Breast Cancer: Targets and Therapy

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ORIGINAL RESEARCH

LINC00917 Promotes Bone Metastasis of Breast Cancer by Targeting the miR-491-5p/FOXP4 Axis

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Purpose: Breast cancer is one of the most common malignant tumors in women. Advanced patients often experience distant metastasis, among which bone metastasis has a relatively high incidence rate, seriously affecting the quality of life and prognosis of patients. LINC00917 may be related to the prognosis of breast cancer patients. This study aims to explore whether LINC00917 plays a significant role in breast cancer bone metastasis by targeting and regulating the expression of miR-491-5p.

Patients and methods: 254 breast cancer patients were recruited. The levels of LINC00917 were examined by RT-qPCR. Furthermore, the association between LINC00917 expression and patient prognosis was evaluated using Kaplan-Meier curves and Cox regression analysis. An in vitro cell model was established, and CCK-8 and Transwell assays were conducted to explore the role of LINC00917 in breast cancer bone metastasis. Additionally, the interaction among LINC00917, miR-491-5p, and FOXP4 were examined using dual-luciferase reporter assays.

Results: LINC00917 was upregulated in breast cancer bone metastasis and was associated with bad prognosis. Additionally, the knockdown of LINC00917 inhibited the function of breast cancer cells, and suppressed osteoclastogenesis while promoting osteoblast differentiation. Moreover, miR-491-5p inhibition counteracted the effects of LINC00917 knockdown on cell models. Furthermore, FOXP4 may be a target gene of miR-491-5p.

Conclusion: LINC00917 is a potential prognostic indicator for breast cancer bone metastasis. It is proposed that LINC00917 may facilitate the bone metastasis process in breast cancer by modulating the miR-491-5p/FOXP4 axis.

Keywords: LINC00917, miR-491-5p, bone metastasis, breast cancer

Introduction

Advanced breast cancer often involves bone metastases, present in 75–85% of women with metastatic disease.[1] Early detection of breast cancer significantly enhances the likelihood of successful treatment outcomes. However, the prognosis for patients deteriorates substantially upon the development of metastatic disease. Specifically, individuals with bone metastases often face a grim outlook, coupled with an elevated risk of skeletal-related complications, referred to as skeletal-related events. These events encompass a range of debilitating conditions, such as pathological fractures, severe bone pain, and impaired mobility.[2,3] Even though bisphosphonates and denosumab are effective in reducing the occurrence of bone-related complications, certain bone metastases will still exhibit primary or acquired resistance to bisphosphonate therapy.[4] Therefore, it is needed to identify novel therapeutic targets for the treatment of breast cancer, particularly in the context of osteolytic bone metastasis, which could potentially contribute to advancements in clinical management of this disease.

Researchers have found that lncRNA plays a crucial role in the prognosis and metastasis of breast cancer. For instance, SNHG1 acts as a regulator of M2 macrophage polarization to promote breast cancer progression.[5] Additionally, elevated PCNAP1 predicts bad prognosis of breast cancer.[6] It is worth noting that multiple lncRNAs mediate the progression of breast cancer bone metastasis, such as SNHG3,[7] TRG-AS1,[8] and MIR193BHG.[9] Study found that LINC00917 is expressed at an enhanced level in patients with intervertebral disc degeneration.[10] Additionally, LINC00917 has potential as a diagnostic

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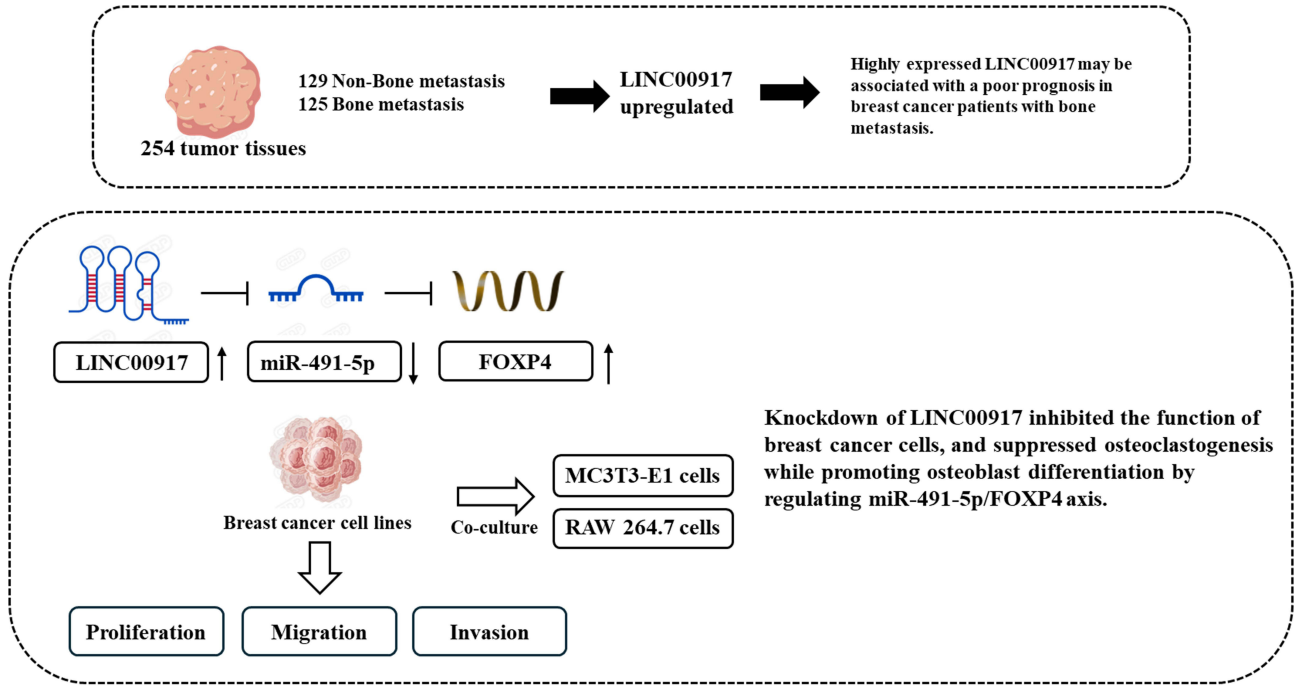
Graphical Abstract



LINC00917 may facilitate the bone metastasis process in breast cancer by modulating the miR-491-5p/FOXP4 axis

biomarker for lung cancer.[11] Moreover, study has reported that LINC00917 may serve as a prognostic factor for the survival of breast cancer patients.[12] However, the role of LINC00917 in breast cancer bone metastasis patients remains unknown.

