

LncRNA-NONHSAT192404.1 Regulates miR-518a-3p to Inhibit Triple-Negative Breast Cancer Progression via the PI3K/AKT Pathway

Huachao Yang¹, Lin Gan¹, Yu Zheng², Jie Min¹, Zhifeng Xiong¹, Ting Wang¹, Shisi Hu¹, Gang Lv^{1,*}

ABSTRACT

Introduction: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer lacking targeted therapies. This study aimed to elucidate the role of the long non-coding RNA (lncRNA) NONHSAT192404.1 in TNBC progression and its regulatory relationship with miR-518a-3p and the PI3K/AKT signaling pathway. **Methods:** Quantitative real-time PCR (qPCR) was used to analyze NONHSAT192404.1 expression in TNBC cell lines. Functional assays, including CCK-8 proliferation, wound-healing, Transwell invasion, flow cytometry, and apoptosis analyses, were performed following NONHSAT192404.1 overexpression. The direct interaction between NONHSAT192404.1 and miR-518a-3p was confirmed using dual-luciferase and RNA pull-down assays. Western blot and qPCR analyses were employed to investigate the regulation of the PI3K/AKT pathway. **Results:** NONHSAT192404.1 was significantly down-regulated in TNBC cell lines. Its overexpression inhibited cell proliferation, migration, and invasion, and induced cell-cycle arrest and apoptosis. A direct molecular interaction between NONHSAT192404.1 and miR-518a-3p was established. Furthermore, NONHSAT192404.1 was shown to negatively regulate the PI3K/AKT pathway in a miR-518a-3p-dependent manner. **Conclusion:** These findings highlight NONHSAT192404.1 as a potential tumor suppressor in TNBC and suggest that the NONHSAT192404.1/miR-518a-3p/PI3K/AKT axis may serve as a promising therapeutic target.

Key words: Triple-Negative Breast Cancer, LncRNA, miR-518a-3p, PI3K/AKT

¹Department of Breast, Chongqing Hospital of Traditional Chinese Medicine, Chongqing 400021, China

²Department of Oncology, Chongqing Hospital of Traditional Chinese Medicine, Chongqing 400021, China

Correspondence

Gang Lv, Department of Breast, Chongqing Hospital of Traditional Chinese Medicine, Chongqing 400021, China

Email: czyfyyrxk@163.com

History

- Received: 19-5-2025
- Accepted: 25-8-2025
- Published Online: 31-8-2025

DOI : 10.15419/8z52aw42



Copyright

© Biomedpress. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.



INTRODUCTION

Breast cancer is the most prevalent malignancy among women worldwide, representing a significant public health challenge due to its heterogeneity and variable clinical outcomes^{1,2}. Among the various subtypes of breast cancer, Triple-Negative Breast Cancer (TNBC) is particularly notorious for its aggressive behavior and poor prognosis^{3,4}. TNBC is defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and HER2 protein expression, which renders conventional endocrine therapies and HER2-targeted treatments ineffective⁵. This leaves TNBC patients with limited therapeutic options, typically relying on surgery, radiation, and chemotherapy, which often result in high recurrence rates and poor overall survival^{6,7}. Given the urgent need for effective targeted therapies in TNBC, there has been increasing interest in the molecular mechanisms that drive TNBC tumorigenesis⁸. Studies indicate that long non-coding RNAs (lncRNAs) and circular RNAs are not only involved in TNBC tumorigenesis but also show potential as targets for personalized treatment, offer-

ing opportunities for improved monitoring of recurrence and resistance⁹. Recent advances in genomics and transcriptomics have revealed that lncRNAs play critical roles in cancer biology¹⁰. LncRNAs, which are transcripts longer than 200 nucleotides that do not encode proteins, have been shown to regulate gene expression at various levels, including chromatin remodeling, transcriptional control, and posttranscriptional processing¹¹. The specificity of lncRNA expression profiles makes them promising for early diagnosis and targeted treatment in TNBC¹². Their dysregulation is associated with various oncogenic processes, including cell proliferation, invasion, and metastasis^{1,13,14}. Studies indicate that lncRNAs like LINC01087 and NRAD1 show promise as diagnostic biomarkers and therapeutic targets in TNBC due to their differential expression and potential roles in cancer progression^{5,15,16}. Additionally, research highlights that lncRNAs interact with miRNAs in TNBC, regulating gene expression and progression, thus presenting potential targets for neoadjuvant chemotherapy^{17,18}. Among these, miR-518a-3p is a microRNA recognized for its

Cite this article : Yang H, Gan L, Zheng Y, Min J, Xiong Z, Wang T, Hu S, Lv G. LncRNA-NONHSAT192404.1 Regulates miR-518a-3p to Inhibit Triple-Negative Breast Cancer Progression via the PI3K/AKT Pathway. *Biomed. Res. Ther.* 2025; 12(8):7633-7643.

tumor-suppressive properties¹⁹, but its interaction with lncRNAs and specific impact on TNBC cell behavior remain poorly understood.

The PI3K/AKT pathway is a critical regulator of cell survival, growth, and metabolism and is frequently activated in various cancers, including TNBC^{20,21}. Dysregulation of this pathway is common in TNBC and plays a central role in promoting cancer cell survival and chemoresistance, making it a key molecular target for developing effective therapies^{22,23}. Research has shown that approximately 25–30 % of TNBC cases feature genetic alterations in the PI3K/AKT/mTOR pathway, suggesting that targeted therapies may be particularly beneficial for these patients²⁴. Specific mutations in PIK3CA and other pathway components have been associated with TNBC progression, indicating the importance of the pathway in this cancer subtype²⁵. Recent findings suggest that certain long non-coding RNAs (lncRNAs) may activate the PI3K/AKT pathway in TNBC, providing potential new targets for therapeutic intervention²⁶. Recent studies have shown that lncRNAs can regulate the PI3K/AKT signaling pathway by acting as molecular sponges for specific miRNAs²⁷. lncRNA H19 promotes PI3K/AKT activation by binding to miR-140-5p in ovarian cancer, while PTENP1 enhances PTEN expression by sponging miR-17 and miR-19b, leading to pathway suppression^{27–29}. These findings support the idea that lncRNA–miRNA interactions play a key role in modulating PI3K/AKT activity in cancer.

lncRNA-NONHSAT192404.1 is a newly identified lncRNA whose role in TNBC has yet to be fully elucidated³⁰. Here, we identified lncRNA-NONHSAT192404.1 as significantly upregulated in TNBC tissues and cell lines, suggesting its potential role in the pathogenesis of this aggressive cancer subtype. Mechanistically, we found that lncRNA-NONHSAT192404.1 interacts with miR-518a-3p to modulate gene expression post-transcriptionally, with subsequent effects on the PI3K/AKT signaling pathway. This study aims to investigate the function and molecular mechanism of lncRNA-NONHSAT192404.1 in TNBC, focusing on its interaction with miR-518a-3p and its downstream regulation of the PI3K/AKT signaling pathway. Our findings are expected to contribute to identifying novel biomarkers and potential therapeutic targets in TNBC.

