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Long noncoding RNA LINC00473 indicates a poor prognosis of breast cancer and accelerates tumor carcinogenesis by competing endogenous sponging miR-497

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Abstract. – OBJECTIVE: Long noncoding RNA LINC00473 (LINC00473) has been reported to be involved in the progression of several tumors. Our present study was conducted to study the potential roles and mechanism of long noncoding RNA LINC00473 (LINC00473) on cells proliferation, migration, invasion, and apoptosis of breast cancer (BC).

PATIENTS AND METHODS: RT-PCR was applied for the analysis of LINC00473 in BC cell lines and tissues samples. The correlations between the LINC00473 levels and clinicopathological parameters were investigated. Kaplan-Meier methods and Cox proportional hazards regression models were explored to reveal potential associations of LINC00473 levels with overall survival of BC patients. The effect of LINC00473 on tumor behavior was evaluated by colony formation, Cell Counting Kit-8, EdU assays, flow-cytometric analysis, wound healing assays and transwell assays. Interactions between LINC00473 and miR-497 were determined using a luciferase reporter assay and RT-PCR assays.

RESULTS: Upregulation of the expression of LINC00473 was found in BC samples and cell lines, in comparison with non-tumor breast tissues and human breast epithelial cells. High expression of LINC00473 was correlated with lymph node metastasis, clinical stage, and poorer outcome in BC patients. Multivariate logistic regression assays further showed LINC00473 as an independent prognostic factor in BC. Lost-of-function assays revealed that knockdown of LINC00473 resulted in the suppression of tumor cell proliferation, promotion of cells apoptosis, inhibition of cells metastasis. Bioinformatics analysis and luciferase reporter assays revealed LINC00473 bonds to miR-497. Further RT-PCR revealed that knockdown of LINC00473 suppressed the expressions of miR-497 in BC cells.

CONCLUSIONS: Our data revealed that LINC00473 acted as a tumor promoter in BC and LINC00473/miR-497 axis may be a novel therapeutic strategy for this tumor.

Key Words
LncRNA, LINC00473, miR-497, Prognosis, Metastasis, Breast cancer.

Introduction

Breast cancer (BC) is the most common neoplasm among women throughout the world and accounts for about 25-30% of female malignant tumors[1,2]. According to the World Health Organization, BC is a heterogeneous tumor that can be classified into four subtypes, namely luminal A, luminal B, ErbB^{2+}, and basal-like[3]. In China, BC is a serious threat to females, and its incidence is increasing annually[4]. With recent success in operative treatment, diagnostic technique, and new chemotherapy methods, the incidence of the disease is increasing, the number of death verge to decrease[5,6]. However, once metastasis occurs, optimal management has remained a challenge, five-year survival rate is less than 25%[7,8]. Thus, the basic exploration of the molecular mechanisms involved in the progression of BC may help investigate the valuable biomarker and therapeutic target for BC. Many studies have described the tumorigenesis with regard to non-coding RNAs[9]. Long noncoding RNAs (lncRNAs) are a class of transcripts that are longer than two hundred nucleotides in length and have no potential of protein-coding[10]. Growing studies showed some functional

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lncRNAs have critical roles in the regulation of complex biological progression including cell growth, cell differentiation, and apoptosis, as well as in some diseases progression[11-13]. Several well-characterized lncRNAs have been confirmed to play a functional role in the regulation on genes expressions at various levels, such as chromatin modification, transcription, and posttranscriptional processing[14,15]. Based on the evidence provided by previous studies that dysregulation of lncRNAs can influence the expression levels of tumor-related genes, such as P53, CKD1, and PTEN[16,17]. It is believed that lncRNAs may act as tumor promoter or oncogenes in different types of tumors[18-20]. In human BC, oncogenic and anti-oncogenic lncRNAs, including lncRNA UICLM[21], lncRNA-ROR[22] and lncRNA HOTAIR[23], have been shown to be dysregulated in breast cell lines and tissues, and contribute to breast carcinogenesis. Therefore, it suggests that lncRNAs may, function as a potential indicator in clinical prognosis of BC patients and useful therapeutic target for the study of new anticancer therapy. Long noncoding RNA LINC00473 (LINC00473) was a recently discovered lncRNA, which was located in the chromosome 6q27. Zhu et al[24] reported that LINC00473 expression was increased in gastric cancer samples by performing Microarray analysis and RT-PCR in both clinical specimens and cells. Following that, other several studies also demonstrated LINC00473 as an overexpressed lncRNA in several tumors, such as lung cancer[25], cervical cancer[26], and mucoepidermoid carcinoma[27]. In addition, a large number of *in vitro* and *in vivo* assays showed the potential oncogenic roles of LINC00473 in these tumors. However, the expression profiles and potential biological in BC have not been investigated. Here, the levels of LINC00473 were also observed to be increased in BC samples. We further explored its clinical significance and potential function in BC.

