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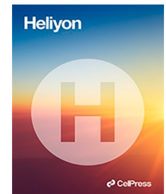

Knockdown of LINC00702 inhibits the growth and induces apoptosis of breast cancer through the Wnt/β-catenin pathway

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ABSTRACT

Long non-coding RNAs (lncRNAs) are essential in many biological areas like cell growth and apoptosis. The role of recently discovered LINC00702 is yet to be explored. Therefore, we wanted to elucidate its role in breast cancer (BC) with bioinformatic and various methods. LINC00702 expression was predicted using bioinformatic analysis and confirmed by RT-qPCR. Furthermore, the impact of LINC00702 knockdown on BC progression was evaluated. High LINC00702 level could lead to a worse outcome in BC patients. Additionally, CCK-8, EdU,and Annexin V-APC7/AAD experiments showed that LINC00702 knockdown inhibited the growth of BT-474 and T-47D cells and promoted their apoptosis. Moreover, in *vivo* experiments showed that shLINC00702-2 significantly reduced tumor sizes and suppressed c-Myc and β-catenin expressions. On the contrary, a rescue assay showed that HLY78, an activator of the Wnt/β-catenin pathway, reversed the cell-inhibiting impact of LINC00702 knockdown. LINC00702 is an oncogenic lncRNA that promotes BC progression by stimulating the Wnt/β-catenin pathway and downstream proteins, making it a promising target for further research on BC treatment.

1. Introduction

Breast cancer (BC) is the leading factor in tumor-related fatalities among global females [1]. By 2070, there will be 4.4 million BC instances, up from the nearly 2.3 million cases and 685,000 fatalities recorded in 2020. Globally, China shows the most BC cases (nearly 18.4%) [2,3], which has increased the burden on families and society. Lifestyle, environmental factors, and genetics are the key risk factors for BC [4,5]. Prior research has revealed that some proteins are vital in its pathogenesis. For example, the nuclear antigen Ki-67, expressed in the interphase and the stage of mitosis, promotes the proliferation of BC cells [6,7]. Proliferating cell nuclear antigen (PCNA), which creates a trimeric ring around DNA, is vital for DNA replication, repair, and damage response and participates in conserved and important processes [8].

Long non-coding RNAs (lncRNAs) are non-coding RNAs having transcript lengths >200 nucleotides [9,10]. Although lncRNAs cannot code for proteins, they control the transcriptional and post-transcriptional stages of gene expression through multiple mechanisms [11]. They are generally divided into antisense, bidirectional, enhancer-related, intergenic, and pseudogenic [10,12–14].

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LINC00702, a recently discovered lncRNA, is strongly associated with the pathogenesis and prognosis of many cancers [15,16]. In malignant meningioma, LINC00702 inhibition inhibits the cell growth through the Wnt/β-catenin pathway [17]. Moreover, this pathway regulates multiple physiological activities like proliferation, differentiation, apoptosis, and invasion [18,19]. The β-catenin is responsible for signal transduction to key effectors in the nucleus, further controlling the transcription of Wnt/β-catenin pathway-specific genes [19]. In most conditions, these regulations are achieved via the impact of β-catenin on cell adhesion and transcriptional activation of target genes [20]. A prior study has revealed that the dysregulation of β-catenin can induce the occurrence of several malignancies, such as colon cancer, melanoma, and ovarian cancer, indicating that it is vital in cell growth and apoptosis [21]. Overall, these findings suggest the involvement of LINC00702, β-catenin, and c-Myc in cancer pathogenesis and development.

Nevertheless, the role of LINC00702 in BC is not yet clear. Therefore, we hoped to unlock its function and molecular mechanism in BC using various techniques.

2. Materials and methods

2.1. Materials

The BC cell lines MCF-7 (Luminal A), T-47D (Luminal A), BT-474 (Luminal B), and MDA-MB-231 (triple-negative) and the normal human breast epithelial cell line MCF-10A were provided by the American Model Culture Library (ATCC, USA). MCF-10A cells were cultivated in special medium (Procell, CM-0525), MCF-7 in MEM (Procell, PM150410), T-47D and BT-474 in RPMI 1640 (Procell, PM150110), and MDA-MB-231 in Leibovitz's L-15 with 10% FBS (WISENT, 085-060) and 1% penicillin-streptomycin (NCM Biotech, c125c5) at 37 °C in a 5% CO_2 incubator.

2.2. Bioinformatic analysis

The large-sample BC gene chip data matrix, GSE54002, was extracted from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo) database of NCBI. Samples were captured from the clinical tissues of patients with BC using laser capture microdissection (LCM), including 417 BC and 16 non-tumor tissues. The detection platform Affymetrix GeneChip, GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 contained 54,675 probes with 25,320 genes. Differentially expressed lncRNAs in BC cells were identified using the limma package (Linear Model for Microarray Data) (version 3.46.0) in R language (version 4.01). KMPLOT (https://kmplot.com/) was used to assess the association of differentially expressed lncRNAs with the patient's outcome. Pan-cancer RNA-seq data ($n = 1090$) were selected for BC overall survival analysis. Finally, genes co-expressed with LINC00702 in the expression matrix of the GSE54002 chip were identified using Pearson's correlation in R software (version 4.01). Protein–protein interaction network was generated with StringDB (https://string-db.org/) (version 11.5). LINC00702-associated protein and genes were annotated in the "biological processes" category of the gene ontology (GO) database.

2.3. Cell transfection

Briefly, cells in the logarithmic phase were digested with 0.25% trypsin, centrifuged for 3 min at 1500 rpm, suspended in 1 mL of medium, and seeded into 6-well plates (1.2×10^6). After adhering to the walls, the cells were transfected with Lv-shNC and Lv-shLINC00702-2, gently mixed, and cultivated in an incubator at 37 °C (8 h). Following 48 h-transfection, cells were continuously screened and expanded with puromycin until the required cell number was reached. Thereafter, the medium was changed to a new one without puromycin (48 h) and changed to another one to be incubated (96 h) in a 5% CO_2 incubator.