METHODS

Cell Culture

Triple-negative breast cancer (TNBC) cell lines MDA-MB-231 (ATCC, HTB-26) and the non-tumorigenic mammary epithelial cell line Hs578Bst (ATCC, HTB-126) were obtained from the American Type Culture Collection (ATCC, USA). TNBC cells and MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Thermo Fisher Scientific, Catalog No. 11965092) supplemented with 10 % fetal bovine serum (FBS; Gibco, Catalog No. 10082147), 1 % penicillin-streptomycin (Gibco, Catalog No. 15140122), and 2 mM L-glutamine (Gibco, Catalog No. 25030081). All cell lines were maintained at 37 °C in a humidified atmosphere containing 5 % CO₂. Cells were passaged at 70–80 % confluence using 0.25 % trypsin-EDTA (Gibco, Catalog No. 25200072).

lncRNA and miR-518a-3p Overexpression and Knockdown

For overexpression of lncRNA-NONHSAT192404.1 and miR-518a-3p, full-length sequences of lncRNA-NONHSAT192404.1 and precursor miR-518a-3p were cloned into the pcDNA3.1(+) vector (Invitrogen, Thermo Fisher Scientific, Catalog No. V79020). For knockdown experiments, small interfering RNAs (siRNAs) targeting lncRNA-NONHSAT192404.1 and antisense oligonucleotides (ASOs) targeting miR-518a-3p were synthesized by GenePharma (Shanghai, China). Cells were transfected with overexpression constructs, siRNAs, or ASOs using Lipofectamine 3000 (Invitrogen, Thermo Fisher Scientific, Catalog No. L3000015) according to the manufacturer's protocol. The sequences of the siRNAs used for knockdown are listed in **Supplementary Table 1**.

Transfection efficiency was confirmed by quantitative real-time PCR (qRT-PCR) 48 h post-transfection. Scrambled siRNA or ASO was used as a negative control.

Quantitative Real-Time PCR (qRT-PCR)

Expression levels of lncRNA-NONHSAT192404.1, NONHSAT148701.1, and miR-518a-3p were measured in TNBC cell lines (MDA-MB-231) and compared with the normal mammary epithelial cell line (Hs578Bst). Total RNA was extracted using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Catalog No. 15596026). miRNA was extracted with the mirVana miRNA Isolation Kit without phenol

(Thermo Fisher Scientific, AM1561). RNA concentration and purity were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Catalog No. ND-2000). cDNA was synthesized from 1 μ g total RNA using the PrimeScript RT Reagent Kit (Takara Bio, Japan, Catalog No. RR037A). qRT-PCR was performed with SYBR Green Master Mix (Applied Biosystems, Thermo Fisher Scientific, Catalog No. 4309155) on a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, Catalog No. 4485690). Expression levels of LncRNA-NONHSAT192404.1, miR-518a-3p, and other genes of interest were normalized to GAPDH or U6 snRNA, respectively, and calculated by the $2^{-\Delta\Delta C_t}$ method. Primer sequences are provided in **Supplementary Table 2**.

Cell Proliferation Assay

Cell proliferation was assessed with the Cell Counting Kit-8 (CCK-8) assay (Dojindo Laboratories, Japan, Catalog No. CK04-11). Cells were seeded in 96-well plates at 2,000 cells/well in 100 μ L complete medium. After overnight adhesion they were transfected as described. At 24, 48, 72, and 96 h post-transfection, 10 μ L CCK-8 solution was added per well and incubated for 2 h at 37 °C. Absorbance was read at 450 nm on a microplate reader (Bio-Rad, USA, Catalog No. 168-1000EDU). Experiments were done in triplicate.

Cell Cycle Analysis

Cell-cycle distribution was analyzed by flow cytometry after propidium iodide (PI) staining. Briefly, MDA-MB-231 cells (transfected as indicated) were harvested 48 h post-transfection, washed twice with ice-cold PBS, and fixed in 70 % ethanol at 4 °C overnight. After washing, cells were incubated with PI (50 μ g/mL) and RNase A (100 μ g/mL) in PBS for 30 min at room temperature in the dark, and DNA content was measured on a flow cytometer. Percentages of cells in G₀/G₁, S, and G₂/M phases were determined with appropriate software.

Apoptosis Detection

Apoptotic cells were quantified by Annexin V-FITC/PI dual staining followed by flow cytometry. Forty-eight hours after transfection, MDA-MB-231 cells were harvested by trypsinization, washed twice with PBS, and resuspended in 100 μ L 1 \times binding buffer. Annexin V-FITC (5 μ L) and PI (5 μ L) from the Annexin V-FITC/PI Apoptosis Detection Kit (KeyGEN BioTECH, China; Cat. No. KGA107) were

added and incubated for 10 min at room temperature in the dark. Samples were analyzed within 1 h. Cells were classified as viable (Annexin V⁻/PI⁻), early apoptotic (Annexin V⁺/PI⁻), and late apoptotic/necrotic (Annexin V⁺/PI⁺).

Cell Invasion Assay

The invasive capacity of TNBC cells was evaluated in Transwell chambers (Corning, USA, Catalog No. 3422) with 8- μ m-pore polycarbonate membranes coated with Matrigel (BD Biosciences, USA, Catalog No. 354234) diluted 1:8 in serum-free DMEM. Hs578Bst was included as a negative control. Cells (5 \times 10) in serum-free medium were seeded in the upper chamber; DMEM with 10 % FBS was placed in the lower chamber as chemoattractant. After 24 h, non-invading cells were removed, and invading cells were fixed with 4 % paraformaldehyde, stained with 0.1 % crystal violet, and counted under a microscope (Olympus, Japan, Catalog No. CX23) in five random fields. Experiments were done in triplicate.

Wound-Healing Assay

Transfected TNBC cells were seeded at 5 \times 10⁵ cells/well in 6-well plates and grown to 90 % confluence. A linear scratch was made with a 200 μ L pipette tip, debris was removed with PBS (Gibco, Thermo Fisher Scientific, Catalog No. 10010049), and cells were cultured in serum-free DMEM. Images were captured at 0, 24, and 48 h with an inverted microscope. Wound width was measured in ImageJ and quantified as previously described³¹.

Luciferase Reporter Assay

To validate the interaction between LncRNA-NONHSAT192404.1 and miR-518a-3p, the pmirGLO dual-luciferase vector (Promega, USA; Cat. No. E1330) was used. A fragment of the TLR4 3'-UTR containing the predicted miR-518a-3p site was cloned downstream of firefly luciferase; a mutant site was generated with the QuikChange II kit (Agilent Technologies, USA; Cat. No. 200523). MDA-MB-231 cells were co-transfected with wild-type, mutant, or empty pmirGLO plus miR-518a-3p mimics (GenePharma, China) using Lipofectamine 3000. After 48 h, luciferase activity was measured with the Dual-Luciferase Reporter Assay System (Promega, USA; Cat. No. E1910). Experiments were done in triplicate.

RNA Pull-Down Assay

Direct interaction between LncRNA-NONHSAT192404.1 and miR-518a-3p was

tested by a biotin-labeled RNA pull-down assay. Wild-type and mutant transcripts of LncRNA-NONHSAT192404.1 were *in vitro* transcribed with 3'-biotin and incubated with streptavidin-magnetic beads using the Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific, USA; Cat. No. PI20164). After incubation with MDA-MB-231 lysates, bound RNA was eluted and miR-518a-3p enrichment quantified by qRT-PCR. Scrambled biotin-RNA served as negative control.

