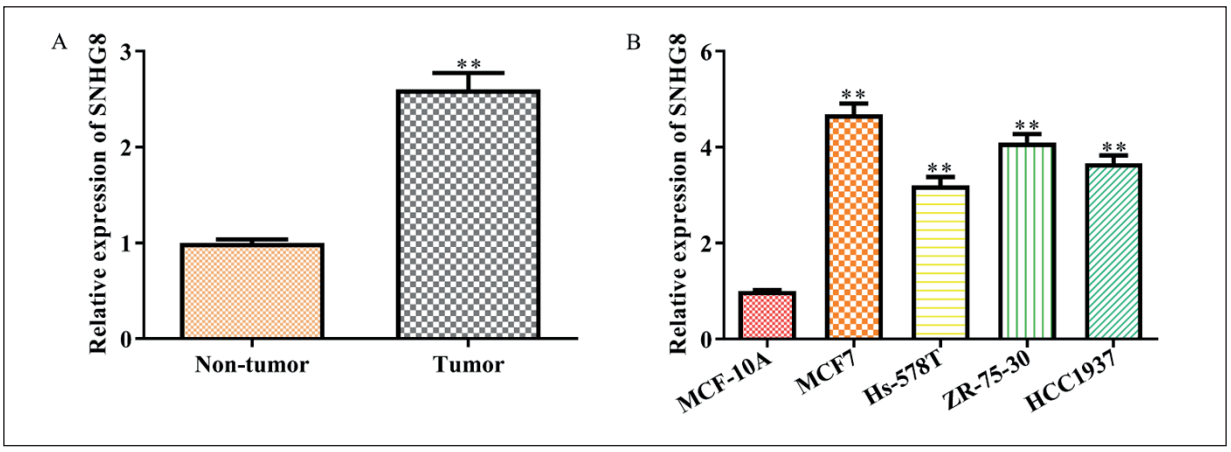

Figure 1. The expression level of lncRNA SNHG8 is upregulated in breast cancer tissues and cell lines. **A,** The expression level of lncRNA SNHG8 in tumor and para-carcinoma tissues. **B,** The expression level of lncRNA SNHG8 in normal breast cells and breast cancer cell lines. Asterisks indicate significant differences from the control (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Student's *t*-test, compared to NC group).