2.4. Tumor formation

The Hunan SJA Laboratory Animal Co. (Changsha, China) provided female BALB/c nude mice aged 5–6 weeks and weighed 18–20 g. They were cultured at 24 °C and 40–60% humidity under a 12-h light/dark cycle and adaptively fed for 1 week. If the mice successfully survived following the injection with the transfected cells, they would be enrolled. Cells transfected with Lv-shNC and Lv-shLINC00702-2 in the logarithmic phase were harvested after digestion, suspended in a mixed solution of culture medium and Matrigel (3:1), adjusted for cell concentration, placed in an icebox, and transferred to the animal room for injection. Mice were equally allocated into the Lv-shNC and Lv-shLINC00702-2 groups at random and were subcutaneously injected under the armpit with 100 μL of cell suspension (5×10^6/mL) of Lv-shNC or Lv-shLINC00702-2 using 1 mL syringe. Mice were maintained, and their tumor size was gauged every five days beginning on day 7. Mice were anesthetized 32 days following injecting of the cells, and samples were gathered in preparation for subsequent research.

2.5. CCK-8 assay

Cell viability was identified with a CCK-8 assay kit. Briefly, transfected cells (5×10^3) were cultured in a 96-well plate having 100 μL of cell culture medium and incubated for 0, 24, 48, and 72 h. Thereafter, 10 μL of CCK8 solution (Sigma, 96992-100TESTS-F) was added to the wells and mixed evenly, followed by incubation in a CO_2 incubator (2 h). The absorbance at 450 nm was quantified with a multifunctional enzyme labeling instrument (PerkinElmer, USA).

2.6. Annexin V-APC 7/AAD assay

Cell apoptosis was evaluated with an Annexin V-APC/7-AAD cell apoptosis detection kit. Briefly, transfected cells at 70% confluency were digested in trypsin without EDTA and centrifuged for 3 min at 1500 rpm. Thereafter, the cell supernatant was rinsed once with PBS after centrifugation for cell counting. Suspension with approximately 1×10^5 cells was centrifuged again, followed by resuspension in 500 μL of binding buffer and the addition of 5 mL each of Annexin V-APC and 7-ADD (KeyGen Bio TECH, KGA1025). Finally, the mixture was incubated in the dark at 22–24° for 15 min. Flow cytometry was done within 1 h (excitation = 633 nm, emission = 660 nm), and the red fluorescence of Annexin V-APC and 7-AAD was identified using the PerCP-H (FL4) and FL3 channels, respectively.

2.7. EdU test

Cell proliferation was assessed with the EdU cell proliferation kit. Briefly, 4×10^3–1×10^5 cells per well were inoculated into 96-well plates, added with 100 μL of 50 μM EdU (RIBOBIO, C10310), and incubated (2 h). Then they were rinsed with PBS, fixed with 50 μL of 4% paraformaldehyde (Solarbio, P1110) for half an hour, added with 50 μL of 2 mg/mL glycine solution (Sigma, G8898), and incubated in a decolorizing shaker for 5 min. Following PBS rewashing, 100 μL of penetrant was added to every well, incubated for 10 min, added with 100 μL of 1 × Apollo staining reaction solution, and incubated in a decolorizing shaker for half an hour. The decolorizing shaker was cleaned twice for 10 min each time using 100 μL of PBS with 0.5% TritonX-100 (Sangon Biotech, A600198). Finally, cells were treated by 100 μL of 1 × Hoechst33342 reaction solution and incubated in a decolorizing shaker for half an hour, then the dyeing reaction solution was taken out. Cells were rinsed thrice with 100 μL of PBS, red fluorescence was observed in EdU-positive cells under green excitation, while blue fluorescence was observed under ultraviolet excitation. EdU positivity was defined as the ratio of EdU-positive to total cells.

2.8. Real-time quantitative PCR (RT-qPCR)

Total RNA was drawn with the TRIzol reagent (Thermo Fisher Scientific, 15596026), and the extracted RNA level was quantified with an enzyme labeling apparatus (PerkinElmer, VICTOR NIVO). cDNA was created from total RNA (2.0 μg) with Oligo dT (1 μL, 60 μm) and random primer (1 μL, 250 μm). cDNA concentration was measured using a microplate reader and diluted to 30 ng/μL. RNA amplification was performed on — PCR system using – reagent and specific primers. The conditions were pre-denaturation at 95 °C (2 min), denaturation at 95 °C (15 s), and annealing extension at 60 °C (30 s for 40 times). The LINC00702 expression was quantified via the $2^{-\Delta\Delta CT}$ method, with the *GAPDH* as an internal control. Table 1 shows the primer sequences.

2.9. Immunohistochemical (IHC) staining assay

Briefly, tissue sections were baked in an incubator at 60 °C (2.5 h) and then dewaxed. Tissue sections in antigen repair solution were heated in a microwave oven (2.5 min) and exposed to room temperature for 5 min and low heat for 24 min. Thereafter, they were soaked in double distilled water (ddH_2O; 10 min), exposed to 100 μL of 3% hydrogen peroxide, and incubated (5 min). Sections were rinsed with PBS thrice, treated with 100 μL of sealing solution (10 min), incubated overnight at 4 °C with antibodies against Ki-67 (Proteintech, 27309-1-AP) and PCNA (Proteintech, 10205-2-AP) at a dilution ratio of 1:5000 and 1:800, respectively. Then the sections were rinsed with PBS thrice, incubated with 100 μL of diluted secondary antibody solution (1:50; 20 min), and treated by 100 μL of horseradish peroxidase-labeled streptavidin (20 min) at room temperature. Thereafter, sections were exposed to 120 μL of DAB, and the reaction was ended with ddH_2O. After dehydration and sealing, slices were sealed with neutral gum, and the results were analyzed after drying.