In recent years, numerous experimental studies have been conducted on the role of miR-491-5p in cancers. Studies indicate that miR-491-5p has the ability to suppress cell growth, invasion, and metastasis.[13] miR-491-5p is reduced in pancreatic cancer.[14] miR-491-5p exerts its tumor suppressor function by inhibiting the biological functions of gastric cells.[15] Additionally, miR-491-5p demonstrates tumor suppressive functions in colorectal cancer,[16] and can effectively prevent the migration and invasion of breast cancer cells.[17] However, the role of miR-491-5p in breast cancer bone metastasis and its relationship with LINC00917 have not yet been verified.

FOXP4 plays a significant role in the progression of various malignancies. For example, it is a key factor in promoting stemness in gastric cancer,[18] advancing ovarian cancer progression,[19] and promoting breast cancer cells migration.[20] Studies have also reported that FOXP4 inversely controls tumor suppressor genes and promotes the progression of thyroid cancer,[21] and the upregulation of FOXP4 in breast cancer promotes migration and invasion.[20] Moreover, miR-491-5p impedes cancer progression by modulating FOXP4 expression in non-small cell lung cancer and osteosarcoma.[22,23] Therefore, we hypothesize that LINC00917 may mediate bone metastasis of breast cancer by regulating miR-491-5p/FOXP4 axis.

Based on the above background, this study aims to explore whether LINC00917 plays a significant role in breast cancer bone metastasis by targeting and regulating the miR-491-5p/FOXP4 axis. This research will provide important clues for breast cancer bone metastasis.

Materials and Methods

Patients and Sample Collection

Sample size calculation was performed by using G*Power 3.1. With α=0.05 and power=0.8, the required sample size was calculated to be 64 per group. A total of 254 breast cancer patients who received surgical treatment at The Third Affiliated Hospital of Kunming Medical University were recruited in this study and were divided into the bone metastasis (NBM, n=129) and bone metastasis (BM, n=125) group. All cases were pathologically and histologically confirmed as breast cancer. All patients with bone metastasis were confirmed to have at least one metastatic lesion, accompanied by pain due to bone metastasis, as verified by imaging examination. In addition, this study excluded patients with advanced breast cancer who already had other metastatic sites at the initial diagnosis, as well as those with primary malignant tumors in other organs. All patients were subjected to a 36-month follow-up period, and their death situations were recorded.

This study has been submitted to the ethics committee of The Third Affiliated Hospital of Kunming Medical University for review and has received formal approval (approval number: kmmu20200723). All patients participating in the study or their legal representatives have signed informed consent forms, clearly indicating their awareness and consent to the study content. This study complies with the Declaration of Helsinki.

Cell Culture and Transfection

MCF-10A cells and breast cancer cells MDA-MB-231 (HTB-26), MCF-7 (HTB-22), BT-549 (HTB-122) and MDA-MB -468 (HTB-132) (ATCC, USA) were selected for research in this study. MDA-MB-468 belongs to the TNBC subtype, MDA-MB-231 is one of the most widely used TNBC cell lines, featuring strong metastatic ability and being frequently applied in bone metastasis research. BT-549 is an invasive TNBC cell. It is often used in the study of the invasion and metastasis mechanisms of breast cancer. MCF-7: an estrogen receptor-positive (ER+) breast cancer cell line, forms a subtype comparison with TNBC cell lines to verify the expression or functional differences of LINC00917 in different breast cancer subtypes. In previous studies, these cell lines were used as subjects for breast cancer or bone metastasis researc.[7,8] BT-549 was grown with RPMI-1640 Medium supplemented with 10% FBS. MDA-MB-468, MDA-MB-231 and MCF-7 cells were cultured in DMEM medium supplemented with 10% FBS. In addition, MC3T3-E1 and RAW264.7 cells were obtained from Shangn Bio (Wuhan, China) and maintained in DMEM and incubated at 37 °C with 5% CO_2.

LINC00917 siRNAs (si-LINC00917, G04009), siRNA negative control (si-NC, A06001), miR-491-5p mimic (B02003), miR-491-5p inhibitor (B03001), and mimic/inhibitor NC (B04002), were procured from GenePharma and their sequences are listed in Supplementary Table 1. Cell transfection was carried out using Lipofectamine 2000 (Invitrogen).

To explore the effect of LINC00917 on bone metastasis of breast cancer, RAW264.7 or MC3T3-E1 cells were co-cultured with the transfected MDA-MB-231 cells in vitro using Transwell chambers to establish a bone metastasis model. MC3T3-E1 cells were cultured in a medium containing 10 mm β-glycerophosphate and 25 mM ascorbic acid for 7 days to induce osteogenic differentiation. In addition, RAW 264.7 cells were treated with a medium containing M-CSF (50 ng/mL) and RANKL (50 ng/mL) for 7 days to induce osteoclast differentiation. MDA-MB-231 cells were starved in serum-free medium for 12 hours. A six-well Transwell insert with 0.4 μm pores was selected for the experiment. RAW264.7 (1×10^4) or MC3T3-E1 (1×10^4) cells were seeded in each well of the lower chamber of the Transwell and 2 mL of complete DMEM medium was added. 1×10^4 MDA-MB-231 cells were seeded in each well of the upper chamber and 2 mL of serum-free DMEM medium was added. The cultures were incubated at 37°C and 5% CO_2 for 48 hours.

