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Increased long noncoding RNA LINP1 expression and its prognostic significance in human breast cancer

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Abstract. – OBJECTIVE: Mounting evidence suggests that the long noncoding RNAs (lncRNAs) function in multiple cancers. Dysregulation of lncRNA in non-homologous end joining (NHEJ) pathway 1 (LINP1) has been reported in breast cancer. However, its clinical significance in breast cancer remains unclear. The aim of this study was to investigate the prognostic relevance of LINP1 in breast cancer.

PATIENTS AND METHODS: Expression of LINP1 in tumor and their matched non-tumor tissues was determined by quantitative Real Time-PCR (qRT-PCR) in breast cancer patients. The relevance of LINP1 expression to the clinicopathological factors was assessed. Kaplan-Meier analysis was performed to evaluate the overall survival and disease-free survival of breast cancer patients with the expression level of LINP1. Cox proportional hazard regression analysis was performed at both univariate and multivariate levels.

RESULTS: The results showed that LINP1 appeared to have higher expression in breast cancer tissues than in adjacent non-tumor tissues ($p < 0.01$). Increased expression of LINP1 was correlated with advanced TNM stage ($p = 0.002$), more lymph node metastasis ($p = 0.000$), and poorer pathological differentiation ($p = 0.004$). Furthermore, clinical assay indicated that patients with high LINP1 expression had a shorter overall survival and disease-free survival compared with the low LINP1 expression group. Finally, Cox regression analysis showed that LINP1 expression was an independent prognosis-predicting factor for breast cancer patients.

CONCLUSIONS: LncRNA-LINP1 may be involved in the progression of the breast cancer and be a novel indicator of poor prognosis in patients with breast cancer.

Key Words:
Long non-coding RNA, LINP1, Prognosis, Breast cancer.

Introduction

Breast cancer is the most frequently diagnosed tumor type and the primary leading cause of cancer deaths in women worldwide[1]. In 2013, 1.8 million incident cases of breast cancer occurred, and the disease caused 464,000 deaths[2]. Despite great progress in therapeutic strategies, including surgery, adjuvant chemotherapy, radiotherapy, and biological therapy, the five-year survival rate for breast cancer patients with distant metastases is still less than 25%[3,4]. Up to date, although various progressions of breast cancer nosogenesis have been achieved, the potential mechanism underlying metastasis of breast cancer remains largely unclear; thus, the development of drug targeting breast cancer is very difficult in clinical progression[5]. On the other hand, in order to improve breast cancer prognosis, identification of suitable biomarkers may help to guide the clinical treatment of individual breast cancer patient.

Recent progressions have revealed a class of long noncoding RNA (lncRNA) with critical roles in various pathophysiological processes[6]. Long non-coding RNAs (lncRNAs) are a class of noncoding RNAs > 200 nucleotides, with limited protein-coding potential[7]. The rapid development of human genomics has highlighted the important role of lncRNAs in diverse biological processes, such as transcriptional regulation, cell growth, and tumorigenesis[8,9]. LncRNAs carry out their functions in a wide range of processes and can regulate gene expression by various mechanisms[10]. Recent evidence has indicated that many lncRNAs play important regulatory roles in the progression of diverse cancers, such as lncRNA ZFAS1[11], lncRNA XIST[12], lncRNA AFAP1-AS1[13], and lncRNA NEAT1[14]. Of note, dysregulation of

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lncRNAs was reported to be associated with overall survival of breast cancer patients, which highlighted the importance of lncRNAs as potential biomarkers for predicting the prognosis of breast cancer patients[15,16].

LncRNA LINP1 (LINP1), located in located in chromosome 10, has been reported to be up-regulated in breast cancer tissues and involved in breast cancer progression[17,18]. The carcinogenic effect of LINP1 has been reported in previous studies. However, its clinical significance in breast cancer patients was rarely reported. In this study, we aimed to identify the clinical significance of LINP1 in 183 clinical breast cancer samples.