Patients and Methods

Clinical Tissue Specimens

BC and non-cancerous tissue specimens were obtained from 122 patients between May 2008 and November 2013 at Cancer Hospital of China Medical University, Liaoning Cancer Hospital and Institute. The written consents were acquired from all subjects and these protocols were approved by the Ethics Committee of Cancer Hospital of China Medical University, Liaoning Cancer Hospital and Institute. After surgery resection, the tissue specimens were frozen and preserved at -80°C. All the patients did not receive any anti-tumor therapy before surgery. The clinicopathological characteristics were shown in Table I.

Cell Lines and Cell Transfection

Human breast cancer cells (MDAMB-231, MDA-MB-453, MCF-7, MDA-MB-468) and MCF-10A (human breast epithelial cells) were obtained from BeNa Culture Collection (Suzhou, Jiangsu, China). RPMI-1640 medium (BioSun, Xuhui, Shanghai, China) containing fetal bovine serum (FBS) (10%) and antibiotics (1%) was used to culture these cells. For cell transfection, we used a LipoFiter Transfect reagent (HANBIO, Pudong, Shanghai, China). In brief, the cells (4×10^5) were planted into plates (6-well) and grown to 60-70% confluent. Then, 50 pmol indicated small interfering RNAs (siRNAs), miRNA mimics or plasmids (4 μg) were mixed with LipoFiter Transfect reagent (12 μl) for about 20 min. Subsequently, the mixture was added into the cells and the medium was changed after 6 h using fresh medium. The siRNAs targeting LINC00473 (si-LINC00473-1, si-LINC00473-2 and si-LINC00473-3), control siRNAs (si-control), and miRNA mimics were purchased from Neo Bioscience Co., Ltd. (Shenzhen, Guangdong, China). LINC00473 overexpressing plasmids were constructed by ShuangLing Biotechnology Co., Ltd. (Nanjing, Jiangsu, China).

Real-Time PCR Detection

Total RNAs were extracted using a Total RNA Isolation kit (Foregene, Chengdu, Sichuan, China), and miRNA was extracted using Qiagen miRNeasy mini kit (YuBio, Yangpu, Shanghai, China). For measuring the expression of LINC00473, RNAs (2 μg) were first reversely transcribed using cDNA Synthesis kits (ShanRan, Fengxian, Shanghai, China). Then, qRT-PCR analysis using a SYBR Green qPCR Mix kit (ZeYe, Songjiang, Shanghai, China) was conducted using the cDNA (2 μl; 1:10 dilution) as templates. For miRNA detection, we applied a miRNA real-time PCR detection kit (Biomics, Nantong, Jiangsu, China). Fold changes in lncRNA or miRNA expression levels were calculated using $2^{-\Delta\Delta Ct}$ method. The expression levels of lncRNA or miRNA were normalized to that of GAPDH. Primers were presented in Table II.

Table I. The correlations between LINC00473 expression and clinicopathological factors of BC patients.

Characteristics	N	High-expression	Low-expression	p
Age (years)				NS
≤ 50	59	28	31	
> 50	63	32	31	
Tumor size (cm)				NS
≤ 2	73	33	40	
> 2	49	27	22	
Differentiation grade				NS
G1 + 2	70	31	39	
G3	52	29	23	
Histological type				NS
Ductal	59	26	33	
Lobular	63	34	29	
ER status				NS
Negative	64	33	31	
Positive	58	27	31	
PR status				NS
Negative	56	26	30	
Positive	66	34	32	
HER-2 status				NS
Negative	68	33	35	
Positive	54	27	27	
Lymph node metastasis				0.030
Absent	90	39	51	
Present	32	21	11	
Clinical stage				0.014
I + II	84	35	49	
III	38	25	31	

Cell Proliferation Evaluation

Cells were first transfected with siRNAs as described above. Next, the treated cells were collected and 2000 cells in 10% serum-containing RPMI-1640 medium were planted into 96-well plates (per well). At the indicated time (0 h, 24 h, 48 h, 72 h and 96 h), 10 μl CCK-8 reagents were placed into the 96-well plates, and the cells were placed at 37°C with 5%CO_2 for 2-3 h. Then, the absorbance values (OD450 nm) were detected by a microplate reader system (318C; PeiOu, Jinshan, Shanghai, China).

Cell Colony Formation Assays

The cells were first transfected with indicated siRNAs and 500 cells were planted in plates (6-well). Cells were then maintained using 10% FBS containing RPMI-1640 medium for 14-21 days. Then, visible colonies were treated using crystal violet solution (0.1%; ShiFeng, Baoshan, Shanghai, China) and photographed using a microscope (PH50; XiPaiKe, Shenzhen, Guangdong, China).

