Proceedings of the 5th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/99/2025.22150

Long Noncoding RNA LINC02487 in the Proliferation and Metastasis of Triple-Negative Breast Cancer Cells

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Abstract: Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer and a leading cause of cancer-related mortality in women. The incidence of TNBC is particularly high in China, yet treatment options remain limited, with chemotherapy—often associated with severe side effects—being the primary therapeutic approach. This study investigates the role of *LINC02487* in TNBC through in vitro experiments to assess its potential as a biomolecular marker for TNBC treatment. RT-qPCR analysis revealed that *LINC02487* expression was significantly upregulated in TNBC. Lentiviral transfection was used to establish *LINC02487* overexpression cell lines. CCK-8 assays and EdU staining demonstrated that *LINC02487* overexpression promoted TNBC cell proliferation at 24, 48, and 72 hours. Conversely, knockdown of *LINC02487* inhibited TNBC cell proliferation, as confirmed by experiments using *LINC02487* knockdown cell lines. Furthermore, Transwell assays indicated that *LINC02487* knockdown suppressed TNBC cell migration and invasion. Overall, this study confirms the high expression of *LINC02487* in TNBC and demonstrates that its knockdown can inhibit cell proliferation and metastasis, thereby slowing TNBC progression. These findings suggest that *LINC02487* has potential as a novel prognostic marker and therapeutic target for TNBC, offering new hope for improved patient outcomes.

Keywords: TNBC, *LINC02487*, noncoding RNA, biomarker, metastasis

1. Introduction

1.1. Triple-Negative Breast Cancer

Triple-negative breast cancer (TNBC) lacks the expression of three key molecular targets: estrogen receptor (ER), progesterone receptor (PR), and HER2. It is one of the most common and deadly cancers in women [1]. Epidemiological surveys show that TNBC accounts for 15%–25% of new breast cancer cases. According to the 2020 Global Cancer Burden Data from the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO), approximately 416,000 new breast cancer cases are diagnosed annually in China, with 60,000–80,000 involving TNBC [2]. Common risk factors for TNBC include family history, a high number of menstrual cycles, late pregnancy, and breastfeeding [3]. Comparing with other subtypes, TNBC has fewer treatment options. Chemotherapy remains the primary treatment, but the recurrence rate is high [3]. Compared to other breast cancer subtypes, TNBC has fewer treatment options. Currently, chemotherapy has been the primary treatment for TNBC patients, but the recurrence rate is extremely high [4]. While PARP inhibitors show targeted therapeutic potential, their effectiveness

DOI: 10.54254/2753-8818/99/2025.22150

is limited to a subset of patients [5]. Overall, TNBC is highly aggressive, often diagnosed at advanced stages, and associated with poor prognosis and a high risk of visceral metastasis. Therefore, developing early diagnostic and treatment strategies is **crucial for improving clinical outcomes.**

1.2. Triple-Negative Breast Cancer Metastasis

Tumor metastasis leads to death of cancer patients, which involves not only changes in tumor cells but also changes in the tumor microenvironment [6]. Metastasis is the process by which tumor cells invade surrounding lymphatic and blood vessels, spread to other tissues, and form new tumor foci. Metastasis can be classified into four types: local spread, lymphatic metastasis, blood metastasis, and distant metastasis [7]. In 1889, Stephen Paget's "seed-soil" theory holds that cancer cell metastasis depends on the interaction between cancer cells ("seeds") and the specific organ microenvironment ("soil"). The tumor-directed metastasis theory indicates that certain tumors preferentially metastasize to specific organs [8]. TNBC is highly prone to early metastasis, with a median overall survival of only 18 months once metastasis occurs. Brain metastases (BM) are particularly life-threatening for TNBC patients [9]. **Therefore, early diagnosis of TNBC patients at high risk of metastasis is crucial for timely treatment and prevention.**

Biomarkers, which include proteins, RNA, and other molecules, are critical for disease diagnosis, especially for tumor metastasis. Biomarkers can detect early, low-level damage, offer early warnings, and provide diagnostic support [10]. Clinically, biomarkers are categorized as diagnostic markers (to confirm disease presence or subtypes), disease severity markers (to assess disease progression), and prognostic markers (to predict clinical events such as recurrence or metastasis) [11]. Thus, biomarkers are essential for diagnosing tumor metastasis.

The biomarkers for TNBC brain metastasis remain unclear. Yunzhu et al. analyzed tissue and cell line samples of TNBC brain metastasis and identified 15 differentially expressed genes. Their results suggest that CXCL8 could serve as a prognostic biomarker for brain metastases in TNBC. Treatment of MDA-MB-231 and Hs578t cell lines with recombinant CXCL8 further confirmed its role [12]. Although CXCL8 may be a potential marker, it has limitations. Tumors evolve rapidly, and detecting markers like CXCL8 often requires invasive biopsies. These procedures, while accurate, pose risks, especially for advanced-stage patients who cannot tolerate repeated procedures. Moreover, no clinical marker for TNBC brain metastasis currently exists, warranting further investigation into new biomarkers. **Therefore, we aim to explore a sensitive, accurate, and less invasive biomarker for early TNBC brain metastasis diagnosis**.

