LncRNA SNHG7 inhibits proliferation and invasion of breast cancer cells by regulating miR-15a expression

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Summary

Purpose: To explore the inhibition of proliferation and invasion of breast cancer cells by LncRNA SNHG7 via regulating the expression of miR-15a and its mechanism.

Methods: The expression of SNHG7 in breast cancer and adjacent tissues and different breast cancer cells was measured by qRT-PCR and the relationship between SNHG7 and clinicopathological parameters of breast cancer patients was analyzed. The interaction of SNHG7 with miR-15a was explored by dual-luciferase reporter assay. The change in the proliferation of breast cancer cells after silencing SNHG7 was examined by cell proliferation assay. The change in the invasion of breast cancer cells after silencing SNHG7 was examined by Transwell invasion assay. Subcutaneous tumor formation in nude mice was detected to record the tumor size and volume of tumor cells.

Results: Compared with adjacent normal tissues, the expression of SNHG7 was significantly increased in breast cancer tissues; the expression of SNHG7 was the highest in breast cancer cells MCF7 and T47D; the expression level of SNHG7 was not notably different among breast cancer patients of different genders and age groups, and the difference was not statistically significant (p>0.05). The expression level of SNHG7 was higher in patients with a higher stage of breast cancer and patients with lymph node metastasis, and the difference was statistically significant. SNHG7 could specifically bind to the 3' UTR of miR-15a. The inhibition of SNHG7 led to constrained proliferation and invasion of breast cancer cells. The tumor volume and weight of the tumor-bearing mice in the si-SNHG7 group were significantly lower than those in the non-specific control (NC) group.

Conclusion: SNHG7 plays an important role in the development of breast cancer. SNHG7 can affect the proliferation and invasion of breast cancer cells through its targeted regulation of miR-15a activity.

Key words: SNHG7, breast cancer, miR-15a, transwell assay

Introduction

Breast cancer is a malignant tumor that originates in breast tissue and the most common cause of malignant tumors-related death in women worldwide [1,2]. Although many molecular inductive factors have been found to be crucial in the development of breast cancer, a number of breast cancer patients show no response to the initial drug chemotherapy. So the exploration of the potential molecular mechanism of breast cancer is currently a research hotspot.

Long non-coding RNA (lncRNA) is a transcribed RNA with more than 200 nucleotides in length but has no notable potential for protein-coding [3]. LncRNA is regarded as a major component of the human genome transcriptome, but little is known about the mode and mechanism of...
action of many lncRNA molecules. LncRNA regulates gene expression and is involved in various biological processes, including regulation of proliferation [4], migration [5], and apoptosis [6]. The specific lncRNAs that play a key regulatory role in the progression of tumor cells are not identified. The expression disorder of these lncRNAs may lead to the pathogenesis of major diseases, especially malignant tumors. Therefore, lncRNAs may inspire new diagnostics and treatments of diseases.

MicroRNAs play different roles in tumorigenesis and progression of breast cancer [7,8]. MicroRNAs (miRs) can act as regulators of oncogenes, tumor suppressors, tumor proliferation and invasion, cell apoptosis, and resistance to therapy [9]. There is increasing evidence that miR-15a is involved in carcinogenesis. The expression of miR-15a is markedly down-regulated in glioma [10], medulloblastoma [11], oral squamous cell carcinoma [12], hepatocellular carcinoma [15], bladder cancer [14] and breast cancer [15]. However, the molecular mechanism by which miR-15a regulates the malignant phenotype of breast cancer cells is unclear.

In this study, lncRNA SNHG7 expression was found to be up-regulated in breast cancer tissues compared to adjacent normal tissues. Abnormal expression of SNHG7 in breast cancer cell lines can directly regulate the expression of miR-15a and thus affect the biological behavior of breast cancer cell proliferation and invasion.