Patients and Methods

Patients and Specimens

A total of 183 paired breast cancer tissue and matched normal tissue samples were obtained from patients that underwent surgical resections at the First People's Hospital of Jining between 2011 and 2014. All samples were confirmed as breast cancer by postoperative histopathological examination. All 183 breast cancer patients were followed-up. The median follow-up was 26 months (range 6-60 months). The clinicopathological characteristics of breast cancer patients were summarized in Table I. Samples were frozen in liquid nitrogen immediately after surgical removal and stored at -80°C prior to RNA isolation and qRT-PCR analysis. This study was approved by the Ethics Committee of the First People's Hospital of Jining. The informed consent was obtained from all patients for sample analysis.

RNA Isolation and Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA from each tissue specimen was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The cDNA was synthesized from 2 ng of total RNAs with a Reverse Transcription Toolkit (Promega Corp., Madison, WI, USA). BANCR expression levels were measured with quantitative Real Time-PCR (qRT-PCR) using an ABI7500 system and the SYBR Green PCR Master Mix (TaKaRa, Otsu, Shiga, Japan). The reaction conditions were 95°C for 5 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec. Glyceraldehyde-3-phosphate dehydrogenase

Table I. Correlation between lncRNA LINP1 expression and clinicopathological characteristics of breast cancer.

Clinicopathological Factors	Cases No.	LINP1 expression		*p*-value
		High	Low	
Age				
< 50	91	41	50	NS
≥ 50	92	49	43	
Tumor size				
< 2.5	110	48	62	NS
≥ 2.5	73	42	31	
ER				
Positive	93	51	42	NS
Negative	90	39	51	
PR				
Positive	105	55	50	NS
Negative	78	35	43	
HER2 status				
Over expressed	112	61	51	NS
Negative	71	29	42	
Lymph node metastasis				
Yes	63	41	22	0.002
No	120	49	71	
TNM stage				
I/II	114	44	70	0.000
III	69	46	23	
Pathological differentiation				
Moderately and highly	124	52	72	0.004
Poorly	59	38	21	

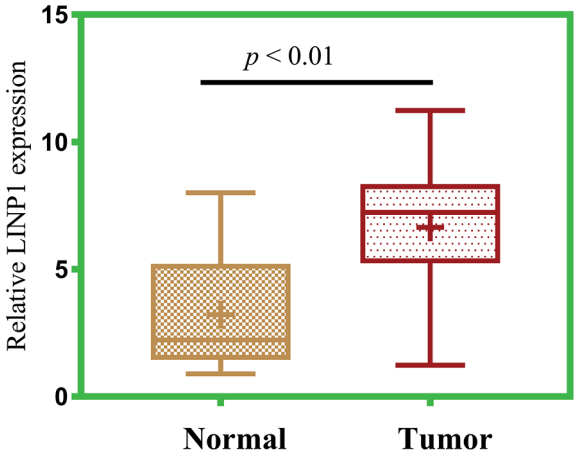

Figure 1. LINP1 expression levels in collected tissues specimens. LINP1 expression levels were significantly higher in breast cancer tissues than that in the non-cancerous tissues ($p < 0.01$).

(GAPDH) was used as an internal control. The primer sequences were shown as follows: LINP1, sense, 5'-TGCCACTGCCATTAGAAGAAC-3', antisense, 5'-GCTCACAGAGGAGCTACCCA-3'; GAPDH, sense, 5'-GCACCACCAACTGCTTAGCA-3', antisense, 5'-GTCTTCTGGGTGGCAGTGATG-3'. The average value in each triplicate was used to calculate the relative amount of LINP1 using $2^{-\Delta\Delta Ct}$ methods. Experiments were repeated at least three times.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 19.0, IBM, Chicago, IL, USA). Data are presented as the mean ± standard deviation. The significance of the differences was evaluated by the Student's *t*-test. Associations between clinicopathological parameters and plasma LINP1 expression were evaluated using the Chi-square test. The overall survival and disease-free survival were estimated using the Kaplan-Meier method. On the basis of the Cox proportional hazards model, univariate and multivariable survival analyses were conducted. A $p < 0.05$ were considered statistically significant.

Results

The Expression Level of LINP1 in Breast Cancer

To determine the expressed levels of LINP1 in breast cancer samples, total RNAs were extracted from breast cancer tissues and matched normal breast cancer tissues, and the expressed levels of LINP1 were analyzed by RT-qPCR. As shown in Figure 1, the expression levels of LINP1 were significantly up-regulated in breast cancer tissues compared with matched normal breast tissues ($p < 0.01$), indicating that dysregulation of LINP1 could be involved in the progression of breast cancer.