Circulating tumor cells (CTCs) are a promising foundation for a novel biomarker for distal metastasis [13]. CTCs originate from primary tumors and are considered key agents of metastasis. They can survive in the bloodstream, evade immune responses, and colonize distant organs [14]. The detection of CTCs offers a real-time liquid biopsy approach for TNBC brain metastasis, enabling early intervention, treatment decisions, and prognosis assessment [15]. **In conclusion, peripheral blood markers may provide a sensitive, accurate, and minimally invasive method for the early diagnosis of TNBC brain metastasis in the future.**

1.3. The Role of Noncoding RNA in Triple-Negative Breast Cancer Metastasis

Non-coding RNA (ncRNA) is an RNA molecule transcribed from the genome that does not encode proteins [16]. There are two main types: structural ncRNA and regulatory ncRNA. Regulatory ncRNA can be further divided into long non-coding RNA (lncRNA) and micro RNA (miRNA). LncRNAs are usually longer than 200 nucleotides and play important roles in epigenetic regulation, cell cycle control, and cell differentiation [17]. MiRNAs are small single-stranded molecules of 20-

DOI: 10.54254/2753-8818/99/2025.22150

24 nucleotides that are derived from a hairpin structure called pre-miRNA. They regulate post-transcriptional gene expression by binding to complementary sequences in the 3' untranslated region (3'UTR) of target mRNAs, resulting in gene silencing [18]. Long intergenic noncoding RNAs (lincRNAs) can regulate gene expression by acting as competitive endogenous RNA (ceRNA) sponges, a mechanism that highlights RNA-RNA interactions [19]. LncRNAs make up a significant portion of human genome transcripts, accounting for up to 62% and existing in large numbers. Further research is needed to fully understand how functional lncRNAs influence tumor recurrence and metastasis, offering potential targets for cancer treatment [20].

LncRNAs play a pivotal role in TNBC metastasis. Research by Po-Shun et al. found that the lncRNA Linc-ZNF469-3 promotes TNBC lung metastasis through the miR-574-5p-ZEB1 signaling axis, potentially serving as a prognostic marker for TNBC metastasis [21]. Yongyin et al. observed elevated levels of LncRNA T376626 in TNBC serum and tissues, with cellular experiments showing that it binds to LAMC2, affecting TNBC cell invasion and migration. This suggests LncRNA T376626 as a diagnostic and prognostic biomarker for TNBC [22].LincRNAs also contribute to distant metastasis through circulating tumor cells (CTCs). Studies have identified DARS-AS1 as a potential therapeutic target for metastatic TNBC. Overexpression of DARS-AS1 promotes migration and invasion of human TNBC by activating the NF-κB/STAT3 pathway through inhibition of miR-129-2-3p and upregulation of CDK1. Treatment with DARS-AS1 siRNA-loaded exosomes effectively slows the growth and liver metastasis of TNBC [23]. The upregulation of lncRNA MLLT4-AS1 in TNBC tissues and Gln-deficient TNBC cell lines also promotes angiogenesis and metastasis by increasing Gln levels via XBP1SBM [24]. **In conclusion, lincRNAs are crucial in TNBC metastasis, but their role in TNBC brain metastasis remains unclear and warrants further study.**

1.4. Role of *LINC02487* in Triple-Negative Breast Cancer Metastasis

Yanli et al. from Peking University People's Hospital conducted a study using 34 fresh breast tumor tissues obtained between June and December 2020 from patients diagnosed with primary breast cancer. These patients underwent surgical resection, and RNA was extracted from the tumor tissue. The cDNA library was sequenced using the NextSeq platform. A comparison of gene expression between TNBC tissue and other breast cancer subtypes identified 273 differentially expressed genes (DEGs) in TNBC, with 172 up-regulated and 101 down-regulated.

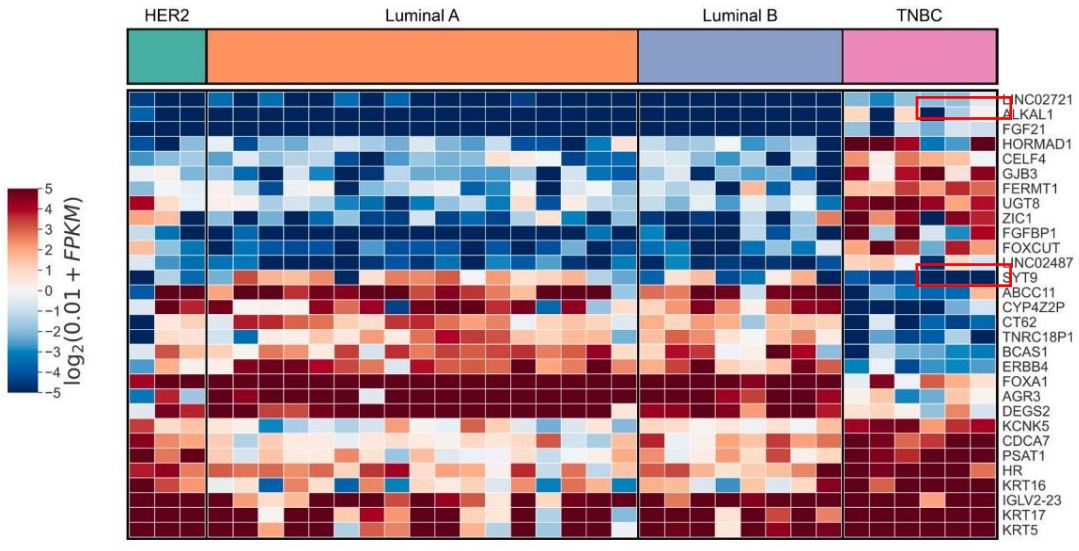

Figure 1: TNBC gene expression difference analysis heat map [25]

DOI: 10.54254/2753-8818/99/2025.22150

Notably, in Figure 1, LINC02487 was significantly up-regulated in TNBC, as shown in the heat map. However, its potential as an early warning marker for brain metastasis remains unclear [25]. Multiple studies suggest that *LINC02487* could serve as an early diagnostic marker for various cancers. Joanna et al. found through bioinformatics analysis that *LINC02487* may be a clinical molecular marker for head and neck squamous cell carcinoma [26]. Yue et al. analyzed clinical lncRNA data from 167 oral squamous cell carcinoma (OSCC) samples and 45 normal tissues, identifying six new lncRNA biomarkers for OSCC [27]. Feng et al. observed a significant reduction in LINC02487 expression in six OSCC cell lines. In a study of 50 OSCC samples from the Chinese population, they found a correlation between *LINC02487* expression levels and cancer metastasis. Further research showed that *LINC02487* interacts with USP17 (a deubiquitinating enzyme) and inhibits OSCC cell migration and invasion through the USP17-snai1 axis [28]. In summary, although *LINC02487* shows potential as a marker for tumor metastasis, its role in TNBC metastasis remains undocumented.