Methods

Tissue collection and cell culture

Breast cancer and adjacent normal tissue samples were obtained from patients undergoing breast cancer surgery in Hexian Memorial Affiliated Hospital of Southern Medical University who gave their signed informed consent. Tumors and corresponding non-tumor fresh specimens were frozen in liquid nitrogen before being cut off and stored at -80°C to extract RNA and protein. This study was approved by the ethics committee of Hexian Memorial Affiliated Hospital of Southern Medical University. Human breast cancer cell lines MCF7 and T47D cells were grown in RPMI 1640 medium containing 10% fetal bovine serum (FBS). The cells were cultured in a humidified atmosphere of 5% CO₂ at 37°C. Lipofectamine-2000, RPMI-1640 cell culture medium, and FBS were purchased from Invitrogen, Carlsbad, USA. Matrigel matrix glue was provided by B&D (Chicago, USA). Dual-luciferase assay kit, reverse transcription kit, and real-time PCR reagent were from Promega (Madison, USA). RNAiso Plus was manufactured by Takara (Kyoto, Japan). CCK-8 kit, total protein extraction kit, bicinchoninic acid (BCA) protein assay kit, and highly sensitive efficient chemiluminescence kit (ECL) were purchased from Beyotime Biotechnology (Shanghai, China).

Quantitative real-time polymerase chain reaction

Total RNA was extracted and amplified according to the instruction of RNAiso Plus kit, followed by the reverse transcription reaction using an RNA reverse transcription kit to obtain a cDNA template. The quantitative polymerase chain reaction (qPCR) mixture (10 mL) was mixed with different primers (0.5 mL for each) to make up a reaction system of 20 mL. The thermal cycling parameters for amplification were as follows: denaturation at 94°C for 2 min, followed by 40 cycles of 94°C for 20 s, 58°C for 20 s, 72°C for 30 s, and incubation at 25°C for 5 min to terminate the reaction. A melting curve was established and the analysis was performed between 62°C and 95°C. The relative expression levels of the target genes were calculated using the formula RQ= 2^{ΔΔCT}. The experiments were repeated three times. SNHG7 primer sequences: 5’-GTGGAGGATCGGATTTTAGCAAACT-3’ (forward) and 5’-CCTATGGGATCGGGCAGTGCAAAGT-3’ (reverse). miR-21 primer sequences: 5’-TTGAGCGAGTGCTTAGGGAAATCG-3’ (forward) and 5’-GCCTATCTTTTAGGGCGAGCA-3’ (reverse).

Dual-luciferase reporter assay

To determine the regulatory relationship between SNHG7 and miR-15a, two databases [TargetScan (www.targetscan.org) and Starbase (starbase.sysu.edu.cn)] were used.

Table 1. Relationship between the expression of SNHG7 and clinicopathological features of breast cancer tissues

<table>
<thead>
<tr>
<th>Clinicopathological data</th>
<th>Number</th>
<th>High expression of SNHG7</th>
<th>Low expression of SNHG7</th>
<th>p</th>
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<tbody>
<tr>
<td>Years</td>
<td></td>
<td></td>
<td></td>
<td>0.863</td>
</tr>
<tr>
<td>≤60</td>
<td>27</td>
<td>17</td>
<td>10</td>
<td></td>
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<tr>
<td>&gt;60</td>
<td>23</td>
<td>16</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Pathological staging</td>
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<td></td>
<td></td>
<td>0.018</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
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<td>II</td>
<td>13</td>
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<td>8</td>
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<tr>
<td>IV</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
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<td></td>
<td></td>
<td>0.021</td>
</tr>
<tr>
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</table>

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used to predict its target genes and found a complementary binding site of the two. The miR-15a-3′UTR containing the complementary site of SNHG7 was amplified from normal human genomic DNA and cloned into the luciferase reporter vector psi-CHECK. The binding site was used as wild-type miR-15a 3′UTR. The mutant miR-15a 3′UTR recombinant plasmid was generated using the QuikChange Site-Directed Mutagenesis Kit. Forty-eight h after the transfection of different plasmid combinations, the luciferase activity was detected using the dual-luciferase assay kit according to the manufacturer’s instructions. The co-transfected Renilla luciferase plasmid was used as an internal control to determine transfection efficiency. Fluorescence intensity was measured by a dual-luciferase reporter assay system (Promega, Madison, USA). The experiments were repeated three times.