RT-qPCR Analysis

Total RNA was isolated using TRIzol reagent (Sigma Chemicals Co). Subsequently, complementary DNA (cDNA) was synthesized with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher). qRT-PCR was conducted on the 7300 Sequence Detection System (Applied Biosystems/Roche) using SYBR Premix Ex Taq mix. GAPDH and U6 served as internal controls. The relative expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Supplementary Table 2.

Dual-Luciferase Assay

Lipofectamine 2000 was utilized to co-transfect cells with a luciferase reporter vector containing either the wild-type or mutant 3' UTR of LINC00917 or FOXP4, along with miR-491-5p mimic or mimic NC, and miR-491-5p inhibitor or inhibitor NC. Luciferase activity was quantified using the Luciferase Assay System (Promega) following 24 hours of transfection.

CCK-8 Assay

Cells were seeded into a 96-well plate following transfection. At each 24-hour interval, the CCK-8 kit was added sequentially to the wells designated for testing at that specific time point, and incubation continued at 37 °C for 2 hours. The OD value of 450 nm was measured using a microplate reader.

Transwell Assays

Cell migration and invasion were conducted using a Transwell chamber. In brief, following washing, the cells were resuspended in serum-free DMEM to generate a single-cell suspension. Subsequently, cell solution was added to the top of the Transwell insert and incubated for 10 minutes. The lower chamber was filled with DMEM medium containing FBS. After incubating for 12 hours, the chamber was removed, and non-migratory cells on the upper surface of the membrane were scraped off. Cells that did not migrate were removed with a cotton swab and migratory cells were fixed with 5% glutaraldehyde at 4°C and stained with crystal violet for 30 min. After fixation and staining, five non-overlapping fields of view were randomly selected from each migration membrane under an optical microscope (magnification 200×) for cell counting. The counting process was independently completed by two experimenters using a double-blind method, and the average value was taken as the number of migrating cells in the sample.

Statistical Analysis

Data were statistically analyzed using SPSS and GraphPad Prism. *T*-test was used for comparing the differences of two groups. When comparing three or more groups, ANOVA was applied. Survival analysis was conducted using the Kaplan-Meier curve. A multivariate Cox proportional hazards regression analysis was performed to determine the risk factors influencing patient survival. All cell experiments were independently repeated three times, and each repeated experiment included three biological replicate samples. Statistical significance was set at a p-value of less than 0.05.

Results

Comparison of Basic Data Between the Two Groups of Patients

No significant differences were found in terms of age, tumor size, menstrual status, and molecular subtypes. However, significant differences were observed in vascular tumor thrombus and lymph node metastasis between the two groups, specifically, the number of patients with vascular tumor thrombus and lymph node metastasis was significantly higher in the BM group (Table 1).

The Expression of LINC00917 in Breast Cancer Patients and Cells

Compared with NBM patients, the expression of LINC00917 was significantly enhanced in bone metastasis patients (Figure 1A). Additionally, the expression of LINC00917 was also upregulated in breast cancer cells, especially showing the most significant difference in the MCF-7 and MDA-MB-231 cell lines (Figure 1B).

Correlation Between LINC00917 Levels and the Prognosis of Breast Cancer Patients with Bone Metastasis

Patients with bone metastasis were categorized into two groups based on the mean level of LINC00917. The findings revealed a significant correlation between LINC00917 and lymph node metastasis in these patients (Table 2). Furthermore, the Kaplan-Meier curve demonstrated a reduced survival rate among patients with high LINC00917 expression (Figure 2A). In addition, lymph node metastasis and LINC00917 may be independent factors influencing bone metastasis (Figure 2B).

Table 1 Clinical and Pathological Data of NBM Group and BM Group

		NBM (n=129)	BM (n=125)	*P* value
Age (years)				0.455
	<50	69	61	
	≥50	60	64	
Menstrual status				0.900
	No	64	63	
	Yes	65	62	
Tumor size (cm)				0.578
	<2	55	49	
	≥2	74	76	
Lymph node metastasis				0.017
	No	76	55	
	Yes	53	70	
Vascular cancer embolus				0.006
	No	118	99	
	Yes	11	26	
Molecular subtype				0.166
	Luminal A	35	40	
	Luminal B	43	52	
	Her2+	25	15	
	TNBC	26	18	

Abbreviations: NBM, non-bone metastasis breast cancer; BM, bone metastasis breast cancer; TNBC, Triple-Negative Breast Cancer.

miR-491-5p Was a Potential Target miRNA of LINC00917

The findings indicated a decreased expression of miR-491-5p in bone metastasis patients (Figure 3A), which was inversely correlated with LINC00917 (Figure 3B). Moreover, experimental results demonstrated a notably reduced expression of miR-491-5p in breast cancer cells when compared to normal cells (Figure 3C). The binding sites between LINC00917 and miR-491-5p were presented in Figure 3D. miR-491-5p overexpression led to a decrease in the luciferase activity of wild-type LINC00917, whereas the inhibition of miR-491-5p resulted in an increase in the luciferase activity of wild-type LINC00917. However, neither the mimic nor the inhibitor had a significant impact on the luciferase activity of the mutant LINC00917 (Figure 3E and F).

