Breast Cancer: Targets and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

LINC01128 Affects Triple-Negative Breast Cancer Progression Through Targeting miR-32-5p

Min Xiong, Quanjun Yang, Le Cheng, Lili Yu, Yili Hu

Department of Oncology, Affiliated Renhe Hospital of China Three Gorges University, Yichang, Hubei, 443000, People's Republic of China

Correspondence: Yili Hu, Department of Oncology, Affiliated Renhe Hospital of China Three Gorges University, No. 410 Yiling Avenue, Yichang, Hubei, 443000, People's Republic of China, Tel +86-13908608166, Email yilihu443000@163.com

Breast Cancer: Targets and Therapy downloaded from https://www.dovepress.com/
For personal use only.

Objective: To clarify the expression and clinical significance of LINC01128 in triple-negative breast cancer (TNBC), investigate whether it regulates the biological behaviors of TNBC cells by targeting miR-32-5p via the ceRNA mechanism, and explore new therapeutic targets.

Methods: Tumor tissues and corresponding adjacent normal tissues from 76 TNBC patients were collected, and the patients' clinicopathological data were gathered. Experiments were conducted using the human normal breast epithelial cell line MCF-12F and multiple TNBC cell lines. Quantitative real-time PCR (qPCR) was used to detect the relative expressions of LINC01128 and miR-32-5p; dual-luciferase reporter assay was performed to verify the targeted binding relationship between the two. CCK-8 assay, flow cytometry, and Transwell assay were used to detect cell proliferation, apoptosis, and migration abilities, respectively. Target gene prediction and GO/KEGG enrichment analyses were carried out by combining databases such as miRDB and miRWalk.

Results: LINC01128 was highly expressed in TNBC tissues and cells ($P<0.01$), and its high expression was an independent risk factor for advanced TNBC (OR=6.635, P=0.001). miR-32-5p was lowly expressed in TNBC ($P<0.01$) and showed a significant negative correlation with LINC01128 ($r=-0.699$, $P<0.001$), with a direct targeted binding between the two. LINC01128 promoted TNBC cell proliferation and migration and inhibited apoptosis by suppressing miR-32-5p (all $P<0.01$). The target genes of miR-32-5p were enriched in tumor-related pathways.

Conclusion: LINC01128 is highly expressed in TNBC and promotes tumor progression by targeting and suppressing miR-32-5p via the ceRNA mechanism, which can serve as a potential molecular marker and therapeutic target for TNBC.

Keywords: LINC01128, miR-32-5p, triple-negative breast cancer, proliferation, apoptosis, migration

Introduction

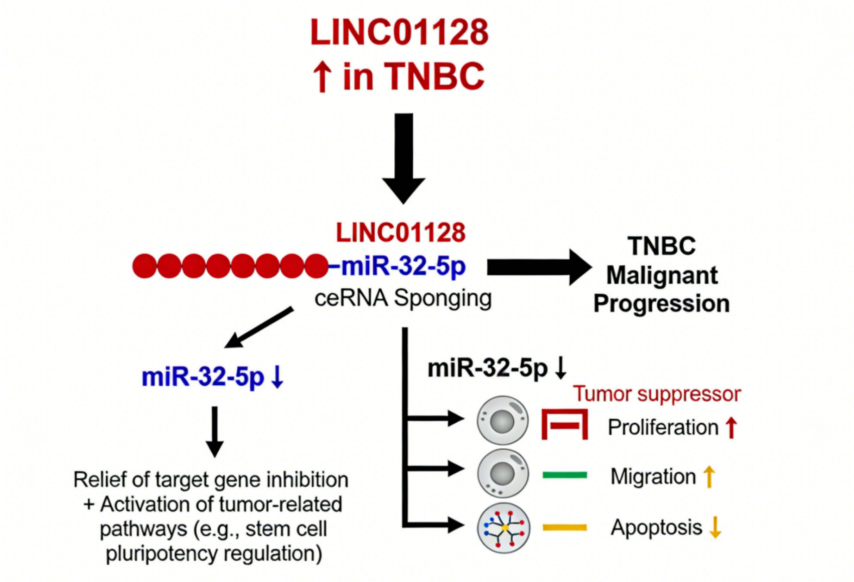
Breast cancer has evolved into the leading malignancy across the globe, exerting a severe impact on human wellbeing. Notably, triple-negative breast cancer (TNBC), a subtype characterized by extensive heterogeneity, comprises around 15% to 20% of all breast cancer incidences.[1–3] Owing to the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression, TNBC displays aggressive biological features, such as an early age of onset, strong metastatic capacity, and an unfavorable prognosis.[4–7] Despite the recent development of novel therapies such as antibody-drug conjugates (ADCs) and immune checkpoint inhibitors, their clinical efficacy remains limited, and chemotherapy remains the first-line treatment. Accordingly, comprehensive dissection of the molecular pathways mediating TNBC progression plays a critical role in uncovering novel therapeutic targets.

Defined as RNA transcripts longer than 200 nucleotides with no protein-coding capacity, long non-coding RNAs (lncRNAs) regulate multiple biological events—such as cancer cell proliferation, apoptosis, and invasion. Accordingly, their dysregulated expression is closely correlated with the initiation and progression of various diseases.[8] Mounting studies have demonstrated that lncRNAs can act as competing endogenous RNAs (ceRNAs) to sponge microRNAs (miRNAs), thereby regulating the expression of miRNA target genes and participating in the progression of breast cancer, particularly TNBC.[9,10] To illustrate, LINC01503 has been reported to drive TNBC cell proliferation and invasion

https://doi.org/10.2147/BCTT.S596228
Received: 14 January 2026
Accepted: 21 March 2026
Published: 16 April 2026

© 2026 Xiong et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v4.0) License (http://creativecommons.org/licenses/by-nc/4.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

Graphical Abstract



by sponging miR-335-5p, which upregulates SPNS2 expression,[11] while LUCAT1 regulates TNBC stem cell properties and apoptosis through the ceRNA network-mediated miR-375/Yap1 pathway.[12] High-throughput sequencing and The Cancer Genome Atlas (TCGA) data analyses have identified numerous differentially expressed lncRNAs between TNBC tissues, normal breast tissues, and non-TNBC tissues, with thousands of significantly dysregulated lncRNA transcripts detected in breast tissues.[13,14] However, the specific mechanisms by which these lncRNAs regulate TNBC progression remain incompletely understood, and investigating their roles holds great significance for improving the diagnosis and treatment of TNBC. While LINC01128 has been characterized in other malignancies such as pancreatic cancer and cervical cancer,[15–17] its functional role in TNBC remains uninvestigated to date. Herein, in our current study, we identified LINC01128 relative expression in tumor tissues from TNBC patients.