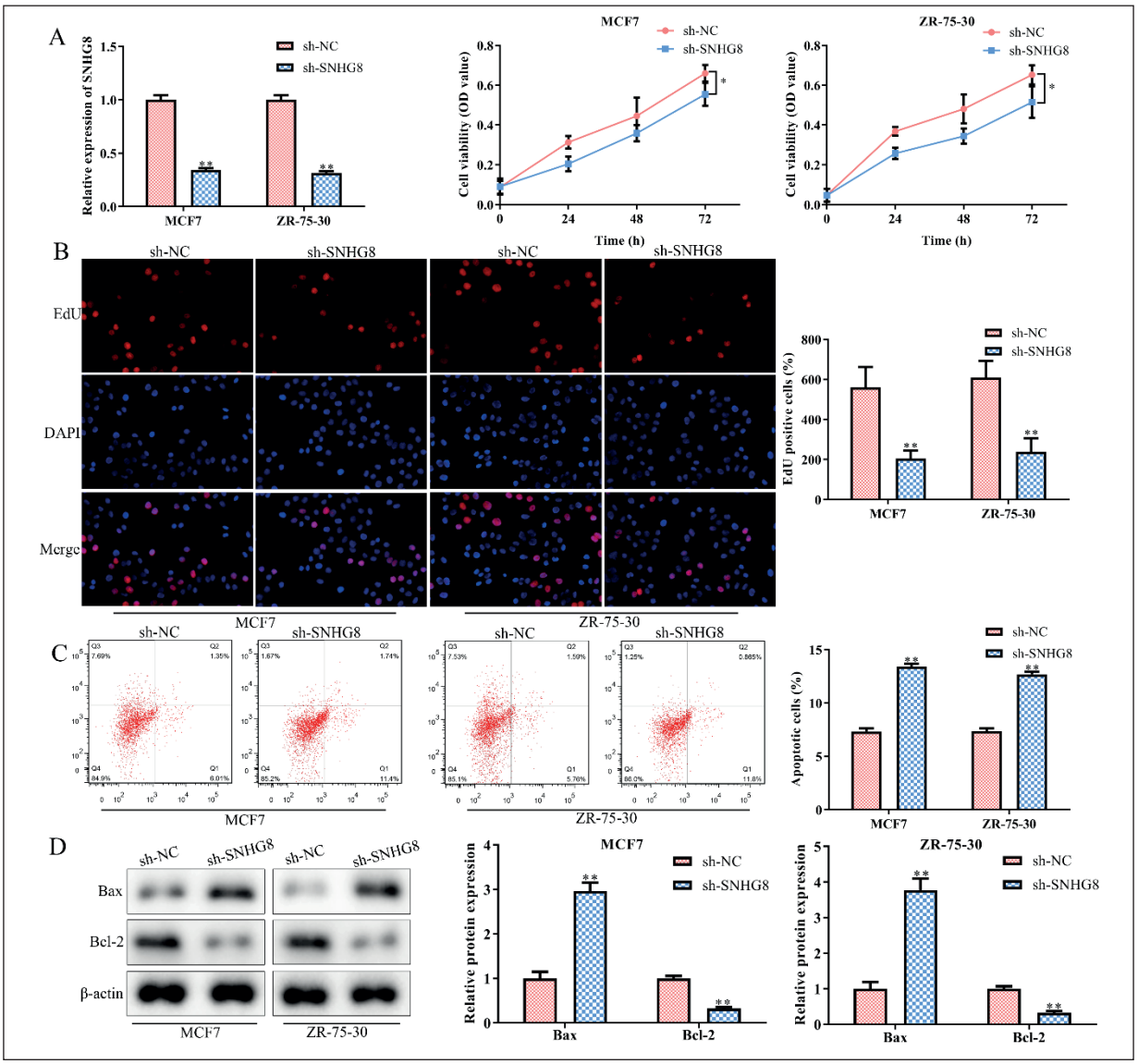

Figure 2. Knockdown of LncRNA SNHG8 inhibited proliferation in breast cancer cells. **A,** The expression level of lncRNA SNHG8 was monitored after transfection with lncRNA SNHG8 mimic by exploring qRT-PCR in MCF-7 and ZR-75-30 cells. **B,** EdU assay was used to detecte the cells' proliferation between NC and SNHG8 groups in breast cancer cells. **C,** Apoptosis of breast cancer cells after transfected with lncRNA SNHG8 (flow cytometry) (× 200). **D,** The expression level of apoptosis related proteins were detected (Western blot) in breast cancers cells between NC and SNHG8 groups. Asterisks indicate significant differences from the control (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Student's *t*-test, compared to NC group).

and normal tissues, the date indicates that the expression of miR-634 in tumor tissues is down-regulated compared to normal tissues (Figure 4C) and in all breast cancer cell lines, the same results are shown in the system (Figure 4D-E). Luciferase reporter gene detection showed that miR-634 inhibited the Luciferase activity of the WT SNHG8 mimic, but it did not inhibit the MUT mimic, indicating that SNHG8 can attach to the miR-634 binding site and inhibit the miR-634 expression. These results demostrate that SNHG8 can directly bind to miR-634, and inhibit miR-634 expression.

Overexpression MiR-634 Suppressed Cell Invasion and Migration in Breast Cancer

In order to investigate the function of miR-634 in breast cancer progression, we transacted