Effects of Inhibiting LINC00917 and miR-491-5p on the Breast Cancer Cells

The siRNAs targeting LINC00917 effectively decreased LINC00917 expression in cell lines (Figure 4A). Furthermore, the siRNA exhibiting the highest transfection efficiency was selected for use in subsequent experimental procedures. However, the inhibition of LINC00917 significantly enhanced the level of miR-491-5p, while transfection with miR-491-5p inhibitor led to a marked decrease in the level of miR-491-5p (Figure 4B). Additionally, the inhibition of LINC00917 resulted in a decline in the proliferation ability of breast cancer cells,

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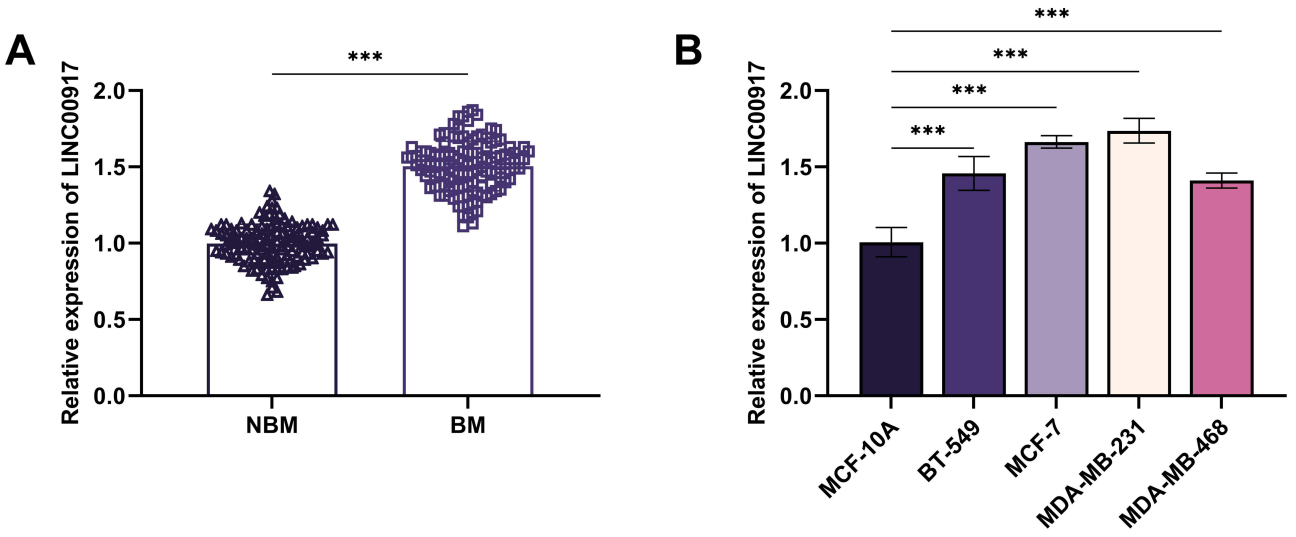

Figure 1 The expression of LINC00917 in breast cancer patients and breast cancer cells. ***P<0.001.The expression of LINC00917 in NBM group and BM group (**A**). The expression of LINC00917 in breast cancer cells (**B**).

this indicates that LINC00917 may have played a key role in promoting the growth of tumor cells, and the miR-491-5p inhibitor could partially counteract this effect (Figure 4C and D). Further studies revealed that LINC00917 inhibition reduced the migration and invasion abilities of cells, suggesting that LINC00917 may be involved in regulating biological processes related to metastasis. But down-regulation of miR-491-5p counteracted these changes, thereby enhancing the migration and invasion numbers of cells (Figure 4E and F). These results further support the hypothesis that LINC00917 affects the invasion and migration ability of breast cancer cells by regulating miR-491-5p.

Table 2 Correlation Between LINC00917 Expression and Clinical Data of BM Breast Cancer Patients

		Patients (n=125)	Linc00917 Expression		*P* value
			Low (n=59)	High (n=66)	
Age (yeas)					0.521
	<50	61	27	34	
	≥50	64	32	32	
Menstrual status					0.327
	No	63	27	36	
	Yes	62	32	30	
Tumor size (cm)					0.074
	<2	49	28	21	
	≥2	76	31	45	
Lymph node metastasis					0.018
	No	56	33	23	
	Yes	69	26	43	

(*Continued*)

Table 2 (Continued).

		Patients (n=125)	Linc00917 Expression		*P* value
			Low (n=59)	High (n=66)	
Vascular cancer embolus					0.059
	No	99	51	48	
	Yes	26	8	18	
Molecular subtype					0.288
	Luminal A	40	15	25	
	Luminal B	52	29	23	
	Her2+	15	8	7	
	TNBC	18	7	11	

Abbreviations: BM, bone metastasis breast cancer; TNBC, Triple-Negative Breast Cancer.

Effects of LINC00917 and miR-491-5p on Osteoblast and Osteoclast Differentiation

We employed RAW264.7 cells as an in vitro model for osteoclast differentiation to assess the impact of LINC00917 on this process. RAW264.7 cells were co-cultured with transfected MDA-MB-231 cells. Data revealed that the down-regulation of LINC00917 led to a decrease in the mRNA levels of transcription factors of osteoclastogenesis (TRAP, NFATc1, and Cathepsin K). Conversely, the inhibition of miR-491-5p resulted in an increase in the mRNA expression of TRAP, NFATc1, and Cathepsin K (Figure 5A).

Furthermore, we further explored the effects of LINC00917 and miR-491-5p on osteoblast differentiation in the tumor microenvironment. The experimental results showed that the inhibition of LINC00917 significantly promoted the expression of OPG mRNA in the co-culture system of MC3T3-E1 and MDA-MB-231, while inhibiting the expression of RANKL mRNA. Additionally, the OPG/RANKL ratio significantly increased. However, miR-491-5p inhibition reversed the regulatory effect of LINC00917 inhibition on the expression levels of OPG and RANKL in osteoblasts (Figure 5B).

