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Research Article
The Role of lncRNA SNHG1 in Breast Cancer Cells by Targeting miRNA-101

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Abstract

Background and Objective: Long non-coding RNA(lncRNA) is involved in various reactions in the body and is closely associated with drug resistance of breast cancer cells. The study was attempted to explore the target interaction of lncRNA SNHG1(SNHG1) and miR-101 in the mechanism of breast cancer progression. **Materials and Methods:** The breast cancer tissues and corresponding counterparts were collected. The expressions of SNHG1 and miR-101 in breast tumour tissues were elevated by qRT-PCR assay and the correlation was analyzed. The target interaction between SNHG1 and miR-101 was predicted by starbase online tool. MDA-MB-231 cells were subjected to co-transfection with SNHG1 shRNA and miR-101 inhibitors (sh-SNHG1 anti-miR-101) or SNHG1 shRNA and inhibitors control (sh-SNHG1 anti-NC). The changes in the proliferation, apoptosis, migrative and invasive ability of MDA-MB-231 cells was detected by MTT, flow cytometry and transwell assay, respectively. Western blot was performed to measure the protein expressions of cleaved Caspase-3 and MMP-2. **Results:** There was an inverse correlation between SNHG1 and miR-101 expression in breast cancer tissues. The binding site of SNHG1 in miR-101 was predicted. The luciferase activity of cells co-transfected with SNHG1-WT and miR-101 mimics was decreased. Meanwhile, the expression of miR-101 was pronouncedly elevated in cells of sh-SNHG1group. Compared with the sh-SNHG1 anti-NC group, cells co-transfected with sh-SNHG1and anti-miR-101 exhibited elevated cell proliferation, declined apoptosis rate and increased migrative and invasive ability of breast cancer cells. Parallelly, the activity of cleaved Caspase-3 was significantly reduced, while the MMP-2 level was significantly elevated in the sh-SNHG1 anti-miR-101 group. **Conclusion:** SNHG1 increased the development and metastasis of breast cancer cells by negatively regulating miR-101 expression.

Key words: Breast cancer, SNHG1, miR-101, invasion, apoptosis, proliferation, target interaction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Breast cancer has attracted public attentiveness for its increasing incidence[1]. The occurrence of breast cancer is closely associated with age, occupation and genetic factors. With the increasing number of breast cancer cases, much attention has been attracted to non-coding RNAs that play key roles in cancer development[2]. LncRNA SNHG1, located at human chromosome 11, is a novel discovered biomarker for the prognosis and treatment for a variety of cancers[3-5]. SNHG1 exerted multiple functions in different tissues by targeting a variant of miRNAs[6-8]. The mechanism of physiological processes and disease progression mediated by SNHG1 is complicated. SNHG1 exerts biological function by complementarily binding to its target miRNAs[9]. A preliminary study revealed that SNHG1 was highly expressed, while miR-101 was down-regulated in breast tumour tissues, in comparison with the corresponding counterparts. SNHG1 knockdown or miR-101 over expression could inhibit the proliferation, migration and invasion of breast cancer cells. Therefore, in the current study, the researcher attempted to verify whether SNHG1 modulated the malignant phenotype of breast cancer cells by targeting miR-101 and to explore its related mechanism.

MATERIALS AND METHODS

Study area: This research project was conducted in Baoding First Hospital Lab China, from February, 2021 to September, 2021.

Patients and clinical sampling: Between December, 2017 and June, 2018, 50 female patients (median age: 42 years old) with breast cancer admitted to First Central Hospital of Baoding were included. The eligible patients had not received any treatment such as radiotherapy, chemotherapy and endocrine therapy before surgery. Fifty pairs of breast tumour tissues and corresponding counterparts (5 cm from the edge of the tumour) were collected and prepared in liquid nitrogen for further experiments.

qRT-PCR analysis: Total RNA was prepared from breast cancer tissue specimens or cells with Trizol reagent. cDNA was synthesized by Transcriptor First Strand cDNA Synthesis Kit (Shanghai Suobao Biological Technology Co., Ltd., Shanghai, China) and miRNA RT kit (TaKaRa). The PCR reaction was performed with a Thermo Realtime PCR Master Mix system. Relative expressions of SNHG1 and miR-101 were calculated by the $2^{-\Delta\Delta Ct}$ method concerning GAPDH and U6, respectively.