Mounting evidence suggests that lncRNAs often regulate downstream functions through interactions with miRNAs.[18] MiRNAs exert diverse regulatory roles in cancer progression. For example, miR-32-5p has been implicated in the regulation of various malignant tumors, including colorectal cancer and cervical cancer, where it modulates tumor cell proliferation, invasion, and other oncogenic processes.[19,20] In TNBC, miR-32-5p serves as a target of lncRNAs: GATA1-activated HNF1A-AS1 sponges miR-32-5p to abrogate its suppression of RNF38, thereby promoting TNBC progression.[21] Additionally, lncRNA WEE2-AS1 influences TNBC cell proliferation and apoptosis via the miR-32-5p/TOB1 axis.[22] Existing studies have verified that miR-32-5p governs TNBC progression through the ceRNA network, while the ENCORI database identifies potential binding sites between LINC01128 and miR-32-5p. However, whether these two molecules interact directly and whether this interaction contributes to the regulation of TNBC biological behaviors remain unclear.

Our current study seeks to elucidate the expression pattern and clinical relevance of LINC01128 in TNBC. Through molecular interaction validation and cellular functional assays, we intend to elucidate whether LINC01128 regulates TNBC cell proliferation, invasion, apoptosis, and other biological behaviors by targeting miR-32-5p via the ceRNA

mechanism. The outcomes of this research may deliver novel understandings of TNBC's pathological mechanisms and pinpoint promising therapeutic targets.

Materials and Methods

Subjects and Sample Collection

Seventy-six patients with pathologically confirmed TNBC were enrolled at our institution between January 2024 and January 2025. All patients met the diagnostic criteria for TNBC, which were defined as follows: the proportion of ER-positive and PR-positive cells was <1% as detected by immunohistochemistry (IHC) SP method; HER2 negativity was defined as IHC 0/1+, or IHC 2+ with a HER2/CEP17 ratio <2.0 confirmed by fluorescence in situ hybridization (FISH).

Tumor tissues and paired adjacent normal breast tissues (≥5 cm from the tumor margin, pathologically confirmed to be free of cancer cell infiltration) were collected from all patients. Right after collection, samples were snap-frozen in liquid nitrogen and stored at −80°C for subsequent analyses. This study obtained ethical clearance from the Institutional Ethics Committee of our hospital (Approval No. 2023-108, December 15, 2023), with all patients signing written informed consent forms voluntarily.

Patient baseline information was extracted from the hospital's electronic health record system, covering three core categories of data: demographic traits (eg, gender, age); imaging and clinicopathological details (including maximal tumor diameter, lymph node metastasis status, distant metastasis status, clinical stage, pathological histologic type, and histological grade); as well as molecular features (ER, PR, HER2 expression status) and treatment regimens (neoadjuvant therapy) For clinical staging, the 8th Edition of the AJCC Cancer Staging Manual was used as the reference; histological grading, meanwhile, followed the NCCN system, which categorizes cases into Grade I (3–5 points), Grade II (6–7 points), and Grade III (8–9 points).

Cell Lines

The human normal breast epithelial cell line MCF-12F and TNBC cell lines (MDA-MB-433, MDA-MB-458, MDA-MB -436, MDA-MB-231, BT20, MDA-MB-453, MDA-MB-468) were all purchased from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China.

Cell Culture and Transfection

MCF-12F cells were maintained in DMEM (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), whereas all TNBC cell lines used RPMI 1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Both media were supplemented with 10% FBS (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Solarbio Life Sciences, Beijing, China), and all cells were routinely incubated in a 37°C, 5% CO_2 constant-temperature incubator. Cell passaging and transfection were performed when cell confluence reached 70%–80%. Transfection was conducted according to the instructions of Lipofectamine 3000 reagent (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA). si-LINC01128, pcDNA-LINC01128, miR-32-5p mimic, miR-32-5p inhibitor, and their corresponding negative controls (synthesized and constructed by GenePharma Co., Ltd., Shanghai, China) were transfected into BT20 and MDA-MB-436 cells (these two high-expression cell lines were selected based on previous LINC01128 expression detection results), respectively. Following transfection, the cells were incubated for 48 hours in preparation for subsequent experimental procedures. Rescue experiment groups included: si-LINC01128 + miR-32-5p inhibitor group, pcDNA-LINC01128 + miR-32-5p mimic group, with corresponding negative control groups and blank control groups set simultaneously.

Quantitative Real-Time PCR (qPCR)

Total RNA was isolated from samples with TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA), followed by cDNA generation via the PrimeScript RT Kit (RR036A, Takara Bio Inc., Dalian, Liaoning, China). The relative expression levels of LINC01128 and miR-32-5p were assessed by qPCR using SYBR Green SuperMix (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA). GAPDH and U6 snRNA served as internal reference genes, where GAPDH was used

for the normalization of LINC01128 expression, and U6 snRNA was used for the normalization of miR-32-5p expression. The relative quantification of target genes was performed using the 2-ΔΔCt method. The primer sequences used in this study were as follows: miR-32-5p, forward: 5′-GCGGCTATTGCACATTACTAAGTTG-3′, reverse: 5′-GTGCAGGGTCCGAGGT-3′; U6 snRNA, forward: 5′-GCGGCGTCGTGAAGCGTTC-3′, reverse: 5′-GTGCAGGGTCCGAGGT-3′; LINC01128, forward: 5′-GCCAGCAAGATGGAGAAGATG-3′, reverse: 5′-GCTGGTGAGTTGAGGAGTGG-3′; GAPDH, forward: 5′-GAAG GTGAAGGTCGGAGTC-3′, reverse: 5′-GAAGATGGTGATGGGATTTC-3′.

Dual-Luciferase Reporter Assay

Using the predicted binding regions between LINC01128 and miR-32-5p (from ENCORI database), we constructed the wild-type LINC01128 reporter vector (WT-LINC01128) and its binding-site mutant counterpart (MUT-LINC01128) via Sangon Biotech Co., Ltd., Shanghai, China. WT/MUT-LINC01128 was co-introduced into HEK293T cells with miR-32-5p mimic, its inhibitor, or respective negative controls; cells were cultured 48 h post-transfection. Following the protocol of the Dual-Luciferase Reporter Assay Kit (Promega Corporation, Madison, WI, USA), a microplate luminometer was used to measure activity- Renilla luciferase served as the internal control to calculate relative luciferase activity.

Cell Proliferation Assay

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8, Beyotime Biotechnology, Nanjing, Jiangsu, China). BT20 and MDA-MB-436 cells (5×10^3 cells/well) from each treatment group were seeded into 96-well plates (6 replicates per group). After 48 h of culture, 10 μL of CCK-8 reagent was added to each well; 2 hours of additional incubation later, a microplate reader measured the optical density at 450 nm (OD450).