Western Blot

MDA-MB-231 cells were transfected as indicated. Total protein was extracted with RIPA buffer (Cell Signaling Technology, USA, Catalog No. 9806S) plus protease/phosphatase inhibitors (Thermo Fisher Scientific, Catalog No. 78440). Protein concentration was determined with the BCA kit (Thermo Fisher Scientific, Catalog No. 23225). Equal protein amounts (30 μ g) were resolved by SDS-PAGE on 4–20 % gradient gels (Bio-Rad, USA, Catalog No. 4561094) and transferred by wet transfer to PVDF membranes (Millipore, USA, Catalog No. IPVH00010). Membranes were blocked with 5 % non-fat milk in TBST (Cell Signaling Technology, Catalog No. 9997S) for 1 h and incubated overnight at 4 °C with primary antibodies (1:1000): TLR4 (Proteintech, 19811-1-AP), AKT (Proteintech, 10176-2-AP), PI3K (Proteintech, 27921-1-AP), PPP2R1A (Proteintech, 15882-1-AP), and GAPDH (Proteintech, 10494-1-AP). After washing, membranes were incubated with HRP-conjugated secondary antibodies (Cell Signaling Technology, Catalog No. 7074S; 1:2000) for 1 h at room temperature. Bands were visualized with ECL substrate (Bio-Rad, Catalog No. 1705061) and imaged on a ChemiDoc XRS+ system (Bio-Rad, USA, Catalog No. 1708265). Densitometry was performed with Image Lab.

Bioinformatics Analysis

Potential miR-518a-3p target genes were predicted with TargetScanHuman 7.2. Functional enrichment (GO terms, KEGG pathways) was analyzed in DAVID v6.8. Protein-protein interaction networks were generated with STRING v11.0 and visualized in Cytoscape v3.

Statistics

Data are presented as mean \pm SD. Two-group comparisons used Student's t-test; multiple groups used one-way ANOVA with Tukey's post-hoc test. $p < 0.05$ was considered significant. Analyses were performed in GraphPad Prism.

RESULTS

Downregulation of LncRNA-NONHSAT192404.1 and NONHSAT148701.1 in TNBC Cells

To evaluate the expression levels of LncRNA-NONHSAT192404.1 and NONHSAT148701.1 in triple-negative breast cancer (TNBC), we conducted quantitative real-time PCR (qRT-PCR) analyses in the TNBC cell line MDA-MB-231 and compared them with normal mammary epithelial cells (Hs578Bst). The results revealed a significant downregulation of LncRNA-NONHSAT192404.1 in TNBC cells relative to Hs578Bst cells (**Figure 1A**). Similarly, LncRNA-NONHSAT148701.1 was found to be significantly downregulated in TNBC cells (**Supplementary Fig. 1A**). These findings imply a potential involvement of LncRNA-NONHSAT192404.1 and NONHSAT148701.1 in the oncogenic processes driving TNBC. We then analyzed survival data from TNBC patients. Kaplan-Meier survival curves were generated to assess the relationship between LncRNA expression levels and patient prognosis. The analysis revealed a significant positive correlation between the expression levels of LncRNA-NONHSAT192404.1 and patient survival, indicating that higher levels of this LncRNA are associated with better outcomes in TNBC patients (**Figure 1B**). Similarly, increased expression of LncRNA-NONHSAT148701.1 was also found to be positively correlated with patient survival, further supporting its potential role as a prognostic biomarker in TNBC (**Supplementary Fig. 1B**).

LncRNA-NONHSAT192404.1 Inhibits TNBC Cell Proliferation and Induces Apoptosis

The influence of LncRNA-NONHSAT192404.1 on TNBC cell proliferation was evaluated using the Cell Counting Kit-8 (CCK-8) assay. Overexpression of LncRNA-NONHSAT192404.1 in MDA-MB-231 cells led to a significant decrease in proliferation (**Figure 2A-C**). In contrast, knock-down of LncRNA-NONHSAT192404.1 resulted in a marked increase in cell proliferation (**Figure 2A-C**), indicating that LncRNA-NONHSAT192404.1 plays a crucial role in inhibiting TNBC-cell proliferation. To understand the mechanistic basis of LncRNA-NONHSAT192404.1's effects, we examined its impact on cell-cycle progression. Flow-cytometry

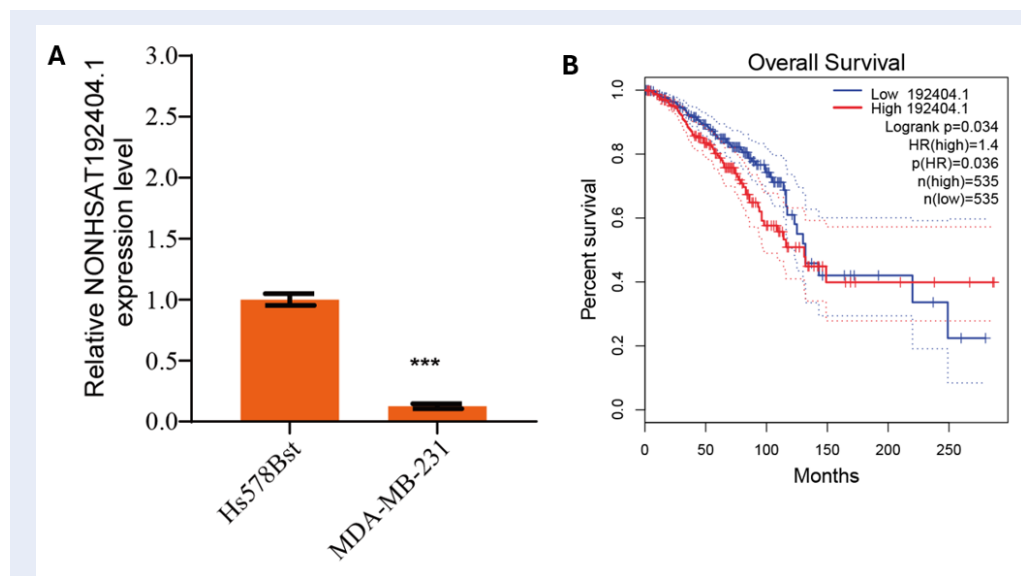


Figure 1: Expression levels of LncRNA-NONHSAT192404.1, NONHSAT148701.1, and miR-518a-3p in TNBC cells and their association with patient prognosis. (A) qRT-PCR analysis of LncRNA-NONHSAT192404.1, NONHSAT148701.1, and miR-518a-3p expression in TNBC cell line (MDA-MB-231) compared to normal mammary epithelial cells (Hs578Bst). NONHSAT192404.1 and NONHSAT148701.1 were significantly downregulated, whereas miR-518a-3p was upregulated in TNBC cells. (n = 3). Error bars represent mean \pm SEM. ***p < 0.001. (B) Kaplan-Meier survival analysis showing better overall survival in TNBC patients with high NONHSAT192404.1 expression. (C) Kaplan-Meier survival analysis showing better overall survival in TNBC patients with high NONHSAT148701.1 expression.

analysis revealed that overexpression of LncRNA-NONHSAT192404.1 led to an increase in the proportion of cells in the S phase, with a concomitant decrease in the M phase, suggesting cell-cycle arrest at S (Figure 2D, E). In addition, overexpression significantly increased apoptosis, as evidenced by a higher percentage of Annexin-V-positive cells, whereas knock-down reduced apoptosis rates (Figure 2F, G).