Table 1: Primer sequences for qRT-PCR analysis

Symbols	Primer sequences
SNHG1	
Forward	5′-CCTAAAGCCACGCTTCTTG-3′
Reverse	5′-TGCAGGCTGGAGATCCTACT-3′
GAPDH	
Forward	5′-ACCCAGAAGACTGTGGAGG-3′
Reverse	5′-TTCTAGACGGCAGGTCAGGT-3′
miR-101	
Forward	5′-TACAGTACTGTGATAACTGAA-3′
Reverse	5′-CTCAACTGGTGTCGTGGA-3′
U6	
Forward	5′-CGCTTCGGCAGCACATATAC-3′
Reverse	5′-TTCACGAATTTGCGTGTCAT-3′

The primers were designed and synthesized by Nanjing KingsRui Company, Nanjing, China) and the primer sequences were displayed in Table 1.

Cell transfection: The breast cancer cell experiments *in vitro* were performed with the MDA-MB-231 cell line. Cells were maintained in RPMI-1640 media (Sigma) supplemented with 10% fetal calf serum, 100 U mL^{-1} penicillin-streptomycin (Sigma). When cells were cultured to the confluence of 50%, cell co-transfection with SNHG1 shRNA and miR-101 inhibitors (Gene Pharma, Shanghai, China) was performed by Lipofectamine 2000 reagent (Invitrogen) for 48 hrs. Cells transfected with SNHG1 shRNA and inhibitors control were set as controls.

Cell viability assay: According to the MTT assay kit (Sangon Biotech, Shanghai, China), a Cell viability assay was performed. Briefly, cells (5×10^3/well) were plated in a 96-well plate. Post transfection for 48 hrs, 10 µL MTT solution was added in cell culture per well and maintained for 4 hrs. Subsequently, the supernatant of cell culture was replaced with 150 µL DMSO solution for 10 min in dark. Finally, the OD values were measured by a microplate reader at 490 nm.

Cell apoptosis assay: FCM was used to evaluate the cell apoptosis. After transfection for 48 hrs, cells were digested by trypsin. Subsequently, cells were suspended at room temperature following treatment with 5 µL Annexin V-FITC and 5 µL PI solution for 15 min. Cell apoptosis percentages were monitored with the application of a flow cytometer.

Dual-luciferase reporter assay: The binding site of SNHG1 on miR-101 was predicted by the starbase online tool. A Dual-luciferase reporter assay was conducted to verify the target interaction between SNHG1 and miR-101. pGL3-SNHG1-WT vector was reconstructed by inserting the binding

sequence of SNHG1. Similarly, the pGL3-SNHG1-MUT vector was constructed by inserting the mutant sequence of SNHG1 in the downstream of luciferase of the pGL3 vector. Co-transfection with miR-101 mimics/mimics control and SNHG1-WT/SNHG1-MUT vector was performed in breast cancer cells. Then, the luciferase activity was evaluated by luciferase activity assay kit.

Protein expressions and western blot: The protein expressions of cleaved Caspase-3 and MMP-2 were detected by western blot assay. The primary antibody incubation was performed with antibodies against MMP-2 (1:1500, cell signaling), cleaved Caspase-3 antibody (1:1500, cell signaling). The visualized protein bands were scanned by an x-ray film scanner. The protein expression was quantitatively analyzed by quantity one:

$$\text{Relative protein expression} = \frac{\text{Grey value of target protein}}{\text{Grey value of GAPDH}}$$

Statistical analysis: All the data were analyzed by SPSS 21.0. The measurement data were represented as Mean±SD. Comparison between 2 groups was performed by t-test and multi-group comparison was achieved by SNK-q in one-way ANOVA. The difference with $p<0.05$ was considered significant.