Cell Apoptosis Assay

For apoptosis detection, BT20 or MDA-MB-436 cells cultured for 48 h after transfection were collected, and a cell suspension of 1×10^5 cells/mL was prepared with pre-cooled buffer from the Annexin V-FITC/PI Apoptosis Detection Kit (BD Biosciences, San Jose, CA, USA). Annexin V-FITC and PI fluorescent dyes from the aforementioned kit were added to the cell suspension, followed by incubation at room temperature in the dark for 15 minutes. Apoptotic cells were counted using a flow cytometer (FACSCalibur, BD Biosciences, San Jose, CA, USA), and the apoptosis rate was analyzed using FlowJo software.

Cell Migration Assay

The Transwell migration assay was performed using 24-well Transwell plates (Corning Inc., Corning, NY, USA; 8 μm pore size). Transfected BT20 or MDA-MB-436 cells (2×10^4 cells/well) were seeded into the upper chamber with serum-free medium, while the lower chamber was filled with medium containing 20% FBS (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) as the chemoattractant. After 24h of incubation, non-migrated cells in the upper chamber were gently wiped off. Migrated cells in the lower chamber were fixed with 4% paraformaldehyde (Solarbio Life Sciences, Beijing, China) for 15min, stained with 0.1% crystal violet (Solarbio Life Sciences, Beijing, China) for 20min, rinsed with distilled water, and air-dried. Subsequently, 5 random fields of view were counted under an Olympus inverted light microscope (Olympus Corporation, Tokyo, Japan), and the average value was used as the indicator of cell migration ability.

Bioinformatics Analysis

By searching for the keywords “triple-negative breast cancer lncRNA miRNA” on PubMed, we identified miR-32-5p. Subsequently, using the Starbase database, we found that LINC01128 can bind to miR-32-5p. We predicted the target genes of miR-32-5p using bioinformatics tools such as miRDB, miRWalk, and Starbase, and screened for overlapping target genes via Venn diagrams. Finally, GO functional annotation and KEGG pathway enrichment analyses of these target genes were performed using the online tool DAVID 6.8.

Statistical Analysis

Statistical analyses were performed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA), and graphs were plotted using GraphPad Prism 10.0 software (GraphPad Software, San Diego, CA, USA). Continuous data are mean ± SD, analyzed by *t*-test or ANOVA for inter-group comparisons. Categorical data are presented as n (%), with chi-square test for group differences (Fisher's exact test for small expected frequencies). Pearson correlation analyzed the correlation between LINC01128 and miR-32-5p expressions. Multivariate Logistic regression identified independent risk factors for advanced TNBC clinical stage. $P < 0.05$ was statistically significant.

Results

Baseline Characteristics of TNBC Patients and Correlation Between LINC01128 Expression and Clinicopathological Features

A total of 76 patients with pathologically confirmed TNBC were enrolled in this study. Their baseline clinical, pathological, molecular biological, and treatment-related characteristics are summarized in Table 1. The enrolled TNBC patients covered different ages, clinical stages, and pathological grades, and their demographic and

Table 1 Baseline Characteristics of 76 Patients with Triple-Negative Breast Cancer (TNBC)

Characteristic Category	Specific Indicators	Number (n=76)	Proportion (%)/Statistical Description
Demographic Characteristics	Gender		
	Female	75	98.7
	Male	1	1.3
	Age (years)		
	Mean ± Standard Deviation		47.6±9.2
	Median (Interquartile Range)		46.5 (38.0–56.0)
	Age Group Distribution		
	≤40 years	21	27.6
	41–50 years	29	38.2
	51–60 years	18	23.7
	>60 years	8	10.5
Clinicopathological Characteristics	Tumor Maximum Diameter (T Stage, AJCC 8th Edition)		
	T1 (≤2cm)	25	32.9
	T2 (2–5cm)	36	47.4
	T3 (>5cm)	11	14.5
	T4 (Invasion of chest wall/skin)	4	5.3
	Lymph Node Metastasis Status (N Stage)		
	N0 (No metastasis)	33	43.4
	N1 (1–3 metastatic nodes)	27	35.5
	N2 (4–9 metastatic nodes)	12	15.8
	N3 (≥10 metastatic nodes)	4	5.3
	Distant Metastasis Status (M Stage)		
	M0 (No distant metastasis)	69	90.8
	M1 (With distant metastasis)	7	9.2
	Clinical Comprehensive Stage		
	Stage I	20	26.3
	Stage II	34	44.7
	Stage III	15	19.7
	Stage IV	7	9.2
	Pathological Histological Type		
	Invasive Ductal Carcinoma	68	89.5
	Invasive Lobular Carcinoma	3	3.9

(*Continued*)

Table 1 (Continued).

Characteristic Category	Specific Indicators	Number (n=76)	Proportion (%)/Statistical Description
	Special Type Carcinoma (Medullary Carcinoma/Carcinosarcoma, etc.)	5	6.6
	Histological Grade (Nottingham System)		
	Grade I (Well-differentiated)	4	5.3
	Grade II (Moderately differentiated)	32	42.1
	Grade III (Poorly differentiated)	40	52.6
Molecular Biological Characteristics	Hormone Receptor and HER2 Expression (Immunohistochemistry)		
	Estrogen Receptor (ER) Negative	76	100.0
	Progesterone Receptor (PR) Negative	76	100.0
	Human Epidermal Growth Factor Receptor 2 (HER2) Negative	76	100.0
	Supplementary Explanation of HER2 Detection		
	IHC 0/1+	65	85.5
	IHC 2+ with no amplification by FISH	11	14.5
Treatment-Related Characteristics	Treatment Status Before Enrollment		
	De Novo Treatment (No anti-tumor treatment received)	64	84.2
	Post-Neoadjuvant Therapy (Chemotherapy ± Immunotherapy)	12	15.8
	Neoadjuvant Treatment Regimens (Neoadjuvant Group Only)		
	Paclitaxel-based + Anthracycline-based Chemotherapy	8	66.7
	Chemotherapy + PD-1/PD-L1 Inhibitors	4	33.3

Notes: Baseline demographic, clinicopathological, molecular biological, and treatment-related characteristics of 76 patients with pathologically confirmed TNBC. Diagnostic criteria for TNBC refer to the Chinese Society of Clinical Oncology (CSCO) Clinical Practice Guidelines for Breast Cancer (2024 Edition): the proportion of estrogen receptor (ER) and progesterone receptor (PR) positive cells is < 1% (detected by immunohistochemical SP method); HER2 negativity is defined as immunohistochemistry (IHC) 0/1+, or IHC 2+ with fluorescence in situ hybridization (FISH) detection showing a HER2/CEP17 ratio < 2.0. Clinical staging is based on the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (8th Edition). Nottingham grading is based on the scoring of gland formation, nuclear pleomorphism, and mitotic count: a total score of 3–5 is Grade I, 6–7 is Grade II, and 8–9 is Grade III.

clinicopathological characteristics were consistent with the overall epidemiological pattern of TNBC, indicating good sample representativeness.