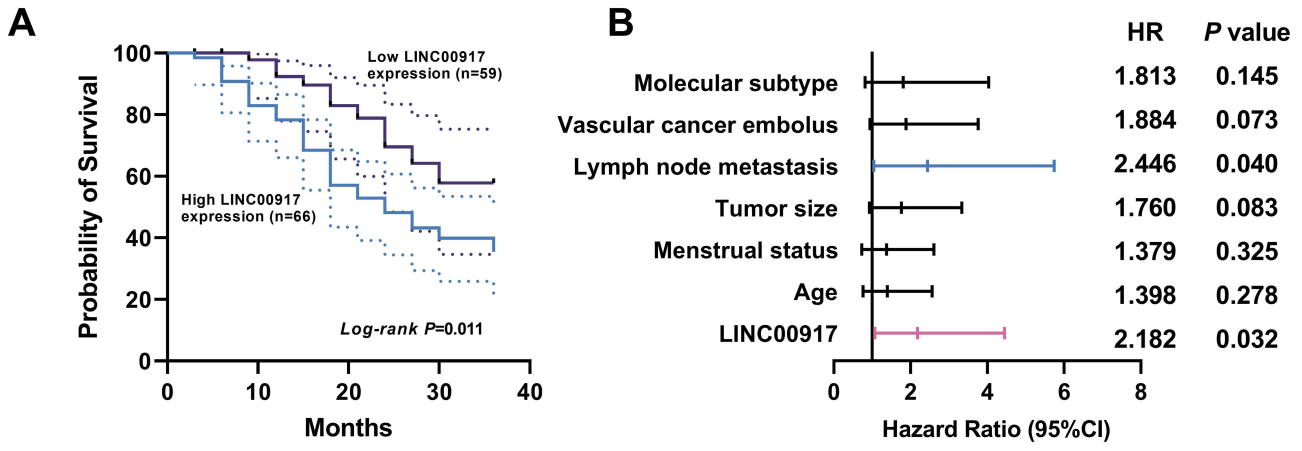

Figure 2 Correlation between LINC00917 levels and the prognosis of breast cancer patients with bone metastasis.Kaplan-Meier curve of the relationship between LINC00917 and prognosis in breast cancer patients with bone metastasis (**A**). Multivariate Cox regression analysis of factors influencing the prognosis of patients with breast cancer bone metastasis (**B**).

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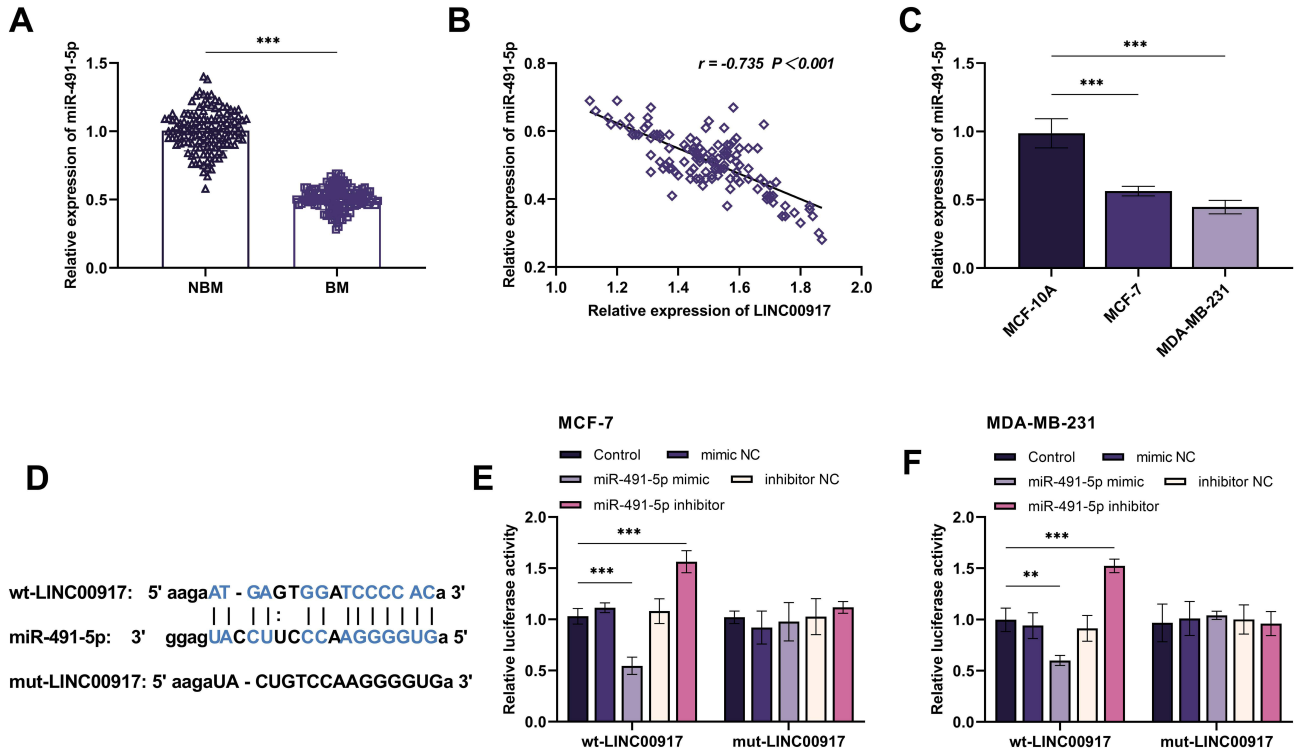

Figure 3 miR-491-5p was a potential target miRNA of LINC00917. ***P*<0.01, ****P*<0.001.The expression of miR-491-5p in NBM group and BM group (**A**). Correlation analysis of the expression levels of LINC00917 and miR-491-5p (**B**). The expression of miR-491-5p in breast cancer cells (**C**). The binding site of LINC00917 and miR-491-5p (**D**). A dual-luciferase reporter gene assay was conducted to verify the binding of LINC00917 and miR-491-5p at the predicted sites (**E** and **F**).