RESULTS

SNHG1 and miR-101 expression in BC tissues: The expression levels of SNHG1 and miR-101 in breast tumour tissues were measured by qRT-PCR. The results showed that the expression level of SNHG1 in cancer tissues was (1.13±0.18), which was significantly higher than that in normal adjacent tissues (0.53±0.15) ($p<0.05$) in Fig. 1a, indicating that SNHG1 was highly expressed in BC. The expression level of miR-101 in cancer tissues was (0.69±0.15), which was significantly lower than that in normal adjacent tissues (1.33±0.21) ($p<0.05$) in Fig. 1b, indicating low expression of miR-101 in BC. The

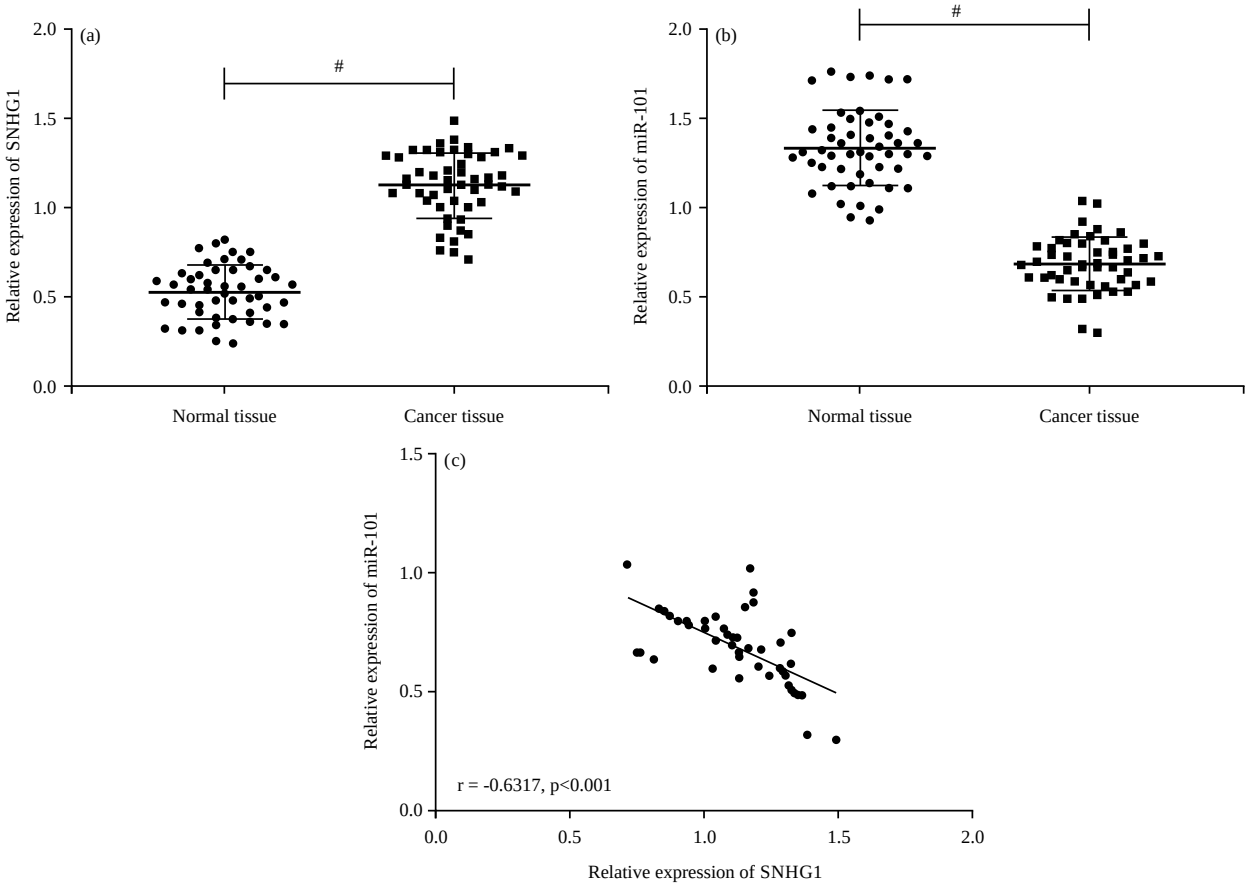

Fig. 1(a-c): Expression of SNHG1 and miR-101 in breast tumour tissues, (a) SNHG1 expression level, (b) miR-101 expression level and (c) Correlation between SNHG1 and miR-101