In conclusion, *LINC02487* is significantly up-regulated in the differential gene expression profile of TNBC. However, the specific role of *LINC02487* in TNBC metastasis is still unknown, suggesting that it could become a promising clinical marker for TNBC metastasis in the future.

1.5. Research Significance

Brain metastasis is the main cause of death in TNBC patients, particularly impacting Chinese women. Currently, there exists no definitive prognostic marker for TNBC brain metastasis through liquid biopsy in clinical practice. This project, based on the gene expression profiles of TNBC patients at Peking University, introduces *LINC02487* as a potential molecular marker for TNBC brain metastasis. Through cytological experiments, the molecular mechanism of *LINC02487* will be further investigated using methods like RT-qPCR and RNA fluorescence detection. Successful completion of this project is anticipated to provide crucial scientific and clinical insights, potentially offering new molecular markers for TNBC brain metastasis.

2. Method

2.1. Patient Samples and Public Databases

The relative expression data of LINC02487 in triple-negative breast cancer (TNBC) patients were acquired from UALCAN, a publicly available cancer lncRNA interaction exploration resource (https://ualcan.path.uab.edu/). We adhered to the website guidelines and utilized datasets from The Cancer Genome Atlas (TCGA) for our analysis.

2.2. Cell Lines and Cell Culture

T-47D cell line (human breast ductal carcinoma cell line) and two triple-negative breast cancer (TNBC) cell lines BT-549 and MDA-MB-231 were obtained from Wuhan Pronocell Biotechnology Co., Ltd. The cell culture conditions and culture medium of this experiment were as follows: T-47D cells were cultured in RPMI 1640 medium; BT-549 cells were cultured in RPMI 1640 medium; MDA-MB-231 cells were cultured in DMEM medium. All culture media were supplemented with 10% (v/v) fetal bovine serum (Gibco) and 1% penicillin-streptomycin (100 μg/mL penicillin and 100 μg/mL streptomycin). After thawing, the cells were resuspended in the corresponding culture medium, centrifuged, and the supernatant was discarded. Fresh medium was added, and the cells were transferred to a culture dish, labeled, and placed in an incubator.

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2.3. Lentiviral-Mediated Overexpression

In this study, the pCMV-MCS-PGK-Puro vector was used to achieve stable overexpression of LINC02487. The full-length *LINC02487* sequence was synthesized and molecularly cloned into the pCMV-puro lentiviral vector (HanYin Biotech). The constructed overexpression vector and the empty pCMV-puro vector (as a control) were transfected into cells using lentivirus. To verify the overexpression efficiency of *LINC02487*, RT-qPCR was performed to measure its expression level.

2.4. Methods for Extracting Total RNA from Cells

Add 500 μl Trizol to each well of a 12-well plate and digest the cells on ice until completely lysed. Pipette the lysate repeatedly to ensure that the cells are completely broken. Transfer the mixture to a 1 ml centrifuge tube, incubate for 5-10 minutes, and then vortex with 500 μl chloroform for 15 seconds. Allow to separate at room temperature (the upper layer is chloroform) and transfer the supernatant to a new tube. Next, add 400 μl isopropanol and centrifuge for 10 minutes. Discard the supernatant and wash the RNA pellet with 1 ml 75% ethanol and centrifuge. Remove the ethanol and air-dry the pellet for 10-15 minutes. Finally, DEPC water was added to dissolve the RNA based on the amount of RNA precipitation.

2.5. RT-qPCR

Total cellular RNA was isolated, and cDNA was synthesized using the TB Green® Premix Ex Taq™ II Kit (Takara). Real-time qPCR was performed on an Applied Biosystems StepOnePlus Real-Time PCR System using the TB Green™ Premix Ex Taq™ PCR Kit (Takara Corporation) following the manufacturer's instructions. The primer sequences used are shown in Table 1:

Table 1: Sequences of RT-qPCR primers

	Positive	Reverse
Internal reference actin	GGGTATTTTGTGCTCCCCCA	CAGGCACTGAAGGTTCGGAT
LINC02487	GGGTATTTTGTGCTCCCCCA	CAGGCACTGAAGGTTCGGAT

2.6. CCK-8

A total of 1,000 cells/well were seeded into 96-well plates. At 3 time points (24, 48, and 72 hours after seeding), 10 μL of CCK-8 reagent (Beyotime, C0039, Cell Counting Kit-8) and 100 μL of culture medium were added to each well and then incubated at 37 °C for 2 hours. The optical density (OD) at 450 nm was measured using a microplate reader. Data are from three independent experiments, each of which was repeated three times.

2.7. DNA Transfection

Before transfection, approximately 200,000 to 700,000 cells per well were seeded into a six-well plate and cultured until the cell density reached 70–90%. Then replace the medium in each well with fresh medium containing serum and no antibiotics. Prepare two clean sterile centrifuge tubes for each well of the six-well plate. Add 125 μl of DMEM medium containing antibiotics and serum to each tube. Next, add 2.5 μg of micro-DNA to one tube and mix gently by pipetting. Add 5 μl of Lipo6000™ transfection reagent to another tube and mix well. Then gently mix the two and incubate at room temperature for 5 minutes. Subsequently, add 250 μl of Lipo6000™-DNA mixture evenly to each well of the six-well plate and mix gently. After 24–48 hours of further incubation, the transfection efficiency was assessed using appropriate methods.