Correlation of LINP1 Expression with Clinicopathological Features of Breast Cancer

For the clinicopathological correlation analysis, using the median LINP1 expression in all 183 osteosarcoma patients as a cutoff, the patients were divided into a high LINP1 expression group and a low LINP1 expression group. As shown in Table I, we found that high LINP1 expression was closely associated with lymph node metastasis ($p = 0.002$), TNM stage ($p = 0.000$) and pathological differentiation ($p = 0.004$). However, there were no significant correlations between LINP1 expression and other clinicopathologic features including patient's age, tumor size, ER, PR, and HER2 status ($p > 0.05$). Thus, our findings revealed that LINP1 may act as an important regulator in the clinical progression of breast cancer patients.

The Correlation Between LINP1 Level and Survival of Breast Cancer Patients

To further study the association between LINP1 expression and clinical outcomes, we determined the prognostic value of the LINP1 expression on overall survival and disease-free survival in breast cancer patients. Kaplan-Meier survival analysis and log-rank tests using patient post-operative

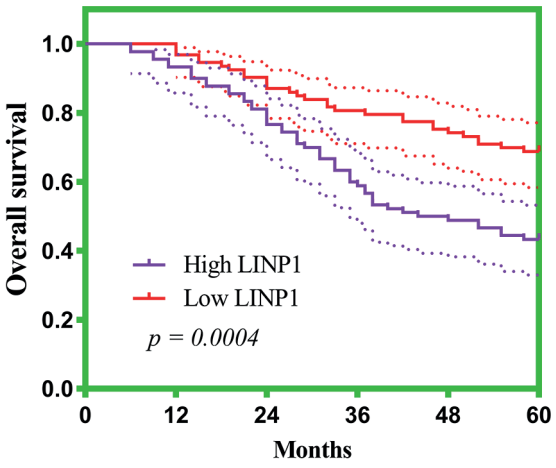

Figure 2. LINP1 as a prognostic factor in breast cancer patients. Breast cancer patients with higher LINP1 expression had poorer overall survival probability ($p = 0.0004$).

survival were performed. As shown in Figure 2A, patients with high LINP1 expression lived shorter than those with low LINP1 expression (p = 0.0004). Similarly, patients with higher LINP1 expression level are associated with poorer disease-free survival (p < 0.0001). Furthermore, univariate Cox proportional hazards regression analysis indicated that LINP1 expression was associated with both overall survival and disease-free survival (Table II). More importantly, in multivariate Cox model, our results revealed that high expression level of LINP1 was a significant independent prognostic factor for overall survival (HR, 3.054; 95% CI, 1.327-5.543; p = 0.0014) and disease-free survival (HR, 3.123; 95% CI, 1.346-6.458; p = 0.006, Table II).

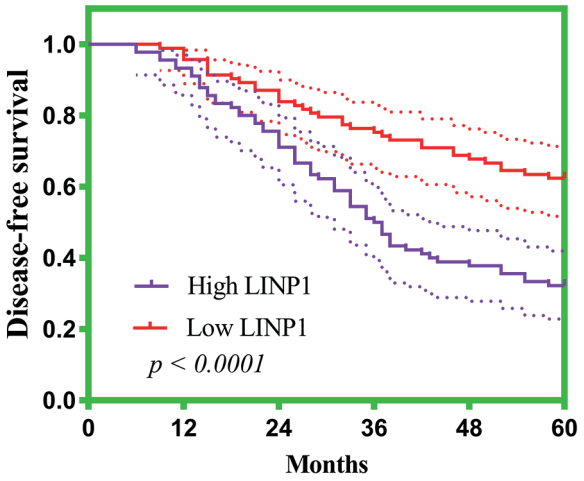

Figure 3. LINP1 as a prognostic factor in breast cancer patients. Breast cancer patients with higher LINP1 expression had poorer disease-free survival probability (p < 0.0001).

Discussion

Breast cancer is one of the major challenges for women health. Although the incidence of the disease is increasing, the number of death verge to decrease, which may be due to the treatment strategies for cancer have changed markedly in clinical therapy[19]. However, the prognosis of the patients with metastasis and diagnosed advanced stages remains poor. Up to date, the choice of therapy in the metastatic setting remains limited[20]. Therefore, clinical indicators that accurately predict breast cancer progression and prognosis are essential for improving patient survival.