There are differences between the groups and #p<0.05

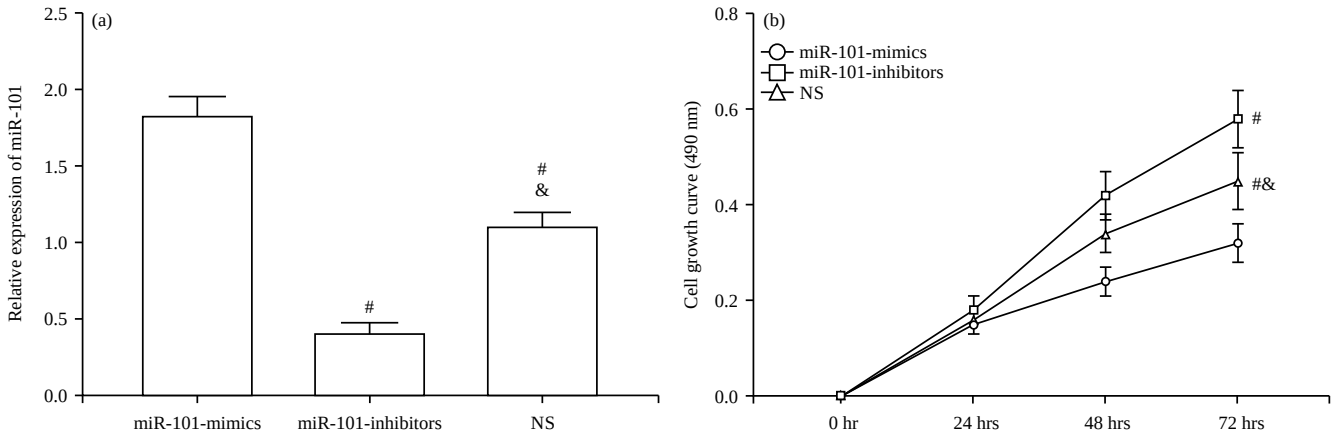

Fig. 2(a-b): Effect of miR-101 on the proliferation of breast cancer cells, (a) PCR verified the success rate of transfection and (b) Cell growth curve

Compared with the miR-mimics group, there is a difference #p<0.05, compared with the miR-inhibitors group and there is a difference, p<0.05

