Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

The expression of lncRNA-MALAT1 in breast cancer patients and its influences on prognosis

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Received December 28, 2019; **Accepted** May 17, 2020; **Published** June 5, 2020

Doi: http://dx.doi.org/10.14715/cmb/2020.66.3.11

Abstract: This study aimed to explore the expression of lncRNA-metastasis associated lung adenocarcinoma transcript 1 (lncRNA-MALAT1) in breast cancer (BC) patients and its influences on the prognosis of the patients. A total of 120 BC patients admitted to our hospital were enrolled as a BC group, of which 58 patients at I/II stage were treated with breast-conserving surgery as an operation group, and the other 62 patients at III/IV stage were treated with neoadjuvant chemotherapy combined with breast-conserving surgery as a combination group. Meantime, 100 healthy people in physical examination during the same period were enrolled as a normal group. The expression of serum lncRNA-MALAT1 in the subjects was determined, and the expression in BC patients and its influences on the patients were analyzed. LncRNA-MALAT1 was over-expressed in patients from the BC group, and the area-under-the-curve (AUC) of it for diagnosing BC was 0.911. After treatment, the expression of lncRNA-MALAT1 in the operation group and the combination group significantly decreased, and the expression of it in patients with good prognosis was greatly lower than that in patients with poor prognosis. The AUC of lncRNA-MALAT1 for predicting poor prognosis was 0.838, and TNM staging, pathological differentiation, tumor diameter, and lncRNA-MALAT1 were independent prognostic factors for poor prognosis of the patients. Furthermore, low expression of lncRNA-MALAT1 was associated with a relatively high 5-year overall survival (OS) of BC patients. The expression of lncRNA-MALAT1 was up-regulated in BC patients, while it was down-regulated in BC patients treated with breast-conserving surgery combined with neo-adjuvant chemotherapy, so lncRNA-MALAT1 can be used as a potential indicator for early diagnosis and prognosis prediction of BC patients.

Key words: LncRNA-MALAT1; Breast cancer; Breast-conserving surgery; Neoadjuvant chemotherapy; Prognosis.

Introduction

Breast cancer (BC) poses a huge potential threat to the health of women, whose morbidity and mortality are both on the rise (1). According to statistics, there were 252,710 new BC patients and 40,610 patients died of BC in the United States in 2017, and the lifetime prevalence rate of BC among women worldwide reaches 12.3% (2, 3). At present, BC screening is cumbersome, expensive and time-consuming, which demands biological diagnostic tools with both sensitivity and specificity (4). Early diagnosis of BC patients is conducive to timely treatment and intervention of them, which can improve the possibility of a good prognosis of patients and further prolong the survival time of them (5). Therefore, it is of great significance to search for biological indicators with high sensitivity and specificity for BC in improving the outcome of BC patients.

Long non-coding RNAs (LncRNAs) belong to the long-chain RNA family, with a regulatory role in chromatin tissue, transcription and post-transcription through interaction with DNA, RNA and protein molecular combinations. Their imbalance is closely related to the development and progress of cancers including BC (6, 7). For example, one study by Shi et al (8) reported that long non-coding RNA-activated by TGF-β (lncRNA-ATB) could accelerate the invasion and metastasis of BC by regulating zinc finger E-box binding homeobox 1 (ZEB1) and zinc finger protein 217 (ZNF-2170), and its high expression was related to the drug resistance of trastuzumab in BC patients, and one other study by Sas-Chen et al (9) pointed out that lncRNA-LIMT could dynamically regulate the migration and metastasis of BC cells by epidermal growth factor (EGF) response, and its low expression was related to the overall survival (OS) and relapse-free survival time (RFS) of BC patients. LncRNA metastasis-associated lung adenocarcinoma transcript 1 (lncRNA-MALAT1), as a member of the lncRNA family, has molecular functions such as alternative splicing and transcriptional regulation. Its abnormal high expression is positively related to the metastasis and progression of cancers such as BC, which indicates that the high expression of lncRNA-MALAT1 may be related to the poor prognosis of BC patients (10, 11). There are many studies on the regulatory mechanism of lncRNA-MALAT1 in BC.