Cell Apoptosis Detection

We used TUNEL kits (Yeason, Pudong, Shanghai, China) to determine the apoptosis of BC cells after transfection of LINC00473 siRNAs. Briefly, the cells were placed in plates and treated using indicated siRNAs as described above. After 24 h, the cells were collected, re-placed in 48-well plates. After attachment, the cells were fixed with paraformaldehyde (4%) and sequentially treated using TUNEL detection reagent (50 μl). After incubation for 35 min, a fluorescence microscope (IX71, Olympus, Tokyo, Japan) was used to take pictures of the apoptotic cells.

Wound Healing Assays

Cells after LINC00473 siRNAs transfection (2×10^5/well) were planted in plates (24-well), cul-

Table II. The primer sequences included in this study.

Name	Primer sequences (5′–3′)
LINC00473: forward	GGCAGCCTCAGGTTACAAAT
LINC00473: reverse	AGGAGCAGGTAGGGAAATGA
miR-497: forward	GTGCAGGGTCCGAGGT
miR-497: reverse	TAGCCTGCAGCACACTGTGGT
GAPDH: forward	CAATGACCCCTTCATTGACC
GAPDH: reverse	GACAAGCTTCCCGTTCTCAG

tured to 100% cell confluence. Thereafter, the wounds were generated by scratching across the cell layers with a pipette tip (200 μl). After removing the cells debris using PBS, the wounds were photographed using a microscope (PH50; XiPaiKe, Shenzhen, Guangdong, China).

Transwell Assays

We used BD Biosciences transwells (YuBio, Fengxian, Shanghai, China) pre-coated with Matrigel to assess the effects of LINC00473 on cellular invasion. In short, the indicated siRNAs-treated cells were re-placed into medium (without FBS), each upper chambers of the transwells were placed with 200 μl cellular suspensions. Besides, 500-600 μl media (15% serum) was placed into each lower chamber of the transwells. Twenty-four hours later, the invaded cells on the lower surfaces of the membranes were treated with crystal violet solution (0.1%; ShiFeng, Baoshan, Shanghai, China). After washing, the stained cells were photographed using a microscope (PH50; XiPaiKe, Shenzhen, Guangdong, China).

RNA-Binding Protein Immunoprecipitation (RIP) Assays

We applied an EZ-Magna RIP assay kit (SC-Bio, Guangzhou, Guangdong, China) to conduct the RIP assays. In brief, the indicated siRNAs-transfected cells were collected and lysed using the RIP lysis buffer. Then, magnetic beads, which conjugated to Abcam anti-Ago2 antibody (Pudong, Shanghai, China) or isotype-matched control antibody provided in the RIP assay kit, was utilized to incubated with the cell lysates. The antibody binding RNAs were then isolated according to the protocols provided in the RIP assay kit. Subsequently, qRT-PCR analyses were conducted to measure the levels of LINC00473 and miR-497 as described above.

Luciferase Detection

LINC00473 wild-type (LINC00473 wt) and LINC00473 mutant (LINC00473 mut) reporter plasmids were constructed by ShuangLing Biotechnology Co., Ltd. (Nanjing, Jiangsu, China). The dual luciferase reporter assays were conducted using a Promega Dual-Glo Luciferase assay kit (TaGene, Xiamen, Fujian, China). Briefly, the cells were first plated into 48-well plates and cultured to 70% confluent. Then, LINC00473 wt or LINC00473 mut reporter plasmids were separately co-transfected with miR-497 or NC mimics as described above. Subsequently, luciferase activities were determined by using Promega luciferase analyses kits (TianPuBio, Xi'an, Shanxi, China) on a microplate reader system (318C; PeiOu, Jinshan, Shanghai, China).

Statistical Analysis

Data analyses in this study were conducted using SPSS 20.0 statistics software (SPSS Inc., Armonk, NY, USA). The comparisons of measurement data between two groups were performed using the *t*-test. Analyses of variance and LSD test were applied for comparisons among multiple groups. Associations between LINC00473 expression level in BC and clinicopathological features were assessed using the χ^2-test. Kaplan-Meier methods (with log-rank test) were carried out to analyze the survival curves. Cox regression analyses were performed to identify the factor with remarkable influence on survivals. Statistically significant was set as p-value < 0.05.

Results

LINC00473 was Upregulated in BC

In order to determine the potential function of LINC00473 BC, the mRNA levels of LINC00473 were evaluated using 122-paired human BC samples and adjacent normal breast specimens using RT-qPCR. As shown in Figure 1A, compared with their corresponding non-tumorous counterparts, LINC00473 expression was significantly upregulated in BC tissues ($p < 0.01$). Furthermore, higher levels of LINC00473 were observed in BC tissues with advanced stages (Figure 1B). In addition to BC tissues, endogenous expression of LINC00473 was detected in a panel of BC cell lines (MDA-MB-468, MDA-MB-453, MCF-7, MDA-MB-468, MDAMB-231) and control cells (MCF-10A). As shown in Figure 1C, it was found that the levels of LINC00473 in BC cells were distinctly increased compared to MCF-10A (Figure 1D). Due to higher levels of LINC00473 in MDA-MB-468 and MCF-7, we chose them for subsequent cells experiments. Overall, our results firstly reported up-regulation of LINC00473 in BC patients.