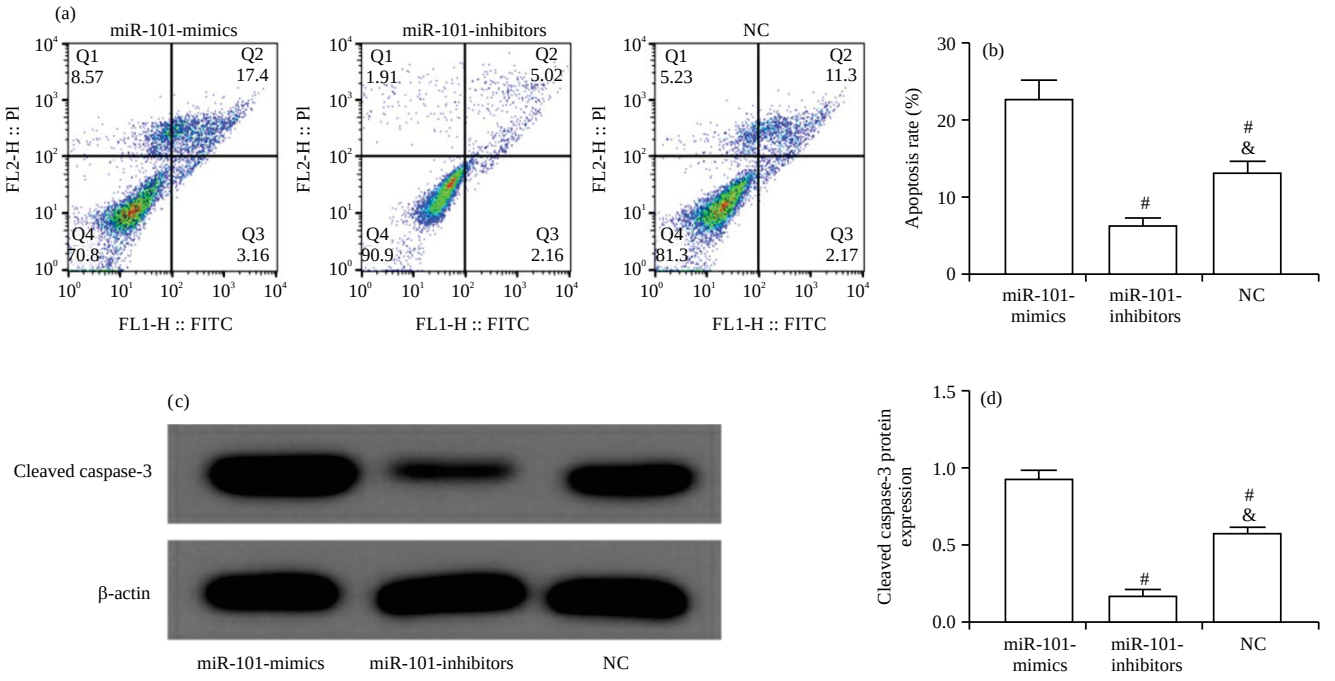

Fig. 3(a-d): Effect of miR-101 on apoptosis of breast cancer cells, (a) Flow cytometry, (b) Apoptosis rate, (c) Western blot map and (d) Caspase-3 protein expression

Interaction between SNHG1 and miR-101 in breast cancer cells, compared with the miR-mimics group, there is a difference #p<0.05, compared with the miR-inhibitors group and there is a difference, p<0.05

correlation between the expression of SNHG1 and miR-101 was detected by pearson correlation analysis and it was found that there was an inverse correlation between the two (p<0.05) Fig. 1c.

Impacts of miR-101 on BC cell viability: First, the success rate of transfection was verified by qRT-PCR. The results showed that the expression level of miR-101 in the miR-mimics group was 1.84±0.12, which was higher than that in the miR-inhibitors group and the NC group (p<0.05), while the expression level of miR-101 in the miR-inhibitors group was 0.42±0.06, lower than that in NC group 1.12±0.08 (p<0.05), Fig. 2a, confirming the successful transfection. Then, the effect of miR-101 on BC cells was analyzed by MTT assay. The results

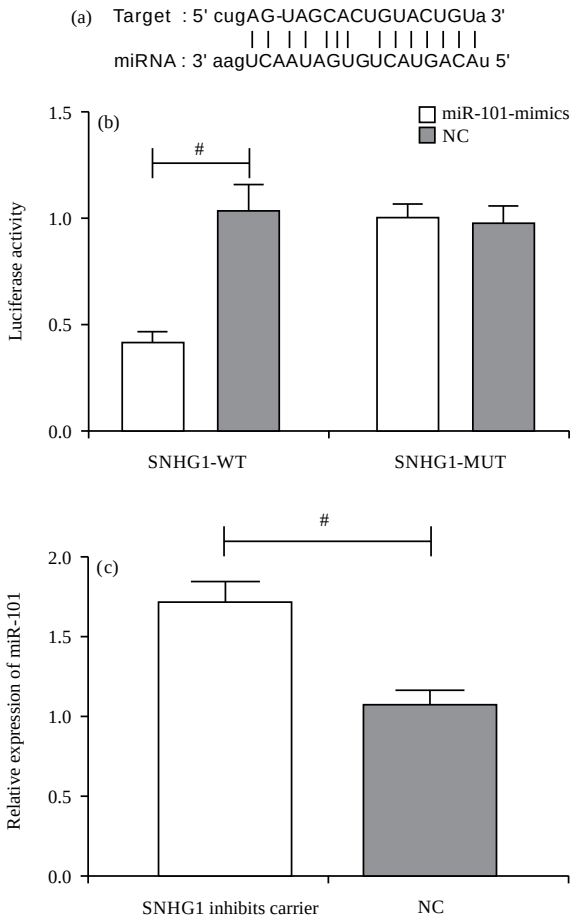

Fig. 4(a-c): Connection between miR-101 and SNHG1 in BC cells, (a) Complementary binding loci of SNHG1 and miR-101, (b) Luciferase activity in cells detected by dual-luciferase reporter assay and (c) Impact of SNHG1 on miR-101 expression