2.10. Western blotting

Briefly, tissue sections were extracted with RIPA Lysis Buffer (Beyotime, P0013B), and the protein content was identified with an enhanced BCA Protein Assay Kit (Beyotime, P0010S). The samples were extracted with Omni-Easy PAGE (Epizyme Biotech, PG212) to be placed on a polyvinylidene fluoride (PVDF) membrane (Millipore, IPVH00010) for 1–2 h. Thereafter, the membranes were mounted with diluted non-fat milk powder and incubated with the primary antibodies against c-Myc (Proteintech, 10828-1-AP) and β-catenin (Proteintech, 17565-1-AP) overnight at 4 °C. They were rinsed thrice with Tris-buffered saline + Tween (TBST) and treated by goat anti-rabbit IgG (Proteintech, 10494-1-AP) or goat anti-mouse IgG (Proteintech, 60004-1-Ig) at 37 °C for 1.5 h. Finally, enhanced

Table 1
List of primer sequences.

Gene	Sequence
GAPDH	Forward: ACAGCCTCAAGATCATCAGC
	Reverse: GGTCATGAGTCCTTCCACGAT
LINC00702	Forward: GAAGCCAGCTCACCACCGT
	Reverse: ACCCAAATCCCTGCCCAT

chemiluminescence (SuperSignal West Pico; Pierce Biotechnology, Rockford, IL, USA) was applied to detect the protein level, and band densities were measured with ImageJ Software.

2.11. Statistical analysis

Data are shown as mean ± standard deviation. Significant differences among groups were determined via a *t*-test or one-way ANOVA. The GraphPad Prism 7.0 software (La Jolla, CA, USA) was applied for data analysis, and with the $p < 0.05$ as a statistical significance. The experiments were done thrice.

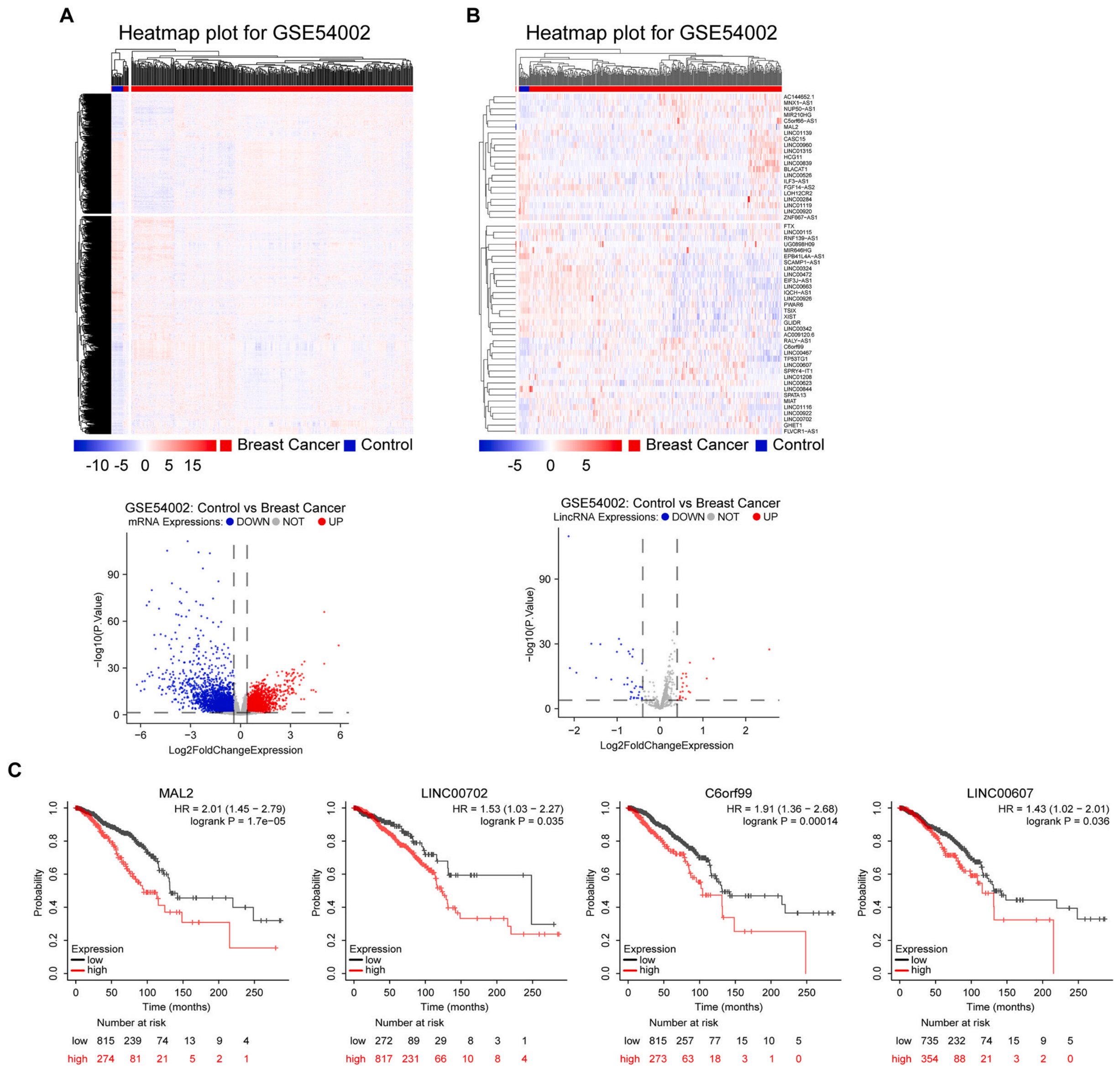


Fig. 1. Screening relevant lincRNAs. (A) GSE54002 dataset, including 417 breast cancer tissues and 16 non-cancer tissues, was uploaded to Affymetrix GeneChip to detect differentially expressed genes (DEGs), and R software was used to detect upregulated and downregulated mRNAs. (B) Heatmap plot for GSE54002 indicating differentially expressed lncRNAs. R software was used to detect upregulated and downregulated lncRNAs. (C) KMPLOT was used to analyze the relationship between 56 differential expressed lncRNAs and the overall survival of patients with breast cancer. The expression levels of MAL2, LINC00702, C6orf99, LINC00607 were upregulated.