LINC00473 High-Expression Predicts Poor Clinical Outcome in BC Patients

Then, to further understand the clinical significance of LINC00473 in BC patients, we divided the 122 BC patients into a high-expressing

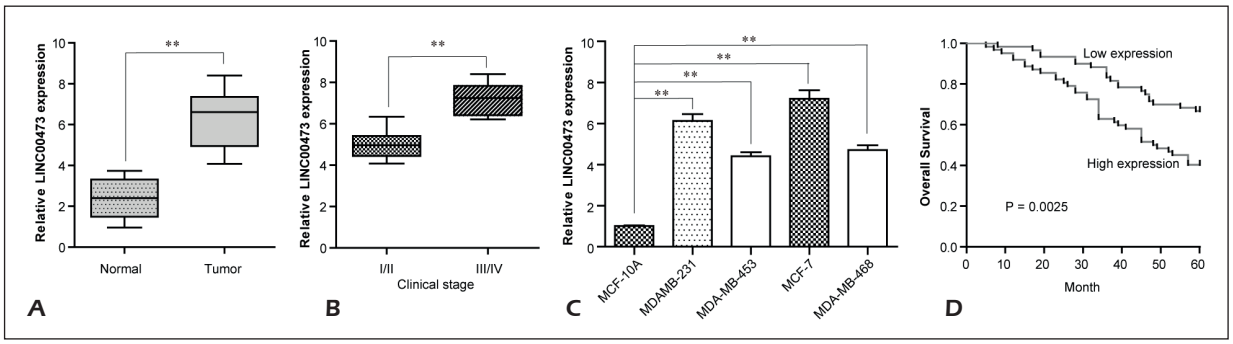

Figure 1. LINC00473 is reduced in BC, which is correlated with poor prognosis. **A**, Real-time PCR analyses of LINC00473 levels in 50 paired primary BC tissues and their adjacent samples. **B**, The expressing levels of LINC00473 were positively associated with advanced clinical stages in BC patients. **C**, The levels of LINC00473 in four BC and control (MCF-10A) cells were detected by qRT-PCR. **D**, Kaplan Meier method analysis predicted the correlation between LINC00473 expression and overall survival rate of BC patients. *p<0.05, **p<0.01.

group (n=60) and a low-expressing group (n=62) according to the median value of LINC00473 expressing levels in BC samples. As presented in Table I, LINC00473 levels were obvious relevance with lymph node metastasis (p = 0.030) and clinical stage (p = 0.014). However, there were no significant correlations of LINC00473 expression with other clinical features such as gender, age, and differentiation grade (all p > 0.05). Moreover, the survival curves were also evaluated to investigate whether LINC00473 expression was relevant to survival. As shown in Figure 1D, we found that the overall survival rates with high LINC00473 levels were significantly lower than that with low LINC00473 levels (p =0. 0025). On the other hand, we also observed that LINC00473 overexpression was an unfavorable prognostic factor in BC patients (RR=3.774, 95% CI: 1.452-5.443, p = 0.004, Table III), regardless of lymph node metastasis and clinical stage. Further analysis in a multivariate assays model demonstrated LINC00473 expression was an independent prognostic indicator for BC patients (RR=3.215, 95% CI: 1.255-4.752, p = 0.0013, Table III). These findings revealed that LINC00473 has potential diagnostic value in predicting the prognosis of BC.

Depressing Expression of LINC00473 Suppressed the BC Cell Proliferation and Promoted Cell Apoptosis

To explore the functional relevance of LINC00473 in BC cells, we first synthesized three siRNAs targeting LINC00473 (si-LINC00473-1, si-LINC00473-2, si-LINC00473-3) and applied qRT-PCR assays to determine the knockdown efficiency of these siRNAs. As the data presented in Figure 2A, the LINC00473 siRNAs, particularly si-LINC00473-1, and si-LINC00473-2, could effectively repress the expressing levels of LINC00473 in BC cells. Hence, we selected si-LINC00473-1 and si-LINC00473-2 to conduct the following studies. After transfecting LINC00473 siRNAs into the BC cells, we next performed CCK-8 assays to determine the changes of cell proliferation, and the results revealed that knockdown of LINC00473 contributed to notably decreased proliferation of BC cells (Figure 2B).

Table III. Univariate and multivariate analysis of overall survival in BC patients.