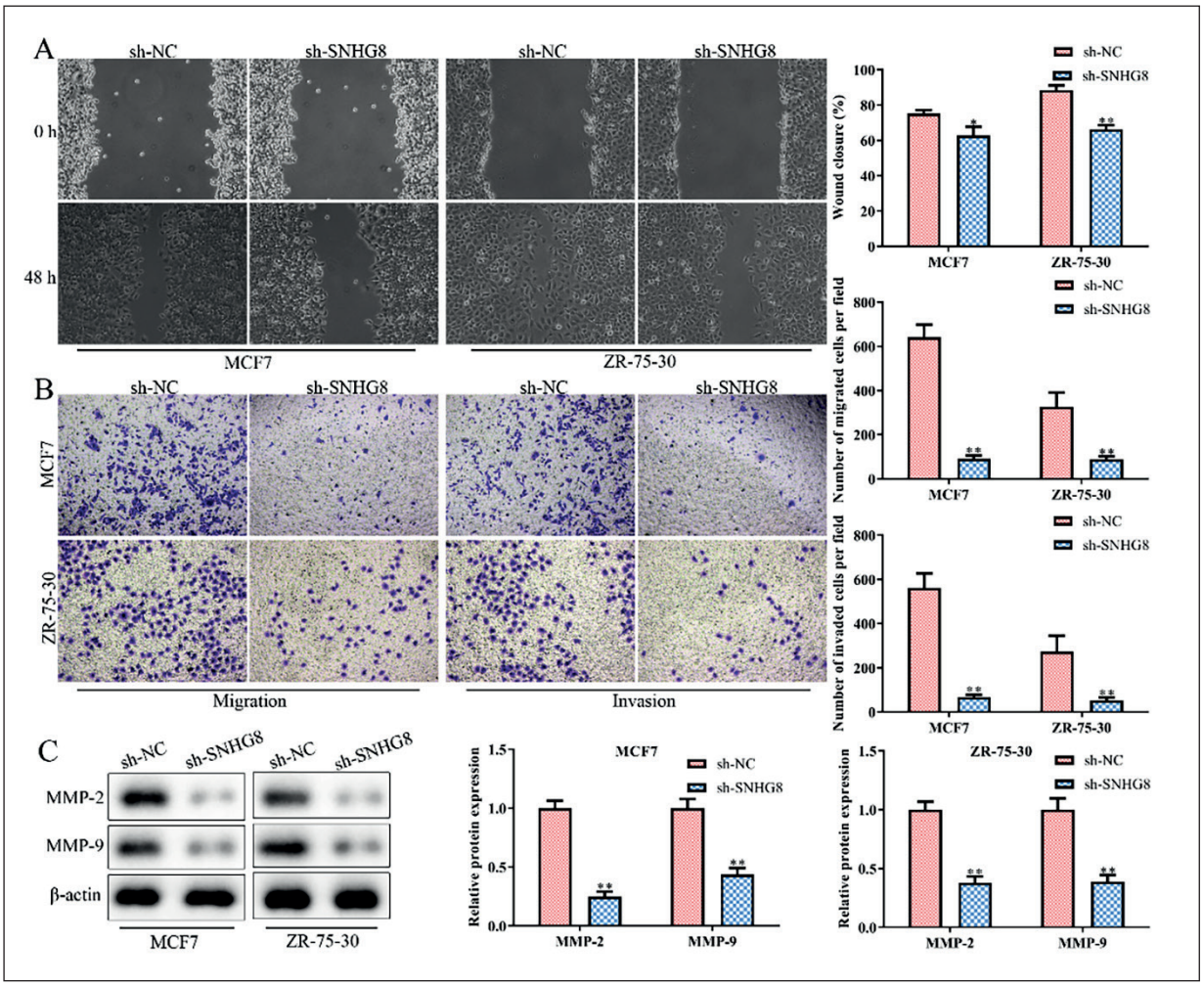

Figure 3. Downregulating the repression level of LncRNA SNHG8 inhibited the migration and invasion in breast cancer cells. **A,** Cells capacity of migration (scratch test) (x 200). **B,** The ability of migration and invasion in breast cancer cells were detected by transwell test (× 200). **C,** The expression level of migration related proteins were test *via* Western blot. Asterisks indicate significant differences from the control (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Student's *t*-test, compared to NC group).

NC-mimics and miR-634 mimics in breast cancer cell lines (MCF9 and ZR-75-30). As shown in Figure 5A-B, compared with the NC mimic group, the expression of miR-634 in cancer cells was significantly upregulated, but the cell viability was downregulated. After that, we used EdU, transit and flow cytometry tests to assess tumor cells proliferation, migration, apoptosis, and invasion. As shown in Figure 5C-E, cells abilities in proliferation, invasion and migration were significantly suppressed after transacted with miR-634 mimics. On the other hand, MCF7/miR-634 mimics and ZR-75-30/miR-634 mimics groups cells apoptosis significantly increased.

SNHG8 Promoted Proliferation and Invasion of Breast Cancer Cells by Targeting the miR-634/ZBTB20 Pathway

The abnormal expression of miR-634 and ZBTB20 were found responsible for many tumors, such as gastric tumor and liver cancer. In our research, we found that the expression level of ZBTB20 was upregulated in breast cancer cell lines, but decreased after overexpressing miR-634 (Figure 6C-E). As mentioned in the previous paragraphs, we also found that miR-634 has a binding site for ZBTB20 (Figure 6A-B). As shown in Figure 7A, MCF7/sh-SNHG8 cells viability increased after transacted with miR-634 mimics and silenced ZBTB20 can offset the