**Cell proliferation assay**

Cell proliferation was analyzed in vitro using the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent. Totally 2000 cells from each group were seeded in fetal bovine serum (FBS) (200 μL) in each well of 5 96-well plates. For analysis of cell proliferation, 20 μL of MTT substrate at a concentration of 2.5 mg/mL in PBS were added to each well. The plates were then returned to the standard tissue culture incubator for an additional 4 h. The medium was then removed and the cells were lysed in 150 μL of dimethyl sulfoxide for colorimetric analysis. The experiments were repeated three times.

**Transwell invasion experiment**

An aliquot of 200 mL of medium (1×10⁴ cells) was inoculated into the upper compartment of each transwell and incubated at 37°C for 24 h to allow the cells to invade through the porous membrane. Forty mL of Matrigel were thawed overnight at 4°C in advance and mixed with a serum-free medium whose volume was three times that of Matrigel before being transferred to a 24-well Transwell chamber (50 μl per well). After incubation for 24 h, the cells remaining on the upper surface of the chamber were completely removed, while cells on the lower surface of the membrane were fixed with 4% paraformaldehyde for 20 min and then stained for 5 min in a 0.5% (w/v) crystal violet solution. After washing, the number of cells in the different groups was determined using Image-Pro Plus 6.0 software. The experiments were repeated three times.

**Nude mouse tumor xenograft model**

Tumor cells of the si-SNHG7 group and the NC group at a concentration of 1×10⁶ were injected into the armpits of 4-6 week old nude mice, respectively. The volume and mass growth of tumor xenografts in nude mice were measured after 8 weeks of feeding. The allograft size was measured according to the following formula: volume = 1/2 (shortest diameter) 2 x (longest diameter).

**Statistics**

SPSS 22.0 statistical package was used in this study. Correlation analysis was performed using the Spearman’s Rank test and statistical analysis was performed using the Student’s t-test. Data were expressed as the mean±standard deviation. A statistical difference was recognized when p<0.05.

**Results**

**Expression of SNHG7 in breast cancer tissues and cell lines**

According to the qRT-PCR detection of SHM-HG7 in different tissue samples (Figure 1), the expression level of SHMHG7 in breast cancer tissues was relatively higher than that in adjacent normal tissues (1.28±0.18 vs. 2.78±0.12, p<0.05), and the difference was statistically significant. According to the qRT-PCR detection of SHMHG7 in different breast cancer cell lines (Figure 1B), the expression of SNHG7 mRNA in MCF7 was relatively higher (3.58±0.18 vs. 3.05±0.28, p<0.05), and the difference was statistically significant.

![Figure 1. Expression of SNHG7 in breast cancer tissues and cell lines. A: The expression level of SNHG7 in breast cancer tissue is higher than that in adjacent normal tissue. B: the expression level of SNHG7 MRNA in MCF7 cell line is higher than that in other cell lines (*p<0.05).](image-url)
Relationship between SNHG7 and clinicopathological parameters of breast cancer patients

Fifty samples of breast cancer tissue and adjacent normal tissues were statistically analyzed. The expression level of SNHG7 was not notably different between patients of different ages (p>0.05; Table 1). The expression level of SNHG7 was higher in patients with a higher stage of breast cancer and patients with lymph node metastasis, and the difference was statistically significant (p<0.05).

Relationship between SNHG7 and miR-15a according to dual-luciferase assay

The bioinformatic analysis was performed to figure out the expression of miRNAs associated with SNHG7 in breast cancer cells. The results showed similar binding sequences between the two, indicating a synergistic association between them (Figure 2A). The results of the dual-luciferase assay demonstrated that the transfection of si-SNHG7 inhibited the luciferase activity of miR-15a in cells and regulated its expression activity and level (Figure 2B).