Variable	Univariate analysis			Multivariate analysis		
	RR	95% CI	p	RR	95% CI	p
Lymph node metastasis Absent vs. Present	3.662	1.346-4.887	0.014	3.237	1.231-4.336	0.031
Clinical stage I-II vs. III	3.427	1.482-4.338	0.021	3.215	1.266-4.1245	0.033
LINC00473 expression High vs. Low	3.774	1.452-5.443	0.004	3.215	1.255-4.752	0.013

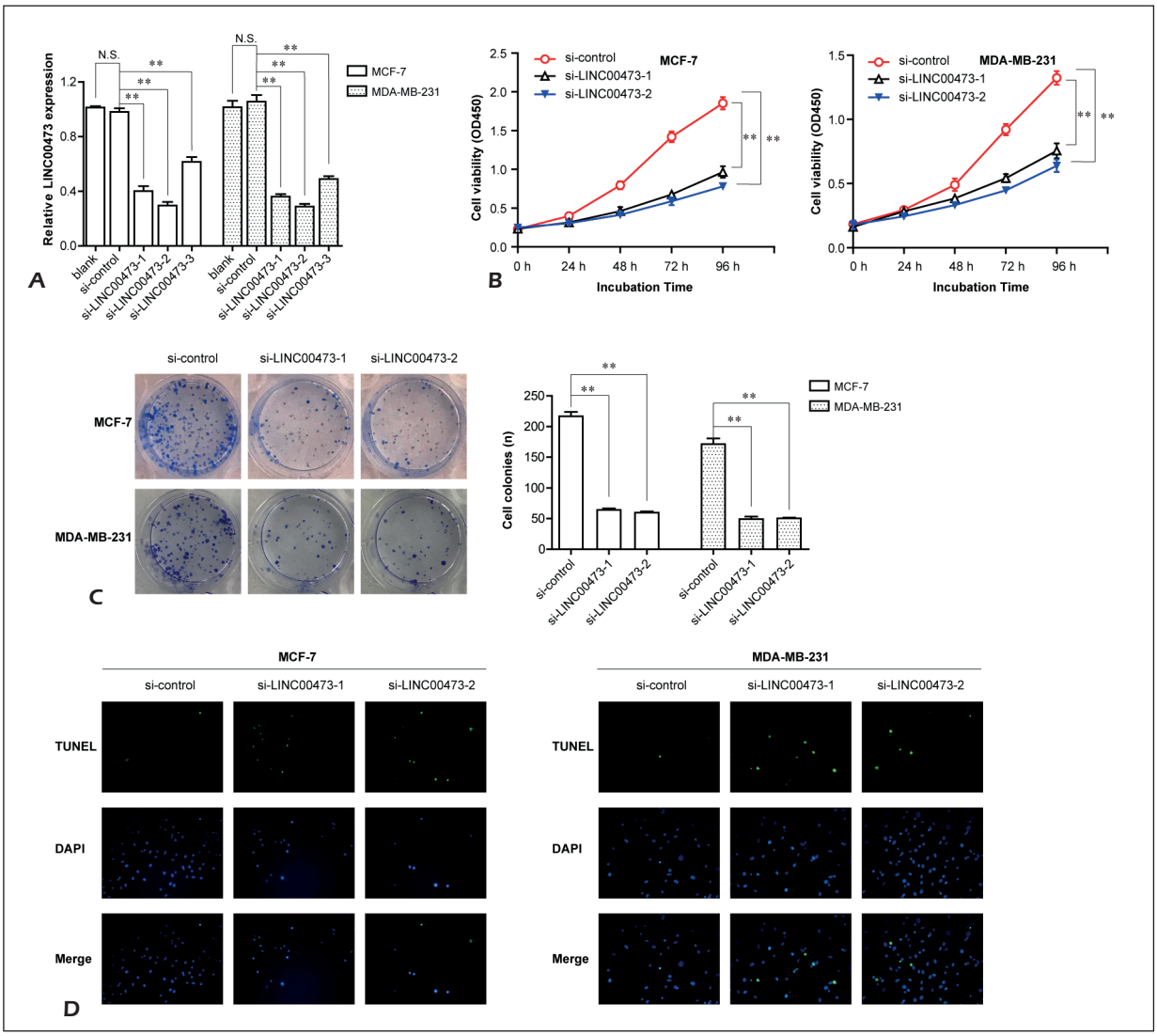

Figure 2. The impact of LINC00473 on the proliferation and apoptosis of BC cells. **A**, The qRT-PCR analyses determined the levels of LINC00473 in BC cells after transfection with LINC00473 siRNAs or negative control siRNAs (si-control). B, The cell viability was assessed by CCK-8 assays. **C**, Transfection of LINC00473 siRNAs reduced the cell colony number of BC cells. **D**, TUNEL assays determined the cell apoptosis of BC cells. The apoptotic cells were labeled with green fluorescence. The cell nuclei were stained using DAPI (blue). *p<0.05, **p<0.01.

Additionally, the colony formation assays demonstrated that silence of LINC00473 significantly reduced the number of cell colonies (Figure 2C). Furthermore, we next carried out TUNEL assays to assess the apoptosis of BC cells after transfecting with LINC00473 siRNAs. The data confirmed that the apoptotic cells were remarkably increased when the cells were transfected with LINC00473 siRNAs, which suggested that LINC00473 depletion was able to accelerate the cell apoptosis. Overall, our data indicated that depression of LINC00473 in BC cells suppressed cell proliferation and induced apoptosis of BC cells.