LncRNA-NONHSAT192404.1 Suppresses TNBC Cell Migration and Invasion

The wound-healing assay further supported these findings, demonstrating that cells overexpressing LncRNA-NONHSAT192404.1 exhibited slower wound closure, while knock-down significantly increased wound-closure ability, suggesting a role in cell migration (Figure 3A, B). To further investigate invasion, Transwell assays were performed. Overexpression of LncRNA-NONHSAT192404.1 significantly inhibited invasion of MDA-MB-231 cells. Conversely, knock-down resulted in a significant elevation in cell invasion (Figure 3C, D).

LncRNA-NONHSAT192404.1 Directly Interacts with miR-518a-3p

Based on bioinformatics predictions using TargetScan, which identified a putative binding site for miR-518a-3p within LncRNA-NONHSAT192404.1, we hypothesized a direct interaction (Figure 4A). miR-518a-3p was significantly up-regulated in MDA-MB-231 relative to normal mammary epithelial cells (Figure 4B). Dual-luciferase assays showed a significant reduction in luciferase activity. Importantly, mutating the miR-518a-3p binding site abolished this reduction, confirming direct binding (Figure 4C). An RNA pull-down assay corroborated these findings: miR-518a-3p was specifically enriched in complexes with LncRNA-NONHSAT192404.1 but not with control RNA (Figure 4D, E). These results demonstrate that LncRNA-NONHSAT192404.1 directly interacts with, and likely sponges, miR-518a-3p in TNBC cells.

Figure 2

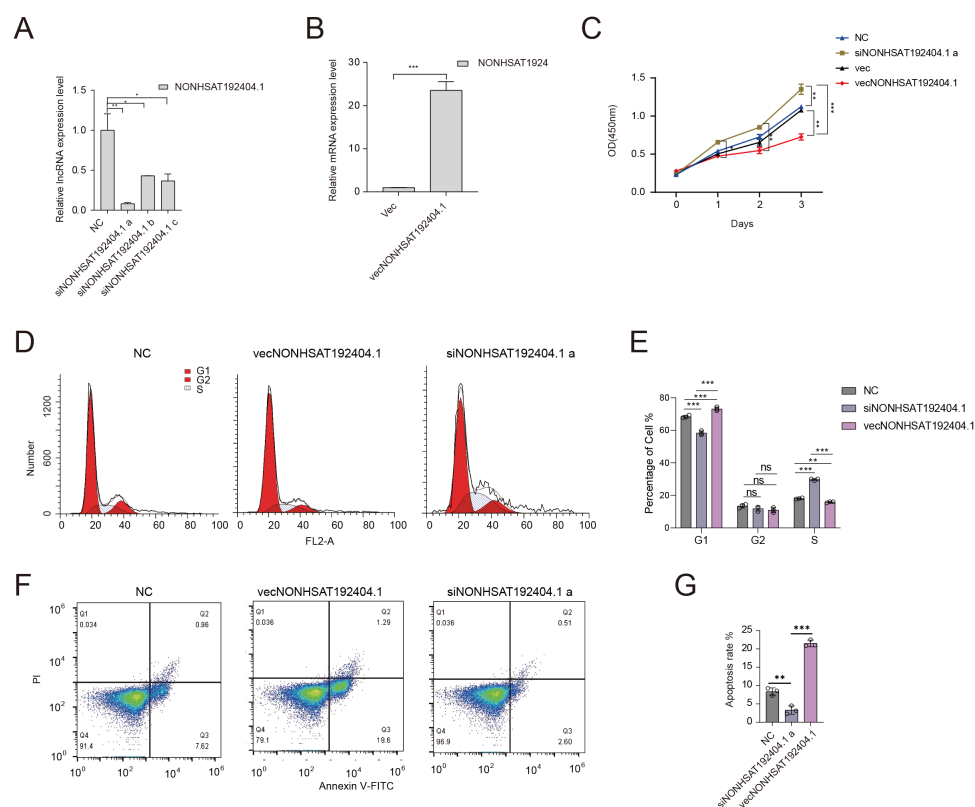


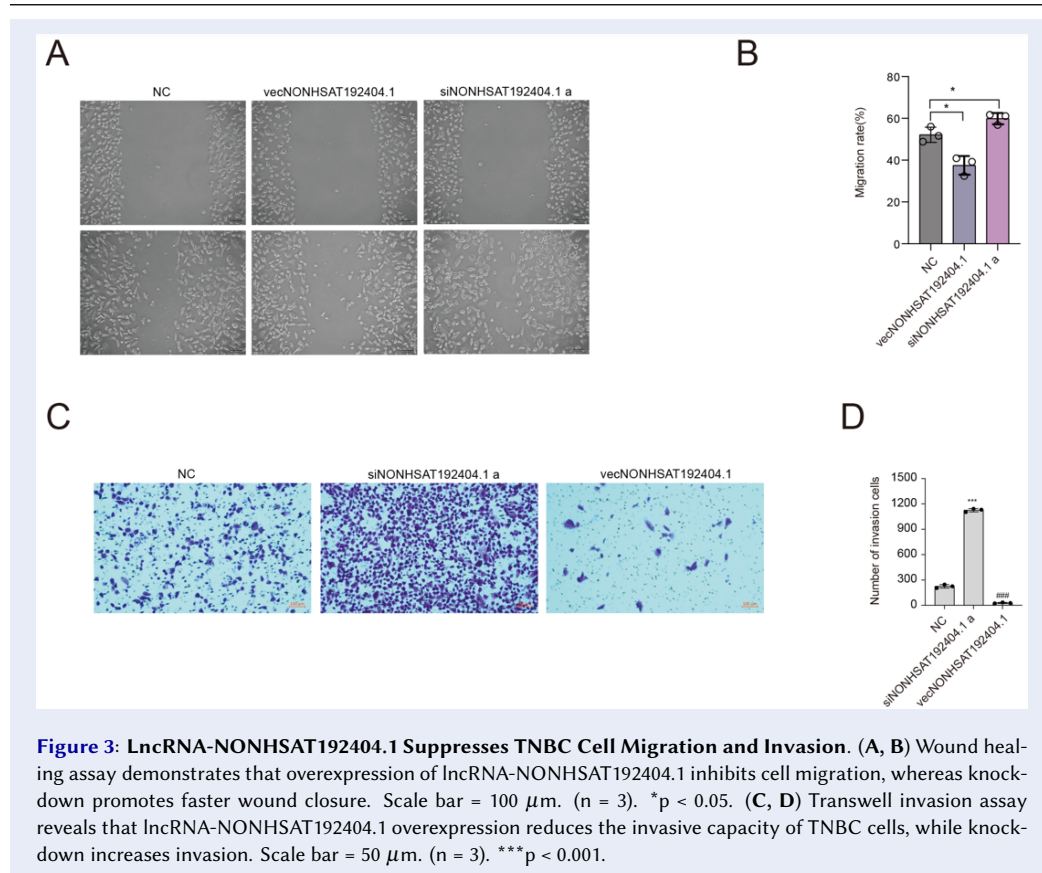
Figure 2: LncRNA-NONHSAT192404.1 Inhibits TNBC Cell Proliferation and Induces Apoptosis. (A-C) Overexpression of lncRNA-NONHSAT192404.1 significantly decreases TNBC cell proliferation, while its knock-down enhances proliferation as shown by the CCK-8 assay. (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001. (D, E) Flow cytometry analysis shows that lncRNA-NONHSAT192404.1 overexpression leads to cell cycle arrest at the S phase, while knockdown decreases S phase cells. (n = 3). p < 0.05. (F, G) Apoptosis analysis shows that lncRNA-NONHSAT192404.1 overexpression increases apoptotic cell death, while its knockdown reduces apoptosis. (n = 3). Error bars represent mean \pm SEM. **p < 0.01, ***p < 0.001.