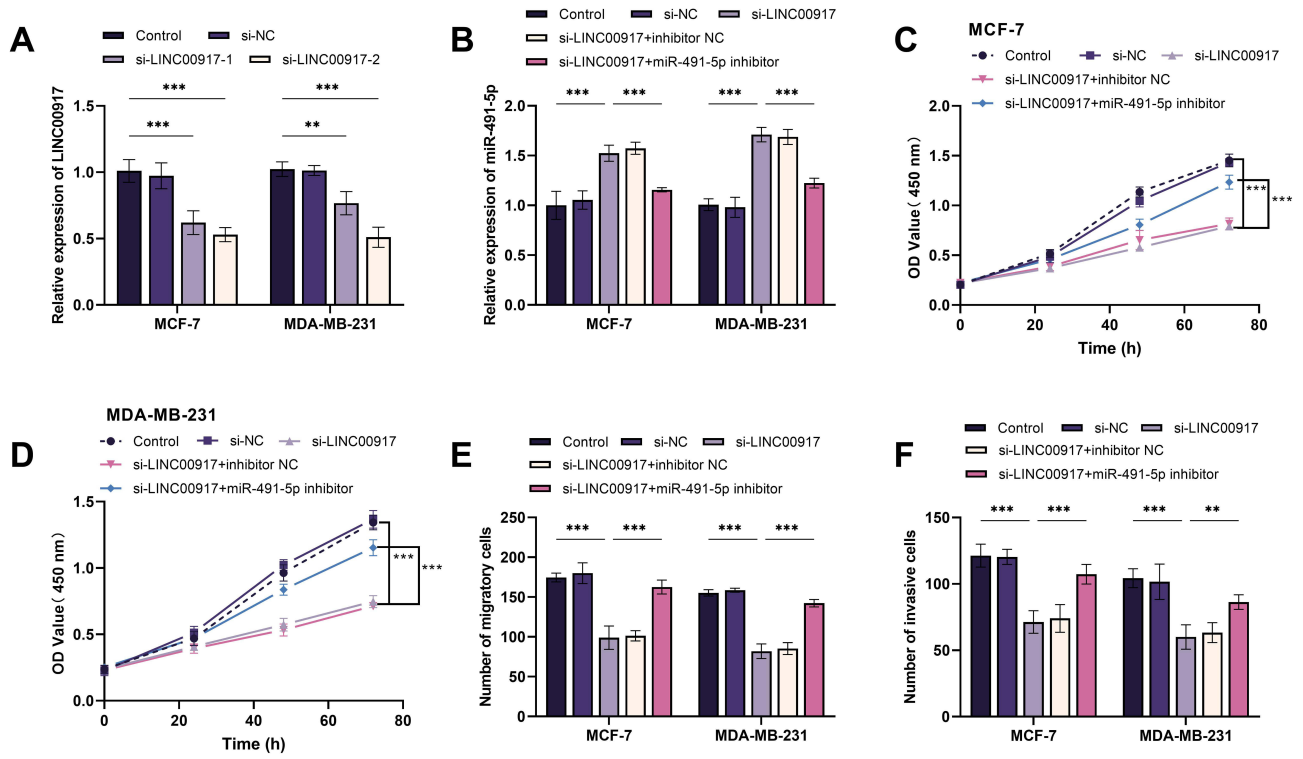

Figure 4 Effects of inhibiting LINC00917 and miR-491-5p on the proliferation, migration and invasion of breast cancer cells. ***P*<0.01, ****P*<0.001.Detection of the transfection efficiency of LINC00917 siRNA. (**A**) The influence of LINC00917 on the expression level of miR-491-5p (**B**). Effects of inhibiting LINC00917 and miR-491-5p on the proliferation of breast cancer cells (**C** and **D**). Effects of inhibiting LINC00917 and miR-491-5p on the migration and invasion of breast cancer cells (**E** and **F**).

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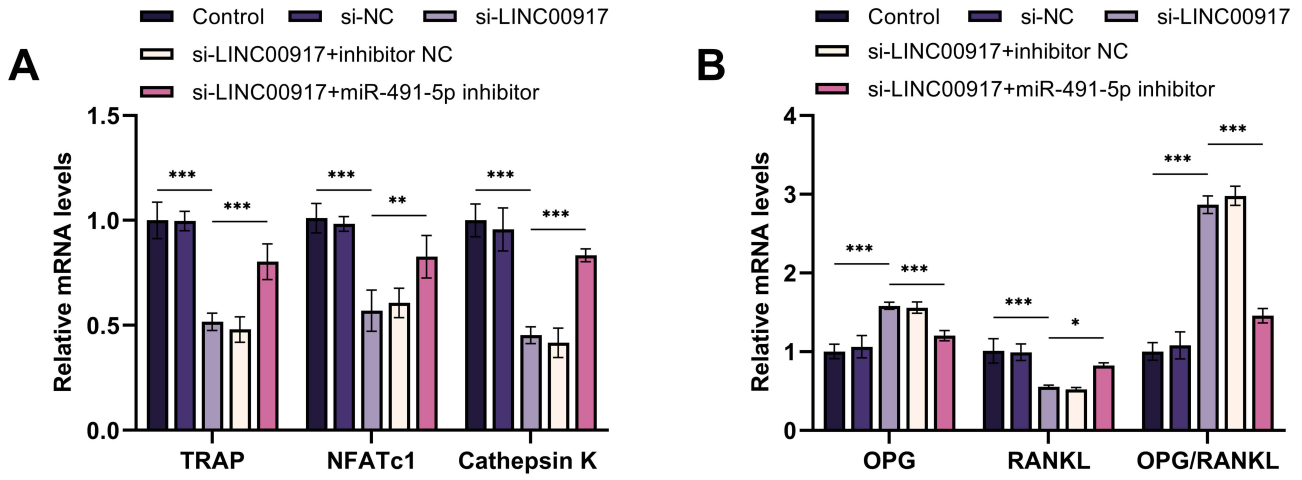

Figure 5 Effects of LINC00917 and miR-491-5p on osteoblast and osteoclast differentiation. **P*<0.05,***P*<0.01, ****P*<0.001.Effects of LINC00917 and miR-491-5p on TRAP, NFATc1, and Cathepsin K mRNA levels (**A**). Effects of LINC00917 and miR-491-5p on OPG, RANKL, and OPG/RANKL ratio (**B**).