Silencing LINC00473 Expression Impeded the Mobility of BC Cells

We investigated whether LINC00473 deficiency was capable to affect the metastatic potentials of BC cells. To this purpose, we first performed wound-healing assays to examine the changes of migration capacity in BC cells after their LINC00473 was knocked down. The results validated that depression of LINC00473 caused significantly inhibitory effects on cellular migration ability (Figure 3A and B). Subsequently, the transwell assays were employed to evaluate the alternation of the invasion in BC cells when they were

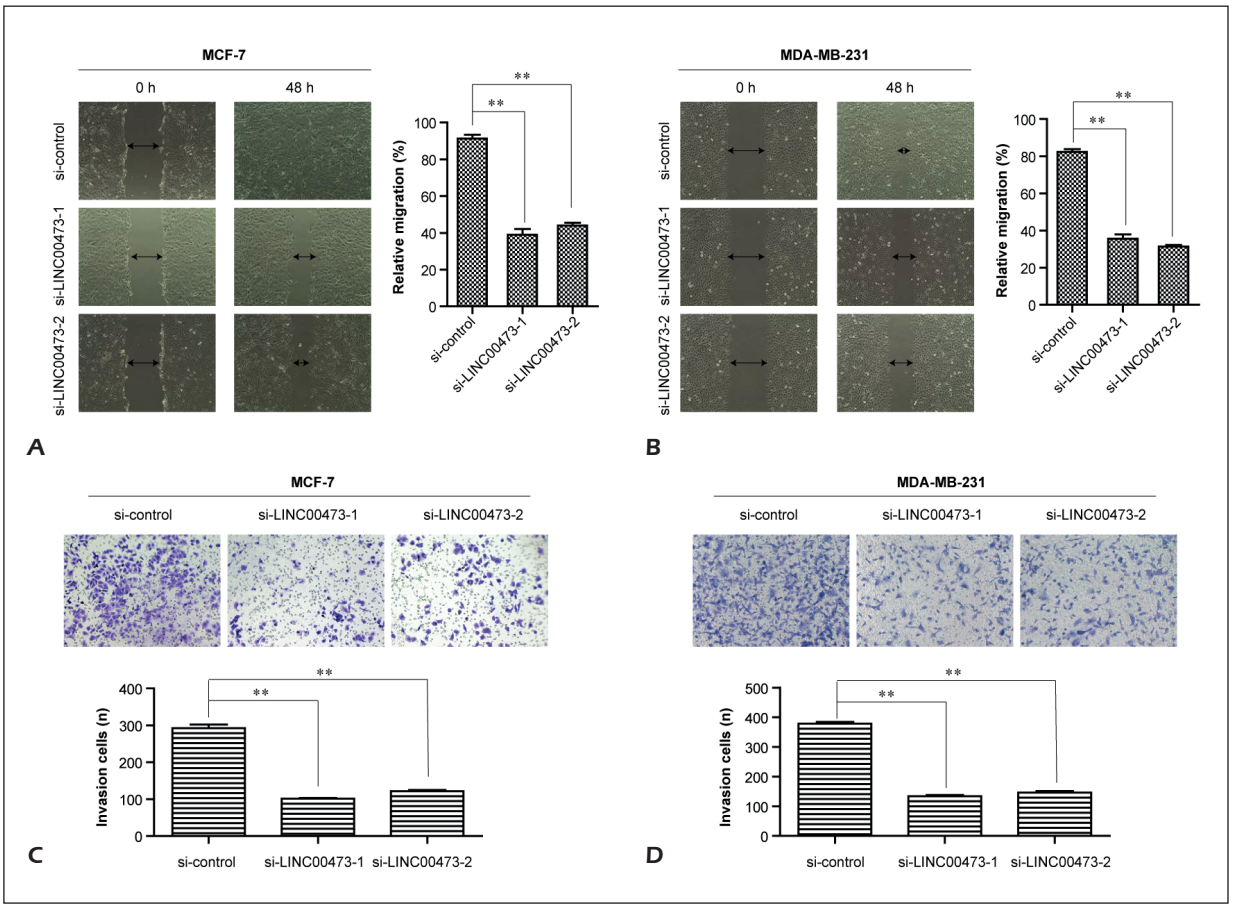

Figure 3. The influence of LINC00473 on the migratory and invasive capacities of BC cells. **A-B**, Wound healing analyses detected the migration of LINC00473 siRNAs or si-control-transfected BC cells. **C-D**, Transwell assay was conducted using BC cells treated with LINC00473 siRNAs or si-control, respectively. $^{**}p<0.05$, $^{**}p<0.01$.

treated with LINC00473 siRNAs. According to the data, the invaded cell number of LINC00473 siRNAs-transfected cells was markedly lower than that of the controls, which indicated that silence of LINC00473 resulted in remarkable decrease of invasive capacity of BC cells (Figure 3C and D). Collectively, these data showed that LINC00473 served as a critical player in regulating the metastasis of BC.