Regulation of PI3K/AKT Signaling by LncRNA-NONHSAT192404.1 Is Reversible by miR-518a-3p

To explore how lncRNA-NONHSAT192404.1 regulates PI3K/AKT signaling, we quantified TLR4, AKT, PI3K and PPP2R1A expression. PI3K, AKT and PPP2R1A were significantly up-regulated in MDA-MB-231 versus Hs578Bst cells, whereas TLR4 was down-regulated (Figure 5A). Western-blotting showed that overexpression of lncRNA-NONHSAT192404.1 increased TLR4 and decreased AKT, PI3K and PPP2R1A (Fig. 5B). Notably, miR-518a-3p expression reversed the PI3K/AKT activation induced by lncRNA-NONHSAT192404.1 (Figure 5B).

DISCUSSION

In this study, we provide compelling evidence that lncRNA-NONHSAT192404.1 and NONHSAT148701.1 are significantly down-regulated in TNBC cells compared with normal mammary epithelial cells. Our data reveal that lncRNA-NONHSAT192404.1 inhibits TNBC cell proliferation, migration, and invasion. Over-expression of this lncRNA suppresses these oncogenic behaviors, whereas its knock-down enhances them, indicating its critical role in TNBC progression. Furthermore, our mechanistic studies suggest that lncRNA-NONHSAT192404.1 exerts its effects, at least in part, by modulating the PI3K/AKT signaling pathway, a well-known pathway implicated in cancer progression.



We identified a direct interaction between lncRNA-NONHSAT192404.1 and miR-518a-3p. Our luciferase reporter and RNA pull-down assays demonstrate that lncRNA-NONHSAT192404.1 can act as a molecular sponge for miR-518a-3p, thereby regulating its availability and activity. This interaction may represent a novel regulatory mechanism by which lncRNA-NONHSAT192404.1 influences TNBC cell behavior.

LncRNA-NONHSAT192404.1 and NONHSAT148701.1 emerge as potential therapeutic targets in TNBC. Targeting these lncRNAs could offer a new strategy to inhibit TNBC cell proliferation and invasion, thereby improving patient outcomes. Future studies should focus on the in-vivo validation of these lncRNAs as therapeutic targets and further exploration of their roles in TNBC pathogenesis.

Our findings align with the growing body of literature describing lncRNA-miRNA crosstalk in regulation of the PI3K/AKT pathway across cancers. For example, several studies have reported that lncRNAs acting as competitive endogenous RNAs (ceRNAs) regulate PI3K/AKT by sequestering oncogenic

miRNAs³². The identification of miR-518a-3p as a functional target of NONHSAT192404.1 adds a novel node to this regulatory network specifically in TNBC. While many ceRNA models involve lncRNAs sequestering miRNAs that target PI3K/AKT pathway members²⁷, our data extend this paradigm by showing that NONHSAT192404.1 itself is regulated by PI3K/AKT-related mechanisms via TLR4. Interestingly, some other TNBC-associated lncRNAs modulate different miRNAs or act through alternate pathways, which may reflect context-dependent expression profiles and binding-site availability, highlighting the importance of cell-specific investigations.

In our study, over-expression of NONHSAT192404.1 led to increased TLR4 expression but reduced PI3K/AKT pathway activity, which seems inconsistent with the classical view that TLR4 activates PI3K/AKT via the MyD88 adaptor to promote cell growth and survival. However, recent studies suggest that PI3K/AKT may also negatively regulate TLR4 signaling. For example, activated AKT can inhibit FOXO1, a transcription factor that promotes TLR4 expression, forming a feedback loop.

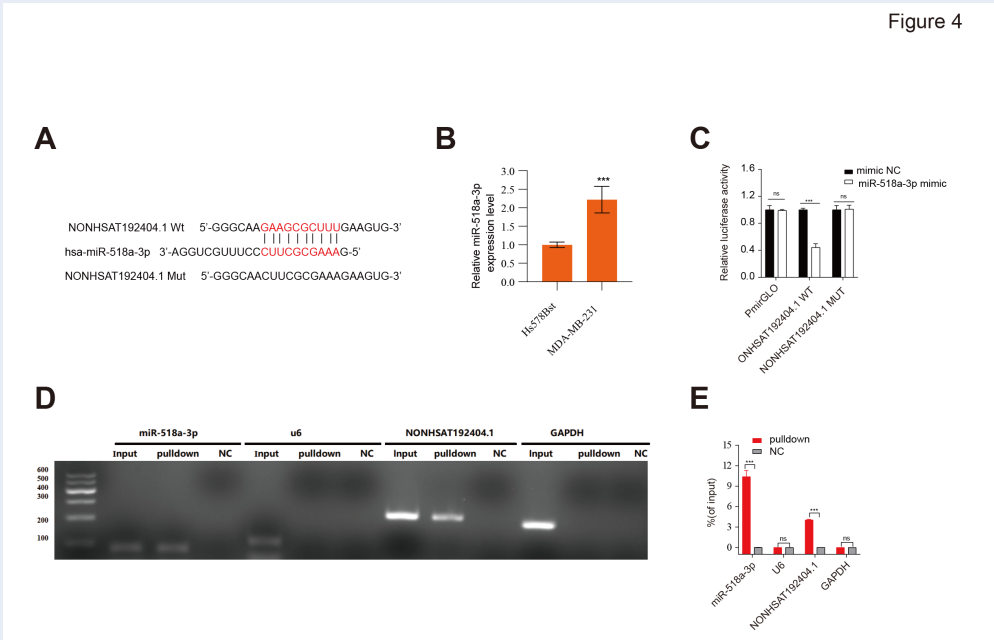


Figure 4: Direct interaction between LncRNA-NONHSAT192404.1 and miR-518a-3p. (A) Schematic representation of the predicted miR-518a-3p binding site within the sequence of NONHSAT192404.1. The wild-type and mutant binding site sequences used for luciferase reporter construction are shown. (B) Luciferase reporter assay confirms direct binding between NONHSAT192404.1 and miR-518a-3p. Mutations in the miR-518a-3p binding site of LncRNA-NONHSAT192404.1 abolish this interaction. (n = 3). Error bars represent mean \pm SEM. ***p < 0.001. (C,D) RNA pull-down assay further validates the direct interaction between LncRNA-NONHSAT192404.1 and miR-518a-3p in TNBC cells. (n = 3). Error bars represent mean \pm SEM. ***p < 0.001.