FOXP4 Might Be a Target Gene of miR-491-5p

In breast cancer patients with bone metastasis, FOXP4 expression was markedly elevated (Figure 6A) and inversely correlated with miR-491-5p levels (Figure 6B). The potential binding sites between FOXP4 and miR-491-5p were identified using the ENCORI database (Figure 6C). Subsequent experiments revealed that in two breast cancer cell lines, miR-491-5p mimic suppressed the luciferase activity of the wild-type FOXP4 construct, whereas the miR-491-5p inhibitor markedly enhanced it (Figure 6D–E). Furthermore, LINC00917 inhibition led to a reduction in FOXP4 expression, while miR-491-5p downregulation resulted in an increased FOXP4 expression (Figure 6F).

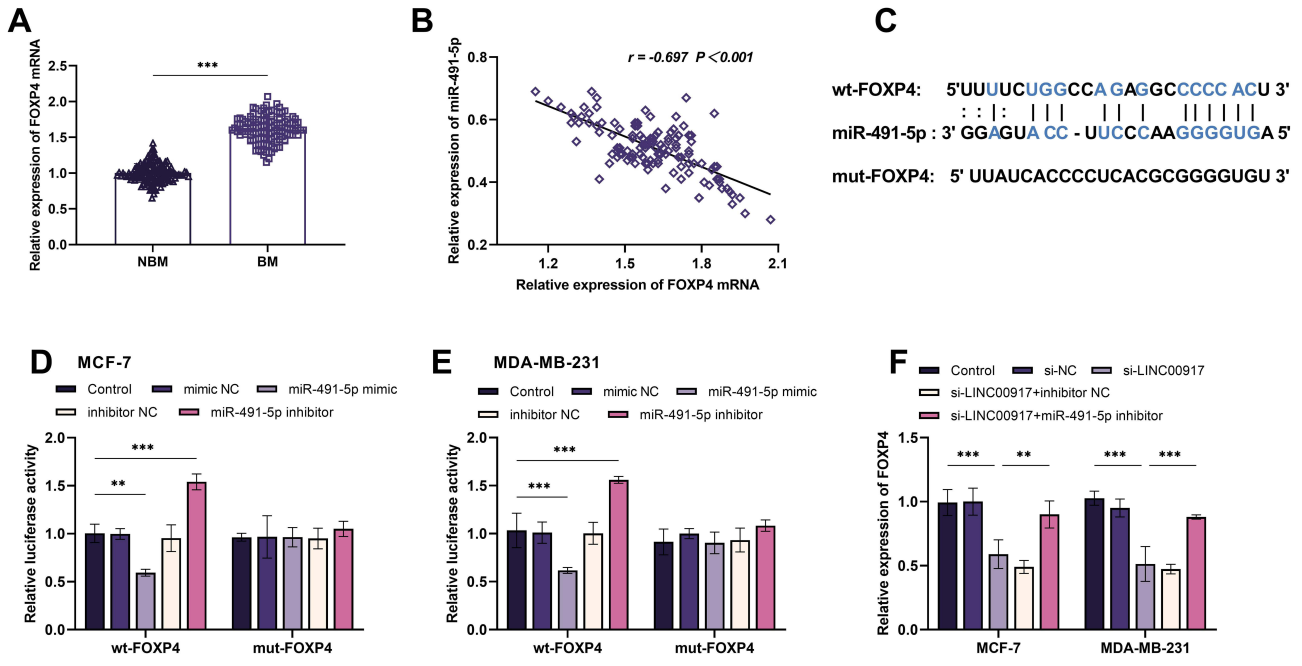

Figure 6 FOXP4 may be a target gene of miR-491-5p. ***P*<0.01, ****P*<0.001. The expression of FOXP4 in NBM group and BM group (**A**). Correlation analysis of the expression levels of FOXP4 and miR-491-5p (**B**). The binding site of FOXP4 and miR-491-5p (**C**). A dual-luciferase reporter gene assay was conducted to verify the binding of FOXP4 and miR-491-5p at the predicted sites (**D** and **E**). The influence of LINC00917 and miR-491-5p on the expression level of FOXP4 (**F**).

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Discussion

Studies have found that abnormal expression of lncRNAs may be a common mechanism in bone metastasis of cancer. For instance, RG-AS1 expression is decreased in MDA-MB-231-BO cells with strong bone metastasis characteristics, potentially inhibiting breast cancer bone metastasis.[8] Decreased MALAT1 expression in osteoclast precursors and mature osteoclasts is linked to osteoporosis and metastatic bone lesions.[24] LINC00263 overexpression significantly promotes breast cancer bone metastasis.[25] This study demonstrated that LINC00917 functioned as a ceRNA by competitively binding to miR-491-5p, leading to the upregulation of FOXP4 expression and consequently promoting breast cancer bone metastasis.

This study found that LINC00917 was abnormally highly expressed in breast cancer patients with bone metastasis. Further analysis revealed that the level of LINC00917 was significantly correlated with the lymph node metastasis status of patients, and patients with high expression of LINC00917 had a poorer prognosis and shorter survival time. This phenomenon further validates the potential value of LINC00917 as a biomarker for poor prognosis in breast cancer. Statistical analysis indicated that the high expression of LINC00917 was the independent risk factors for poor prognosis in patients, meaning that even after excluding the influence of other known risk factors, the expression level of LINC00917 still significantly predicts the survival status of patients. This observation aligns with the findings reported in prior research, suggesting that LINC009 might be a risk factor for the prognosis of breast cancer.[12] These findings revealed the connection between LINC00917 and breast cancer bone metastasis, providing direction for future studies and establishing a foundation for its potential clinical application.