LINC00473 Acted as a ceRNA Sponge of miR-497 in BC Cells

In the next study, we aimed to uncover the detail molecular mechanisms by which LINC00473 exerted its oncogenic roles in BC. Since lncRNAs were known to modulate cellular phenotypes via "sponging" other non-coding RNAs, particularly miRNAs, we searched an online bioinformatics tool: starBase, to predict the target miRNA of LINC00473. As the data shown in Figure 4A, miR-497, which was found to be a tumor suppresser in various cancer types, was predicted as a potential target of LINC00473. Indeed, we performed qRT-PCR assays to examine the expressing levels of miR-497 in BC tissue samples and found that miR-497 was down-regulation in cancerous tissues (Figure 4B). Therefore, we next aimed to verify whether LINC00473 directly interacted with miR-497. To achieve that, dual luciferase reporter analyses were performed, and the data revealed that co-transfection of the LINC00473 wild-type (LINC00473 wt) plasmids and miR-497 mimics resulted in remarkable decrease of luciferase activity in BC cells. Whereas, the luciferase activity was not changed in cells co-transfected with the LINC00473 mutant (LINC00473 mut) plasmids and miR-497 mimics or control mimics (Figure 4C). Furthermore, the results of RIP assays indicated that LINC00473 and miR-497 were markedly enriched in Ago2-containing beads when compared with the input groups (Figure 4D). Moreover, we over-

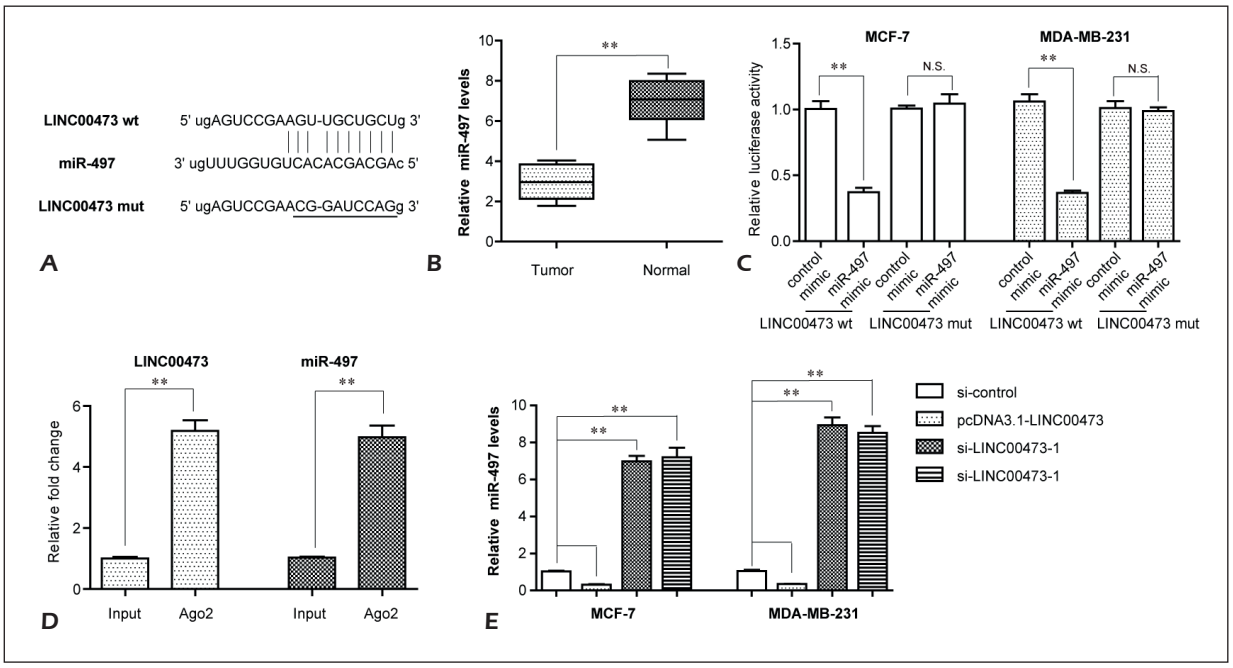

Figure 4. MiR-497 was directly interacted by LINC00473 in BC cells. **A**, Bioinformatics tool "starBase" predicted the binding site. **B**, The expressing levels of miR-497 in BC and paired normal specimens were examined by real-time PCR assays. **C**, The luciferase activities of BC cells were determined by dual-luciferase reporter assays. **D**, RIP assays evaluated the enrichment of LINC00473 and miR-497. **E**, Relative levels of miR-497 in BC cells after transfection with si-control, pcDNA3.1-LINC00473 plasmids, si-LINC00473-1 or si-LINC00473-2. *p<0.05, **p<0.01.

expressed LINC00473 or reduced LINC00473 expression in BC cells and subsequently applied qRT-PCR analysis to measure the expression of miR-497. As the data shown in Figure 4E, overexpressing LINC00473 significantly reduced the miR-497 levels, while knockdown of LINC00473 notably increased the expression of miR-497. Taken together, these results demonstrated that LINC00473 directly interacted with miR-497.