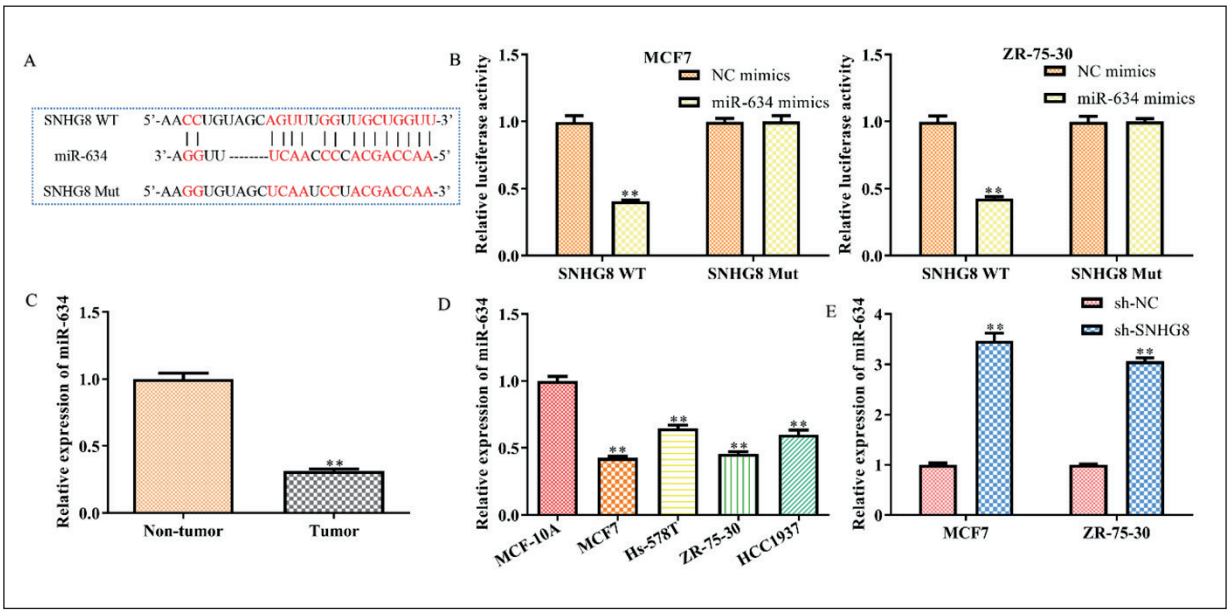

Figure 4. LncRNA SNHG8 is a target gene of miR-634. **A,** The predicted SNHG8 binding sites in the region of miR-634 and the corresponding mutant sequence was shown. **B,** Relative values of luciferase signal. **C** The expression level of miR-634 both in tumor and normal breast tissues. **D,** The expression level of miR-634 in normal breast cells and breast cancer cell lines. **E,** The expression level of miR-634 both in MCF7 and R-75-30 cells after transfected with NC mimics and SHNG8 minics respectively. Asterisks indicate significant differences from the control (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Student's *t*-test, compared to NC group).

effects. EdU, transit and flow cytometry tests' results (Figure 7B-D) showed the same conclusion that SNHG8 promoted proliferation and invasion of breast cancer cells by targeting the miR-634/ZBTB20 pathway.

Discussion

Besides skin cancer, breast cancer is the most common cancer in women worldwide. Over the past few decades, studies[17-19] have shown that a large amount of lncRNA is abnormally expressed in breast cancer. Therefore, elucidating the role of lncRNA in breast cancer progression is important for the diagnosis and clinical treatment of breast cancer.

In this study, we first detected the expression level of lncRNA SNHG8 in breast cancer tissues and a group of breast cancer cell lines. In addition, the effects of SNHG8 inhibition on the malignant biological characteristics of breast cancer cell lines, including cell viability, migration, invasion and apoptosis, were also examined. Our research indicates that the downregulation of SNHG8 expression inhibits the proliferation and invasion of breast cancer cells, suggesting that SNHG8 has a role in promoting tumor growth in breast cancer.

In cancer research, lncRNA SNHGs can reverse the effects induced by miRNAs in tumor progression[20-22] and it has been observed that UCA1 is implicated in tumor progression by sponging different miRNAs such as miR-26a/b, miR-193a and miR-214 in gastric cancer[23]. At present, the expression level of miR-634 has been clearly showed downregulated in many tumors' tissues[24-26]. Upregulating the expression level of miR-634 effectively decreased the invasion and proliferation activities of tumor cells[24,26,27].

In this research, we found that SNHG8 is a target gene of miR-634. In addition, the expression of miR-634 in breast cancer tissues and cell lines was downregulated and negatively correlated with the expression of SNHG8. Luciferase reporter assays revealed that miR-634 can bind to the 3′-UTR of SNHG8 and decrease its Luciferase activity of MCF-7/SNHG8WT and ZR-75-30/SNHG8WT cell lines. On the other hand, we observed that miR-634 overexpression can inhibited the viability and invasion in breast cancer cell lines. These results indicate that SNHG8 acts as a