3. Results

3.1. LINC00702 is overexpressed in BC

In total, 6823 DEGs were identified in cancer cells, with 3371 downregulated DEGs (log2FC < -0.4, $p < 0.05$) and 3452 upregulated DEGs (log2FC > 0.4, $p < 0.05$) (Fig. 1A). Additionally, 552 lncRNA were detected, among which 56 were differentially expressed, with 21 upregulated (log2FC > 0.40, $p < 0.05$) and 35 downregulated (log2FC < -0.40, $p < 0.05$) (Fig. 1B). The KMPLOT (https://kmplot.com/) analysis showed that 17 differential lncRNAs were associated to overall survival. Additionally, four significantly upregulated (log2FC > 1, $p < 0.05$) lncRNAs (MAL2, LINC00702, C6orf99, and LINC00607) were risk factors for the patient's overall survival (Fig. 1C).

Moreover, LINC00702 was significantly upregulated (log2FC $= 0.4678$, $p = 5.65\text{e-}03$) in the BC tissue expression chip (GSE54002) (Fig. 2A). High LINC00702 expression could induce shorter survival time (hazard ratio $= 1.9$, $p = 0.0081$) in the GEPIA database (http://gepia.cancer-pku.cn/detail.php?gene=LINC00702) (Fig. 2B). The RT-qPCR revealed that a markedly increased LINC00702 expression ($p < 0.05$) in BC cell lines than in the normal ones (Fig. 2C). Further experiments to identify the cellular location of LINC00702 showed that LINC00702 expression was high in the cytoplasm but low in the nucleus (Fig. 2D). The above results suggest that LINC00702 is located in the cytoplasm and induce a poor prognosis in BC cases.

3.2. LINC00702 knockdown suppresses growth and promotes apoptosis in BC cells

BT-474 and T-47D cells were transfected with shLINC00702-1 and shLINC00702-2 to elucidate the function of LINC00702 in BC. Transfection with shLINC00702-1 and shLINC00702-2 significantly reduced ($p < 0.01$) LINC00702 expression, with shLINC00702-2 exerting a better knockdown efficiency (Fig. 3A). Compared with the controls, LINC00702 knockdown significant inhibited the

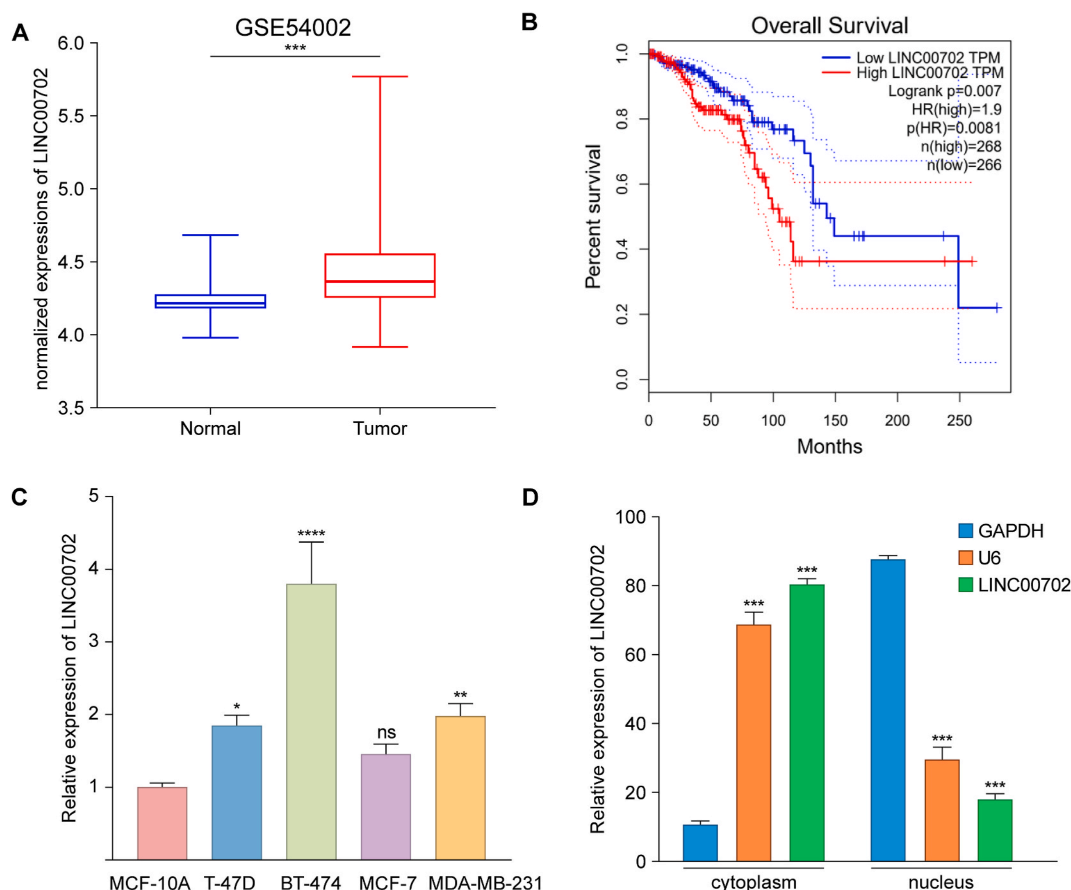


Fig. 2. LINC00702 expression in breast cancer tissues and cells. (A) The expression of LINC00702 was analyzed in GSE54002. (B) The ratio of overall survival of patients with breast cancer was detected. The sample grouping method is quartile, which is a comparison between the top 25% of patients with the highest expression and the bottom 25% of patients with the lowest expression. (C). RT-qPCR was used to examine the expression of LINC00702 in different breast cancer cell lines. (D). lncRNA subcellular localization analysis was performed to identify the expression of LINC00702 in the cytoplasm and nucleus. *$p < 0.05$; **$p < 0.01$; ns = not significant.

