Effects of Long Non-coding RNA SNHG3 on Proliferation, Migration, and Invasion of Human Breast Cancer MCF-7 Cells Postprint

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Abstract

Objective: To investigate the effects of long non-coding RNA SNHG3 on the proliferation, migration, and invasion of human breast cancer MCF-7 cells. Methods: SNHG3 overexpression plasmid was constructed. The experiment was set up with a negative control group (pcDNA-3.1+) and an SNHG3 gene overexpression group (pcDNA-3.1+/SNHG3). MCF-7 cells were transfected with control plasmid and SNHG3 overexpression plasmid. Real-time quantitative PCR was employed to detect SNHG3 mRNA transcription levels, Western blot was used to detect MMP9 and EMT-related protein levels; colony formation assay was performed to assess MCF-7 cell proliferation capacity; scratch wound healing assay was conducted to evaluate MCF-7 cell horizontal migration capacity; Transwell chamber assay was utilized to examine MCF-7 cell vertical migration capacity and invasion capacity. Results: Following SNHG3 overexpression, the mRNA level of SNHG3 in MCF-7 cells was significantly elevated ($P<0.001$); the in vitro proliferation capacity of MCF-7 cells was markedly increased ($P<0.01$), and both migration ($P<0.01$) and invasion capacities ($P<0.001$) were also significantly enhanced. Real-time quantitative PCR and Western blot results demonstrated that SNHG3 could activate EMT-related pathways. Conclusion: Overexpression of SNHG3 may promote the proliferation, migration, and invasion of breast cancer MCF-7 cells by activating the EMT pathway.

Full Text

The Effects of Long Non-Coding RNA SNHG3 on the Proliferation, Migration, and Invasion of Human Breast Cancer MCF-7 Cells

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Abstract

Objective: To investigate the effects of long non-coding RNA SNHG3 on the proliferation, migration, and invasion of human breast cancer MCF-7 cells. **Methods:** SNHG3 overexpression plasmids were constructed, and experiments were divided into two groups: a negative control group (pcDNA-3.1+) and a SNHG3 overexpression group (pcDNA-3.1+/SNHG3). MCF-7 cells were transfected with control plasmids and SNHG3 overexpression plasmids. Real-time quantitative PCR was used to detect SNHG3 mRNA transcription levels, while Western blot was employed to measure MMP9 and EMT-related protein levels. Colony formation assays assessed MCF-7 cell proliferation capacity, scratch wound healing assays evaluated horizontal migration ability, and Transwell chamber assays examined both vertical migration and invasion capabilities. **Results:** Following SNHG3 overexpression, SNHG3 mRNA levels in MCF-7 cells increased significantly ($P<0.001$). The in vitro proliferation capacity of MCF-7 cells was markedly enhanced ($P<0.01$), and both migration ($P<0.01$) and invasion ($P<0.001$) abilities were significantly strengthened. Real-time quantitative PCR and Western blot results demonstrated that SNHG3 could activate EMT-related pathways. **Conclusion:** Overexpression of SNHG3 may promote the proliferation, migration, and invasion of breast cancer MCF-7 cells by activating the EMT pathway.

Keywords: Breast cancer; LncRNA SNHG3; Proliferation; Migration; Invasion

Breast cancer is one of the most common malignant tumors in women. According to the latest global cancer statistics, breast cancer accounts for 10% of all cancer incidence worldwide, 25-30% of female malignant tumor incidence, and 15% of female tumor-related mortality. In 2015, there were 2.4 million new breast cancer cases and 533,000 breast cancer deaths, making breast cancer the fifth leading cause of cancer-related life loss across both sexes from 2005 to 2015. However, the molecular mechanisms mediating breast cancer are not fully understood, making it crucial to investigate the molecular mechanisms of breast cancer development and identify new therapeutic targets.

With advances in genome sequencing and other biotechnologies, it has been

confirmed that only a small portion of the human genome encodes proteins, while over 97% of transcriptional products do not have protein-coding capacity. These are known as non-coding RNAs (ncRNAs), which mainly include long non-coding RNAs (LncRNAs) and small non-coding RNAs (microRNAs, miRNAs). LncRNAs, exceeding 200 nucleotides in length, are longer than microRNAs. Initially considered "transcriptional noise," LncRNAs play important roles in cellular function and tumorigenesis through various mechanisms including post-translational modification, translational suppression, and chromatin remodeling. Accumulating evidence indicates that lncRNAs participate in numerous biological processes, including proliferation, metastasis, differentiation, inflammation, angiogenesis, and metabolism.