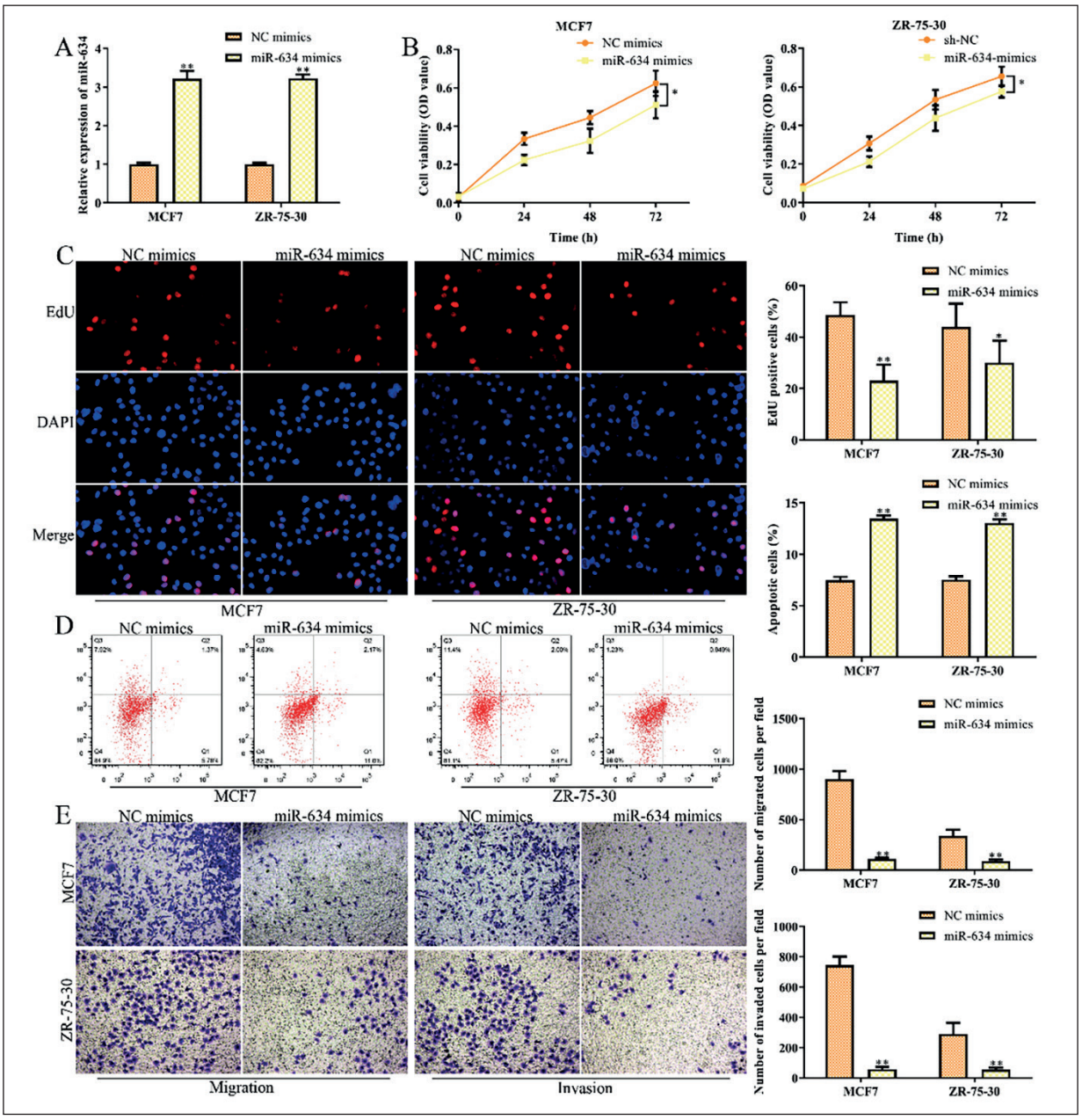

Figure 5. Upregulating the expression level of miR-634 inhibited the migration and invasion in breast cancer cells. **A,** The expression level of miR-634 in breast cancer cells after transfected with miR-634 mimic. **B,** The cells viability after transfection with miR-634 mimic in breast cancer cells (CKK-8 assay). **C,** EdU test was used to detect the breast cancer cells' proliferation ability in both NC and miR-6354 mimic groups (x 200). **D,** Apoptosis of breast cancer cells after transfected with miR-634 (flow cytometry). **E,** The ability of migration and invasion in breast cancer cells were revealed by transwell test (x 200). Asterisks indicate significant differences from the control (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Student's *t*-test, compared to NC group).

sponge for miR-634 to promote breast cancer cell viability and invasion.