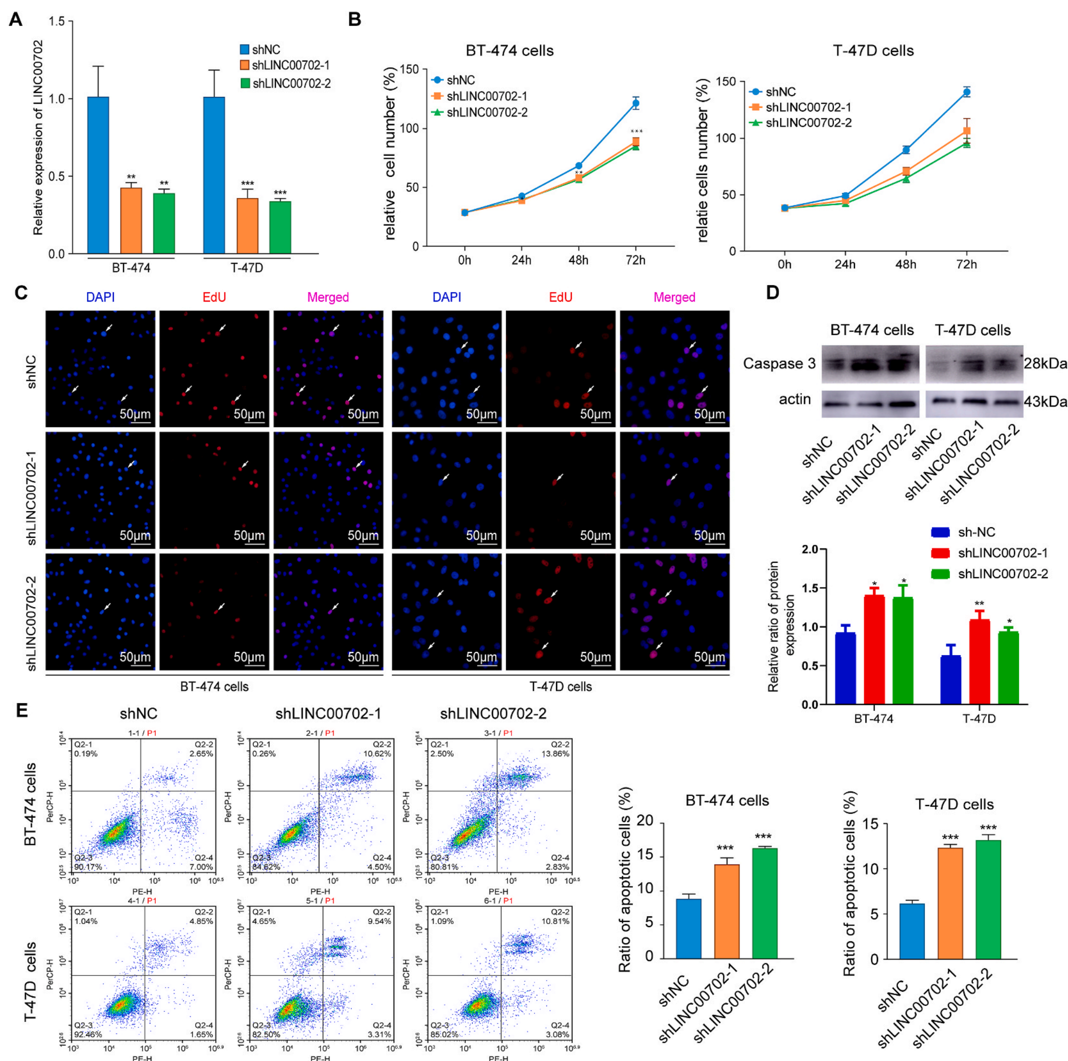


Fig. 3. Effects of shLINC00702 on proliferation, growth, and apoptosis in breast cancer cells. (A) The expression of LINC00702 in BT-474 and T-47D cells was detected following LINC00702 knockdown using RT-qPCR. (B) CCK8 assay was used to assess the relative number of T-47D and BT474 cells following transfection with shNC, shLINC00702-1, and shLINC00702-2. (C) The proliferation of BT-474 and T-47D cells after LINC00702 knockdown was detected using EdU assay, with red spots for LINC00702 and blue spots for DAPI (Scale bar = 50 μm). (D) Western blotting analysis was used to detect the expression Caspase 3. (E) Apoptosis in two cell lines was assessed following LINC00702 knockdown using Annexin V-APC7/AAD staining. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

viability and growth of BC cells after 24 h (both $p < 0.05$; Fig. 3B and C). Moreover, LINC00702 knockdown largely raised the Caspase 3 level in BC cells and promoted ($p < 0.05$) cell apoptosis compared with the normal ones (Fig. 3D and E). Overall, these results suggest that LINC00702 knockdown suppresses proliferation rate and causes apoptosis in BC cells.

3.3. LINC00702 knockdown inhibits the BC cell proliferation

To examine the influence of LINC00702 on BC in *vivo*, female nude mice were grafted with shLINC00702-2-transfected cells, and tumor growth was examined. Significantly smaller tumor sizes were observed in mice injected with shLINC00702-2-transfected cells than in the control mice ($p < 0.001$; Fig. 4A). Additionally, RT-qPCR showed a markedly decreased ($p < 0.01$) LINC00702 level in the knockdown group (Fig. 4B). Moreover, IHC showed a decreased concentration of the proliferation-related proteins Ki-67 and PCNA in the Lv-shLINC0702-2-injected group (Fig. 4C). Collectively, the above findings revealed that LINC0702 knockdown suppresses the BC cell growth in *vivo*.