Patients were divided into high and low expression groups based on the median expression of LINC01128 (38 cases in each group, Table 2). LINC01128 expression was significantly correlated with tumor size (T stage), lymph node metastasis, clinical stage, and histological grade (all $P < 0.05$), but not with age, pathological type, or treatment status (all $P > 0.05$).

Table 2 Correlation Between LINC01128 Expression Levels and Clinicopathological Characteristics of Patients with Triple-Negative Breast Cancer (TNBC)

Clinicopathological Characteristics	Grouping Criteria	High Expression Group (n=38)	Low Expression Group (n=38)	P
Age Group	≤40 years	12	9	0.774
	41–50 years	14	15	
	51–60 years	8	10	
	>60 years	4	4	
Tumor Size (T Stage, AJCC 8th Edition)	T1–T2 (≤5cm)	24	31	0.029
	T3–T4 (>5cm/Chest Wall Invasion)	14	7	
Lymph Node Metastasis Status (N Stage)	N0 (No metastasis)	11	22	0.003
	N1–N3 (With metastasis)	27	16	
Clinical Comprehensive Stage	Stage I–II (Early stage)	15	29	<0.001
	Stage III–IV (Advanced stage)	23	9	
Histological Grade (Nottingham System)	Grade I–II (Well/Moderately Differentiated)	12	24	0.002
	Grade III (Poorly Differentiated)	26	14	

(*Continued*)

Table 2 (Continued).

Clinicopathological Characteristics	Grouping Criteria	High Expression Group (n=38)	Low Expression Group (n=38)	P
Pathological Histological Type	Invasive Ductal Carcinoma	34	34	0.990
	Other Types (Invasive Lobular Carcinoma/Special Type Carcinoma)	4	4	
Treatment Status Before Enrollment	De Novo Treatment (No anti-tumor treatment received)	32	32	1.000
	Post-Neoadjuvant Therapy (Chemotherapy ± Immunotherapy)	6	6	

Notes: Correlation between LINC01128 expression and clinicopathological characteristics in TNBC patients. Patients were divided into high-expression and low--expression groups according to the median value of LINC01128 expression (n=38 per group). Statistical analysis was performed using Chi-square test (χ^2 test); Fisher's exact test was applied when the expected frequency of any cell was < 5 (eg, Grade I with only 4 cases). Bilateral P < 0.05 was considered statistically significant, and statistical software SPSS 26.0 was used for analysis.

Multivariate Logistic regression analysis (Table 3) showed that high LINC01128 expression (OR=6.635, 95% CI: 2.352–18.701, P=0.001) and lymph node metastasis (OR=3.078, 95% CI: 1.098–8.615, P=0.036) were independent risk factors for advanced clinical stage in TNBC patients, while tumor size and histological grade were not statistically significant.

High Expression of LINC01128 in TNBC Tissues and Cells

LINC01128 expression in tissues and cells by qPCR showed that the relative expression level of LINC01128 in TNBC tissues was significantly higher than that in adjacent normal tissues (Figure 1A, **P<0.01). In cell lines, compared with the normal breast epithelial cell line MCF-12F, the expression of LINC01128 was increased to varying degrees in TNBC cell lines (such as MDA-MB-433, MDA-MB-458), with the most significant increase in BT20 and MDA-MB-436 cells (Figure 1B, *P<0.05, **P<0.01).

Targeted Regulatory Relationship Between LINC01128 and miR-32-5p in TNBC

We detected the expression of miR-32-5p in TNBC tissues. The results showed that the relative expression level of miR-32-5p in TNBC tissues was significantly lower than that in adjacent normal tissues (Figure 2A, **P<0.01). Correlation analysis revealed a significant negative correlation between LINC01128 and miR-32-5p expressions in TNBC tissues (Figure 2B, r=−0.699, P<0.001).

Figure 2C shows the potential binding sites between LINC01128 and miR-32-5p predicted by ENCORI. Further verification by Dual-Luciferase Reporter Assay demonstrated that compared with the control group, miR-32-5p mimic significantly reduced the luciferase activity of wild-type LINC01128 (WT-LINC01128) but had no significant effect on that of mutant-type LINC01128 (MUT-LINC01128); miR-32-5p inhibitor significantly increased the luciferase activity of WT-LINC01128 (Figure 2D, ***P<0.001). These results confirmed the direct targeted binding interaction between LINC01128 and miR-32-5p.

Table 3 Multivariate Logistic Regression Analysis of LINC01128 Expression and Advanced Clinical Stage in TNBC Patients

Features	OR	(95% CI)	P
LINC01128	6.635	2.352–18.701	0.001
Tumor Size	2.679	0.938–7.651	0.079
Lymph Node Metastasis	3.078	1.098–8.615	0.036
Histological Grade	2.399	0.845–6.806	0.118

Notes: Multivariate Logistic regression analysis was performed to identify independent risk factors for advanced clinical stage in TNBC patients.

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

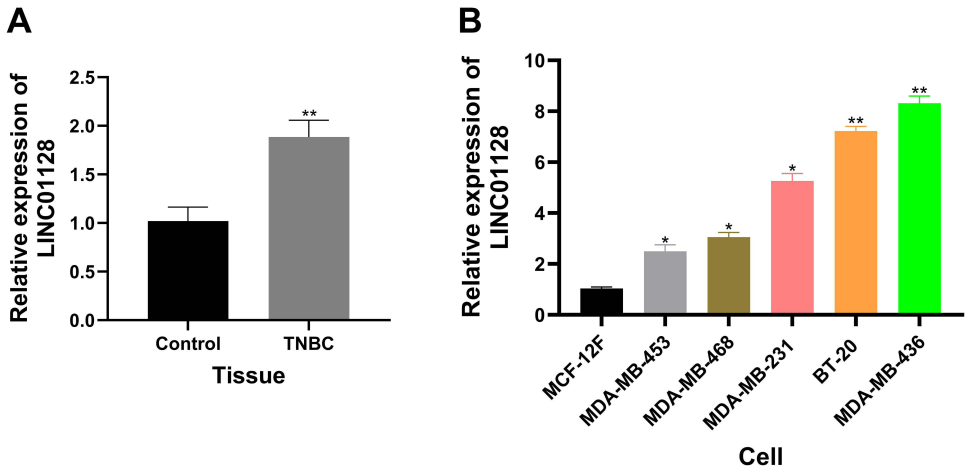


Figure 1 Expression characteristics of LINC01128 in triple-negative breast cancer (TNBC) tissues and cell lines. (**A**) Relative expression level of LINC01128 in TNBC tissues and adjacent normal tissues (Control) detected by qPCR (**P<0.01); (**B**) Relative expression level of LINC01128 in normal breast epithelial cell line MCF-12F and various TNBC cell lines detected by qPCR (*P<0.05, **P<0.01).