MiR-634 has been proved to be a new tumor suppressor, which plays an important role in inducing tumor cells apoptosis, especially in liver cancer and hypophysoma[28-30]. In nasopharyngeal carcinoma, it upregulates cell proliferation by targeting p-MEK and p-ERK[31]. It is worth noting that ZBTB20 was predicted to be a target gene of miR-634 by bioinformatics. In the current study, we discovered that miR-634 was downregulated in breast cancer tissues and cell lines, and had a

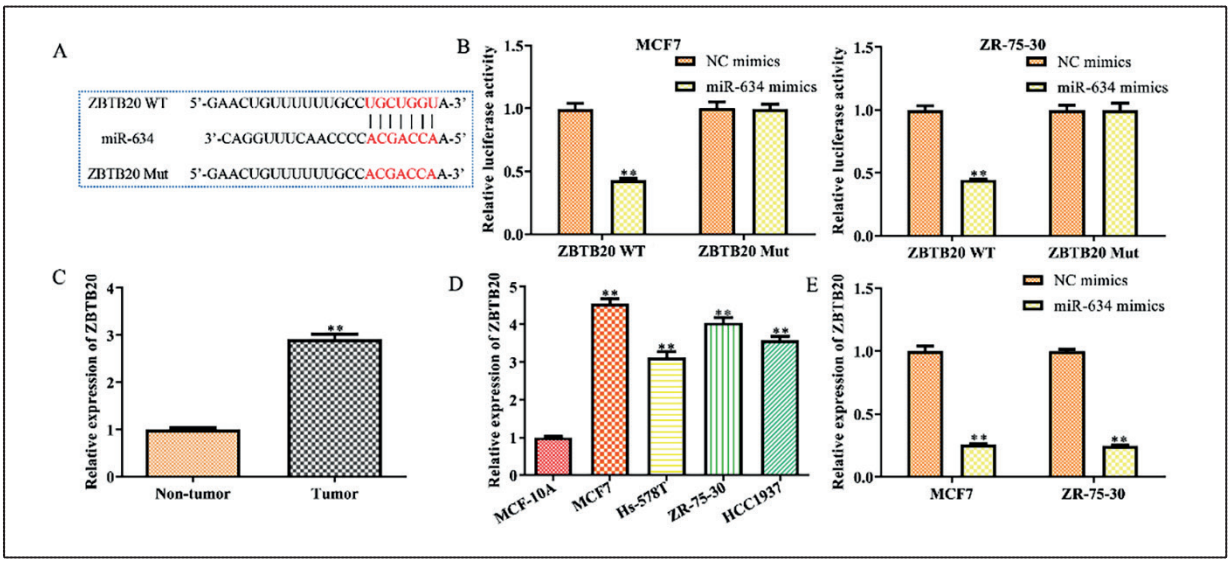

Figure 6. MiR-634 is a target gene of ZBTB20. **A,** The predicted mir-634 binding sites in the region of ZBTB20 and the corresponding mutant sequence was shown. **B,** Relative values of luciferase signal. **C,** The expression level of ZBTB20 in breast cancer and normal breast tissues. **D,** The expression level of ZBTB20 in normal breast cells and breast cancer cell lines. **E,** The expression level of ZBTB20 in MCF7 and R-75-30 cells after transfected with NC mimics and miR-634 mimics respectively. Asterisks indicate significant differences from the control (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Student's *t*-test, compared to NC group).

negative correlation with ZBTB20 expression but a positive correlation with SNHG8 expression. MiR-634 inhibitors can increase cell proliferation and invasion, but upregulated ZBTB20 expression, while the miR-634 mimic can inhibited these effects and downregulated ZBTB20 expres-