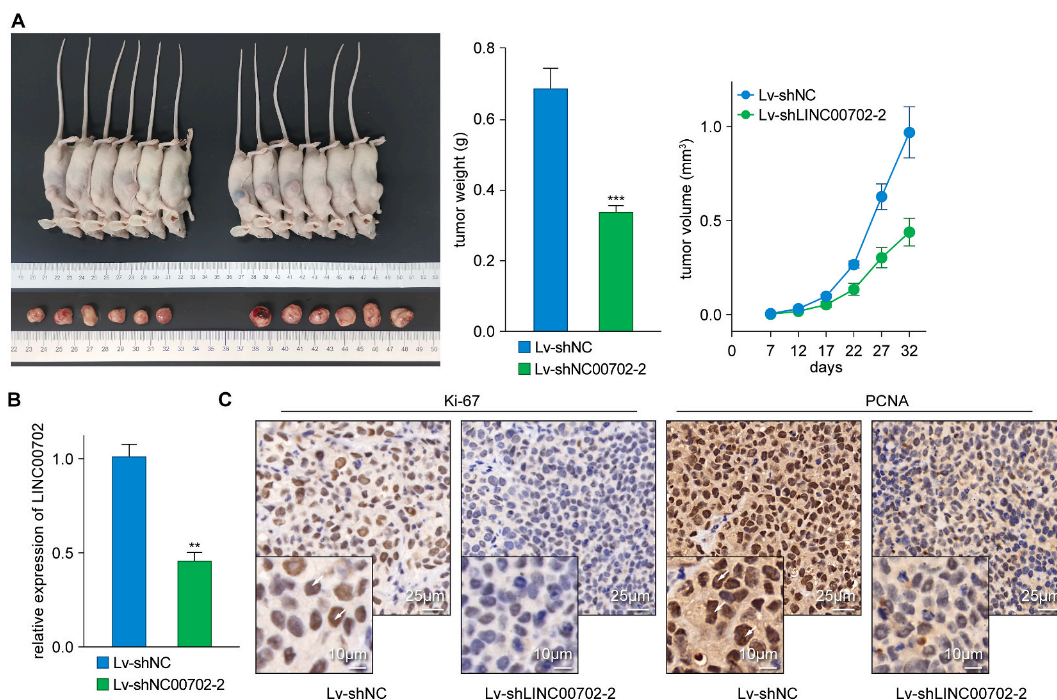


Fig. 4. Injection with shLINC00702-transfected cells decreased the proliferation rate of breast cancer cells in *vivo*. shLINC00702-2-transfected cells were injected into nude mice to observe the effects of LINC00702 in *vivo*. (A) Tumor weight and volume in nude mice were assessed. (B) RT-qPCR analysis was performed to detect LINC00702 expression in *vivo*. (C) Immunohistochemistry was performed to detect the expression of the proliferation-related proteins Ki-67 and PCNA; the arrow is pointed to the target protein (Scale bar = 25 μm). $^{**}p < 0.01$.

3.4. The role of LINC00702 functions in BC via the Wnt/β-catenin pathway

To elucidate the impact of LINC00702 on BC development, GO functional annotation was performed for LINC00702 and associated genes in the PPI network. GO annotation showed that largely enriched genes related to LINC00702 ($p = 0.0226$) in the Wnt signaling pathway and tissue morphogenesis and cell adhesion (Fig. 5A). Therefore, we assessed the expression of two downstream proteins β-catenin and c-Myc. Compared with the shNC group, LINC00702 knockdown markedly suppressed ($p < 0.05$) the activity of the two factors in cancer cells (Fig. 5B), with similar trends observed in *vivo* (Fig. 5C).

Furthermore, the cells were exposed to HLY78 (an activator of the Wnt/β-catenin pathway) to examine the related protein expressions. HLY78 treatment significantly reversed LINC00702 knockdown-induced decrease in β-catenin and c-Myc expression in BT-474 cells (Fig. 6A). Additionally, EdU and CCK8 assays showed that HLY78 treatment significantly reversed LINC00702 knockdown-induced decrease in BT-474 cell viability, growth, and proliferation (Fig. 6B and C). Moreover, Annexin V-APC 7/AAD staining showed that HLY78 treatment significantly suppressed LINC00702 knockdown-induced increase in apoptosis in BT-474 cells (Fig. 6D); and WB showed the increased level of Caspase 3, HLY78 reversed the effects (Fig. 6E) Overall, these results confirm that LINC00702 knockdown decreases the two factor expressions to regulate the BT-474 cell cycle.

4. Discussion

BC is mostly accompanied by uncontrolled cell growth and can be divided into hormone receptor-positive (approximately 70%), ERBB2-positive (approximately 15–20%), and triple-negative (15%) BC [22]. Although BC-related mortality has been on a decrease owing to improvements in treatment technology, its incidence rate is increasing. BC remains a contributor to the disease burden in women [23], and its treatment includes surgery, chemotherapy, and radiation therapy [24]. Recently, research attention has focused on identifying and validating molecular biomarkers with potential prognostic and therapeutic value in BC. Particularly, it is important to identify novel targets to offer a reliable basis to precisely treat BC and improve prognosis.

lncRNAs were once considered transcriptional byproducts that lack coding ability. Recently, high-throughput sequencing technology has revealed a group of non-coding elements that mostly overlap with the lncRNA genome in mammalian genomes [25]. lncRNAs participate in several biological processes as biomarkers and are potential therapeutic targets. However, the potential mechanisms of action of lncRNAs in diseases are largely unknown, and limited studies have been performed on LINC00702 [26]. LINC00702 stimulates the progress of malignant meningioma via the Wnt/β-catenin pathway, indicating that LINC00702 may be responsible for the outcome of malignant meningioma [17]. Additionally, LINC00702 is upregulated in ovarian cancer cells and promotes ovarian tumor development by interacting with EZH2 and inhibiting KLF2 [16]. In contrast, LINC00702 is expressed at low