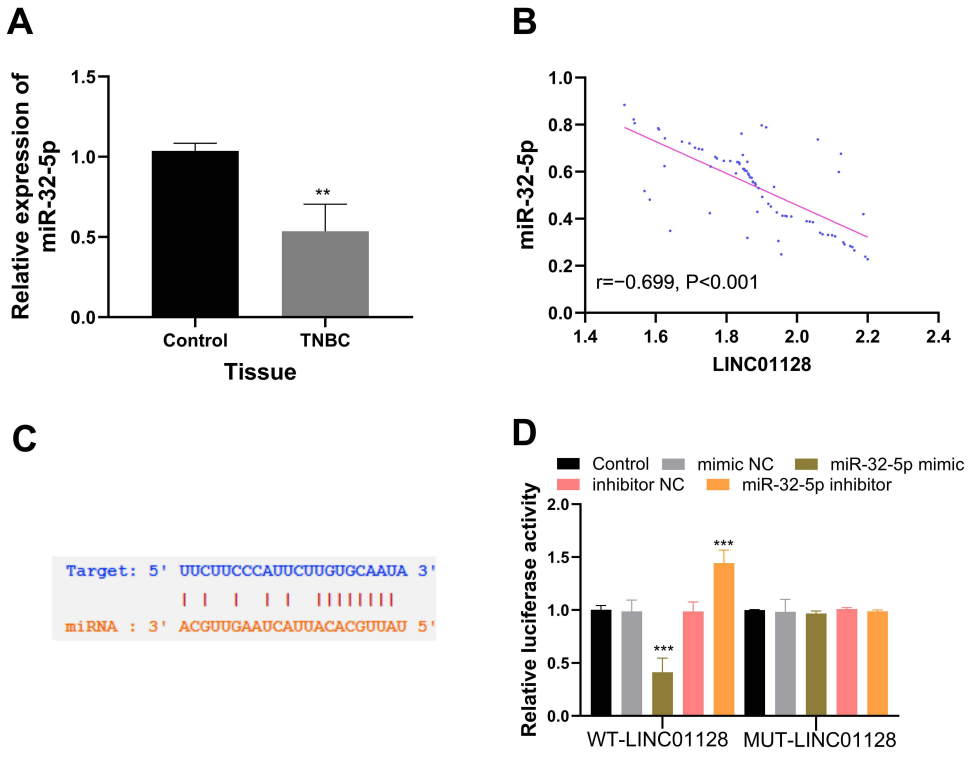


Figure 2 Verification of the targeted regulatory relationship between LINC01128 and miR-32-5p. (**A**) Relative expression level of miR-32-5p in TNBC tissues and adjacent normal tissues (Control) detected by qPCR (**P<0.01); (**B**) Scatter plot showing the correlation between LINC01128 and miR-32-5p expressions in TNBC tissues (r= −0.699, P<0.001); (**C**) Sequences of complementary binding sites between LINC01128 and miR-32-5p predicted by bioinformatics; (**D**) Results of dual-luciferase reporter assay (***P<0.001); WT-LINC01128: wild-type LINC01128 reporter vector; MUT-LINC01128: LINC01128 reporter vector with mutated binding sites.

LINC01128 Affects Proliferation, Apoptosis, and Migration of TNBC Cells by Regulating miR-32-5p

BT20 and MDA-MB-436 cells were selected for subsequent experiments, as LINC01128 expression was most significantly upregulated in these two TNBC cell lines among all tested ones (Figure 1B), making them ideal for expression intervention and functional validation.

https://doi.org/10.2147/BCTT.S596228

Regarding expression regulation, LINC01128 knockdown (si-LINC01128) markedly elevated miR-32-5p levels (**P<0.01) compared to the negative control (NC) group, while LINC01128 overexpression (pcDNA-LINC01128) notably reduced miR-32-5p expression (***P<0.001). Rescue assays confirmed that co-transfecting si-LINC01128 with miR-32-5p inhibitor reversed miR-32-5p upregulation ($^{###}$P<0.001), and co-transfecting pcDNA-LINC01128 with miR-32-5p mimic abrogated miR-32-5p downregulation ($^{###}$P<0.001) (Figure 3A–D).

For cellular functions, si-LINC01128 decreased cell proliferation (lower OD450 value, **P<0.01), inhibited migration (fewer migrated cells, **P<0.01), and promoted apoptosis (higher apoptosis rate, **P<0.01) relative to the NC group. In contrast, pcDNA-LINC01128 exerted the opposite effects (***P<0.001 for all). Rescue experiments further demonstrated that co-transfection with miR-32-5p inhibitor or mimic reversed the respective effects of si-LINC01128 and pcDNA-LINC01128 on cell proliferation, apoptosis, and migration ($^{###}$P<0.001 for all) (Figure 3E–J).

Collectively, these results indicate that LINC01128 promotes TNBC cell proliferation and migration while suppressing apoptosis by targeting and inhibiting miR-32-5p.

Bioinformatics Analysis of Target Genes Related to the LINC01128/miR-32-5p Regulatory Axis

To explore the downstream mechanisms by which the LINC01128/miR-32-5p regulatory axis affects TNBC progression, we combined three miRNA target gene databases (miRDB, miRWalk, and ENCORI) to screen out 164 common intersection target genes of miR-32-5p (Figure 4A). This high-confidence gene set served as the core for subsequent analyses.

KEGG pathway enrichment analysis was performed on these 164 target genes. The results showed that they were significantly enriched in tumor-related pathways such as “Signaling Pathways Regulating Stem Cell Pluripotency”, “Viral Carcinogenesis”, and “Role of Proteoglycans in Cancer” (Figure 4B). GO functional enrichment analysis (Figure 4C) revealed the following: at the biological process level, the genes were mainly enriched in core tumor processes including “regulation of cell proliferation” and “signal transduction”; at the cellular component level, they were concentrated in functional locations such as “cytoplasm” and “membrane-bound organelles”; at the molecular function level, they were mainly involved in “nucleic acid binding” and “protein binding” (Figure 4C).

Discussion

TNBC is characterized by complex pathogenesis and limited therapeutic options, leading to unsatisfactory clinical outcomes. Thus, in-depth exploration of its underlying molecular mechanisms is urgently needed. Mounting research has established that lncRNAs exert crucial regulatory functions in breast cancer progression.[23] LncRNAs acting as ceRNAs to regulate miRNAs mediate a critical regulatory pathway in tumor progression.[9] All core findings of this study are fully consistent with our experimental data, completely achieve the preset research objectives, and align well with the established research background of TNBC molecular regulation and targeted therapy. This study marks the first investigation into the role of LINC01128 in TNBC, delivering novel understandings of its pathogenic mechanisms.

qPCR confirmation revealed that LINC01128 shows a marked increase in expression in TNBC tissues as well as relevant cell lines (eg, BT20, MDA-MB-436) This oncogenic expression pattern is consistent with its reported role in pancreatic cancer and cervical cancer.[15,16] Additionally, LINC01128 has been shown to promote prostate cancer progression by regulating miR-27b-3p,[17] suggesting that it may possess pan-cancer oncogenic properties. Clinical correlative analysis verified that elevated LINC01128 expression is closely linked to increased tumor size, lymph node involvement, and progressive clinical stage in TNBC cases, while also constituting an independent risk factor for advanced lesions. It is worth noting that there was no substantial correlation identified between LINC01128 expression and patients’ age, pathological subtype, or therapeutic status. These findings indicate that the dysregulation of LINC01128 is specifically involved in the malignant progression of TNBC, rather than being affected by individual baseline characteristics or treatment interventions, supporting its potential as a biomarker for auxiliary diagnosis and prognostic risk stratification of TNBC.