LncRNA-MALAT1 can play the role of the endogenous regulator in BC through the MALAT1-miR-124-CDK4/E2F1 pathway and can regulate the progression of malignant triple-negative BC via the MALAT1-miR-129-5p pathway. Moreover, the high expression of lncRNA-MALAT1 is linked to the poor prognosis of triple-negative BC. The above conclusions indicate that lncRNA-MALAT1 can participate in the pathological

mechanism of BC through various molecular pathways (12, 13). Therefore, we selected lncRNA-MALAT1 to study its diagnostic and prognostic value in BC patients.

At present, there are few studies on the value of lncRNA-MALAT1 in serum-based diagnosis and prognosis of BC. Therefore, we determined the expression of serum lncRNA-MALAT1 in BC patients, and analyzed the correlation between the expression and BC, to provide a clinical reference for diagnosis and treatment of BC patients.

Materials and Methods

General materials

A total of 120 BC patients admitted to our hospital from January 2014 to March 2016 were enrolled as a BC group, of which 58 patients (30-69 years old) at I/II stage were treated with breast-conserving surgery as an operation group, and the other 62 patients at III/IV stage were treated with neoadjuvant chemotherapy combined with breast-conserving surgery as a combination group. Meantime, 100 healthy people between 31 and 67 years old in physical examination during the same period were enrolled as a normal group. All subjects and their family members signed informed consent forms after understanding this study. This study did not violate ethics and morality, and it was carried out under the permission of the Ethics Committee of our hospital.

Inclusion and exclusion criteria

The inclusion criteria of the patients were as follows: Patients meeting the diagnostic criteria of BC (14), patients meeting the clinical TNM staging criteria (15), patients without contraindications for the surgery and drugs, and those without surgery history within the latest half-year. The exclusion criteria of them were as follows: Patients who had taken drugs affecting the results of this study, patients comorbid with other malignant tumors or severe dysfunction in organs including heart, lung, and kidney, pregnant women, and patients with cognition impairment or communication disorder. The inclusion criteria applied to the BC group, and the control group consisted of healthy people in physical examination.

Treatment methods

Breast-conserving surgery

Tissues within the range of about 3 cm around the tumor was cut out along the mammary areola arc through an opening site selected according to the tumor. A titanium clip was inserted locally, and the tissues were cut out according to the mammary gland of the patient. The removal tissues were processed and detected. If the detection results were positive, the resection scope would be expanded. Then the tissues resected from a larger scope were detected, and if the detection results were still positive, the resection scope would be expanded again until the detected results were negative. Afterward, the lymph nodes of the patient were examined, and if the examination results were negative, the patient was given breast-conserving surgery. During the operation, attention should be paid to adjusting nipple shape, keeping normal arc, placing drainage tube, suturing and binding (16).

Neo-adjuvant chemotherapy combined with breast-conserving surgery

The patient was given 60 mg/m^2 epirubicin (Shanghai Caiyou Industrial Co., Ltd., China, K533014) through intravenous drip on the first day, and then given 150 mg/m^2 paclitaxel (Shanghai Caiyou Industrial Co., Ltd., China, 33069-62-4) through intravenous drip for continuous 3 hours on the second day. One cycle consisted of three weeks of the above intravenous drip, and the patient was treated with 2-4 courses of treatment according to the patient's conditions. Breast-conserving surgery was carried out to the patient after the above treatment (17).

Determination methods

Elbow venous blood (5 mL) was sampled from each subject at 8 a.m., placed in EDTA-K2 vacuum test tubes, and centrifuged at 1500 x g and 4 ℃ for10 min to take the supernatant. The supernatant was stored in new EP tubes at -70 ℃ for later analysis. The total RNA of the serum was extracted using a Trizol reagent (Shanghai Mingjin Biotechnology Co., Ltd., China, 5301100) according to the operating instructions, dissolved in 20 μL of diethylpyrocarbonate (DEPC) water, and then done with reverse transcription using a reverse transcription kit (Shanghai Kanglang Biotechnology Co., Ltd., China, KL266) in 15 μl of total reaction volume containing 1 μl of M-MLV, 1μl of Olig (d T), 0.5 μl of RNA enzyme inhibitor, 1 μl of NTPs, and RNAse free water to adjust the volume. The RNA was incubated at 38°C for 60 min. cDNA (1 μl) was taken and synthesized at 85°C for 5 s. The synthesized cDNA was used as a template for qRT-PCR amplification. The PCR reaction system was prepared with 25 μl of total reaction volume containing 2.5 μl of 10×PCR buffer, 1 μl of d NTPs, 1 μl of upstream and downstream primers, respectively, 0.25 μl of Taq DNA Polymerase, and dd H2O to adjust the volume. Reaction conditions were as follows: Pre-denaturation at 95 °C for 15 min, followed by 35 cycles of denaturation at 95 °C for 15 s and annealing at 58 °C for 30 min, and then followed by extension at 72 °C for 15 min. Three replicate wells were set for each sample, and the experiment was repeated three times. GAPDH was adopted as an internal reference of lncRNA-MALAT1. After the reaction, the amplification curve and dissociation curve of Real-Time PCR were drawn, and the relative quantity of target genes was calculated based on the resulting parameters. The relative quantity of target genes was calculated using $2^{-\Delta Ct}$.