Long non-coding RNA SNHG3 (Small Nucleolar RNA Host Gene 3) belongs to the SNHGs family, located on the positive strand of chromosome 1, overlapping with three exons at the 5' end of the protein-coding gene RCC1. SNHG3 contains two snoRNAs—U17a and U17b—within its introns. Previous studies have reported that LncRNA SNHG3 expression is significantly upregulated in hepatocellular carcinoma (HCC), and its expression level correlates significantly with HCC malignancy and poor prognosis. Analysis of breast cancer clinical samples through the Oncomine database (https://www.oncomine.org) revealed that SNHG3 is highly expressed in breast cancer patients compared to healthy individuals, suggesting that SNHG3 may be involved in breast cancer development and progression. However, the function and mechanism of SNHG3 in breast cancer have rarely been reported. Therefore, this study investigated the effects of SNHG3 overexpression on tumor cell proliferation, migration, and invasion capabilities in human breast cancer MCF-7 cells.

1.1 Cells and Reagents

Human breast cancer MCF-7 cells were preserved by the College of Laboratory Medicine, Chongqing Medical University. DMEM high-glucose medium was purchased from HyClone (USA). Fetal bovine serum (FBS) was obtained from Gibco (USA). Transwell chambers were purchased from Corning. TRIzol reagent and Lipofectamine 2000 transfection reagent were from Invitrogen. Reverse transcription and PCR-related reagents, PrimeSTAR® HS high-fidelity enzyme, DNA fragment purification kit, and DNA marker -EcoT14 I digest were purchased from Takara. BamHI and HindIII restriction endonucleases and T4 ligase were from NEB. Gel extraction and plasmid extraction kits were from Omega. Western blot and protein extraction-related reagents were from Beyotime Biotechnology (Shanghai). ECL luminescent solution was from Millipore. Rabbit anti-human Vimentin and Snail antibodies, mouse anti-human -actin antibody were from Cell Signaling Technology (USA). Rabbit anti-human MMP9 was from Abcam. Secondary antibodies (goat anti-rabbit IgG/HRP-labeled, goat anti-mouse IgG/HRP-labeled) were from Sino Biological (Beijing). Primers and full-length SNHG3 fragments were synthesized by GenScript. The pcDNA-3.1+ empty plasmid was preserved by the College of Laboratory

Medicine, Chongqing Medical University.

1.2.1 Cell Culture

Breast cancer cells were cultured in DMEM high-glucose medium (containing 100 ml/L FBS, 1000 IU/ml ampicillin, and 100 mg/ml kanamycin) in an incubator at 37°C with 50 ml/L CO . Cells were passaged with trypsin digestion every 2 days.

1.2.2 Construction of SNHG3 Overexpression Plasmid

Primers for amplifying the full-length SNHG3 fragment are shown in Table 1, with a fragment length of 2238 bp. Amplification was performed using PrimeSTAR® HS high-fidelity enzyme with the following PCR program: denaturation at 98°C for 10 s, annealing at 55°C for 5 s, and extension at 72°C for 2 min 30 s, for 35 cycles, followed by gel extraction purification of the target fragment. Both pcDNA-3.1+ plasmid and SNHG3 target fragment were digested with HindIII and BamHI restriction endonucleases at 37°C for 2 h. After purification of both digestion systems, T4 ligase was used for overnight connection at 16°C for 16 h. Ten microliters of the ligation product was transformed into competent TOP10 bacteria and evenly spread on plate medium (containing ampicillin). After incubation at 37°C for 12 h, single positive colonies were selected to prepare bacterial suspension. Colony PCR screening primers were SNHG3-1 (Table 1) with a fragment length of 400 bp. Fifty microliters of PCR-positive bacterial liquid was inoculated into LB medium containing 100 ng/mL ampicillin, cultured for 14 h, and plasmids were extracted. Double digestion with HindIII and BamHI at 37°C for 2 h was performed for electrophoresis identification. After sequencing confirmation, the plasmid was named pcDNA3.1+/SNHG3.