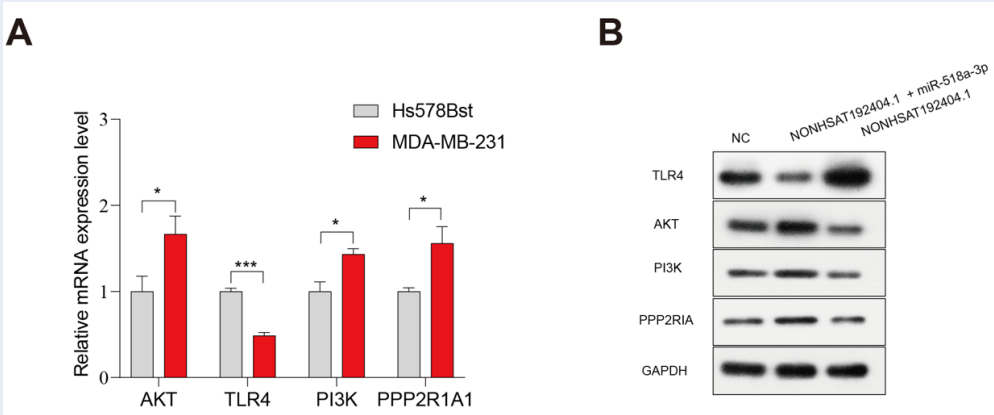


Figure 5: Regulation of the PI3K/AKT signaling pathway by LncRNA-NONHSAT192404.1 is reversible by miR-518a-3p. (A) qPCR analysis of PI3K, TLR4, AKT, and PPP2R1A expression in MDA-MB-231 cells relative to normal mammary epithelial cells (Hs578Bst, used as control). (n = 3). *p < 0.05, ***p < 0.001. (B) Western blot analysis showing the protein levels of PI3K, AKT, TLR4, and PPP2R1A in MDA-MB-231 cells following overexpression or knockdown of LncRNA-NONHSAT192404.1. Co-transfection with miR-518a-3p mimics reversed the effects of LncRNA overexpression, while miR-518a-3p inhibitors reversed the effects of LncRNA knockdown. GAPDH was used as a loading control.

In macrophages, PI3K/AKT also limits TLR4/NF- κ B-mediated inflammation, indicating a complex interaction^{33,34}. In cancer, although TLR4 over-expression often enhances NF- κ B activity and tumor progression, PI3K/AKT signaling can still be suppressed by other mechanisms, such as miRNA targeting or PTEN activation. This suggests that high TLR4 expression does not always lead to increased AKT activity³⁵⁻³⁸. Our results support this complexity: the relationship between TLR4 and PI3K/AKT is not strictly linear but is influenced by multiple layers of regulation.

Although the downstream targets of miR-518a-3p within the PI3K/AKT pathway have not yet been definitively characterized, recent evidence suggests that miR-518a-3p may regulate the PI3K/AKT pathway by targeting key components such as PI3K subunits, AKT isoforms, or upstream modulators like PIK3IP1. While the exact targets of miR-518a-3p remain to be fully identified, studies on similar miRNAs (e.g., miR-19a-3p and miR-520a-3p) have shown direct inhibition of PI3K/AKT signaling²⁷. In our system, it is likely that miR-518a-3p also suppresses PI3K/AKT activity by binding to relevant pathway molecules. In future studies, we will further investigate the downstream targets of miR-518a-3p in the PI3K/AKT pathway.

Our findings indicate that restoring NONHSAT192404.1 or inhibiting miR-518a-3p may suppress PI3K/AKT-mediated tumor progression in TNBC, highlighting NONHSAT192404.1 as a potential biomarker or therapeutic target. However, this study was limited to the MDA-MB-231 cell line, and further validation in additional TNBC models and patient samples is necessary. Future research should also assess its in-vivo relevance, examine downstream signaling events such as phosphorylation, and explore upstream regulatory mechanisms to better understand its role in TNBC pathogenesis.

CONCLUSIONS

Our study characterizes a novel regulatory mechanism by which lncRNA-NONHSAT192404.1 attenuates TNBC aggressiveness through interaction with miR-518a-3p and modulation of PI3K/AKT signaling. These findings contribute to a deeper understanding of noncoding RNA regulation in TNBC and provide a foundation for translational research targeting this axis.

ABBREVIATIONS

ASO: Antisense Oligonucleotide, **ATCC**: American Type Culture Collection, **BCA**: Bicinchoninic Acid, **cdNA**: Complementary DNA, **CCK-8**: Cell Counting Kit-8, **ceRNA**: Competitive Endogenous RNA, **CO₂**: Carbon Dioxide, **DAVID**: Database for Annotation, Visualization and Integrated Discovery, **DMEM**: Dulbecco's Modified Eagle Medium, **ECL**: Enhanced Chemiluminescence, **EDTA**: Ethylenediaminetetraacetic acid, **ER**: Estrogen Receptor, **FBS**: Fetal Bovine Serum, **FITC**: Fluorescein Isothiocyanate, **FOXO1**: Forkhead Box O1, **GAPDH**: Glyceraldehyde 3-Phosphate Dehydrogenase, **GO**: Gene Ontology, **HER2**: Human Epidermal Growth Factor Receptor 2, **HRP**: Horseradish Peroxidase, **KEGG**: Kyoto Encyclopedia of Genes and Genomes, **lncRNA**: Long Non-Coding RNA, **miRNA/miR**: MicroRNA, **mTOR**: Mammalian Target of Rapamycin, **MyD88**: Myeloid Differentiation Primary Response 88, **NF- κ B**: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells, **PBS**: Phosphate-Buffered Saline, **PI**: Propidium Iodide, **PI3K**: Phosphoinositide 3-Kinase, **PIK3CA**: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha, **PIK3IP1**: Phosphoinositide-3-Kinase Interacting Protein 1, **PR**: Progesterone Receptor, **PPP2R1A**: Protein Phosphatase 2 Scaffold Subunit Aalpha, **PTEN**: Phosphatase and Tensin Homolog, **PVDF**: Polyvinylidene Fluoride, **qPCR/qRT-PCR**: Quantitative Real-Time Polymerase Chain Reaction / Quantitative Reverse Transcription Polymerase Chain Reaction, **RIPA**: Radioimmunoprecipitation Assay, **RNA**: Ribonucleic Acid, **SD**: Standard Deviation, **SDS-PAGE**: Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis, **siRNA**: Small Interfering RNA, **STRING**: Search Tool for the Retrieval of Interacting Genes/Proteins, **TBST**: Tris-Buffered Saline with Tween 20, **TLR4**: Toll-Like Receptor 4, **TNBC**: Triple-Negative Breast Cancer, **UTR**: Untranslated Region

ACKNOWLEDGMENTS

We thank all the members of Gang Lv's Laboratory for their assistance throughout this research.

AUTHOR'S CONTRIBUTIONS

H.Y. and G.L. was responsible for the study's conception, design, and funding acquisition. L.G. performed the experiments and data analysis. Y.Z. contributed to data interpretation and manuscript revision. J.M. assisted with the experiment setup and

provided critical feedback during manuscript preparation. Z.X. and T.W. contributed to the experimental design and data collection. S.H. helped with statistical analysis and manuscript revision. All authors have read and approved the final manuscript.

FUNDING

This work was supported by the Special Project for Performance Incentives and Guidance of Scientific Research Institutions in Chongqing (cstc2022jxjl120038), the Chongqing Hospital of Traditional Chinese Medicine College “unveiling and commanding” project (JBGS2024-003), the Chongqing Municipal Health Commission Traditional Chinese Medicine Key Discipline Construction Project (YZY [2021] No. 16).

AVAILABILITY OF DATA AND MATERIALS

Data is provided within the manuscript or supplementary information files.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Chongqing Hospital of Traditional Chinese Medicine (approval number 2023-ky-52).