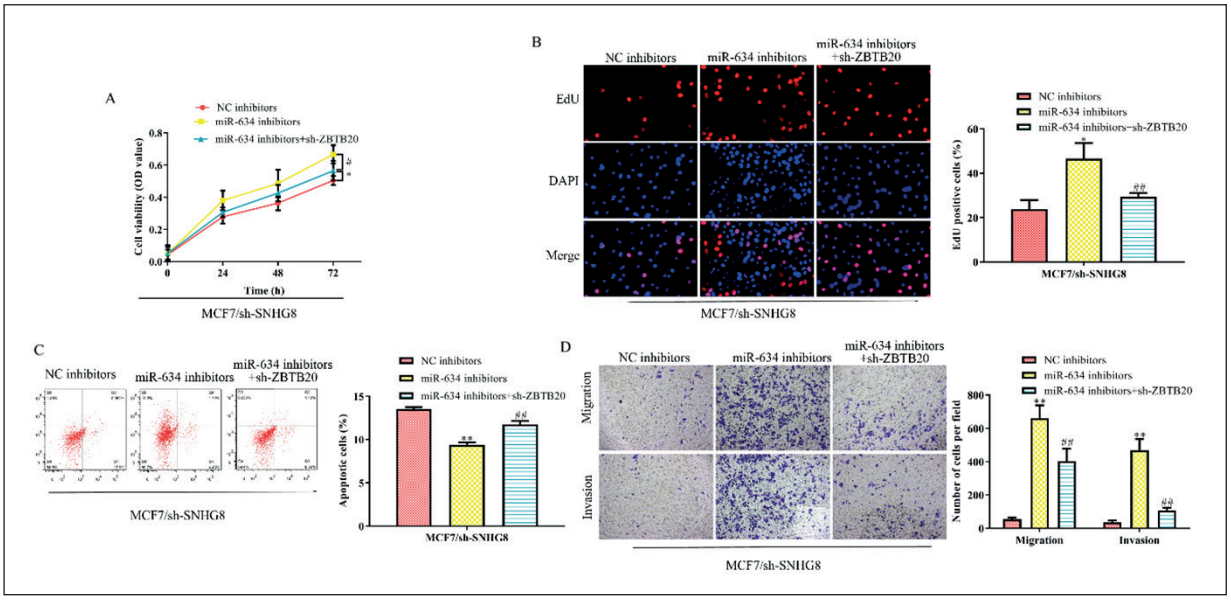

Figure 7. SNHG8 promotes breast cancer cells by targeting the miR-634/ZBTB20 axis. **A,** Cells viability were compared between NC, miR-634 inhibitors and miR-634 inhibitors+sh-ZBTB20 groups in MCF/sh-SNGH8 cells. **B,** EdU assay was applied to explore difference in cells proliferation between NC, miR-634 inhibitors and miR-634 inhibitors+sh-ZBTB20 groups in MCF/sh-SNGH8 cells (x 200). **C-E,** The apoptosis, migration and invasion ability of MCF7/sh-SNGH8 cells were evaluated by the flow cytometry, transwell migration and invasion assays, respectively (x 200). Asterisks indicate significant differences from the control (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Student's *t*-test, compared to NC group).

sion in breast cancer cell lines. These results suggest that SNHG8 may act as a sponge for miR-634, resulting in increased expression of ZBTB20 and promoting breast cancer progression.

Conclusions

Our research demonstrated that the expression level of SNHG8 is upregulated in breast cancer tissues, as well as cell lines. Moreover, the knockdown of SNHG8 expression inhibits breast cancer cell proliferation and invasion. In addition, we found that miRNA-634 is downregulated in breast cancer tissues, while upregulated expression of miR-634 inhibits breast cancer cell progression induced by SNHG8. Further investigation revealed that SNHG8 promotes the proliferation and invasion of breast cancer cells by targeting the miR-634/ZBTB20 axis. Due to the limitation of the study, we failed to clarify the specific relationship between SNHG8 expression and breast cancer TNM stage and distant metastasis. Related capacity is worthy of further study in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020; 70: 7-30.
2) Konner M. Progress in the treatment of breast cancer. N Engl J Med 2020; 382: e4.
3) Rozeboom B, Dey N, De P. ER+ metastatic breast cancer: past, present, and a prescription for an apoptosis-targeted future. Am J Cancer Res 2019; 9: 2821-2831.
4) Solinas C, Aiello M, Migliori E, Willard-Gallo K, Emens LA. Breast cancer vaccines: heeding the lessons of the past to guide a path forward. Cancer Treat Rev 2019; 84: 101947.
5) Banik K, Ranaware AM, Choudhary H, Thakur N, Girisa S, Deshpande V, Fan L, Nalawade SP, Sethi G, Kunnumakkara AB. Piceatannol: a natural stilbene for the prevention and treatment of cancer. Pharmacol Res 2020; 153: 104635.
6) Akhade VS, Pal D, Kanduri C. Long noncoding RNA: genome organization and mechanism of action. Adv Exp Med Biol 2017; 1008: 47-74.
7) Luo N, Zhang K, Li X, Hu Y. ZEB1 induced-upregulation of long noncoding RNA ZEB1-AS1 facilitates the progression of triple negative breast cancer by binding with ELAVL1 to maintain the stability of ZEB1 mRNA. J Cell Biochem 2020. doi: 10.1002/jcb.29572. [Epub ahead of print].
8) Guo S, Jian L, Tao K, Chen C, Yu H, Liu S. Novel breast-specific long non-coding RNA LINC00993 acts as a tumor suppressor in triple-negative breast cancer. Front Oncol 2019; 9: 1325.
9) Li J, Li Z, Zheng W, Li X, Wang Z, Cui Y, Jiang X. LncRNA-ATB: an indispensable cancer-related long noncoding RNA. Cell Prolif 2017; 50. doi: 10.1111/cpr.12381.
10) Ferre F, Colantoni A, Helmer-Citterich M. Revealing protein-lncRNA interaction. Brief Bioinform 2016; 17: 106-116.
11) Liu Y, Sharma S, Watabe K. Roles of lncRNA in breast cancer. Front Biosci (Schol Ed) 2015; 7: 94-108.
12) Zhang P, Li S, Chen Z, Lu Y, Zhang H. LncRNA SNHG8 promotes proliferation and invasion of gastric cancer cells by targeting the miR-491/PDGFRA axis. Hum Cell 2020; 33: 123-130.
13) Song Y, Zou L, Li J, Shen ZP, Cai YL, Wu XD. LncRNA SNHG8 promotes the development and chemo-resistance of pancreatic adenocarcinoma. Eur Rev Med Pharmacol Sci 2018; 22: 8161-8168.
14) Dong J, Teng F, Guo W, Yang J, Ding G, Fu Z. LncRNA SNHG8 promotes the tumorigenesis and metastasis by sponging miR-149-5p and predicts tumor recurrence in hepatocellular carcinoma. Cell Physiol Biochem 2018; 51: 2262-2274.
15) Zhuo LA,Wen YT,Wang Y, Liang ZF,Wu G, Nong MD, Miao L. LncRNA SNHG8 is identified as a key regulator of acute myocardial infarction by RNA-seq analysis. Lipids Health Dis 2019; 18: 201.
16) Yamamoto-Shimojima Keiko, Imaizumi Taichi, Akagawa Hiroyuki, Kanno Hitoshi, Yamamoto Toshiyuki. Primrose syndrome associated with unclassified immunodeficiency and a novel ZBTB20 mutation. Am J Med Genet A 2020; 182: 521-526.
17) Zhang L, Yan X, Yu S, Zhong X, Tian R, Xu L, Bian X, Su J. LINC00365-SCGB2A1 axis inhibits the viability of breast cancer through targeting NF-kappaB signaling. Oncol Lett 2020; 19: 753-762.
18) Zhu Q, Li Y, Dong X, Yang Y, Wang H, Guo S. LincOIP5 loss regulates migration and invasion in MDA-MB-231 breast cancer cells by inhibiting YAP1/JAG1 signaling. Oncol Lett 2020; 19: 103-112.
19) Qiu P, Dou Y, Ma LZ, Tang XX, Liu XL, Chen JW. Long non-coding RNA TTN-AS1 promotes the metastasis in breast cancer by epigenetically activating DGCR8. Eur Rev Med Pharmacol Sci 2019; 23: 10835-10841.
20) Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing miRNA-lncRNA interactions. Methods Mol Biol 2016; 1402: 271-286.
21) Chen L, Zhou Y, Li H. LncRNA, miRNA and lncRNA-miRNA interaction in viral infection. Virus Res 2018; 257: 25-32.