Table 1 Primer sequences

Gene	Forward primer (5' →3')	Reverse primer (5' →3')
SNHG3	CAAGCTTGATTCTCTAACTGCC[illegible]	[illegible]TCCTTCAGAAAAAAATCACTTTA
SNHG3-1	GGCCACTTTTGTATGATTTCTA[illegible]	[illegible]GGATACAACCTCCCGTTGCTAC
SNHG3-2	TTGTAATTTACC	CATCGATTTTT
Snail	TCGCTTCTTCTCCTTGGATTTG	AGGCATGAAATGCACCTCAA
Vimentin	CCCTTGTGCTCTTCCCTGGA	TCTGCCACCCGAGTGTAACC
-actin	TCCAGCAGCCCTACGACCAG	AGGCCGAGGTGGACGAGAA
	GTGATCTCCTTCTGCATCCTGT	

1.2.3 Plasmid Transfection

Experiments were divided into control group (transfected with pcDNA-3.1+ empty plasmid) and experimental group (transfected with pcDNA3.1+/SNHG3

plasmid). MCF-7 cells were seeded in six-well plates at 2.0×10 cells per well. When cell confluence reached approximately 75%, plasmid transfection was performed. Three micrograms of plasmid was transfected per well, and the medium was changed to serum-free, antibiotic-free medium. The transfection procedure followed the reagent instructions. After 4-6 h, the medium was replaced with serum-containing medium, and cells were collected for subsequent experiments after 24-48 h.

1.2.4 Real-Time Quantitative PCR Detection of SNHG3 Overexpression

Total RNA from each group of cells was extracted using TRIzol reagent. Two micrograms of RNA was reverse-transcribed into cDNA, and qRT-PCR was used to detect and analyze the expression levels of SNHG3, MMP9, Snail, Vimentin, and -actin, with -actin serving as an internal reference control.

1.2.5 Western Blot Detection of MMP9 Protein and EMT Pathway Expression Levels

MCF-7 cells from the control and experimental groups were collected after 48 h treatment. Total cellular protein was extracted, and protein concentration was measured using BCA assay. Thirty-five micrograms of protein was separated by 10% SDS-PAGE electrophoresis and transferred to PVDF membrane at constant current (210 mA). After blocking with 5% BSA at 37°C for 2 h, primary antibodies (MMP9/Vimentin/Snail/ -actin 1:1000) were added. Following TBST washes, HRP-labeled secondary IgG antibodies (1:5000) were incubated at 37°C for 1 h. The membrane was washed with 1× TBST for 10 min × 3, and HRP chemiluminescent solution was used for development. Grayscale values were analyzed using Quantity One 4.6.2 software, with relative protein expression levels calculated as target protein grayscale value/internal reference protein grayscale value.

1.2.6 Colony Formation Assay

MCF-7 cells were transfected with pcDNA3.1+ and pcDNA3.1+/SNHG3 plasmids, then cultured in serum-free and antibiotic-free DMEM medium. After 4-6 h, the medium was changed to DMEM containing 10% fetal bovine serum. After 48 h, cells were digested with trypsin and collected. Cells were seeded in 6-well plates at a density of 1×10^3 cells per well. After 8 days of culture, cells were fixed with paraformaldehyde and stained with crystal violet solution to observe colony formation.

1.2.7 Scratch Wound Healing Assay

MCF-7 cells were seeded in 6-well plates at 2×10 cells per well. After 12 h, cells were transfected with pcDNA3.1+ and pcDNA3.1+/SNHG3 plasmids. Six hours after transfection or when confluence reached over 95%, a 10 L pipette tip

was used to create a scratch in the center of each well. After PBS washing, 2 mL of medium containing 10% serum was added and photographed at 0 h. After 24 h, photographs were taken at the same positions for each group. Adobe Illustrator software was used to calculate the distances at 0 h and 24 h, and the average scratch healing rate was determined.

1.2.8 Transwell Migration and Invasion Assays

For migration assays, cell suspension was directly added to the upper chamber. For invasion assays, Matrigel was first applied to the upper chamber, diluted 1:5 with serum-free medium. Thirty microliters of diluted Matrigel solution was added to each chamber, which was then placed steadily in a 37°C incubator for 1 h before proceeding. MCF-7 cells were transfected with empty pcDNA3.1+ and pcDNA3.1+/SNHG3 overexpression plasmids for 48 h, then digested with trypsin and resuspended in serum-free medium to prepare single-cell suspensions at a density of 3×10 cells/mL. Three hundred microliters of cell suspension was added to the upper chamber (with or without Matrigel coating), and 600 L of medium containing 10% fetal bovine serum was added to the lower chamber. For migration assays, chambers were removed after 24 h; for invasion assays, after 36 h. After PBS washing, cells on the lower surface were fixed with 4% paraformaldehyde and stained with 0.05% crystal violet. Following PBS washes, cells on the upper surface were gently wiped away with cotton swabs. After drying, cells on the lower surface were photographed and counted under an inverted microscope.