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2.8. SiRNA Transfection

For each well of the six-well plate to be transfected, two centrifuge tubes were prepared. Add 125 μl of DMEM medium containing antibiotics and serum to each tube, add 100 pmol siRNA to one tube and mix thoroughly, and add 5 μl of Lipo6000™ transfection reagent to the other tube and mix thoroughly. The remaining steps were the same as for DNA transfection.

2.9. EdU Staining

EdU staining experiments were performed using the BeyoClick™ EdU-488 Cell Proliferation Assay Kit (C0071S, Beyotime). An appropriate amount of cells was cultured in a six-well plate and a 2×EdU working solution was prepared. An equal volume of preheated (37 °C) 2×EdU working solution (20 μM) was added to the six-well plate to ensure a final concentration of 1×EdU and incubated for 2 hours. After EdU labeling, the culture medium was removed, 1 ml of fixative was added, and the cells were fixed at a constant temperature for 15 minutes. The fixative was then removed and the cells were washed three times with 1 ml of wash solution for 3 to 5 minutes each time. Next, 1 ml of permeabilization solution was added and the cells were incubated at room temperature for 10 to 15 minutes.

2.10. Transwell Experiment

4×10^4 cells were suspended in 100 μL of DMEM without FBS and seeded into the upper chamber of a 24-well Transwell chamber (BD Pharmingen) with an 8 μm pore size membrane. The lower chamber was filled with medium containing 10% FBS as a chemoattractant. After 24 h of incubation, the non-migrated cells on the upper side of the membrane were removed with a cotton swab. The cells that migrated to the bottom of the membrane were fixed and stained with crystal violet solution (Beyotime Biotech), then counted and imaged under a microscope. Eight fields of view were randomly selected in each chamber to count the number of cells, and the average was calculated. Each experiment was repeated three times. The cell counting index was defined as the number of migrated cells.

2.11. Statistical Analysis

Statistical analysis was performed using GraphPad 7. The relative expression levels of RNA were analyzed using the $2^{-\Delta\Delta CT}$ method. All data are presented as the mean ± SD of three or more independent experiments. Two groups of data were compared using a two-sided Student's t test, with a significance level of $P < 0.05$.

3. Result

3.1. *LINC02487* Being Significantly Upregulated in TNBC Cell Lines

RT-qPCR was performed to investigate the expression of *LINC02487* in TNBC. The results in Figure 2A showed that *LINC02487* was significantly upregulated in two TNBC cell lines, BT-549 ($p < 0.001$) and MDA-MB-231 ($p < 0.0001$), compared to the T-47D human breast ductal carcinoma cell line. The expression level in the MDA-MB-231 cell line was 10-fold higher than that in BT-549, prompting the selection of MDA-MB-231 for subsequent experiments. To validate these findings, the relative expression of *LINC02487* in the TCGA data was analyzed (as shown in Figure 2B), and significantly higher expression was observed in 115 TNBC patients compared to controls, suggesting a potential association between *LINC02487* expression and cancer proliferation and metastasis.

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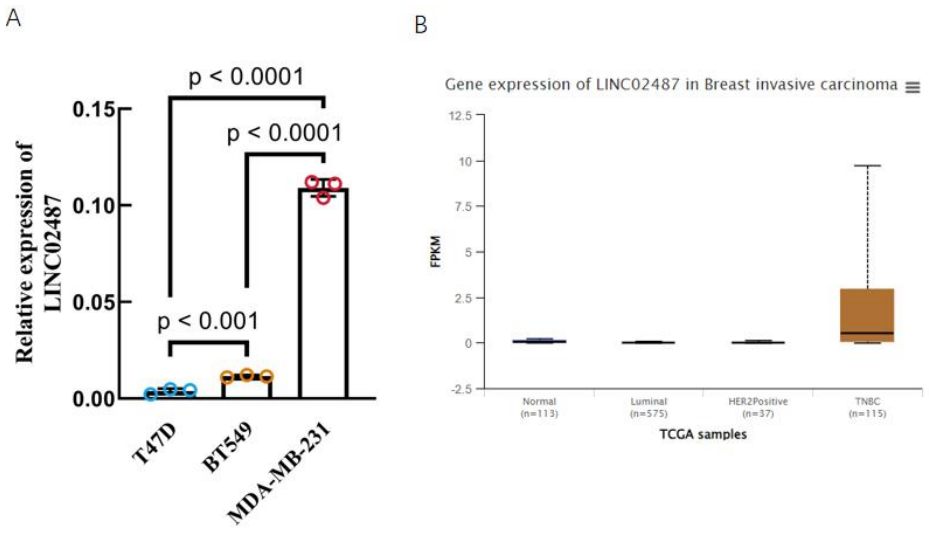

Figure 2: Expression level of *LINC02487* in TNBC.

A | RT-qPCR was used to detect the expression level of LINC02487 in TNBC cell lines BT 549 and MDA-MB-231.

B | LINC02487 expression level of TNBC clinical samples in TCGA database.

3.2. *LINC02487* Overexpression Promotes TNBC Cell Proliferation

To study the function of *LINC02487* in TNBC, we established an MDA-MB-231 TNBC cell line overexpressing *LINC02487* via lentiviral transfection of the pCMV-puro plasmid. RT-qPCR results showed that *LINC02487* was stably overexpressed more than 3000 times after transfection into MDA-MB-231 cells as shown in Figure 2A. Next, we compared the proliferation phenotype changes of MDA-MB-231 cells in the *LINC02487* overexpression group, empty vector group, and control group. The CCK-8 experiment found that the cell viability of *LINC02487* overexpressing cells was significantly increased. Further testing was performed on cells from each group at three time points: 24 h, 48 h, and 72 h. The results showed that the empty vector overexpression group was significantly better than the control group. There was no significant difference in cell activity among the groups, but the activity of cells overexpressing *LINC02487* was significantly higher than that of cells in the empty vector group, as shown in Figure 4A-C.

