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Expression of long non-coding RNA linc-ITGB1 in breast cancer and its influence on prognosis and survival

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Abstract. – OBJECTIVE: Long noncoding RNA linc-ITGB1 (linc-ITGB1) was reported to serve as a tumor promoter in breast cancer (BC). However, the clinical significance of linc-ITGB1 has not been reported. The present study aimed to determine the relationship between linc-ITGB1 expression and clinicopathological features and survival.

PATIENTS AND METHODS: qRT-PCR was used to quantify the expression levels of linc-ITGB1 in BC and adjacent non-cancerous breast tissues. The X2 test was performed to determine the associations between linc-ITGB expression and the clinicopathological characters. The overall survival time (OS) and disease-free survival (DFS) were collected by follow-up and analyzed by Kaplan-Meier analysis. Multivariate Cox regression analysis was used to identify the independent risk factors for BC.

RESULTS: The results showed that linc-ITGB1 levels were lower in tumor tissues of BC patients in comparison to adjacent non-cancerous breast tissues ($p < 0.001$). Linc-ITGB1 expression was significantly associated with lymph node metastasis, pathological differentiation and TNM stage (all $p < 0.05$). Furthermore, Kaplan-Meier analysis demonstrated that high-linc-ITGB1 expression level was associated with poorer OS ($p = 0.006$) and DFS ($p = 0.003$). Cox proportional hazards risk analysis demonstrated that linc-ITGB1 was an independent predictor for both OS ($p = 0.004$) and DFS ($p = 0.002$) in BC.

CONCLUSIONS: These results indicated, for the first time, that linc-ITGB1 be a potential biomarker in the prognosis of BC.

Key Words:
Linc-ITGB1, Breast cancer, Prognostic marker.

Introduction

Breast cancer (BC) is the most prominent health problem for women worldwide, with over 1,300,000 breast cancer cases and 450,000 breast cancer deaths annually worldwide[1,2]. Over the last years, although various treatments for breast cancer, such as chemotherapy, radiation and hormone therapy, have significantly reduced mortality of breast cancer patients, the prognosis of the patients with distal metastasis remains poor[3-5]. Despite the advancement in pathogenesis of BC, the complex molecular mechanisms in BC pathogenesis remain largely unclear. Thus, new biomarkers are needed to improve the detection and prognostic outcome of BC.

Long non-coding RNAs (lncRNAs) are defined as noncoding transcripts with over 200 nucleotides in length[6]. Increasing studies indicated that the disorders of lncRNA are closely related to human diseases, including various kinds of cancer[7,8]. For instance, lncRNA AB073614 has been identified as a tumor promoter in glioma by targeting affecting epithelial-mesenchymal transition[9]. LncRNA MALAT1 promotes the proliferation, invasion and migration of melanoma cells by sponging miR-22[10]. Deregulated expression of lncRNA NEAT1 was associated with poor prognosis and aggressive phenotype of colorectal cancer, and knockdown of lncRNA NEAT1 significantly inhibited growth and facilitated apoptosis via regulation of Akt signaling[11]. Notably, Yan et al[12] reported that linc-ITGB1 is upregulated in BC and promoted cell proliferation and metastasis in breast tumorigenesis. However, whether linc-ITGB1 was associated with the prognosis of BC patients

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remains unknown. In this study, we focused on linc-ITGB1 and analyzed the association of linc-ITGB1 with clinicopathological factors or BC patient prognosis.

Patients and Methods

Patients

This work included 224 patients with breast carcinoma who underwent surgical treatment at our hospital between September 2009 and December 2011. Fresh sample was snap frozen in liquid nitrogen immediately after resection and stored at -80°C for RNA and proteins extraction. The range of patients' age at the time of diagnosis was 29-81 years (median, 51 years). All diagnoses were confirmed by postoperative pathological examinations. Complete clinical data was available for each patient. Data from the patients treated without preoperative therapy were used for the analysis of metastasis and prognosis. This study was conducted according to the principles of the Helsinki Declaration. This study was approved by the Hospital's Ethics Committee, and informed consent was obtained from each patient.

RNA Extraction and qRT-PCR

Total RNA was extracted by Trizol reagent (Invitrogen, Carlsbad, CA, USA). followed by cDNA synthesis using the SuperScript first-strand synthesis system (Invitrogen, Carlsbad, CA, USA). Relative levels of linc-ITGB1 were examined using SYBR green Real-time quantitative reverse transcription PCR (qRT-PCR) and were normalized to levels of GAPDH mRNA. The comparative $2^{-\Delta\Delta Ct}$ method was used for relative quantification and statistical analysis. The sequences of the primers that were used were as follows: for linc-ITGB1, 5'-CCTCTCAGCCTCCAGCGTTG-3'(forward) and 5'-TGCTCTTGCTCACTCACACTCC-3' (reverse); for GAPDH, 5'-ATTCAACGGCACAGTCAAGG-3' and 5'-GCAGAAGGGGCGGAGATGA-3'.