A

Statistics of Pathway Enrichment

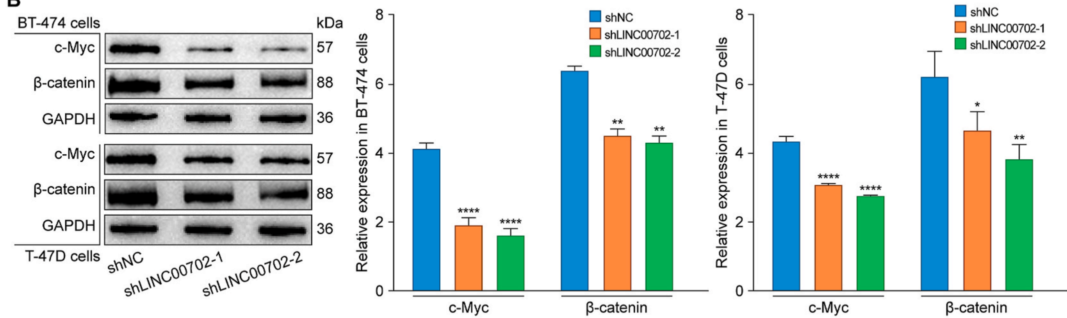
Wnt signaling pathway
vasculature development
tube morphogenesis
tube development
tissue development
system development
skeletal system development
regulation of Wnt signaling pathway
regulation of cellular response to growth factor stimulus
positive regulation of Wnt signaling pathway
multicellular organismal process
multicellular organism development
extracellular matrix organization
developmental process
collagen fibril organization
circulatory system development
cell adhesion
blood vessel morphogenesis
blood vessel development
biological adhesion
animal organ morphogenesis
animal organ development
angiogenesis
anatomical structure morphogenesis
anatomical structure development

0.4 0.8 1.2 1.6
Rich factor

gene_count
30
60
90
120

−log10(fdr)
0.4
0.2
0.0
−0.2

B



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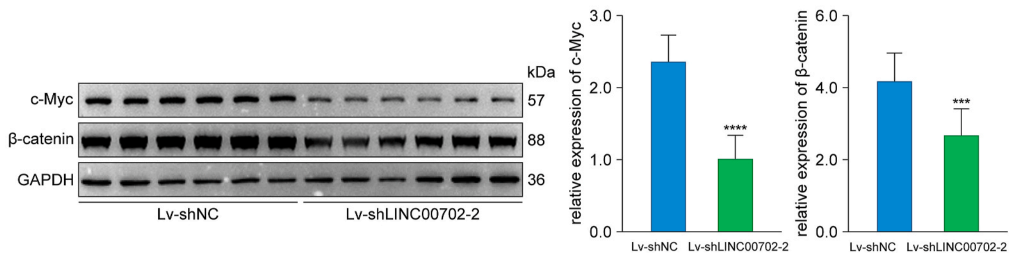

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Fig. 5. Effect of LINC00702 knockdown on c-Myc and β-catenin expression. (A) Gene ontology (GO) enrichment analysis was performed to identify LINC00702-related biological pathways. (B) Western blotting was performed to detect β-catenin and c-Myc expression in BT-474 and T-47D following LINC00702 knockdown using shLINC00702-1 and shLINC00702-2. (C) Western blotting analysis was performed to detect the expression of β-catenin and c-Myc in breast cancer tissues of mice. *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$.