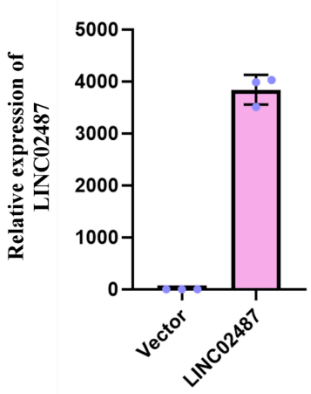

Figure 3: The relative expression level of *LINC02487* after transfection with lentiviral plasmid overexpression vector

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3.3. *LINC02487* Knockdown Significantly Inhibits the Proliferation of TNBC Cells

To further confirm the function of *LINC02487* in TNBC, we also examined the effect of knocking down *LINC02487* on cell proliferation in MDA-MB-231 cells. The CCK-8 test found that the cell viability of cells after *LINC02487* knockdown was significantly reduced, as observed in Figure 4A-C. Among them, cells were taken for testing at three time points: 24 h, 48 h, and 72 h. The results showed that the activity of *LINC02487* knockdown cells significantly decreased over time, and the difference with the control group was most obvious at 48 h. Statistical analysis in Figure 4 at 48 h revealed that cell activity in the *LINC02487* knockdown group was significantly inhibited compared to the control group ($P < 0.0001$), and was also significantly lower than in the empty vector control group ($P < 0.0001$).

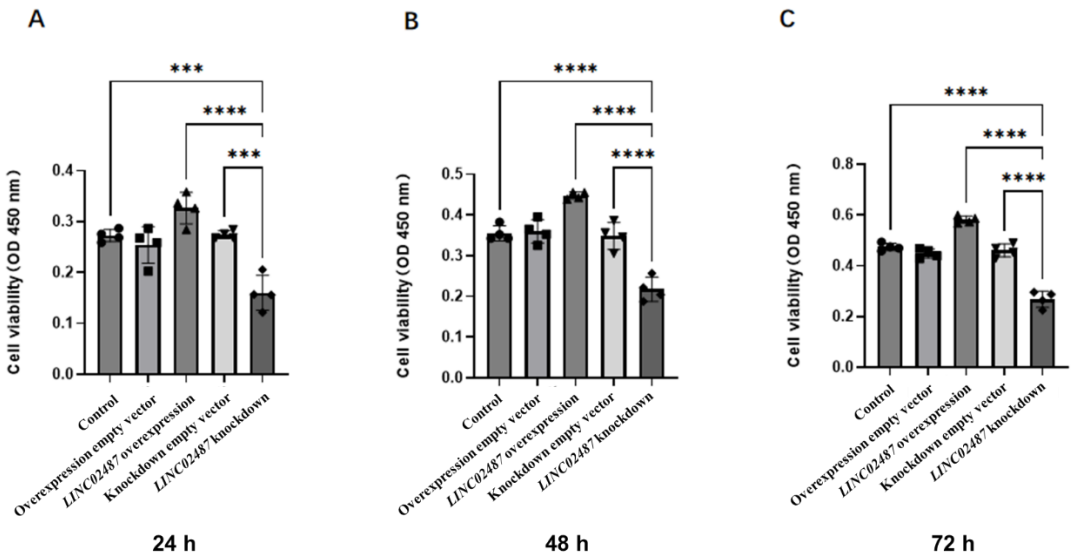

Figure 4: Effects of *LINC02487* overexpression and knockdown on MDA-MB-231 Effects on cell proliferation

A-C | CCK-8 assay detected the changes in cell proliferation at different time points of 24 h, 48 h, and 72 h after *LINC02487* overexpression or knockdown. **P < 0.01, ***P < 0.001, ****P < 0.0001.

3.4. EDU staining to detect *whether LINC02487* affects TNBC cell viability

EDU (5-ethynyl-2'-deoxyuridine) is a novel thymidine analog that can replace thymidine and is incorporated into newly synthesized DNA during DNA replication. It is commonly used as a marker to detect cell activity. To further confirm the effect of *LINC02487* on TNBC cell activity, we stained four groups of cells—overexpressing empty vector, overexpressing *LINC02487*, knocking down empty vector, and knocking down *LINC02487*—with EDU to assess cell activity. The results in Figure 5 showed that, compared to the empty vector control group, the cell activity in the *LINC02487* overexpression group was the highest, while the *LINC02487* knockdown group exhibited the lowest activity. What's more, we used a double staining method, marking cells with HOECHST nuclear staining to detect apoptosis and clarify the results.