Statistical Analysis

All statistical analyses were performed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The χ^2 test was used to analyze the relationship between linc-ITGB1 expression and clinicopathological characteristics. Disease free survival (DFS) and overall survival (OS) curves were performed by the Kaplan-Meier survival plot. Univariate analysis and multivariate analysis were performed by Cox proportional hazard regression model. All statistical values were considered significant at the p level of < 0.05. All of the experiments have been repeated at least three times.

Results

Upregulation of Linc-ITGB1 in BC Tissues

We first measured the expression of linc-ITGB1 by qRT-PCR in 224 BC tissue specimens and matched non-cancerous breast tissue specimens. As shown in Figure 1, we found that mean linc-ITGB1 level in BC tissues was significantly higher than that in normal breast tissues ($p < 0.01$).

Overexpression of linc-ITGB1 Associated with Aggressive Clinicopathologic Features of BC

Next, we analyzed the correlation between linc-ITGB1 expression level and clinicopathological features. Patients with BC were divided into high and low expression group by the mean expression level of linc-ITGB1. Table I presented the patients' clinicopathologic characteristics. The results showed that high linc-ITGB1 expression were significantly correlated with advanced TNM stage ($p = 0.013$), lymph node metastasis ($p = 0.002$), and high-grade pathological differentiation ($p = 0.008$). However, the level of linc-ITGB1 expression in BC tissues was not associated with the age, tumor size, LNM, ER, PR (all $p > 0.05$, Table I).

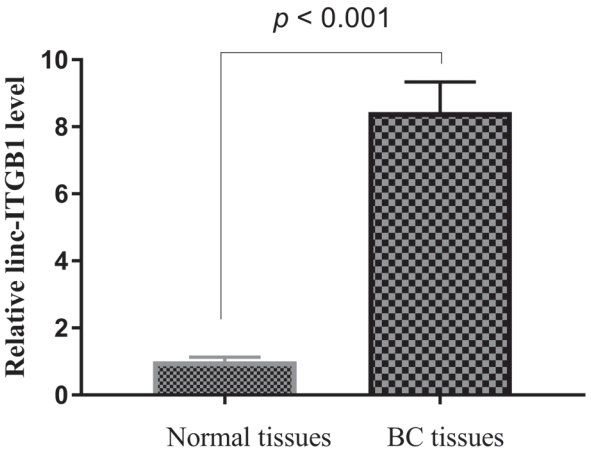

Figure 1. The expression level of linc-ITGB1 in 224 pairs of cancerous and matched normal tissue samples.

Table I. The correlation between linc-ITGB1 and clinicopathological parameters.

Parameter	Patients, n	Linc-ITGB1 expression		p value
		High, n (%)	Low, n (%)	
Age, years				0.375
< 50	96	46	50	
≥ 50	128	69	59	
Tumor size, cm				0.600
< 2	115	61	54	
≥ 2	99	54	55	
LNM				0.467
Negative	85	41	44	
Positive	139	74	65	
ER				0.342
Positive	108	59	49	
Negative	116	56	60	
PR				0.301
Positive	101	48	53	
Negative	123	67	56	
Lymph node metastasis				0.002
Yes	60	41	19	
No	164	74	90	
TNM stage				0.013
I/II	155	71	84	
III	69	44	25	
Pathological differentiation				0.008
Well + moderate	165	76	89	
Poor	59	39	20	

Survival Analysis and Prognostic Significance of Linc-ITGB1 Expression in BC

The association between linc-ITGB1 expression status and OS and DFS in BC patients was studied by log-rank test. We found that patients with higher linc-ITGB1 expression levels had dramatically shorter OS (Figure 2, $p = 0.0006$) and DFS (Figure 3, $p = 0.0003$) than were observed in those with lower levels. In multivariate DFS analysis, we observed that linc-ITGB1 expression (HR, 3.131; 95% CI, 1.893-6.136; $p = 0.002$), lymph node metastasis (HR, 4.324, 95% CI, 1.733-6.331; $p = 0.002$), TNM stage (HR, 3.235, 95% CI, 1.691-5.231; $p = 0.004$) and pathological differentiation (HR, 3.892, 95% CI, 2.139-6.631; $p = 0.006$) retained significance as a prognostic factor of a short OS (Table II). In multivariate OS analysis, we showed that linc-ITGB1 expression (HR, 2.891; 95% CI, 1.672-5.231; $p = 0.004$), lymph node metastasis (HR, 3.891, 95% CI, 1.477-5.721; $p = 0.005$), TNM stage (HR, 2.489, 95% CI, 1.399-4.891; $p = 0.007$) and pathological differentiation (HR, 3.271, 95% CI, 1.879-5.391; $p = 0.009$) were an independent predictor of poor prognosis.