Effect of SNHG7 on the proliferation of breast cancer cells

The MTT proliferation experiment (Figure 3) revealed a lower cell proliferation rate of the si-SNHG7 group than the NC group (MCF-7 0.81±0.19 vs. 0.58±0.08, p<0.05; T47D 0.82±0.17 vs. 0.53±0.11, p<0.05), and the difference was statistically significant. Inhibited SNHG7 led to the suppressed proliferation of breast cancer cells.

Effect of SNHG7 on the invasion of breast cancer cells

The results of Transwell invasion assay displayed (Figure 4) that the number of breast cancer cell line (MCF-7 cells) passing through Matrigel in the si-SNHG7 group was 176.35±14.32, markedly higher than that in the NC group (35.54±5.32, p<0.05); the number of breast cancer cell line (T47D cells) passing through Matrigel in the si-SNHG7 group was 195.92±12.75, markedly higher than that in the NC group (62.5±7.17, p<0.05). Such results suggest that inhibited SNHG7 led to suppressed invasion of breast cancer cells.

**Figure 2.** Dual-luciferase assay to detect the relationship between SNHG7 and miR-15a. After transfection with SI-SNHG7, the luciferase expression of miR-15a in breast cancer cells was inhibited. *p<0.05.

**Figure 3.** Effect of SNHG7 on the proliferation of breast cancer cells. **A:** The cell proliferation rate of the SI-SNHG7 experimental group was lower than that of the control group. **B:** the proliferation of breast cancer cells decreased after SNHG7 inhibition. (*p<0.05).
**Effect of SNHG7 on the growth of xenografts in nude mice**

The mean tumor volume and weight of xenografts in the si-SNHG7 group were lower than those in the NC group (volume 802.3±32.1 vs. 386.1±18.25 mm, p<0.05; 563.3±31.5 vs. 283.3±13.5 mg, p<0.05) (Figure 5), indicating that inhibition of SNHG7 expression controlled breast cancer cell growth in vivo.

**Discussion**

A previous study reported that lncRNA acts as a tumor suppressor or oncogene in cancers [16]. LncRNA SNHG7 was found to be capable of inhibiting cell proliferation by targeting miRNAs in breast cancer [17]. However, the specific mechanism of lncRNA SNHG7 expression remains unclear.

In this study, lncRNA SNHG7 expression was significantly higher in breast cancer tissues than...
in adjacent non-tumor tissues and breast cancer cells. Cell proliferation was greatly constrained after the transfection of lncRNA SNHG7-siRNA into MCF-7. Latest studies have unveiled the involvement of lncRNA SNHG7 in tumor progression. In the present study, lncRNA SNHG7 promoted the expression of miR-15a in breast cancer, which further suggests the inhibition of the growth of breast cancer cells in vitro and in vivo by miR-15a. MiR-124 was reported to reversely inhibit lncRNA SNHG7 levels in cervical cancer cells, but it is not clear whether this reverse inhibition occurs in breast cancer [18]. Wu et al [19] found that lncRNA SNHG7 regulated the abnormal expression of the cell cycle regulatory proteins cyclinD1 and CDK6 in laryngeal carcinoma.

MiR-15a is a candidate gene of breast cancer [20]. Also, miR-15a has been identified as a key factor in the regulation of EMT regulation [21]. MiR-15a participates in the process of breast cancer invasion and metastasis and the activation of miR-15a has a certain influence on the proliferation and invasion of breast cancer cells [22]. This study confirmed that lncRNA SNHG7 may directly regulate the expression of miR-15a and directly participate in the proliferation and invasion of breast cancer cells. We plan to conduct subsequent studies to investigate the reverse regulation of miR-15a on lncRNA SNHG7 and the targeted regulation. This study provides a reference for elaborating the role of lncRNA-SNHG7 and miR-15a in breast cancer.

In conclusion, data of this study demonstrate that lncRNA SNHG7 inhibits cell proliferation and invasion by reducing miR-15a expression in breast cancer. Therefore, we speculated that the SNHG7-miR-15a signaling pathway may be involved in the regulation of breast cancer cell proliferation, providing new insights into the progression of breast cancer.

Conflict of interests

The authors declare no conflict of interests.

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