LncRNAs were previously reported to be dysregulated in multiple types of breast cancer and had been proposed to be a promising indicator for prognosis of breast cancer[21]. For instance, Cai et al[22] reported that lncRNA CCAT2 was highly expressed in both breast cancer tissues and cell lines, and its suppressing decreases cell proliferation and invasion *in vitro* by regulating the Wnt signaling pathway. Huan et al[23] found that lncRNA CRNDE expression was significantly up-regulated in breast cancer and associated with

Table II. Univariate and multivariate Cox regression analyses of overall survival and disease-free survival in breast cancer patients.

	Overall survival			Disease-free survival		
Variable	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Univariate analyses						
Age	1.243	0.457-2.231	0.464	1.126	0.833-2.316	0.167
Tumor size	1.678	0.734-3.122	0.168	1.326	0.548-2.784	0.114
ER	1.346	0.847-2.446	0.157	1.255	0.785-2.784	0.317
PR	1.548	0.893-2.641	0.136	1.232	0.744-2.317	0.169
HER2 status	1.564	0.755-2.438	0.117	1.215	0.954-2.134	0.089
Lymph node metastasis	**2.967**	**1.655-5.238**	**0.011**	**2.568**	**1.785-6.428**	**0.007**
TNM stage	**2.857**	**1.215-5.219**	**0.014**	**2.326**	**1.117-6.644**	**0.017**
Pathological differentiation	**2.458**	**1.234-4.562**	**0.017**	**2.149**	**1.044-4.128**	**0.026**
LINP1 expression	**3.341**	**1.557-6.637**	**0.008**	**3.451**	**1.664-7.213**	**0.004**
Multivariate analyses						
Lymph node metastasis	**2.678**	**1.347-4.653**	**0.015**	**2.755**	**1.377-5.237**	**0.009**
TNM stage	**2.563**	**1.456-4.861**	**0.017**	**2.548**	**1.384-4.784**	**0.101**
Pathological differentiation	**2.367**	**1.427-4.006**	**0.031**	**2.143**	**1.238-3.885**	**0.033**
LINP1 expression	**3.054**	**1.327-5.543**	**0.014**	**3.123**	**1.346-6.458**	**0.006**

advanced clinical stages and prognosis. Furthermore, they suggested that lncRNA CRNDE promoted the proliferation of breast cancer cells *in vitro* by acting as a molecular sponge of microRNA-136. Li et al[24] showed that linc-ITGB1 was down-regulated in breast cancer and associated with distant tumor metastasis; furthermore, they suggested that ITGB1 as an independent prognostic factor for breast cancer. LINP1, a novel lncRNA that was recently identified by Zhang et al[17], has been demonstrated to be overexpressed in human triple-negative breast cancer. Liang et al[18] further reported that overexpression of LINP1 was significantly associated with poor prognosis in breast cancer. *In vitro* assay indicated that LINP1 knockdown inhibited migration and invasion in breast cancer cells. However, the clinical significance of LINP1 was rarely reported.

In this study, we collected breast cancer samples and performed RT-PCT, finding that LINP1 expression was significantly up-regulated in breast cancer tissues compared with matched normal tissues. Moreover, it was found that the increased expression of LINP1 in breast cancer tissues was significantly correlated with aggressive clinicopathological features. Although the previous study had reported the prognostic value of LINP1 in breast cancer patients, a larger sample size and longer follow-up time needed to be further investigated. Our results of Kaplan-Meier method indicated that patients with a high level of LINP1 expression had significantly shorter overall survival and disease-free survival. These results were consistent with previous findings. Notably, we also performed multivariate analyses to explore whether LINP1 could a novel prognostic biomarker for patients with this disease. As expected, both the univariate and multivariate analyses showed that LINP1 was an independent prognostic marker for breast cancer.

Conclusions

Our investigation further confirmed that LINP1 expression was increased in breast cancer and associated with tumor progression. We also provided evidence that LINP1 expression may be associated with clinical prognosis of breast cancer patients. The present study also provides new insights into the role of LINP1 in the development of breast cancer, and it might be a potential prognostic biomarker for breast cancer prognosis.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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