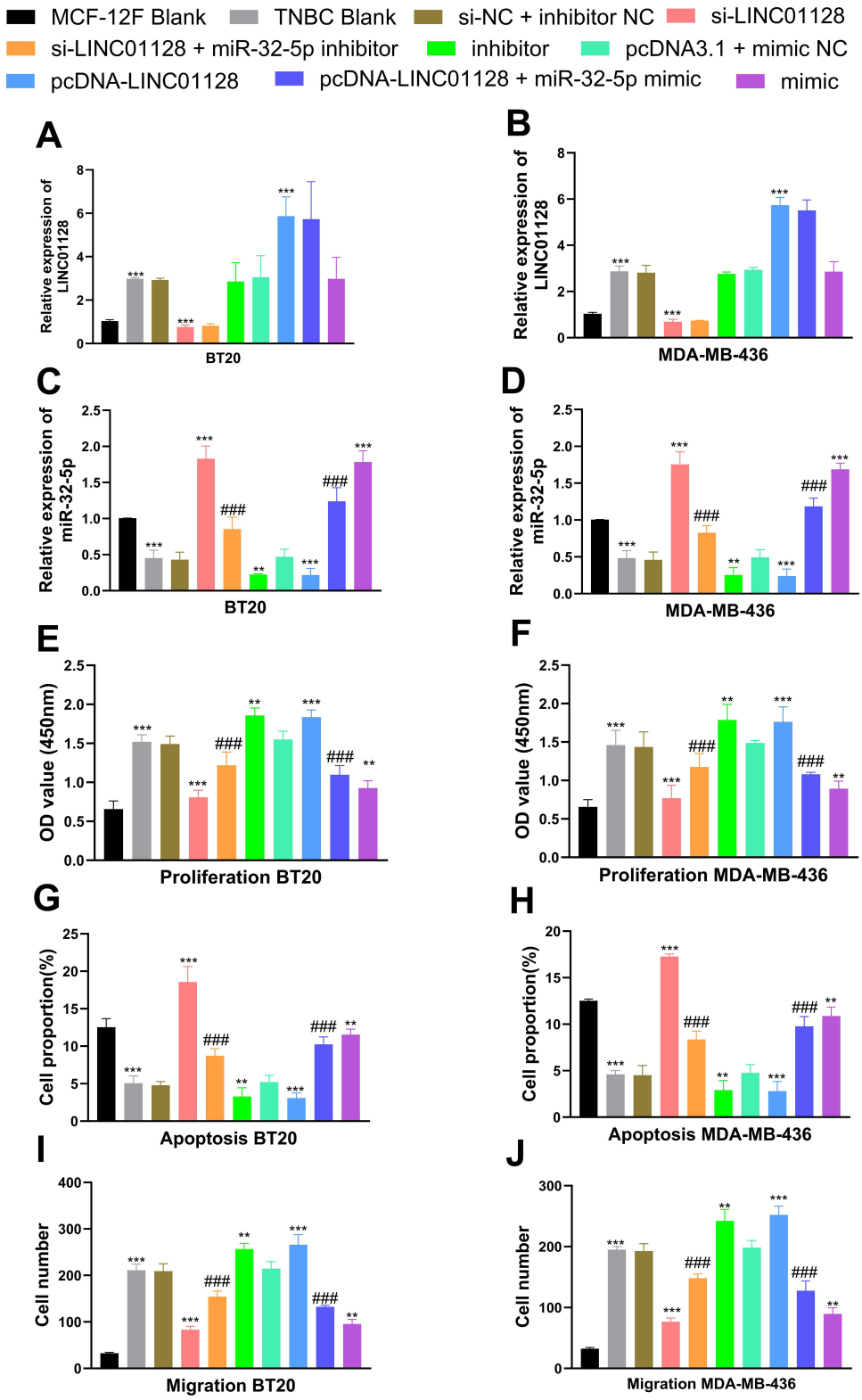


Figure 3 LINC01128 affects the proliferation, apoptosis, and migration of TNBC cells by regulating miR-32-5p. (**A**–**D**) Relative expression levels of LINC01128 and miR-32-5p in BT20 (**A** and **C**) and MDA-MB-436 (**B** and **D**) cells detected by qPCR; (**E** and **F**) Proliferative capacity of BT20 (**E**) and MDA-MB-436 (**F**) cells detected by CCK-8 assay (expressed as OD450 value); (**G** and **H**) Apoptotic rate of BT20 (**G**) and MDA-MB-436 (**H**) cells detected by flow cytometry; (**I** and **J**) Number of migrated BT20 (**I**) and MDA-MB-436 (**J**) cells detected by Transwell assay.

Notes: **P<0.01, ***P<0.001 compared with the blank group/negative control group; ###P<0.001 compared with the si-LINC01128 group or pcDNA-LINC01128 group; si-LINC01128: LINC01128 knockdown vector; pcDNA-LINC01128: LINC01128 overexpression vector; miR-32-5p mimic: miR-32-5p mimic; miR-32-5p inhibitor: miR-32-5p inhibitor.