Expression level of miR-101 in the sh-SNHG1 group was higher than that in the NC group, there are differences between the groups and #p<0.05

showed that the OD of the miR-mimics group was 0.32±0.04 at 72 hrs, which was lower than that of the miR-inhibitors group and the NC group (p<0.05), while the OD of the miR-inhibitors group was 0.58±0.06 at 72 hrs, higher than that of the NC group (0.45±0.06) (p<0.05) in Fig. 2b. It suggests that up-regulating the expression of miR-101 could inhibit the proliferation of BC cells while silencing miR-101 could promote the growth of BC cells.

Impacts of miR-101 on BC cell apoptosis: First of all, the effect of miR-101 on the apoptosis rate of BC cells were detected by FCM and the experimental results are shown in Fig. 3a. The apoptosis rate of the miR-mimics group (22.81±2.27%) was

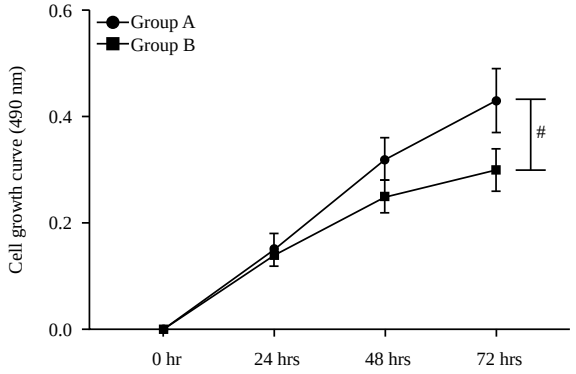

Fig. 5: Cell growth curve

Cell proliferation ability of group A was higher than that of group B, there are differences between the group and #p<0.05

higher than that of the miR-inhibitors group and the NC group, while that of the miR-inhibitors group (6.42±0.84%) was lower compared with the NC group (13.21±1.42 %) (p<0.05) in Fig. 3b. Caspase-3 is a classic pro-apoptotic protein and its expression level can sensitively reflect the apoptotic ability of cells. Therefore, we also detected the protein expression of Caspase-3 in cells by Western blot for verification and the results are shown in Fig. 3c. The expression of Caspase-3 protein in the miR-mimics group was 0.94±0.05, higher than that in the miR-inhibitors group and the NC group, while that in the miR-inhibitors group (0.18±0.03) was lower compared with the NC group (0.58±0.04) (p<0.05) in Fig. 3d. Therefore, up-regulating miR-101 could promote the apoptotic ability of BC cells, while silencing miR-101 led to the opposite results.

Interaction between miR-101 and SNHG1 in BC cells: The binding loci of SNHG and miR-101 predicted by starbase can be found in Fig. 4a. DLR assay suggested that the cells luciferase activity was lowered by co-transfection of SNHG-WT+miR-101 mimics in comparison with those transfected with SNHG-WT and miR-NC (p<0.05) Fig. 4b, indicating the targeted complementary binding relationship between SNHG and miR-101. miR-101 expression was quantified by qRT-PCR after the transfection of the SNHG1 inhibitory vector into the cells. After transfection with SNHG1 inhibitory vector, the expression level of miR-101 in BC cells was (1.73±0.12), which was higher than that in the NC group (1.08±0.09) in Fig. 4c.

Impacts of SNHG1 knockdown on BC cell multiplication were reversed by miR-101 down-regulation: The effect of SNHG1 targeting miR-101 on BC cell proliferation was analyzed by MTT assay. The results showed that the OD value