Follow-up

The patients were followed up for 3 years since the end of the first treatment through telephone and outpatient medical records inquiry in March, June, September and December each year to understand their survival. OS refers to the time from confirmed diagnosis to death or the last follow-up day.

Statistical analysis

In this study, the data were analyzed statistically using SPSS22.0 (Beijing EASYBIO Technology Co., Ltd., China). Enumeration data were expressed as the number of cases / percentage (n / %). Inter-group comparison in terms of enumeration data was carried out using

the chi-square test. Data with theoretical frequency in the chi-square test less than 5 were analyzed using the continuity correction chi-square test. Measurement data were expressed as the mean ± standard error of the mean (mean ± SEM), and inter-group comparison in terms of them was carried out using the independent-samples T-test, and comparison within groups was carried out using the paired t-test. Receiver operating characteristic (ROC) curves were adopted to analyze the value of lncRNA-MALAT1 in diagnosing BC patients and predicting prognosis of them, and multivariate Cox regression analysis was conducted to analyze risk factors for poor prognosis of BC patients. P<0.05 suggested a dramatic significance.

Results

Baseline data

There was no significant difference between the two groups of patients in menopause, age, average age, hypertension history, diabetes mellitus history, drinking history, and smoking history (all P> 0.05) (Table 1).

The expression of lncRNA-MALAT1 in BC patients and its diagnostic value

The relative expression of lncRNA-MALAT1 in the BC group was significantly higher than that in the normal group ((1.16±0.35) vs. (0.63±0.23), P<0.05). We drew a ROC curve of lncRNA-MALAT1 for diagnosing BC, and it came out that the area under the curve (AUC) of lncRNA-MALAT1 for diagnosing BC was 0.911 (95% CI: 0.874-0.948) and the best cut-off value, sensitivity, and specificity of it were 0.88, 82.50%, and 87.00% respectively (Figure 1).

Expression of lncRNA-MALAT1 in BC patients before and after treatment

After treatment, the expression of lncRNA-MALAT1 in the operation group and the combination group significantly decreased (P< 0.05). See Figure 2.

The value of lncRNA-MALAT1 in predicting prognosis of treated BC patients

According to the survival of the patients within 3 years, the patients were classified as follows: Dead patients were regarded as patients with poor prognosis (n=20), and surviving patients were regarded as patients with good prognosis (n=100). It was found that the expression of lncRNA-MALAT1 in patients with poor prognosis was significantly higher than that in patients with good prognosis (P< 0.05). We drew a ROC curve of lncRNA-MALAT1 for predicting BC, and it came out that the AUC of lncRNA-MALAT1 for predicting poor prognosis of treated BC patients was 0.838 (95% CI: 0.766-0.909) and the best cut-off value, sensitivity, and specificity of it were 0.94, 77.00%, and 95.00%, respectively (Figure 3).

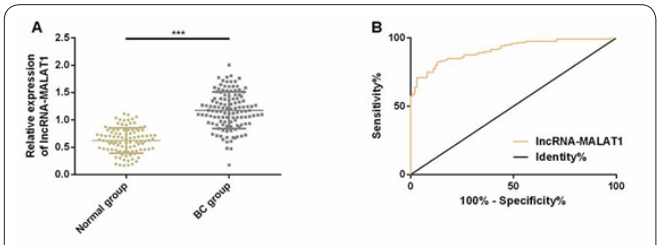

Figure 1. The expression of lncRNA-MALAT1 in BC patients and its diagnostic value. (A) LncRNA-MALAT1 was over-expressed in BC patients. (B) ROC curve of lncRNA-MALAT1 in diagnosing BC patients. Note: *** indicates P< 0.001.