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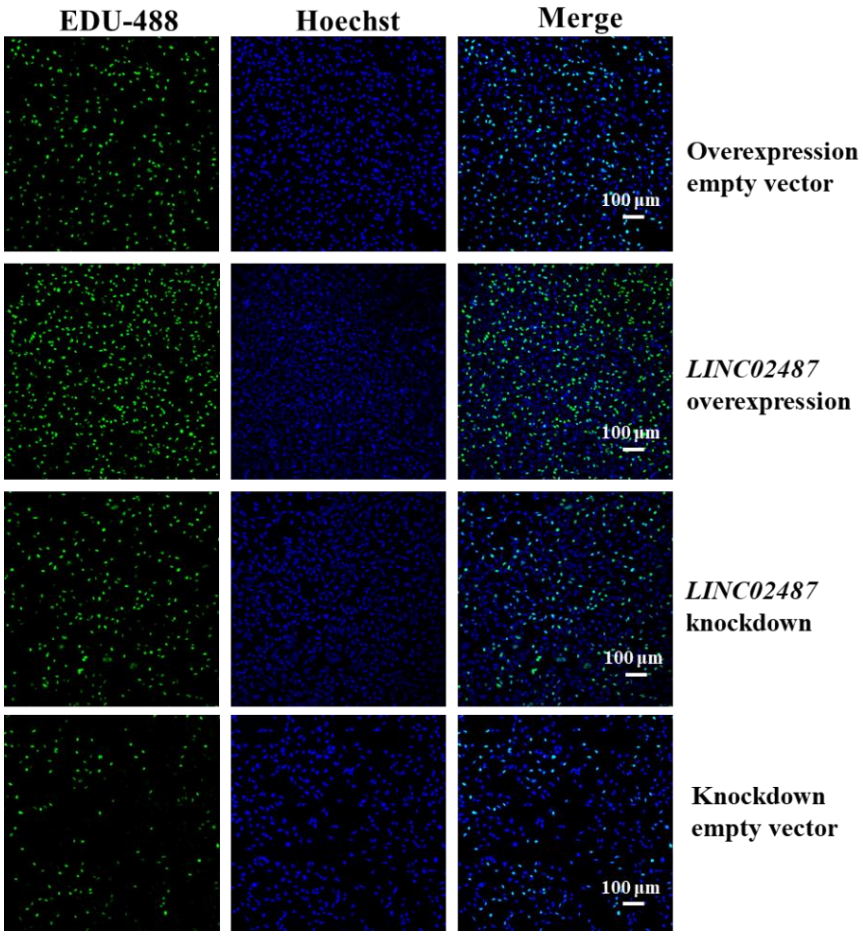

Figure 5: EDU staining to detect the effect of *LINC02487* on TNBC cell viability

The four groups of cells in the figure are *LINC02487* knockdown, *LINC02487* overexpression, knockdown empty vector, and overexpression empty vector; green fluorescence represents EDU staining; blue fluorescence represents HOECHST staining

3.5. Transwell Assay to Detect *Whether LINC02487* Affects the Migration and Invasion of TNBC Cells

We examined the effect on the migration and invasion abilities of MDA-MB-231 cells to further explore the function of *LINC02487*. We performed Transwell experiments, with or without Matrigel-coated upper chambers, on four groups of cells. The results in Figure 6A showed that both migration and invasion were significantly inhibited in *LINC02487* knockdown cells. Comparison of the *LINC02487* overexpression group with the empty vector overexpression group revealed a significant increase in both migration and invasion of TNBC cells. Similarly, comparing the *LINC02487* knockdown group with the empty vector knockdown group in Figure 6A and B showed a significant improvement in migration and invasion, with values significantly higher than those in the *LINC02487* overexpression group.

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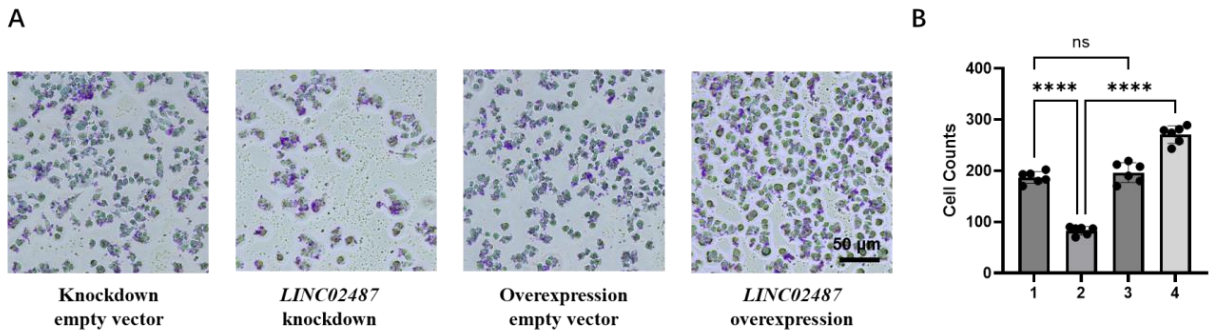

Figure 6: Transwell assay detects that *LINC02487* affects the migration and invasion of TNBC cells.

A | Using Transwell assay to detect cell migration in *LINC02487* knockdown, *LINC02487* overexpression, knockdown empty vector, and overexpression empty vector.

B | Statistical data of cell numbers in the above four groups. ns, not significant; *P < 0.05, **P < 0.01, ***P < 0.001.

4. Discussion

In this study, based on second-generation sequencing results from five TNBC patients published by Peking University People's Hospital, we discovered that the expression of two long non-coding RNAs, *LINC02487* and *LINC02721*, was specifically upregulated in TNBC. We first demonstrated that the expression of *LINC02487* was significantly upregulated in both TNBC cell lines and in 115 TNBC patients from the TCGA public database. To further explore the involvement of *LINC02487* in regulating the pathogenesis of TNBC, particularly in proliferation and distant metastasis, we used genetic engineering methods to construct in vitro models of TNBC with *LINC02487* overexpression and knockdown. CCK-8 assays showed that *LINC02487* overexpression significantly increased proliferation in the MDA-MB-231 cell line, while *LINC02487* knockdown significantly inhibited proliferation and delayed TNBC progression. Finally, we confirmed that *LINC02487* significantly influences the proliferation and metastasis of the MDA-MB-231 TNBC cell line. EDU staining results further validated the CCK-8 findings at the cellular level, showing that knockdown of *LINC02487* significantly inhibited proliferation in MDA-MB-231 cells. Transwell assays confirmed that *LINC02487* knockdown inhibited the migration and invasion of TNBC cells, delaying disease progression, while *LINC02487* overexpression promoted migration and invasion, increasing the risk of distant metastasis [29]. To explore the function of *LINC02487* in TNBC, we used StarBase to predict its target genes and, based on existing literature, speculated on the potential molecular mechanisms through which *LINC02487* promotes proliferation and metastasis in TNBC. Taken together, these findings suggest that *LINC02487* may be a potential prognostic biomarker for TNBC progression and metastasis, playing a novel role in TNBC pathogenesis.