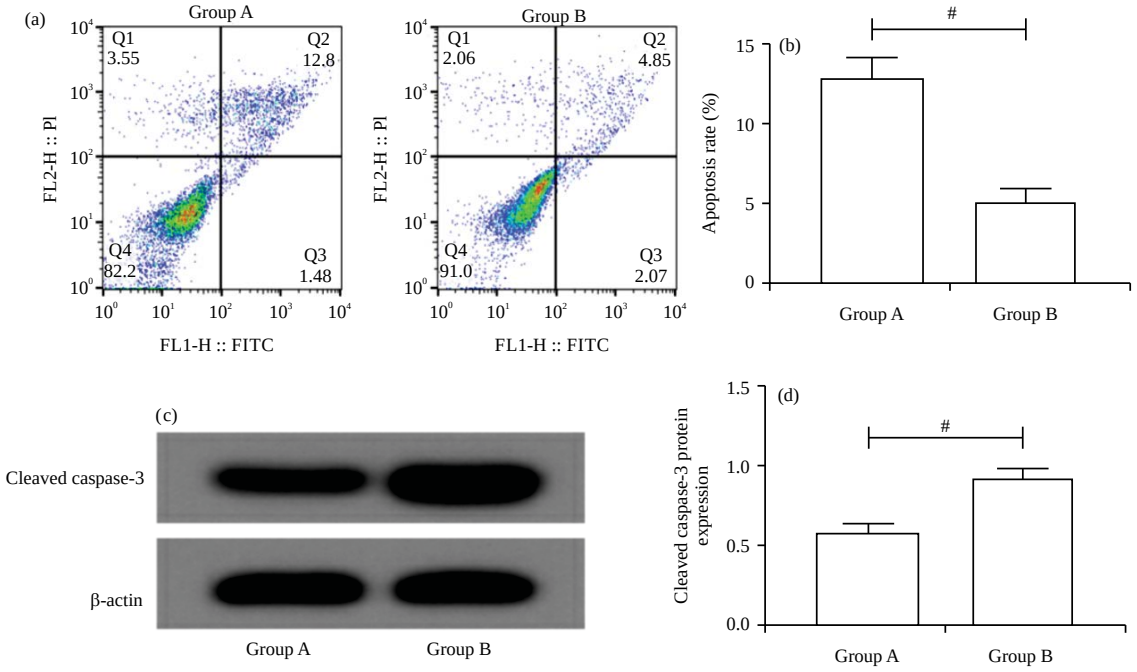

Fig. 6(a-d): miR-101 down-regulation abolished the impact of SNHG1 knockdown on BC cell apoptosis, (a) Flow cytometry, (b) Apoptosis rate, (c) Western blot map and (d) Caspase-3 protein expression

Apoptosis rate of group A was higher than that of group B, the Caspase-3 protein expression in group A was lower than that in group B, there are differences between the groups and $^{\#}p<0.05$

of cells in group A was 0.43±0.06 at 72 hrs, which was higher than that of cells in group B (0.30±0.04) ($p<0.05$) in Fig. 5. These results indicated that the effect of SNHG1 silencing on BC cell activity was reversed by simultaneous inhibition of miR-101.

MiR-101 down-regulation abolished the impact of SNHG1 knockdown on BC cell apoptosis: Similarly, the effect of SNHG1 targeting miR-101 on the apoptosis rate of BC cells was detected by FCM and the experimental results are shown in Fig. 6a. The apoptosis rate in group A was 12.81±1.34%, which was higher than that in group B (5.08±0.86%) ($p<0.05$) in Fig. 6b. The detection results of Caspase-3 protein expression in cells are shown in Fig. 6c. The expression of Caspase-3 protein in group A was 0.58±0.06, which was lower than that in group B (0.92±0.06) ($p<0.05$) in Fig. 3d. The above results also showed that the effect of silencing SNHG1 on the activity of BC cells was reversed by simultaneous inhibition of miR-101.

DISCUSSION

The progression of BC may result from expression abnormalities of cancer-related genes, including the activation of proto-oncogenes and the degradation of suppressor genes. Cancers of different stages show differential gene expression profiles[10-12]. LncRNAs mediated a plethora of cellular functions involving multiple mechanisms, such as interactions with multiple miRNAs[13]. Current studies on the role of SNHG1 show that SNHG1 plays a variety of biological functions in different tumour tissues by interacting with its target miRNAs such as miR-154[8], miR-338[14], miR-326[15] and miR-137[16]. It is reported that miR-101 is poorly expressed in several tumour tissues and its up-regulation has excellent anti-tumour effects[17,18]. However, the role of miR-101 in BC has not been verified, except that the possible expression of miR-101 in BC was preliminarily mentioned in the previous studies[19]. It is also unclear whether there is a relationship between miR-101 and SNHG1. At present, researchers have found that the mechanism of lncRNAs' influence on tumour cells is carried out by regulating and targeting downstream genes[20]. Therefore, to thoroughly understand whether SNHG1 has significance and application in the diagnosis and treatment of BC in the future, it is necessary to confirm its downstream pathway. A study has pointed out that SNHG1 can affect osteoblast differentiation by regulating the expression of miR-101 in mesenchymal stem cells[21] but there are no other related studies. Given that the role and relationship between