Discussion

Dubey et al[28] published in 2015 reported that approximately 439,100 new cancers cases and 273,4100 cancer deaths occurred in China. Although current therapeutic tools have improved BC outcome to a certain extent, the five-year survival rate remains relatively low[29]. Clinical studies revealed that cancer biomarkers, which have relative high sensitivity and specificity in the identification of tumors likely to metastasize, are key to improve BC patient survivals[30,31]. Despite great advances and enthusiastic progress toward identifying novel biomarkers for early diagnosis, clinical outcome, and clinical management, identification of biomarkers for BC remains an uncommon challenge. Recently, increasing attention was given to the effects of lncRNAs on progression of tumors[32,33]. In this research, we focused on a new lncRNA named LINC00473.

First, we detected the expression of LINC00473 in BC tissues and cell lines, finding that LINC00473 was overexpressed in BC, especially in BC tissues with advanced stages. Then, we observed high expression of LINC00473 was associated with lymph node metastasis and clinical stage, suggesting that LINC00473 may act as a clinical regulator in this disease. Moreover, clinical survival assays suggested that higher LINC00473 expression predicted shorter survival relative to the low-expression group. More importantly, in a multivariate Cox model, we found that LINC00473 expression was an independent prognostic indicator for five-year overall survival in BC. Based on our findings, we provided important evidence that dysregulation of LINC00473 was involved in the clinical progress and the detection of the levels of LINC00473 may have potential useful values in diagnosis and prognosis of BC patients. However, the number of patients in this study was relatively small, whether similar results can be also confirmed on a great number of patients remains unclear. Thus, further investigations with more patients are needed to solve this problem.

LINC00473 as an oncogene has been reported to be overexpressed in several tumors and predict poor prognosis in patient. For instance, Shi et al[26] suggested that LINC00473 was highly expressed in cervical cancer tissues and was correlated with poor clinical prognosis of tumor patients. In addition, overexpression of LINC00473 was found to promote the tumor cells growth via suppressing ILF2 degradation in this tumor. Chen et al[34] showed LINC00473 was overexpressed in human hepatocellular carcinoma tissues, which was associated with vascular invasion and unfavorable prognosis. Functional assays revealed that up-regulation of LINC00473 promoted cells proliferation and invasion and induced EMT process in hepatocellular carcinoma. In addition, the up-regulation of LINC00473 and its tumor-promotive roles were also reported in several other tumors[35-37]. However, to our best knowledge, the effects of LINC00473 in BC remain unclear. In this work, the biological function of LINC00473 in BC cell lines was investigated using siRNA to knockdown LINC00473. Further functional assays suggested that silencing LINC00473 resulted in the suppression of the proliferation, migration, and invasion. Consistent with previous results, we also provided evidences that LINC00473 might exhibit oncogenic activity in BC.

MiRNAs as important regulators in tumor progression have been well-studied in various studies[38]. Recently, the crosstalk of RNA transcripts with each other by shared miRNA response elements was found in several new biological mechanisms, including the competing endogenous RNA (ceRNA) hypothesis[39,40]. Growing evidences indicated that lncRNAs could harbor miRNAs to display its biological function in tumor progression via modulating the enrichment of miRNAs. This is the well-known miRNA 'sponge'. For example, lncRNA-UCA1 was reported to contribute to biological progress of hepatocellular carcinoma via suppression of miR-216b[41]. LncRNA SNHG15 was highly expressed in BC and promote human BC proliferation and metastasis by sponging miR-211-3p[42]. Bioinformatics software was used to predict the possible miRNAs of LINC00473, and miR-497 was found to contain putative binding regions in LINC00473 sequences. Previous researches have reported that miR-497 levels were up-regulated in various tumors and acted as tumor promoter[43,44]. Also, this miRNA was found to suppress cell growth and invasion by targeting cyclin E1 in BC[45]. Here, luciferase reporter and qRT-PCR experiments were conducted and the observation concluded that LINC00473 served as miR-497 sponge to modulate its levels. In addition, forced LINC00473 expression inhibited the levels of miR-497, while its knockdown promoted the levels of miR-497. Taken together, these data are consistent with our hypothesis and revealed LINC00473 influenced the biological characteristic of BC cells by suppression of miR-497.

Conclusions

We showed that LINC00473 functioned as a functional oncogene in BC, and the overexpression of LINC00473 was associated with advanced stages and clinical prognosis of BC patients. LINC00473 contributed to the progression of BC by working as a ceRNA to sponge miR-497. Our results revealed that a strategy for targeting LINC00473 as a potential biomarker and a therapeutic target in BC patients.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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