https://doi.org/10.2147/BCTT.S596228

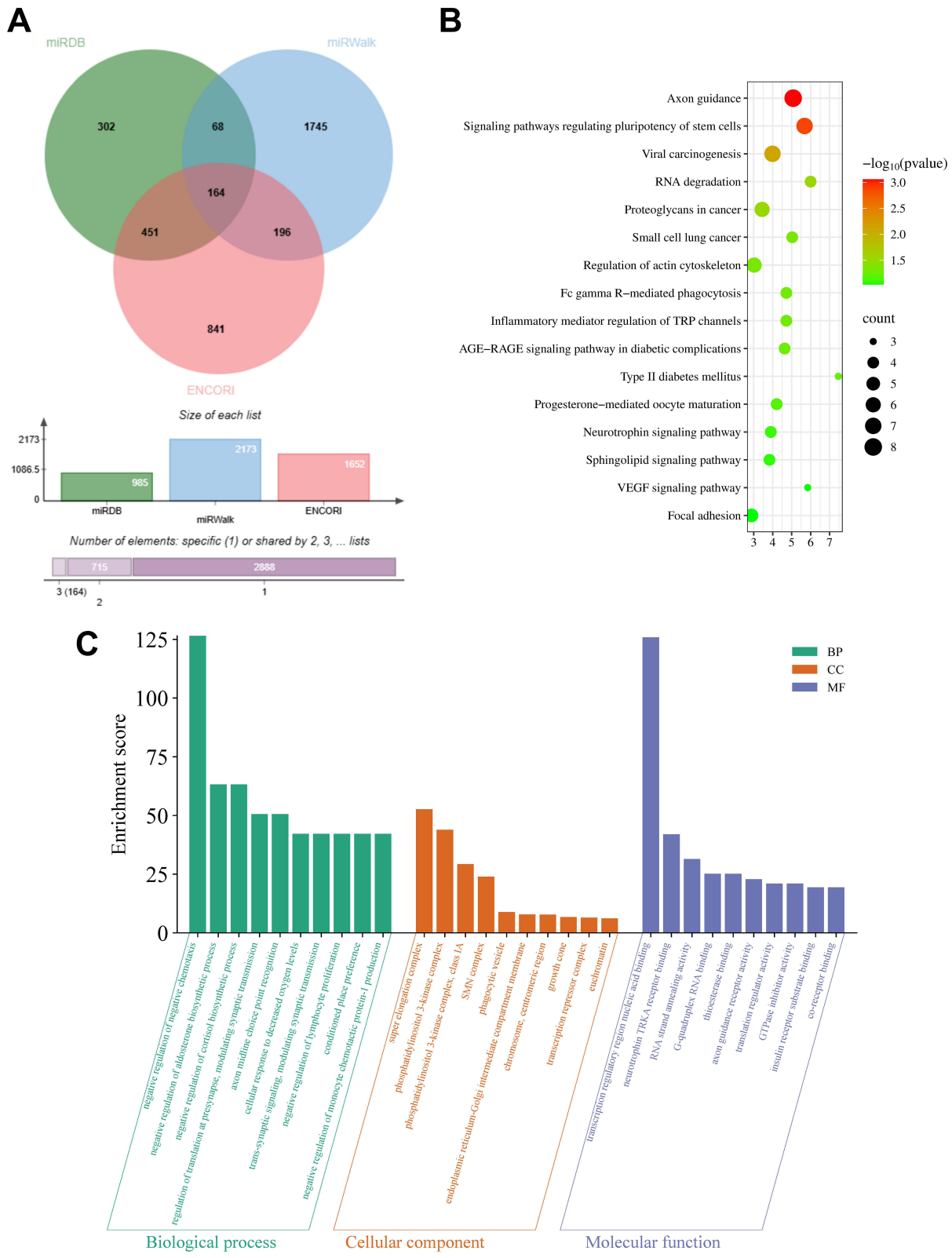


Figure 4 Bioinformatics analysis of target genes in the LINC01128/miR-32-5p regulatory axis. (**A**) Venn diagram showing the intersection of miR-32-5p target genes predicted by miRDB, miRWalk, and ENCORI databases; (**B**) KEGG pathway enrichment analysis diagram of the intersection target genes of miR-32-5p (the bubble color corresponds to the P value; the darker the color, the higher the enrichment significance; the bubble size corresponds to the number of target genes contained in the pathway); (**C**) GO functional enrichment analysis diagram of the intersection target genes of miR-32-5p (the ordinate represents the enrichment score).
Abbreviations: BP, Biological Process; CC, Cellular Component; MF, Molecular Function.

https://doi.org/10.2147/BCTT.S596228

At the molecular level, miR-32-5p shows reduced expression in TNBC tissues, and a strong inverse association was observed between its expression levels and LINC01128. Through bioinformatics predictions and dual-luciferase reporter assays, a direct targeting interaction between LINC01128 and miR-32-5p was confirmed — this augments the existing ceRNA regulatory network of miR-32-5p in TNBC.[21,22] Prior studies have established that lncRNAs (eg, HNF1A-AS1 and WEE2-AS1) regulate the progression of TNBC by sequestering miR-32-5p[21,22] Our present research characterizes LINC01128 as another crucial ceRNA of miR-32-5p, indicating that miR-32-5p expression may be coordinately modulated by multiple lncRNAs, providing a new upstream target to restore the tumor suppressor function of miR-32-5p in TNBC.

Cellular functional experiments showed that LINC01128 drives proliferation and migration of BT20 and MDA-MB-436 cells and inhibits their apoptosis by targeting and suppressing miR-32-5p. Rescue experiments further validated the specificity of this regulatory relationship. This functional phenotype is consistent with the enrichment of miR-32-5p target genes in pathways related to "regulation of cell proliferation" and "signal transduction", suggesting that the LINC01128/miR-32-5p axis may regulate TNBC cell biological behaviors by activating these pathways. This finding provides a promising actionable therapeutic target for TNBC, which lacks effective targeted treatment options, and supports the development of therapeutic strategies targeting LINC01128 or miR-32-5p mimetics to block TNBC progression.

To unravel the downstream regulatory mechanisms of the LINC01128/miR-32-5p axis, we collectively identified 164 high-confidence target genes of miR-32-5p by leveraging three databases: miRDB, miRWalk, and ENCORI. Functional enrichment analyses of GO and KEGG indicated that these genes show marked enrichment in cancer-related signaling pathways such as "signaling pathways regulating stem cell pluripotency", with biological processes concentrated in "regulation of cell proliferation" and "signal transduction", and molecular functions mainly involving "nucleic acid binding" and "protein binding". These analytical outcomes suggest that miR-32-5p may impact TNBC cell stemness, proliferative activity, and invasive ability through the modulation of these target gene pathways. Functioning as a ceRNA, LINC01128 may sequester miR-32-5p, thus abrogating its suppressive influence on downstream target genes and triggering oncogenic signaling pathways. Notably, this enrichment result suggests the axis may participate in regulating TNBC chemoresistance and recurrence via stem cell-related pathways, offering a new intervention direction to improve chemotherapy efficacy. We speculate that this regulatory axis may promote tumor progression by influencing stem cell-related pathways closely associated with TNBC drug resistance and recurrence.[4] In future studies, key target genes in the "signaling pathways regulating stem cell pluripotency" should be prioritized for screening, and their regulatory relationships with the LINC01128/miR-32-5p axis should be verified through Western blot, immunohistochemistry, and other experiments to clarify the specific functional pathways and improve the molecular regulatory network of TNBC.

The present study has several limitations. First, the 76 cases from a single center lack long-term follow-up and multicenter validation. Second, only in vitro experiments were performed using two TNBC cell lines, and in vivo validation in animal models was not conducted, precluding the clarification of the actual in vivo effects of the regulatory axis. Third, the critical downstream target genes of miR-32-5p, as well as the mechanisms underlying pathway crosstalk, remain elusive.