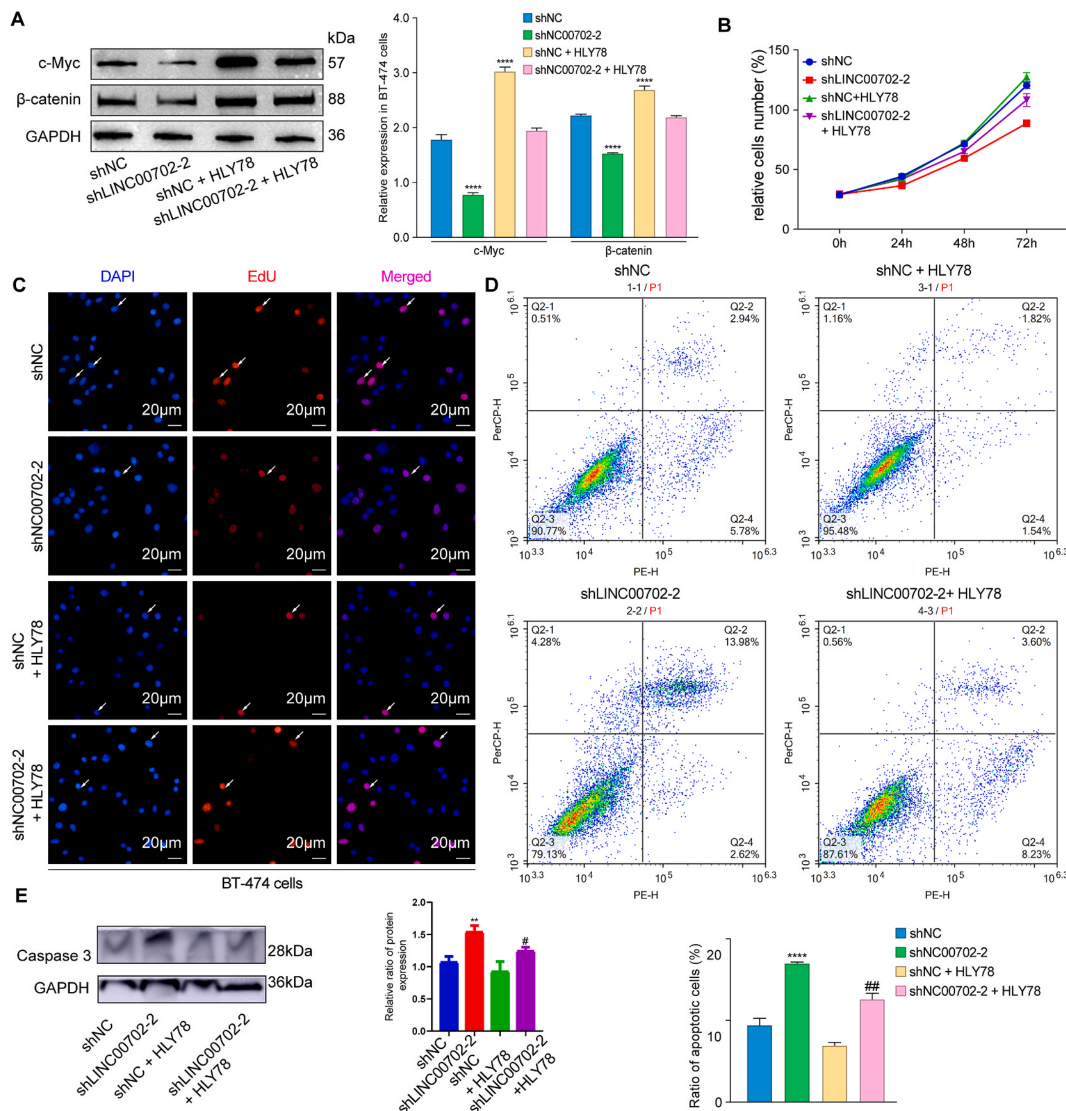

Fig. 6. Wnt/β-catenin is involved in cell proliferation and apoptosis in breast cancer. shLINC00702-2-transfected BT-474 cells were treated with the Wnt/β-catenin pathway activator HLY78, and the expression of the downstream proteins and cell proliferation and apoptosis were examined. (A) The expression levels of β-catenin and c-Myc in each group were determined using western blotting analysis. (B) Cell viability was observed using an CCK8 assay. (C) EdU assay kit was used to assess cell proliferation. (D) Cell apoptosis was determined in each group using Annexin V-APC7/AAD staining. (E) Western blotting was performed to detect the expression of Caspase 3. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs shNC.#$p < 0.05$, ##$p < 0.01$ vs shLINC00702-2.

levels and suppresses the proliferation of non-small-cell lung and colorectal cancers by controlling the PTEN axis [27,28]. Moreover, LINC00702 mediates DUSP1 transcription to prevent bladder cancer progression [15]. Therefore, it could be concluded that although LINC00702 expression level varies with cell lines, it may be a target for tumor therapy.

In the present study, we detected differentially expressed lncRNAs in BC tissues using bioinformatics and showed that LINC00702 was markedly upregulated and closely related to the patient's outcome. Additionally, its level was largely raised in the cytoplasm of BC cells, which was similar to findings in malignant meningioma and ovarian cancer cells. Moreover, LINC00702 knockdown significantly decreased cancer cell growth but induced cell apoptosis. Importantly, in vivo experiments revealed a markedly reduced tumor size in

nude mice injected with shLINC00702-2-transfected cells. Collectively, the above findings confirm that LINC00702 can be classified as an oncogenic lncRNA that promotes proliferation and suppresses apoptosis in BC.

The location and specific interactions of lncRNAs with DNA, RNA, and proteins are of critical importance in various cancer-related processes, with further control of chromatin function, modification of the stability and translation of cytoplasmic mRNAs, and disruption of signaling cascades [29]. Wnt/β-catenin and c-Myc can alter key cell functions. Moreover, the abnormal structure of β-catenin and its signal characteristics can frequently induce cancers [30]. The activated c-Myc is an typical event in tumor occurrence, and high levels of c-Myc are closely associated with tumor survival [31,32]. As the downstream gene of the Wnt/β-catenin pathway, c-Myc and β-catenin drive the process of proliferation and apoptosis in various diseases. For instance, RNF8 promotes the growth of colon cancer cells by stimulating their nuclear translocation and enhancing the expression of c-Myc [33]. Additionally, the Wnt/β-catenin pathway raises the c-Myc level and promotes the growth of human Sertoli cells [34]. Consistent with previous studies, this research showed that LINC00702 knockdown downregulated β-catenin and c-Myc levels, inhibited cell activity, and promoted apoptosis in BC. In a rescue experiment, treatment with the activator HLY78 upregulated the β-catenin and c-Myc expressions, increased cell growth, and decreased apoptosis. This result indicates that the Wnt/β-catenin pathway can regulate the growth and apoptosis of BC cells.

Despite the positive findings, this study has several limitations. For instance, bioinformatic analysis was used to enrich the possible pathway related to LINC00702, and the impact of its knockdown on the downstream proteins of Wnt/β-catenin was examined. However, it is important to also examine the impact of its overexpression on the related factors.

Conclusively, this study revealed that LINC00702 is a potential oncogenic lncRNA highly related to the survival of BC patients. Mechanistically, Knockdown of LINC00702 suppresses BC progression by decreasing growth and promoting apoptosis in BT-474 and T-47D cells via the Wnt/β-catenin pathway, making it a new therapeutic target. Nevertheless, more clinical investigations need to validate the our findings.

Funding

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Ethics approval

The Scientific Research Ethics Committee of the General Hospital of Ningxia Medical University (2020-057) approved the experimental procedure.

CRediT authorship contribution statement

Dahai Chai: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing. **Chunli Yang:** Data curation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **Yaobang Liu:** Conceptualization, Resources, Software. **Hong Li:** Conceptualization, Software, Visualization, Writing – review & editing. **Bin Lian:** Investigation, Methodology, Project administration, Writing – review & editing. **Zhengyang Bai:** Data curation, Validation. **JinPing Li:** Conceptualization, Data curation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20651.

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