the 2 in BC have yet to be confirmed by exact studies, we carried out the relevant analysis. First studied the levels of miR-101 and SNHG1 in BC tissues and their noncancerous counterparts. The results identified down-regulated SNHG1 and up-regulated miR-101 in tumour tissues. And an inverse association was determined between SNHG1and miR-101. In previous studies, found that clinical attention to miR-101 was mainly focused on its role in ovarian cancer, cervical cancer and other diseases and miR-101 was also significantly under-expressed in these diseases[22,23], which on the one hand proves the accuracy of our experimental results and on the other hand, shows the important significance of miR-101 in gynaecological tumours. Second, there are also studies indicating that DSKA-AS1 and PTAR were involved in the development of osteosarcoma and lung cancer through miR-101[24,25]. Thus, speculated that SNHG1 could modulate the progression of BC by directly regulating miR-101. But further experiments are needed for confirmation.

Subsequently, through transfection of different miR-101 sequences and biological behaviour detection, found that inhibiting miR-101 can promote BC cell viability and reduce apoptosis, while over expression of miR-101 can accelerate BC cell apoptosis, which verifies the involvement of miR-101 in BC cells. In previous studies, also found that low expression of miR-101 can promote the activity of liver cancer cells[26], which can confirm the results of this study. Therefore, combined with the results of the above experiments, we can preliminarily verify our initial view that both SNHG1 and miR-101 play a part in BC carcinogenesis and progression and there is a certain regulatory relationship between them. Besides, the complementary binding loci of SNHG1 and miR-101 were predicted by Starbase, an online bioinformatics tool. The DLR assay verified that SNHG1 negatively regulated miR-101 expression. To further determine the effect of SNHG1 and miR-101 interaction in modulating the malignant phenotype of BC cells, we co-transfected BC cells with SNHG1 inhibitory expression vector and miR-101 inhibitory sequence. The experimental results demonstrated that suppressing miR-101 expression could completely reverse the effect of inhibition of SNHG1 on BC cell multiplication and apoptosis, indicating the presence of a targeted regulatory relationship between SNHG1 and miR-101. Although previous studies have found that lncRNA SPRY4-IT1 and MALAT1 affect the biological behaviour of tumour cells such as osteosarcoma and oral cancer through miR-101[27,28], this study is the first to confirm the role of miR-101 in BC and its targeted regulation relationship with SNHG1, which lays a foundation for molecular targeted therapy from the perspective of SNHG1 in the future and provides a more accurate reference for subsequent studies. Of course, the previous studies also fully indicate that there are other upstream target genes of miR-101 besides SNHG1. While based on miR-101, can also analyze other upstream lncRNAs with the unclear relationship with BC, which will be the focus and direction of subsequent studies.

CONCLUSION

Based on the above experiments can preliminarily understand the action pathway of SNHG1 on BC cells. However, miR-101 is not the only downstream target gene of SNHG1, so it is not excluded that SNHG1 may also affect BC cells in other ways. In addition, the downstream targeting proteins and related signalling pathways in the process of SNHG1 targeting miR-101 are also directions worthy of further study. In conclusion, SNHG1 was involved in the development and metastasis of breast cancer cells by reversely regulating miR-101 expression.

SIGNIFICANCE STATEMENT

This study discovered the pathway of SNHG1 on BC cells that can be beneficial for cancer research. This study will help the researchers to uncover the critical areas of miR-101 that affect breast cancer that many researchers were not able to explore. Thus a new theory on -miRNA and lncRNA regulating the tumour therapy may be arrived at.

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