Conclusion

To summarize, the current research verifies that LINC01128 shows high expression in TNBC and boosts tumor cell proliferation and migration while curbing apoptosis by targeting and downregulating miR-32-5p through the ceRNA regulatory pathway. These results not only complement the non-coding RNA regulatory network of TNBC, but also clarify the potential clinical application value of the LINC01128/miR-32-5p axis as a diagnostic/prognostic biomarker and therapeutic target. These results offer new molecular regulatory perspectives on TNBC's pathogenesis. Subsequent research ought to include large-cohort clinical studies, in vivo functional assays, and verification of downstream target genes to enhance its clinical translational potential.

Data Sharing Statement

Corresponding authors may provide data and materials.

Ethical Approval

All the studies were approved by the Ethics Committee of Affiliated Renhe Hospital of China Three Gorges University (Approval No. 2023-108, December 15, 2023), in line with the Declaration of Helsinki.

Consent for Publication

All patients provided written informed consent.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2):87–108. doi:10.3322/caac.21262
2. Zhang X, Li F, Zhou Y, et al. Long noncoding RNA AFAP1-AS1 promotes tumor progression and invasion by regulating the miR-2110/Sp1 axis in triple-negative breast cancer. *Cell Death Dis*. 2021;12(7):627. doi:10.1038/s41419-021-03917-z
3. Hwang SY, Park S, Kwon Y. Recent therapeutic trends and promising targets in triple negative breast cancer. *Pharmacol Ther*. 2019;199:30–57. doi:10.1016/j.pharmthera.2019.02.006
4. Karaayvaz M, Cristea S, Gillespie SM, Patel AP, Mylvaganam R, Luo CC. Unravelling subclonal heterogeneity and aggressive disease states in TNBC through single-cell RNA-seq. *Nat Commun*. 2018;9(1):3588. doi:10.1038/s41467-018-06052-0
5. Shah SP, Roth A, Goya R, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature*. 2012;486 (7403):395–399. doi:10.1038/nature10933
6. Bianchini G, De Angelis C, Licata L, Gianni L. Treatment landscape of triple-negative breast cancer - expanded options, evolving needs. *Nat Rev Clin Oncol*. 2022;19(2):91–113. doi:10.1038/s41571-021-00565-2
7. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res*. 2020;22(1):61. doi:10.1186/s13058-020-01296-5
8. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet*. 2009;10(3):155–159. doi:10.1038/nrg2521
9. Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing MiRNA-LncRNA Interactions. *Methods Mol Biol*. 2016;1402:271–286.
10. Yu X, Qian F, Zhang X, et al. Promotion effect of FOXCUT as a microRNA sponge for miR-24-3p on progression in triple-negative breast cancer through the p38 MAPK signaling pathway. *Chin Med J*. 2024;137(1):105–114. doi:10.1097/CM9.0000000000002700
11. Weng W, Huang H. LINC01503 promotes the cell proliferation, migration and invasion of triple-negative breast cancer as a ceRNA to elevate SPNS2 expression by sponging miR-335-5p. *Heliyon*. 2024;10(17):e36531. doi:10.1016/j.heliyon.2024.e36531
12. Verma D, Siddharth S, Yende AS. LUCAT1-mediated Competing Endogenous RNA (ceRNA) network in triple-negative breast cancer. *Cells*. 2024;13(22):1918. doi:10.3390/cells13221918
13. Reiche K, Kasack K, Schreiber S, et al. Long non-coding RNAs differentially expressed between normal versus primary breast tumor tissues disclose converse changes to breast cancer-related protein-coding genes. *PLoS One*. 2014;9(9):e106076. doi:10.1371/journal.pone.0106076
14. Fan CN, Ma L, Liu N. Comprehensive analysis of novel three-long noncoding RNA signatures as a diagnostic and prognostic biomarkers of human triple-negative breast cancer. *J Cell Biochem*. 2019;120(3):3185–3196. doi:10.1002/jcb.27584
15. Zhong M, Fang Z, Ruan B, Xiong J, Li J, Song Z. LINC01128 facilitates the progression of pancreatic cancer through up-regulation of LDHA by targeting miR-561-5p. *Cancer Cell Int*. 2022;22(1):93. doi:10.1186/s12935-022-02490-5
16. Hu Y, Ma Y, Liu J, Cai Y, Zhang M, Fang X. LINC01128 expedites cervical cancer progression by regulating miR-383-5p/SFN axis. *BMC Cancer*. 2019;19(1):1157. doi:10.1186/s12885-019-6326-5
17. Zhao Y, Zhang Z, Zheng Y, et al. LncRNA LINC01128 promotes prostate cancer cell proliferation, metastasis, and epithelial-mesenchymal transition by modulating miR-27b-3p. *J Cancer Res Clin Oncol*. 2025;151(3):98. doi:10.1007/s00432-025-06153-6
18. Cai Y, Yu X, Hu S, Yu J. A brief review on the mechanisms of miRNA regulation. *Genomics Proteomics Bioinf*. 2009;7(4):147–154. doi:10.1016/S1672-0229(08)60044-3
19. Liang H, Tang Y, Zhang H, Zhang C. MiR-32-5p regulates radiosensitization, migration and invasion of colorectal cancer cells by targeting TOB1 gene. *Onco Targets Ther*. 2019;12:9651–9661. doi:10.2147/OTT.S228995
20. Liu YJ, Zhou HG, Chen LH, et al. MiR-32-5p regulates the proliferation and metastasis of cervical cancer cells by targeting HOXB8. *Eur Rev Med Pharmacol Sci*. 2019;23(1):87–95. doi:10.26355/eurrev_201901_16752

21. Yang J, Niu H, Chen X. GATA1-activated HNF1A-AS1 facilitates the progression of triple-negative breast cancer via sponging miR-32-5p to upregulate RNF38. *Cancer Manag Res*. 2021;13:1357–1369. doi:10.2147/CMAR.S274204
22. Wang R, Huang Z, Qian C, et al. LncRNA WEE2-AS1 promotes proliferation and inhibits apoptosis in triple negative breast cancer cells via regulating miR-32-5p/TOB1 axis. *Biochem Biophys Res Commun*. 2020;526(4):1005–1012. doi:10.1016/j.bbrc.2020.01.170
23. Venkatesh J, Wasson MD, Brown JM, Fernando W, Marcato P. LncRNA-miRNA axes in breast cancer: novel points of interaction for strategic attack. *Cancer Lett*. 2021;509:81–88. doi:10.1016/j.canlet.2021.04.002

Breast Cancer: Targets and Therapy

Dovepress
Taylor & Francis Group

Publish your work in this journal

Breast Cancer - Targets and Therapy is an international, peer-reviewed open access journal focusing on breast cancer research, identification of therapeutic targets and the optimal use of preventative and integrated treatment interventions to achieve improved outcomes, enhanced survival and quality of life for the cancer patient. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/breast-cancer—targets-and-therapy-journal