CONSENT FOR PUBLICATION

Not applicable.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that they have not used generative AI (a type of artificial intelligence technology that can produce various types of content including text, imagery, audio and synthetic data. Examples include ChatGPT, NovelAI, Jasper AI, Rytr AI, DALL-E, etc) and AI-assisted technologies in the writing process before submission.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

- Wang Q, Gao S, Li H, Lv M, Lu C. Long noncoding RNAs (lncRNAs) in triple negative breast cancer. *Journal of Cellular Physiology*. 2017;232(12):3226–33. PMID: 28138992. Available from: <https://doi.org/10.1002/jcp.25830>.
- Tang Z, Wei Y, Liang Y, Zhu X, Tang J, Sun Y. Breast cancer burden among young women from 1990 to 2021: a global, regional, and national perspective. *European Journal of Cancer Prevention : The Official Journal of the European Cancer Prevention Organisation (ECP)*. 2025;34(2):130–9. PMID: 39137116. Available from: <https://doi.org/10.1097/CEJ.0000000000000909>.
- Beltrán-Anaya FO, Romero-Córdoba S, Rebollar-Vega R, Arrieta O, Bautista-Piña V, Dominguez-Reyes C. Expression of long non-coding RNA ENSG00000226738 (lncKLHDC7B) is enriched in the immunomodulatory triple-negative breast cancer subtype and its alteration promotes cell migration, invasion, and resistance to cell death. *Molecular Oncology*. 2019;13(4):909–27. PMID: 30648789. Available from: <https://doi.org/10.1002/1878-0261.12446>.
- Yang F, Liu YH, Dong SY, Yao ZH, Lv L, Ma RM. Co-expression networks revealed potential core lncRNAs in the triple-negative breast cancer. *Gene*. 2016;591(2):471–7. PMID: 27380926. Available from: <https://doi.org/10.1016/j.gene.2016.07.002>.
- Shen X, Xie B, Ma Z, Yu W, Wang W, Xu D. Identification of novel long non-coding RNAs in triple-negative breast cancer. *Oncotarget*. 2015;6(25):21730–9. PMID: 26078338. Available from: <https://doi.org/10.18632/oncotarget.4419>.
- Lv M, Xu P, Wu Y, Huang L, Li W, Lv S. lncRNAs as new biomarkers to differentiate triple negative breast cancer from non-triple negative breast cancer. *Oncotarget*. 2016;7(11):13047–59. PMID: 26910840. Available from: <https://doi.org/10.18632/oncotarget.7509>.
- Zhu S, Sun J, Liu X, Shao H, Feng C, Wang Z, et al. CTCF-Induced lncRNA C5orf66-AS1 Facilitates the Progression of Triple-Negative Breast Cancer via Sponging miR-149-5p to Up-Regulate CTCF and CTNNB1 to Activate Wnt/ β -Catenin Pathway. *Molecular and Cellular Biology*. 2022;42(6):e0018821. PMID: 35499320. Available from: <https://doi.org/10.1128/mcb.00188-21>.
- Xu J, Wu KJ, Jia QJ, Ding XF. Roles of miRNA and lncRNA in triple-negative breast cancer. *Journal of Zhejiang University Science B*. 2020;21(9):673–89. PMID: 32893525. Available from: <https://doi.org/10.1631/jzus.B1900709>.
- Palcau AC, Brandi R, Mehterov NH, Botti C, Blandino G, Pulito C. Exploiting Long Non-Coding RNAs and Circular RNAs as Pharmacological Targets in Triple-Negative Breast Cancer Treatment. *Cancers (Basel)*. 2023;15(16):4181. PMID: 37627209. Available from: <https://doi.org/10.3390/cancers15164181>.
- Han J, Han B, Wu X, Hao J, Dong X, Shen Q. Knockdown of lncRNA H19 restores chemo-sensitivity in paclitaxel-resistant triple-negative breast cancer through triggering apoptosis and regulating Akt signaling pathway. *Toxicology and Applied Pharmacology*. 2018;359:55–61. PMID: 30244121. Available from: <https://doi.org/10.1016/j.taap.2018.09.018>.
- Chen C, Li Z, Yang Y, Xiang T, Song W, Liu S. Microarray expression profiling of dysregulated long non-coding RNAs in triple-negative breast cancer. *Cancer Biology & Therapy*. 2015;16(6):856–65. PMID: 25996380. Available from: <https://doi.org/10.1080/15384047.2015.1040957>.
- Singh DD, Lee HJ, Yadav DK, on Long Non-Coding RNAs in Triple-Negative Breast Cancer RCA. *Recent Clinical Advances on Long Non-Coding RNAs in Triple-Negative Breast Cancer*. *Cells*. 2023;12(4):674. PMID: 36831341. Available from: <https://doi.org/10.3390/cells12040674>.
- Nandagopal S, Misra S, Sankanagoudar S, Banerjee M, Sharma P, Pane SE. Long Non Coding RNA in Triple Negative Breast Cancer: A Promising Biomarker in Tumorigenesis. *Asian Pacific Journal of Cancer Prevention : APJCP*. 2023;24(1):49–59. PMID: 36708551. Available from: <https://doi.org/10.31557/APJCP.2023.24.1.49>.
- Kapinova A, Kubatka P, Zubor P, Golubnitschaja O, Dankova Z, Uramova S, et al. The hypoxia-responsive long non-coding RNAs may impact on the tumor biology and subsequent management of breast cancer. *Biomedicine & Pharmacotherapy*. 2018;99:51–8. PMID: 29324312. Available from: <https://doi.org/10.1016/j.biopha.2017.12.104>.