LncRNA expression can serve as a marker for identifying biomarkers related to the prognosis of TNBC and is crucial for improving the biological understanding of the mechanisms driving TNBC progression [29]. LncRNAs may function as epigenetic, transcriptional, and post-transcriptional regulators in various cancer types [30]. The results show that the expression level of *LINC02487*, compared to the T47D human breast duct carcinoma cell line, is significantly upregulated in two TNBC cell lines (BT-549 and MDA-MB-231), with a more prominent upregulation observed in MDA-MB-231 cells. Yanli et al. from Peking University People's Hospital collected clinical samples from five TNBC patients and found, through second-generation sequencing, that the expression of *LINC02487* was significantly upregulated in Chinese TNBC patients, which is consistent with our findings [25]. In Yu's study, it was also found that *LINC02487* and FOXCUT

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are specifically highly expressed in basal-like breast cancer tissues. The increased expression of *LINC02487* may be positively correlated with a poorer prognosis. Their study further supports the idea that *LINC02487* may be highly expressed in TNBC [31]. Although our data shows that *LINC02487* is highly expressed in TNBC, studies have also reported that *LINC02487* is poorly expressed in oral squamous cell carcinoma (OSCC) and cervical cancer (CC), which contrasts with our results. Feng et al. confirmed that the expression level of *LINC02487* was significantly reduced in six independent OSCC cell lines. Further studies found that *LINC02487* expression was significantly downregulated compared to paired adjacent normal tissues in 50 OSCC samples from the Chinese population, and this may be related to tumor metastasis [27, 28]. Ru et al.'s study found that, similar to OSCC, *LINC02487* expression was downregulated in CC cells and tissues, and it may inhibit the progression of CC by upregulating PTEN expression and inhibiting the Akt/mTOR signaling pathway [32].

We found that the expression level of LINC02487 is inconsistent with its expression trend in OSCC and CC, suggesting that during tumor proliferation and metastasis, the mode of action of *LINC02487* differs significantly between TNBC, OSCC, and CC. This difference may be due to distinct targets and pathways involved in each cancer type. We also analyzed the expression of *LINC02487* across various cancer types in the TCGA database, as shown in Figure 7. Compared to OSCC samples, *LINC02487* expression was significantly upregulated in normal tissues. Additionally, figure 7 shows that the expression level of *LINC02487* was significantly upregulated in colorectal cancer, cholangiocarcinoma, rectal cancer, and esophageal cancer samples. Thus, the trend of *LINC02487* expression may vary across different cancer types.

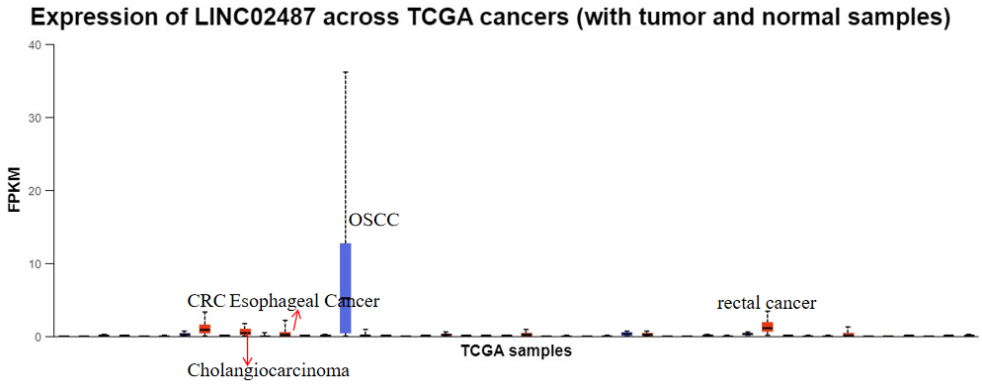

Figure 7: Expression levels of *LINC02487 in pan-cancers* in TCGC public data

LncRNA expression has important implications for the transcriptome and is dysregulated in many cancer types, and often serves as a prognostic marker. Upregulation of *LINC02487* may act as a prognostic marker for TNBC metastasis. To verify this hypothesis, we examined the relationship between high *LINC02487* expression in breast cancer and overall survival (OS) in TCGA data, as being presented in Figure 8. Our findings show that the OS of breast cancer patients with high *LINC02487* expression is not significantly reduced. While high *LINC02487* expression in breast cancer does not lower overall survival, it does not rule out a potential link with TNBC patient survival. Future studies could explore the correlation between high *LINC02487* expression and survival rates in TNBC patients, providing further evidence for its use as a prognostic marker.

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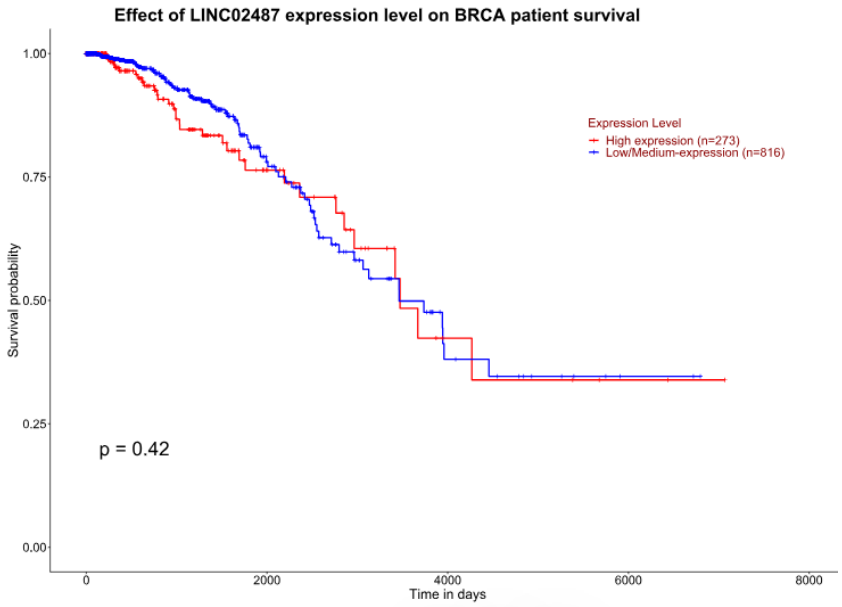