2.1 High Expression of LncRNA SNHG3 in Breast Cancer

Analysis of the Richardson Breast 2 breast cancer subset database within the Oncomine tumor-related gene database (https://www.oncomine.org) revealed that SNHG3 expression in 40 breast cancer tissue samples was significantly higher than in 7 normal breast tissue samples (Figure 1 [Figure 1: see original paper]). This suggests that SNHG3 may be involved in breast cancer development and progression.

Figure 1 Differential expression of SNHG3 in breast cancer and mammary tissue in Richardson Breast 2 database. $P < 0.01$ (Breast vs. Breast Carcinoma)

2.2 Successful Overexpression of SNHG3 in MCF-7 Cells

qRT-PCR results showed that SNHG3 mRNA levels in breast cancer MCF-7 cells were significantly elevated in the overexpression group compared to the control group ($P < 0.001$, Figure 3 [Figure 3: see original paper]). These results confirm that the recombinant plasmid successfully achieved SNHG3 overexpression in MCF-7 cells.

Figure 2 [Figure 2: see original paper] SNHG3 overexpressed in breast cancer MCF-7 cells. Note: The expression of SNHG3 mRNA in MCF-7 cells was

detected by qRT-PCR. ***: P < 0.001 (pcDNA3.1+ vs. pcDNA3.1+/SNHG3)

2.3 Overexpression of SNHG3 Promotes Proliferation of Breast Cancer MCF-7 Cells

Colony formation assays demonstrated that the colony-forming capacity of breast cancer MCF-7 cells was significantly increased in the SNHG3 overexpression group compared to the control group (P < 0.01, Figure 4 [Figure 4: see original paper]). Microscopic observation revealed that individual colonies formed by SNHG3-overexpressing MCF-7 cells were also larger than those in the control group, confirming that SNHG3 can promote the proliferation capacity of breast cancer MCF-7 cells.

Figure 3 SNHG3 promoted the proliferation of MCF-7 cells. The MCF-7 cells proliferation were determined by colony forming test. **: P < 0.01 (pcDNA3.1+ vs. pcDNA3.1+/SNHG3)

2.4 Overexpression of SNHG3 Promotes Migration of Breast Cancer MCF-7 Cells

Scratch wound healing assays showed that the 24 h scratch healing rate of SNHG3-overexpressing MCF-7 cells was significantly higher than that of the control group (P < 0.01, Figure 5a [Figure 5: see original paper]). Transwell chamber assays demonstrated that the number of MCF-7 cells passing through the membrane was significantly higher in the SNHG3 overexpression group compared to the control group (P < 0.001, Figure 5b), indicating that SNHG3 can promote both horizontal and vertical migration capabilities of breast cancer MCF-7 cells.

Figure 4 SNHG3 promoted the migration of MCF-7 cells. (a), (b) The MCF-7 cells migration were determined by wound healing test and Transwell migration assay. **: P < 0.01 (pcDNA3.1+ vs. pcDNA3.1+/SNHG3);** *: P < 0.001 (pcDNA3.1+ vs. pcDNA3.1+/SNHG3)

2.5 Overexpression of SNHG3 Promotes Invasion of Breast Cancer MCF-7 Cells

In Transwell invasion assays, the number of cells invading to the lower chamber was (70 ± 5) in the control group and (205 ± 11) in the experimental group, with a statistically significant difference between the two groups (P < 0.001). These results demonstrate that SNHG3 overexpression can promote the invasive ability of breast cancer cells.

Figure 5 SNHG3 promoted the invasion of MCF-7 cells. The MCF-7 cells invasion were determined by Transwell invasion assay. ***: P < 0.001 (pcDNA3.1+ vs. pcDNA3.1+/SNHG3)

2.6 Overexpression of SNHG3 Promotes Epithelial-Mesenchymal Transition in Breast Cancer

qRT-PCR and Western blot results showed that after SNHG3 overexpression, both mRNA and protein levels of EMT-related markers Vimentin and Snail, as well as invasion-related marker MMP9, were significantly increased in MCF-7 cells. These results suggest that SNHG3 may promote the proliferation, migration, and invasion of breast cancer MCF-7 cells by activating EMT-related pathways.