Discussion

In 2013, BC accounted for 87,034 mortalities with a mortality rate of 13.42 mortalities per 100,000 individuals in China[13]. Despite of the great progress in diagnosis and multimodality treatment in the past decades, the prognosis of metastatic BC is still poor [14]. The pre-

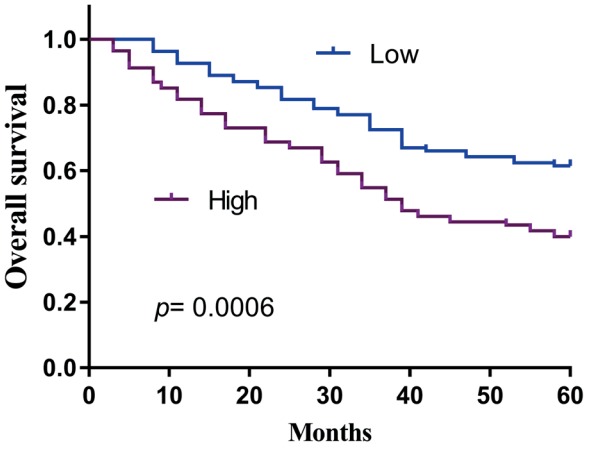

Figure 2. Correlation between linc-ITGB1 expression and OS time in BC patients.

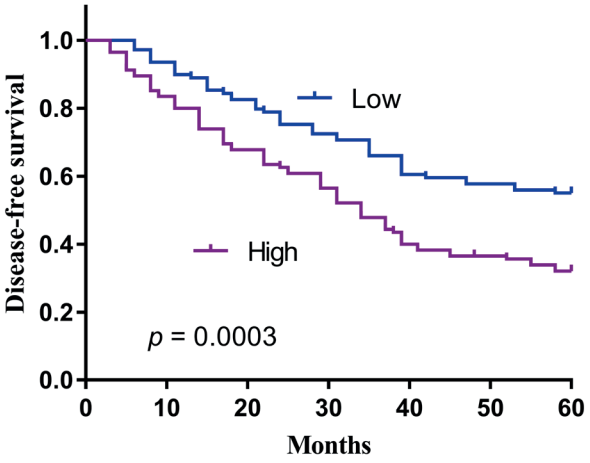

Figure 3. Correlation between linc-ITGB1 expression and DFS time in BC patients.

vention and therapy of breast cancer remain a major public health concern. More studies[15,16] have recognized that the appropriate therapies require effective biomarkers to guide. Recently, growing papers reported that changes in expression of lncRNAs can be used as robust and important biomarkers for cancer risk, diagnosis, and prognosis[17-19]. Based on previous findings, our attention focused on the linc-ITGB1.

Linc-ITGB1 is a newly discovered lncRNA. To our best knowledge, only two papers reported the specific role of linc-ITGB1 in tumors, including gallbladder cancer and BC. Wang et al[20] firstly reported that expression of linc-ITGB1 was upregulated in gallbladder cancer cells. Moreover, they confirmed that linc-ITGB1 was a promoter of gallbladder cancer metastasis due to its regulation of epithelial-to-mesenchymal transition (EMT). Yan et al[12] found that linc-ITGB1 was overexpressed in BC tissues and cell lines, and its over-expression significantly inhibits cell proliferation, migration and invasion, while its knockdown exert the contrary role. Furthermore, constant with previous study, they also identified that inc-ITGB1 served as a tumor promoter by affecting EMT. Those findings informed that linc-ITGB1 played an important role in progression and development of gallbladder cancer and BC. However, their research didn't study the clinical significance of linc-ITGB1 in patients.

In the present work, we firstly determine the expression levels of linc-ITGB1 in BC and matched normal tissues and observed significantly increased expression of linc-ITGB1 in the BC tissues than in the adjacent normal breast tissues. We further found the expression level of linc-ITGB1 was significantly associated with lymph node metastasis, pathological differentiation and TNM stage, suggesting linc-ITGB1 contributed to progression of this tumor. In addition, Kaplan-Meier analysis demonstrated that high linc-ITGB1 expression level was associated with poorer OS and DFS. Based on these data, we performed multivariate analysis and the results indicated that linc-ITGB1 was an independent prognostic factor for predicting OS and DFS of BC patients. On the basis of the previous reports, we suggested hypothesized that the contribution of high linc-ITGB1 expression to the poor prognosis of BC patients might be caused by its facilitative efficiency on BC cell proliferation and metastasis.

Conclusions

We revealed, for the first time, that linc-ITGB1 may represent a valuable independent prognostic indicator for BC. Further study to discover the molecular mechanism of linc-ITGB1 about tumor migration and invasion is currently in progress.

Table II. Multivariate analyses for DFS and OS by Cox regression model.

Variable	DFS			OS		
	Hazard ratio	95% CI	*p*-value	Hazard ratio	95% CI	*p*-value
Age	0.921	0.622-1.762	0.421	–	–	–
Tumor size	1.142	0.561-1.923	0.219	–	–	–
LNM	1.428	0.783-1.884	0.183	–	–	–
ER	1.732	0.672-2.135	0.211	–	–	–
PR	1.677	0.824-2.421	0.188	–	–	–
Lymph node metastasis	4.324	1.733-6.331	0.002	3.891	1.477-5.721	0.005
TNM stage	3.235	1.691-5.231	0.004	2.489	1.399-4.891	0.007
Pathological differentiation	3.892	2.139-6.631	0.006	3.271	1.879-5.391	0.009
Linc-ITGB1 expression	3.131	1.893-6.136	0.002	2.891	1.672-5.231	0.004

Conflict of Interest

The Authors declare that they have no conflict of interests.

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