To investigate the molecular mechanism by which LINC00917 affects bone metastasis in breast cancer, we conducted in vitro experiments. We also observed that LINC00917 was abnormally overexpressed in breast cancer cells. Abnormalities of lncRNA have been confirmed to exhibit tumor suppressor or oncogenic effects and play a significant role in the development of tumors.[26] In MCF-10A cells, LINC00917 may play a role in maintaining normal intracellular homeostasis, as lncRNAs in non-cancerous cells typically regulate fundamental cellular processes. In contrast, in MCF-7 and MDA-MB -231 cells, LINC00917 shows an upregulated trend and is associated with pro-metastatic functions. Additionally, we predicted the downstream miRNAs regulated by LINC00917, and found that LINC00917, as a ceRNA of miR-491-5p, affects the cellular functions of breast cancer. Knocking down LINC00917 suppressed cell proliferation, invasion, and migration. However, miR-491-5p inhibitor counteracted this effect. This further indicates that LINC00917 may promote breast cancer processes by regulating miR-491-5pxpression.

The development of bone metastasis is associated with a multitude of factors, involving a complex interplay among cancer cells, the bone matrix, osteoclasts and osteoblasts.[27] Specifically, breast cancer cells secrete cytokines and growth factors that stimulate bone resorption, thereby accelerating osteoclast development and leading to osteolytic metastasis.[28] Our study demonstrated that the knockdown of LINC00917 suppressed the expression of transcription factors regulating osteoclastogenesis in an in vitro model. This finding suggests that LINC00917 inhibition may offer protective effects against the pathological progression of breast cancer bone metastasis. Additionally, osteoblasts can express RANKL to bind to the RANK receptor on osteoclast precursor cells, promoting their differentiation into mature osteoclasts. Conversely, osteoblasts can secrete OPG to inhibit the RANK/RANKL pathway.[29] We further observed that LINC00917 inhibition enhanced OPG expression while suppressing RANKL expression, thereby modulating the OPG/RANKL balance. This might indicate that inhibiting LINC00917 helps to enhance the function of osteoblasts.

Previous studies have shown that FOXP4 upregulation may counteract the tumor-suppressive effects of miR-491-5p.[22,23] Consistent with these findings, our study showed that FOXP4 expression is upregulated in breast cancer bone metastasis and confirms FOXP4 as a target gene of miR-491-5p. LINC00917 functions as a ceRNA by sponging miR-491-5p, thereby upregulating FOXP4 expression. These results suggest that the upregulation of FOXP4 during the process of breast cancer bone metastasis may interfere with the function of miR-491-5p, thereby relieving its inhibitory effect on the growth of cancer cells. It has been reported that the upregulation of FOXP4 in breast cancer promotes cell migration and invasion.[30] Additionally, FOXP4 promotes cell proliferation and metastasis in prostate cancer.[31] Therefore, the upregulation of FOXP4 may interfere with the inhibitory effect of miR-491-5p on the metastasis of breast cancer cells, thereby promoting the malignant behavior of tumor cells. Based on these observations, we propose that LINC00917 may regulate the miR-491-5p/FOXP4 axis, thereby influencing the development of breast cancer bone metastasis.

In recent years, numerous studies have revealed the crucial roles of lncRNAs in the process of bone metastasis. LINC00263 significantly promotes breast cancer-related osteolytic bone metastasis by inducing the generation of osteoclasts and inhibiting their ferroptosis process.[25] MALAT1 can promote the osteogenic differentiation of bone marrow mesenchymal stem cells, which may affect the remodeling and repair process of the bones.[32] LncRNA TEX41 participates in regulating the autophagy process by upregulating the expression level of the transcription factor RUNX2, thereby influencing the colonization and growth of tumor cells in bone tissues.[33] These studies have revealed the regulatory mechanisms of various molecules in the process of bone metastasis from different perspectives. Therefore, the mechanism by which LINC00917 affects breast cancer bone metastasis requires more experiments to be investigated.

Research has found that three years after surgery, the overall distant recurrence risk of ER+ tumors is significantly higher than that of ER-negative (ER-) tumors, and ER status is an important prognostic risk factor for late skeletal recurrence.[34] In this study, LINC00917 knockdown significantly inhibited migration and invasion abilities in both ER+ (MCF-7) and triple-negative breast cancer (MDA-MB-231) cells. Although the inhibition was greater in MDA-MB-231 cells (migration: 43% vs 47%; invasion: 41% vs 42%), the difference between the two cell lines was small. This may indicate that LINC00917 plays a key role in both ER+ and TNBC, suggesting that its regulatory mechanism may be independent of ER signaling pathway. Additionally, it may be due to the fact that in vitro migration/invasion experiments may not fully simulate the in vivo microenvironment. In future research, more in vivo experiments may be needed to explore the association between ER status and LINC00917 in bone metastasis. Additionally, we analyzed the relationship between the expression level of LINC00917 and the different subtypes of breast cancer patients, and found that the expression of LINC00917 in bone metastasis patients had no significant correlation with the molecular subtypes of the patients. This might be due to the insufficient number of patients included in this study.

One significant limitation of this study is that it did not specifically investigate the relationship between ER status and LINC00917 in bone metastasis. Therefore, in future experiments, more patients should be included and classified to systematically explore the association among LINC00917, ER status and bone metastasis of breast cancer. Moreover, in vivo experiments are also needed to investigate the relationship between ER status and bone metastasis. Additionally, LINC00917 may participate in the regulation of other signaling pathways, the precise mechanisms of which remain to be fully elucidated. Further experimentation and in-depth investigation are necessary to unravel its comprehensive functional network. Furthermore, the specific role of FOXP4 in cellular functions warrants further validation. Future study should integrate both in vivo and in vitro experiments to further corroborate the conclusions drawn from this study.

Conclusion

LINC00917 was upregulated in breast cancer bone metastasis and was associated with bad prognosis. Additionally, the knockdown of LINC00917 inhibited the function of breast cancer cells, and suppressed osteoclastogenesis while promoting osteoblast differentiation. Moreover, miR-491-5p inhibitor counteracted the effects of LINC00917 on cell models. Furthermore, FOXP4 may be a target gene of miR-491-5p. Therefore, the LINC00917/miR-491-5p/FOXP4 axis may be involved in the pathogenesis of breast cancer.

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Disclosure

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