15. De Palma FD, Monaco VD, Pol JC, Kremer M, D'Argenio V, Stoll G, et al. The abundance of the long intergenic non-coding RNA 01087 differentiates between luminal and triple-negative breast cancers and predicts patient outcome. *Pharmacological Research*. 2020;161:105249. PMID: 33068730. Available from: <https://doi.org/10.1016/j.phrs.2020.105249>.
16. Vidovic D, Huynh TT, Konda P, Dean C, Cruickshank BM, Sultan M. ALDH1A3-regulated long non-coding RNA NRAD1 is a potential novel target for triple-negative breast tumors and cancer stem cells. *Cell Death and Differentiation*. 2020;27(1):363–78. PMID: 31197235. Available from: <https://doi.org/10.1038/s41418-019-0362-1>.
17. Ma Y, Liang L, Zhang Y, Wang M, Jiang W, Zhao F. Upregulated lncRNA WTAPP1 in Triple Negative Breast Cancer Predicts Survival. *Critical Reviews in Eukaryotic Gene Expression*. 2022;32(1):67–78. PMID: 35377982. Available from: <https://doi.org/10.1615/CritRevEukaryotGeneExpr.2021039983>.
18. Xing Z, Zhang M, Liu J, Liu G, Feng K, Wang X. Overexpression of lncRNA SAMMSON Promotes Triple-Negative Breast Cancer Cell Proliferation by Interacting with p53. *Critical Reviews in Eukaryotic Gene Expression*. 2021;31(6):1–8. PMID: 34936287. Available from: <https://doi.org/10.1615/CritRevEukaryotGeneExpr.2021039534>.
19. Jiang YZ, Liu YR, Xu XE, Jin X, Hu X, Yu KD. Transcriptome Analysis of Triple-Negative Breast Cancer Reveals an Integrated mRNA-lncRNA Signature with Predictive and Prognostic Value. *Cancer Research*. 2016;76(8):2105–14. PMID: 26921339. Available from: <https://doi.org/10.1158/0008-5472.CAN-15-3284>.
20. Kansara S, Pandey V, Lobie PE, Sethi G, Garg M, Pandey AK. Mechanistic Involvement of Long Non-Coding RNAs in Oncotherapeutics Resistance in Triple-Negative Breast Cancer. *Cells*. 2020;9(6):1511. PMID: 32575858. Available from: <https://doi.org/10.3390/cells9061511>.
21. Wang R, Huang Z, Qian C, Wang M, Zheng Y, Jiang R. lncRNA WEE2-AS1 promotes proliferation and inhibits apoptosis in triple negative breast cancer cells via regulating miR-32-5p/T0B1 axis. *Biochemical and Biophysical Research Communications*. 2020;526(4):1005–12. PMID: 32307083. Available from: <https://doi.org/10.1016/j.bbrc.2020.01.170>.
22. Amato O, Buisseret L, Gebhart G, Plouznikoff N, Larsimont D, Awada A, et al. PIK3CA copy-number gain and inhibitors of the PI3K/AKT/mTOR pathway in triple-negative breast cancer. *Cold Spring Harbor Molecular Case Studies*. 2023;9(2):a006255. PMID: 36863843. Available from: <https://doi.org/10.1101/mcs.a006255>.
23. Nguyen TT, Qu Z, Nguyen VT, Nguyen TT, Le TT, Chen S. Natural Prenylated Xanthenes as Potential Inhibitors of PI3K/Akt/mTOR Pathway in Triple Negative Breast Cancer Cells. *Planta Medica*. 2022;88(13):1141–51. PMID: 34963183. Available from: <https://doi.org/10.1055/a-1728-5166>.
24. Arnold KM, Pohlig RT, Sims-Mourtada J. Co-activation of Hedgehog and Wnt signaling pathways is associated with poor outcomes in triple negative breast cancer. *Oncology Letters*. 2017;14(5):5285–92. PMID: 29142600. Available from: <https://doi.org/10.3892/ol.2017.6874>.
25. Cossu-Rocca P, Orrù S, Mironi MR, Sanges F, Sotgiu G, Ena S, et al. Analysis of PIK3CA Mutations and Activation Pathways in Triple Negative Breast Cancer. *PLoS One*. 2015;10(11):e0141763. PMID: 26540293. Available from: <https://doi.org/10.1371/journal.pone.0141763>.
26. Tu Z, Hu Y, Raizada D, Bassal MA, Tenen DG, Karnoub AE. Long noncoding RNA-mediated activation of PROT1/PRR5-AKT signaling shunt downstream of PI3K in triple-negative breast cancer. *Proceedings of the National Academy of Sciences of the United States of America*. 2022;119(43):e2203180119. PMID: 36269860. Available from: <https://doi.org/10.1073/pnas.2203180119>.
27. Ghafouri-Fard S, Abak A, Anamag FT, Shoori H, Majidpoor J, Taheri M. The emerging role of non-coding RNAs in the regulation of PI3K/AKT pathway in the carcinogenesis process. *Biomedicine & Pharmacotherapy*. 2021;137:111279. PMID: 33493969. Available from: <https://doi.org/10.1016/j.biopha.2021.111279>.
28. Xu H, Ding Y, Yang X. Overexpression of Long Noncoding RNA H19 Downregulates miR-140-5p and Activates PI3K/AKT Signaling Pathway to Promote Invasion, Migration and Epithelial-Mesenchymal Transition of Ovarian Cancer Cells. *BioMed Research International*. 2021;2021(1):6619730. PMID: 34250088. Available from: <https://doi.org/10.1155/2021/6619730>.
29. Wang Y, Gao WJ. Long non-coding RNA-H19 promotes ovarian cancer cell proliferation and migration via the microRNA-140/Wnt1 axis. *The Kaohsiung Journal of Medical Sciences*. 2021;37(9):768–75. PMID: 34002485. Available from: <https://doi.org/10.1002/kjm2.12393>.
30. Yang R, Xing L, Wang M, Chi H, Zhang L, Chen J. Comprehensive Analysis of Differentially Expressed Profiles of lncRNAs/mRNAs and miRNAs with Associated ceRNA Networks in Triple-Negative Breast Cancer. *Cellular Physiology and Biochemistry*. 2018;50(2):473–88. PMID: 30308479. Available from: <https://doi.org/10.1159/000494162>.
31. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 2012;9(7):671–5. PMID: 22930834. Available from: <https://doi.org/10.1038/nmeth.2089>.
32. Li Z, Cai B, Abdalla BA, Zhu X, Zheng M, Han P. lncIRS1 controls muscle atrophy via sponging miR-15 family to activate IGF1-PI3K/AKT pathway. *Journal of Cachexia, Sarcopenia and Muscle*. 2019;10(2):391–410. PMID: 30701698. Available from: <https://doi.org/10.1002/jcsm.12374>.
33. Jiang H, Zhang Y, Hu G, Shang X, Ming J, Deng M. Innate/Inflammatory Bioregulation of Surfactant Protein D Alleviates Rat Osteoarthritis by Inhibiting Toll-Like Receptor 4 Signaling. *Frontiers in Immunology*. 2022;13. PMID: 35865531. Available from: <https://doi.org/10.3389/fimmu.2022.913901>.
34. Xu X, Liu X, Yang Y, He J, Jiang M, Huang Y. Resveratrol Exerts Anti-Osteoarthritic Effect by Inhibiting TLR4/NF-κB Signaling Pathway via the TLR4/Akt/FoxO1 Axis in IL-1β-Stimulated SW1353 Cells. *Drug Design, Development and Therapy*. 2020;14:2079–90. PMID: 32581510. Available from: <https://doi.org/10.2147/DDDT.S244059>.
35. Sun Y, Wu C, Ma J, Yang Y, Man X, Wu H. Toll-like receptor 4 promotes angiogenesis in pancreatic cancer via PI3K/AKT signaling. *Experimental Cell Research*. 2016;347(2):274–82. PMID: 27426724. Available from: <https://doi.org/10.1016/j.yexcr.2016.07.009>.
36. Li J, Yin J, Shen W, Gao R, Liu Y, Chen Y. TLR4 Promotes Breast Cancer Metastasis via Akt/GSK3β/β-Catenin Pathway upon LPS Stimulation. *The Anatomical Record : Advances in Integrative Anatomy and Evolutionary Biology*. 2017;300(7):1219–29. PMID: 28296189. Available from: <https://doi.org/10.1002/ar.23590>.
37. Hashemi M, Etemad S, Rezaei S, Ziaolhagh S, Rajabi R, Rahmani P, et al. Progress in targeting PTEN/PI3K/Akt axis in glioblastoma therapy: revisiting molecular interactions. *Biomedicine & Pharmacotherapy*. 2023;158:114204. PMID: 36916430. Available from: <https://doi.org/10.1016/j.biopha.2022.114204>.
38. Ibrahim HM, Ismail MB, Ammar RB, Ahmed EA. Thidiazuron suppresses breast cancer via targeting miR-132 and dysregulation of the PI3K-Akt signaling pathway mediated by the miR-202-5p-PTEN axis. *Biochemistry and Cell Biology*. 2021;99(3):374–84. PMID: 33103467. Available from: <https://doi.org/10.1139/bcb-2020-0377>.