Figure 6 [Figure 6: see original paper] SNHG3 activated the EMT signaling pathway of MCF-7 cells. (a) The expression of MMP9 and EMT mRNA were detected by qRT-PCR. (b) The expression of MMP9 and EMT protein were detected by Western blot. *: $P < 0.05$ (pcDNA3.1+ vs. pcDNA3.1+/SNHG3); **: $P < 0.01$ (pcDNA3.1+ vs. pcDNA3.1+/SNHG3)

Current data indicate that approximately 15% of new female malignant tumor cases in China are breast cancer, which has become the most prevalent malignant tumor among Chinese women and the leading cause of cancer death in women under 45. One in 14 women will develop breast cancer between birth and age 79, making it the primary cause of cancer death among women worldwide. In China, influenced by factors such as childbearing age, number of births, dietary structure, and living environment, both the incidence and mortality of breast cancer are increasing, seriously threatening women' s health and lives. Therefore, research on the mechanisms of breast cancer growth and metastasis is particularly important.

Long non-coding RNAs are a class of RNA molecules longer than 200 bp that do not encode proteins. Numerous studies have reported that lncRNAs play important roles in tumor progression and metastasis. For example, HOTAIR can promote breast cancer metastasis by inducing chromatin rearrangement, lncRNA-MALAT1 plays a key regulatory role in lung cancer cell metastasis, and lncRNA-NKILA can inhibit breast cancer metastasis by blocking IkB phosphorylation. LncRNAs are RNA molecules with certain tissue- and temporal-specific expression patterns, closely related to many human diseases, especially cancer. Recent studies have shown that lncRNAs function as oncogenes or tumor suppressors in many tumor types, participating in the regulation of tumor cell proliferation, apoptosis, invasion, migration, and angiogenesis through mechanisms such as epigenetic activation/inhibition, alternative splicing, competing endogenous RNA (ceRNA), protein interaction, and genetic variation.

Long non-coding RNA SNHG3 belongs to the SNHGs family. SNHG3 is located on the positive strand of chromosome 1 (chr1:28832455-28837404), overlaps with three exons at the 5' end of the protein-coding gene RCC1, and contains a polyA tail. Previous studies have reported that lncRNA SNHG3 expression is significantly upregulated in hepatocellular carcinoma, and its expression level positively correlates with HCC malignancy. SNHG3 is also associated with fatty acid metabolism pathways and participates in energy metabolism in ovar-

ian cancer by regulating miRNAs and eIF4AIII, which have target sites for PKM, PDHB, IDH2, and UQCRH in glycolysis, the Krebs cycle, and oxidative phosphorylation. In this study, analysis of the Oncomine database revealed that SNHG3 expression in breast cancer tissues was significantly higher than in normal breast tissues, suggesting that high SNHG3 expression in human breast tissues may promote carcinogenesis. Therefore, this study selected MCF-7 cells with relatively low malignancy and poor metastatic/invasive capacity for SNHG3 overexpression. Colony formation assays demonstrated that SNHG3 overexpression promoted breast cancer cell proliferation. Scratch wound healing assays showed that SNHG3 overexpression enhanced the horizontal migration capacity of breast cancer cells. Transwell results indicated that both vertical migration and invasion capabilities were significantly increased in the experimental group, suggesting that breast cancer cells with high SNHG3 expression are more likely to metastasize to distant organs.

Long non-coding RNAs have been confirmed to regulate important tumor biological functions such as epithelial-mesenchymal transition (EMT), proliferation, invasion, and metastasis. EMT is a critical process in tumor development and progression, where non-migratory epithelial cells redifferentiate into mesenchymal-like migratory cells, leading to infiltration of malignant breast cancer cells to secondary sites—a characteristic of advanced metastatic cancer stages. Certain cancer cell subtypes survive and develop into metastases in distal tissues, contributing to a more aggressive breast cancer phenotype. Our study found that after SNHG3 overexpression in breast cancer MCF-7 cells, both mRNA and protein levels of the invasion-related marker MMP9 were significantly elevated. Transcriptional and translational levels of the EMT-related marker Vimentin increased, while the EMT transcription factor Snail level also rose significantly, indicating that SNHG3 overexpression may activate EMT-related pathways in breast cancer cells, thereby affecting their migration and invasion capabilities.

In summary, this study demonstrated that SNHG3 overexpression in human breast cancer MCF-7 cells can promote proliferation, migration, and invasion capabilities, and this promotional effect may be achieved through the EMT pathway. Next steps will involve further investigation into the deeper mechanisms underlying these changes in migration and invasion capabilities.

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Note: Figure translations are in progress. See original paper for figures.