Table 1. Baseline data of two groups of patients [n(%), mean ± SD].

Factor	n	The normal group (n=100)	The BC group (n=120)	χ^2/t	P-value
Menopause or not?				0.296	0.586
No	99	43 (43.00)	56 (46.67)		
Yes	121	57 (57.00)	64 (53.33)		
Age (Y)				0.593	0.441
<50	95	46 (46.00)	49 (40.83)		
≥50	125	54 (54.00)	71 (59.17)		
Average age (Y)	220	51.03±7.28	52.55±7.79	1.484	0.139
Hypertension history				3.171	0.075
No	131	66 (66.00)	65 (54.17)		
Yes	89	34 (34.00)	55 (45.83)		
Diabetes mellitus history				2.225	0.136
No	101	44 (44.00)	41 (34.17)		
Yes	119	56 (56.00)	79 (65.83)		
Drinking history				0.933	0.334
No	122	59 (59.00)	63 (52.50)		
Yes	98	41 (41.00)	57 (47.50)		
Smoking history				0.031	0.860
No	135	62 (62.00)	73 (60.83)		
Yes	85	38 (38.00)	47 (39.17)		
TNM staging					
I and II stages	58	-	58 (48.33)		
III and IV stages	62	-	62 (51.67)		
Pathological differentiation					
Low differentiation	51	-	51 (42.50)		
Moderate and high differentiation	69	-	69 (57.50)		
Tumor diameter (cm)					
<5	65	-	65 (54.17)		
≥5	55	-	55 (45.83)		

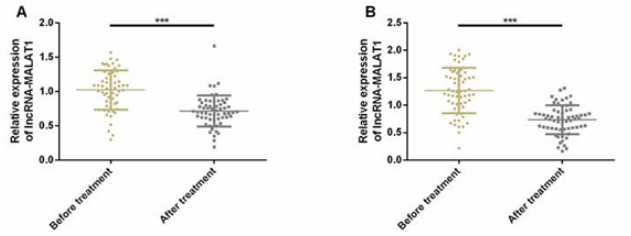

Figure 2. The expression of lncRNA-MALAT1 in treated BC patients. (A) The expression of lncRNA-MALAT1 in the operation group decreased significantly after treatment; (B) The expression of lncRNA-MALAT1 in the combination group decreased significantly after treatment. Note: *** indicates P<0.001.

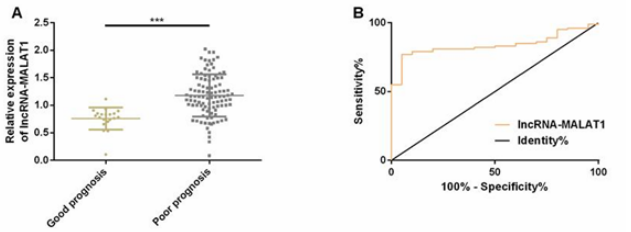

Figure 3. The value of lncRNA-MALAT1 in predicting prognosis of treated BC patients. (A) The expression of lncRNA-MALAT1 in patients with poor prognosis was significantly higher than that in patients with a good prognosis. (B) ROC curve of lncRNA-MALAT1 for predicting poor prognosis of treated BC patients. Note: *** indicates P< 0.001.

Factors affecting the poor prognosis of treated BC patients

There were 20 patients with poor prognosis and 100 patients with a good prognosis in this study. We compared the clinical parameters and related indicators of the two kinds of patients, finding that there was no significant difference between them in menopause, age, average age, hypertension history, diabetes mellitus history, drinking history, smoking history, and treatment method (all $P> 0.05$), while there were significant differences between them in TNM staging, pathological differentiation, tumor diameter, and lncRNA-MALAT1 (all $P< 0.05$). We carried out a multivariate Cox regression analysis for factors with differences, and it came out that TNM staging (P=0.006), pathological differentiation degree (P= 0.017), tumor diameter (P=0.023), and lncRNA-MALAT1 (P = 0.035) were independent prognostic factors affecting the poor prognosis of BC patients, and high TNM staging, low pathological differentiation, long tumor diameter, and high lncRNA-MALAT1 expression could increase the risk of poor prognosis of BC patients. (Tables 2-4).