22) Jiang S, Liu Y, Xu B, Zhang Y, Yang M. Noncoding RNAs: new regulatory code in chondrocyte apoptosis and autophagy. Wiley Interdiscip Rev RNA 2020; 10: e1584.

23) Gong P, Qiao F, Wu H, Cui H, Li Y, Zheng Y, Zhou M, Fan H. LncRNA UCA1 promotes tumor metastasis by inducing miR-203/ZEB2 axis in gastric cancer. Cell Death Dis 2018; 9: 1158.

24) Fujiwara N, Inoue J, Kawano T, Tanimoto K, Kozaki K, Inazawa J. MiR-634 activates the mitochondrial apoptosis pathway and enhances chemotherapy-induced cytotoxicity. Cancer Res 2015; 75: 3890-3901.

25) van Jaarsveld MT, van Kuijk PF, Boersma AW, Helleman J, van IWF, Mathijssen RH, Pothof J, Berns EM, Verweij J, Wiemer EA. MiR-634 restores drug sensitivity in resistant ovarian cancer cells by targeting the Ras-MAPK pathway. Mol Cancer 2015; 14: 196.

26) Tan Z, Zhao J, Jiang Y. MiR-634 sensitizes glioma cells to temozolomide by targeting CYR61 through Raf-ERK signaling pathway. Cancer Med 2018; 7: 913-921.

27) Peng X, Cao P, He D, Han S, Zhou J, Tan G, Li W, Yu F, Yu J, Li Z, Cao K. MiR-634 sensitizes nasopharyngeal carcinoma cells to paclitaxel and inhibits cell growth both in vitro and in vivo. Int J Clin Exp Pathol 2014; 7: 6784-6791.

28) Xu D, Yu J, Gao G, Lu G, Zhang Y, Ma P. LncRNA DANCR functions as a competing endogenous RNA to regulate RAB1A expression by sponging miR-634 in glioma. Bioscience reports 2018; 38: BSR20171664.

29) Shao B, He L. Hsa_circ_0001742 promotes tongue squamous cell carcinoma progression via modulating miR-634 expression. Biochem Biophys Res Commun 2019; 513: 135-140.

30) Guo J, Zhang CD, An JX, Xiao YY, Shao S, Zhou NM, Dai DQ. Expression of miR-634 in gastric carcinoma and its effects on proliferation, migration, and invasion of gastric cancer cells. Cancer Med 2018; 7: 776-787.

31) Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y, Ma J. Nasopharyngeal carcinoma. Lancet 2019; 394: 64-80.