Figure 8: Correlation between high expression *of LINC02487 and OS of breast cancer patients* in TCGC public data

The StarBase v2.0 database is used to predict the potential downstream target genes of *LINC02487* in TNBC, being shown in Figure 9. Our analysis in Figure 9 identified *AIF1L* (Allograft Inflammatory Factor 1 Like), *PCGF2* (Polycomb Group RING Finger Protein 2), and *MIAT* (Myocardial Infarction Associated Transcript) as potential targets. According to Pei et al., *AIF1L* is downregulated and hypermethylated in breast cancer patients, and its ectopic expression inhibits MDA-MB-231 cell migration and invasion. Further evidence showed that AIF1L overexpression inhibited cell spreading, altered cell shape, and reduced protrusion formation because of decreased expression of focal adhesion kinase (FAK) and RhoA [33]. A cohort study from Shanghai found altered expression of *PCGF2* in TNBC patients, though no further mechanistic studies were conducted [34]. *PCGF2*, a transcriptional repressor often located in the nucleus, participates in DNA-binding transcription factor activity and ubiquitin protein transferase activity. It has tumor suppressor activity and may regulate cancer cell proliferation. Studies have shown that *LINC02487* is located in the cytoplasm and accumulates near the nuclear membrane [28]. Therefore, we hypothesize that *LINC02487* may regulate TNBC-related pathogenic gene transcription in the nucleus through *PCGF2*. The expression of *MIAT* is significantly higher in TNBC compared to normal or adjacent tissues, which may be linked to tumor-infiltrating immune cells in the tumor microenvironment [35]. Future experiments will further verify the binding and functionality of *LINC02487* with *AIF1L*, *PCGF2*, and *MIAT* through ChIRP, WB, and RIP assays. Based on this bioinformatics analysis combined with literature evidence, we conclude that the cellular functions regulated by *LINC02487* may be mediated through *PCGF2*.

DOI: 10.54254/2753-8818/99/2025.22150

ceRNAgeneID	ceRNAname	ceRNAType	GeneID	GeneName	GeneType	HitMiRnum
ENSG00000203688	LINC02487	lncRNA	ENSG00000224004	ATP5F1CP1	processed_pseudogene	3
ENSG00000203688	LINC02487	lncRNA	ENSG00000126878	AIF1L	protein_coding	5
ENSG00000203688	LINC02487	lncRNA	ENSG00000139651	ZNF740	protein_coding	5
ENSG00000203688	LINC02487	lncRNA	ENSG00000219712	AL357054.1	processed_pseudogene	2
ENSG00000203688	LINC02487	lncRNA	ENSG00000277258	PCGF2	protein_coding	4
ENSG00000203688	LINC02487	lncRNA	ENSG00000136908	DPM2	protein_coding	4
ENSG00000203688	LINC02487	lncRNA	ENSG00000225783	MIAT	lncRNA	4
ENSG00000203688	LINC02487	lncRNA	ENSG00000144711	IQSEC1	protein_coding	5
ENSG00000203688	LINC02487	lncRNA	ENSG00000115825	PRKD3	protein_coding	5

Figure 9: Target genes of *LINC02487* predicted by starbase v2.0 database

Table 2: Functions of potential target genes of *LINC02487* in TNBC

Gene	Function	Signaling pathways in TNBC	References
AIF1L	Regulates actin filament binding activity and participates in cytoskeletal movement	Ectopic expression of AIF1L inhibited the migration and invasion of MDA-MB-231 cells, confirming that AIF1L overexpression inhibited cell spreading, changed cell shape, and reduced protrusion formation, which was associated with decreased expression of focal adhesion kinase (FAK) and RhoA.	[33]
PCGF2	Transcription repressor. DNA-binding transcription factor activity and ubiquitin-protein transferase activity. May inhibit tumor activity. Plays a role in controlling cancer cell proliferation.	PCGF2 specifically binds to the DNA sequence 5'-GACTNGACT-3'. Participates in the p38 MAPK signaling pathway	[34]
MIAT	Encoding spliced long noncoding RNAs that may constitute components of the nuclear matrix.	unknown	[35]

Due to limited resources, this experiment only involved cell-based assays to verify our hypothesis, with no animal experiments or clinical trials conducted. As the study was primarily in vitro, the results may differ in a human environment. In the future, we plan to conduct animal experiments to enhance the project. By constructing a TNBC animal model and testing drug efficacy on cancer tissue, we aim to gather in vivo data.

5. Conclusion

We used genetic engineering to construct in vitro TNBC models with *LINC02487* overexpression and knockdown. CCK-8 assays showed that *LINC02487* overexpression significantly increased the

proliferation of the MDA-MB-231 cell line, whereas *LINC02487* knockdown significantly inhibited its proliferation and delayed TNBC progression. We also determined that *LINC02487* significantly affects the proliferation and metastasis of MDA-MB-231 cells. EDU staining further validated the CCK-8 results at the cellular level, confirming that *LINC02487* knockdown significantly inhibited MDA-MB-231 proliferation. Transwell assays showed that *LINC02487* knockdown inhibited migration and invasion of TNBC cells, while overexpression promoted these processes. Using StarBase, we predicted the target genes of *LINC02487* and, in combination with literature, speculated on the potential molecular mechanisms underlying its role in TNBC proliferation and metastasis. In summary, *LINC02487* may serve as a potential prognostic biomarker for TNBC progression and metastasis and play a novel role in its pathogenesis.

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