Relationship between lncRNA-MALAT1 and 5-year OS of treated BC patients

All the 120 BC patients were successfully followed up for 5 years, and it was turned out that the 5-year OS

Table 2. Univariate analysis of factors affecting the poor prognosis of BC patients [n(%), mean ± SD].

Factor	n	Patients with good prognosis (n=100)	Patients with poor prognosis (n=20)	χ^2/t	P-value
Menopause or not?				0.429	0.513
No	56	48 (48.00)	8 (40.00)		
Yes	64	52 (52.00)	12 (60.00)		
Age (Y)				0.172	0.678
<50	49	40 (40.00)	9 (45.00)		
≥50	71	60 (60.00)	11 (55.00)		
Average age (Y)	120	52.57±7.88	51.13±7.42	0.753	0.453
Hypertension history				3.551	0.060
No	65	58 (58.00)	7 (35.00)		
Yes	55	42 (42.00)	13 (65.00)		
Diabetes mellitus history				0.185	0.667
No	41	35 (35.00)	6 (30.00)		
Yes	79	65 (65.00)	14 (70.00)		
Drinking history				0.060	0.806
No	63	53 (53.00)	10 (50.00)		
Yes	57	47 (47.00)	10 (50.00)		
Smoking history				2.525	0.112
No	73	64 (64.00)	9 (45.00)		
Yes	47	36 (36.00)	11 (55.00)		
TNM staging				7.715	0.006
I and II stage	58	54 (54.00)	4 (20.00)		
III and IV stages	62	46 (46.00)	16 (80.00)		
Pathological differentiation				4.972	0.026
Low differentiation	51	38 (38.00)	13 (65.00)		
Moderate and high differentiation	69	62 (62.00)	7 (35.00)		
Tumor diameter (cm)				5.646	0.018
<5	65	59 (59.00)	6 (30.00)		
≥5	55	41 (41.00)	14 (70.00)		
lncRNA-MALAT1				4.220	0.040
<0.94	42	39 (39.00)	3 (15.00)		
≥0.94	78	61 (61.00)	17 (85.00)		
Treatment methods					
Breast-conserving surgery	58	50 (50.00)	8 (40.00)	0.667	0.414
Neo-adjuvant chemotherapy + breast-conserving surgery	62	50 (50.00)	12 (60.00)		

Table 3. Assignment in multivariate Cox regression analysis.

Factor	Variable	Assignment
TNM staging	X1	II stage =0, III/IV stage =1
Pathological differentiation	X2	Moderate/high differentiation=0, low differentiation=1
Tumor diameter	X3	<5cm=0, ≥5cm=1
LncRNA-MALAT1	X4	<0.94=0, ≥0.94=1

Table 4. Multivariate analysis on factors for poor prognosis of BC patients.

Factor	β	S.E	Wald	P-value	OR	95% CI
TNM staging	1.788	0.651	7.533	0.006	5.976	1.667-21.423
Pathological differentiation	1.382	0.582	5.647	0.017	0.251	0.080-0.785
Tumor diameter	1.342	0.588	5.207	0.023	3.827	1.208-12.123
lncRNA-MALAT1	1.504	0.714	4.439	0.035	4.501	1.111-18.242

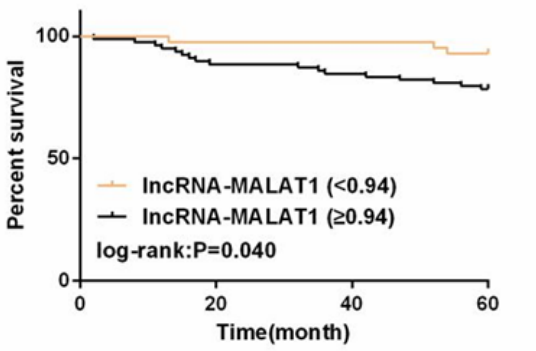

Figure 3. High lncRNA-MALAT1 expression was associated with a relatively low 5-year OS of treated BC patients.

of them was 83.33% (100/120), and low lncRNA-MALAT1 expression was associated with a relatively high 5-year OS of treated BC patients ($P< 0.05$) based on Figure 4.

Discussion

BC is one of the major causes of female deaths worldwide, and an effective diagnosis of it is crucial to improve the survival of BC patients (18). Neo-adjuvant chemotherapy is a standard treatment for patients with advanced BC, which can not only shrank tumors but also increase the possibility of breast-conserving surgery for patients (19, 20). Breast-conserving surgery is a treatment suitable for early BC patients, and its breast resection rate and re-resection rate are only 6.2 and 6.0%, while it contributes to an OS and disease-free survival (DFS) of the patients of 95.0 and 90.0%, so it is a treatment bringing about relatively low complication rate, with high safety (21). Therefore, this study applied the breast-conserving surgery to treat early BC patients, and both neoadjuvant chemotherapy and breast-conserving surgery to treat patients with mid-term or advanced BC.

LncRNA-MALAT1 is not only a member of the lncRNA family related to the pathological mechanism of BC but also a pro-inflammatory factor of the typical inflammatory reaction of cancer (22). One study revealed that lncRNA-MALAT1 participated in the inflammatory cascade of postoperative fever in BC patients, and it was associated with tumor recurrence and metastasis in BC patients (23). In this study, the relative expression of lncRNA-MALAT1 in the BC group was very high, and the AUC of serum lncRNA-MALAT1 for diagnosing subjects in the BC group and the normal group was as high as 0.911, which indicated that serum lncRNA-MALAT1 as a potential indicator for early diagnosis of BC. One study by Miao et al (24) revealed that the high expression of lncRNA-MALAT1 in BC patients was related to lymphatic metastasis and poor 5-year DFS of BC patients, and the AUC of serum lncRNA-MALAT1 for diagnosing BC and benign breast diseases was 0.833, implying that the high expression of lncRNA-MALAT1 was related to adverse clinical outcomes, and the diagnostic value of serum lncRNA-MALAT1 was high, which was similar to the results of our study. In our study, the expression of serum lncRNA-MALAT1 in BC patients from the operation group and the combination group decreased by different degrees after treatment, which suggested that lncRNA-MALAT1 may be involved in the development and progression of BC. In a study by Chou et al (25), lncRNA-MALAT1 could affect the division cycle of BC cells through targeted binding to miR-1, thus promoting the migration and invasion of BC cells, which suggested that silencing the expression of lncRNA-MALAT1 may inhibit the malignant biological behaviors of BC cells, and lncRNA-MALAT1 may be a potential therapeutic target for BC.

Many scholars have studied the prognosis of BC patients. For example, Farquhar et al (26) have uncovered that high-dose chemotherapy and autografting were linked to poor prognosis of early BC patients. In addition, some studies have revealed that telomere length is positively correlated with the development of benign BC tumors, and the neutrophil-lymphocyte ratio (NLR) level was negatively correlated with prognosis of them (27, 28). In this study, the high level of lncRNA-MALAT1 was linked to the poor prognosis of BC patients, and the AUC of lncRNA-MALAT1 for predicting the poor prognosis of BC patients was 0.838, which indicated that lncRNA-MALAT1 also had high predictive value for the prognosis of BC patients. We analyzed factors for the prognosis of BC patients, finding that high TNM staging, low pathological differentiation, long tumor diameter, and high lncRNA-MALAT1 expression could increase the risk of poor prognosis of BC patients. A study by Carmichael et al (29) showed that obesity was one of the risk factors for poor prognosis of BC patients, and its risk is especially reflected in postmenopausal

BC patients. At the end of this study, we analyzed the relationship between lncRNA-MALAT1 and the 5-year OS of BC patients. It came out that the high lncRNA-MALAT1 level was associated with poor 5-year OS, which suggested that knockdown of lncRNA-MALAT1 expression may help to improve the 5-year OS of BC patients and improve their survival outcomes (29-40).

To sum up, this study has verified that lncRNA-MALAT1 can be used as a potential indicator for diagnosis and prognosis prediction of BC patients. Cancer has many factors and components that need to be carefully evaluated (41-49). However, there is still room for improvement in this study. First of all, we can study the role of lncRNA-MALAT1 in the biological functions of BC cells and explore its specific regulatory mechanism and potential drug resistance mechanism. Secondly, we can also expand the sample size to investigate the diagnostic value of lncRNA-MALAT1 in BC relapse or metastasis. We will conduct experiments from the above improvement points in the future.

Authors' contributions

ZS designed the study and drafted the manuscript. JqL was responsible for the collection and analysis of the experimental data. JtL revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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