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Original Article

Serum MALAT1 expression predicts clinical outcome in breast cancer patients receiving cyclophosphamide based treatment

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Abstract: Cyclophosphamide based treatment is one of the most common used chemotherapeutic strategies in breast cancer, however large proportion of patients finally become chemoresistant. The metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is known to be associated with metastasis and is a favorable prognostic factor for lung cancer. Whether MALAT1 plays a critical role in chemoresistance in breast cancer remains unclear. In this study, we found that MALAT1 was significantly upregulated in breast cancer specimens and associated with tumor size, lymph node status, estrogen receptor expression (ER) and TNM stage. By using the RT-qPCR directly applied in serum (RT-qPCR-D) method, we revealed the diagnostic and prognostic value of serum MALAT1 in breast cancer patients. Moreover, serum MALAT1 was significantly up-regulated in patients non-responding to cyclophosphamide treatment compared with responding patients, and the proportion of patients who not responding to chemotherapy was significantly higher in the high MALAT1 expressing group than in the low group. Finally, the Kaplan-Meier survival data showed that serum MALAT1 is an independent prognostic factor for chemoresponse to cyclophosphamide therapy among breast cancer patients. To conclude, our study indicates that high level of circulating MALAT1 expression was correlated with enhanced malignant potential and poor prognosis of breast cancer patients. Furthermore, high serum MALAT1 was associated with poor response to cyclophosphamide based chemotherapy in breast cancer patients. Thus, suppression of MALAT1 could be a future direction to enhance chemosensitivity to cyclophosphamide based chemotherapy regimens.

Keywords: MALAT1, cyclophosphamide, chemoresistance, cell-free, prognosis, breast cancer

Introduction

Breast cancer is a leading cause of cancer-related deaths in women worldwide [1]. A major obstacle to effective anti-cancer treatments is the resistance to chemotherapy remains, which finally results in relapse and progression in breast cancer. The essential factors that cause recurrence remain unknown. Researchers have long searched for prognosis-related markers, such as gene expression profiling. However, the efficiency has proven to be limited because it is unable to define subgroups with similar prognostic and therapeutic characteristics. Thus, it is urgent to find new prognostic and therapeutic target to improve the clinical outcome of breast cancer patients.

Long noncoding RNAs (lncRNAs) are defined as transcripts >200 nucleotides in length and are transcribed but non-translated noncoding RNAs in human genome [2]. They have emerged as new gene regulators and prognostic markers in several cancers [3, 4]. Altered expression of several lncRNAs has recently been attributed to pathogenesis of some malignant neoplasia, including breast cancer [5]. However, the biological roles of most lncRNAs in carcinogenesis and chemoresistance are almost unknown at present.

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is an abundantly expressed nuclear lncRNA with a length of approximately 8000 nucleotides. It was firstly identified by Ji et al as the lncRNA associated with metastasis and survival in non-small cell lung cancer [6]. Aberrant expression of MALAT1 was also observed in a broad range of human malignant tumors including cervical cancer, hepato-

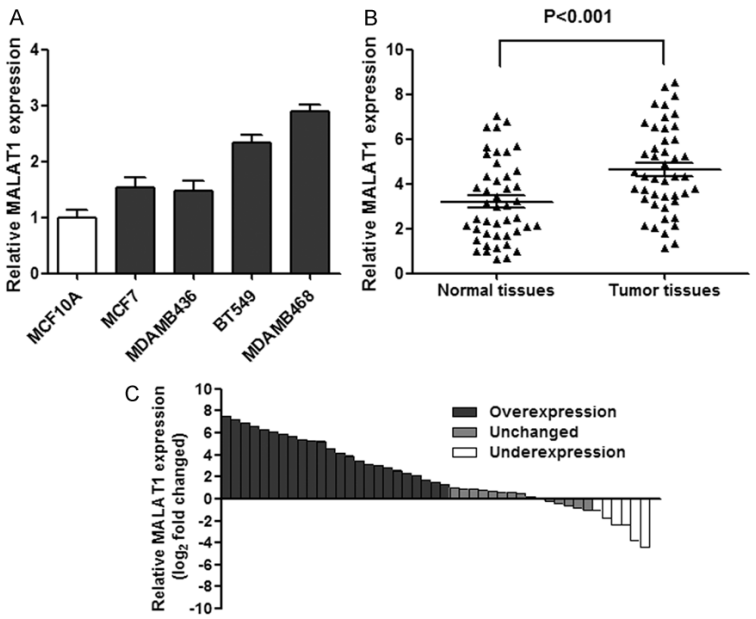


Figure 1. MALAT1 expression was significantly increased in breast cancer cells and primary tissues. A. The expression of MALAT1 was significantly increased in breast cancer cell lines compared with breast epithelia cell line MCF10A. B. MALAT1 expression was significantly increased in primary breast cancer tissues compared with adjacent normal tissues. C. 53.3% (24 of 45) cases had at least 2-fold higher expression of MALAT1 in the breast cancer tissues compared with adjacent normal tissues.

cellular cancer, bladder cancer and breast cancer [7-10]. Huang et al reported that MALAT1 interacts with estrogen receptor and predicts poor survival in breast cancer [11]. However, another study demonstrated by Xu et al indicated that MALAT1 is down-regulated in breast cancer and MALAT1 knockdown induces epithelial-to-mesenchymal transition via the PI3K-AKT pathway in breast cancer cells [12]. These contradictory conclusions indicate that more research is needed to investigate the role of MALAT1 in breast cancer. Moreover, the role of MALAT1 in breast cancer chemoresistance is largely unknown and relevant research is also needed.

Early detection and removal of cancerous or precancerous lesions is thought to be of critical importance for reducing the incidence and improve the prognosis of breast cancer [13]. The development of noninvasive and convenient method which can complement and improve on current breast cancer screening strategies has become a major challenge. Despite of high concentration of ribonucleases in peripheral blood, studies indicated that it is feasible to detect circulating RNA in serum of tumor patients [14]. At present, more and more researchers focus on circulating biomarkers, and a specific marker is urgently needed for increasing the early detection rate and decreasing the mortality rate in breast cancer patients [15]. On the other hand, predictive biomarkers are better blood-based, as blood is easily available and provides the chance to monitor chemotherapy response. To conclude, it is important to identify cell-free markers that predict a patient's responsiveness to chemotherapy, which may allow for the development of targeted therapies for overcoming chemoresistance.

In this study, we determined the expression of MALAT1 in breast cancer specimens and cell lines, and analyze the association between MALAT1 and clinical pathological factors. Moreover, we also investigated the prognostic role of serum MALAT1 in breast cancer patients who had received cyclophosphamide based chemotherapy. Our date indicated that MALAT1 was upregulated in breast cancer and high serum MALAT1 expression was associated with poor response to cyclophosphamide based treatment in breast cancer patients.

Materials and methods

Patients and samples

For clinical parameter analysis, 66 serum samples from patients with breast cancer, as well as 40 serum samples from healthy volunteers were collected at the Xiangyang Hospital between 2009 and 2011. After collection, the serum samples were immediately subjected for isolation of cell-free nucleic acids to prevent contamination from cellular nucleic acids. At the same time, 45 primary breast cancer tissues and paired adjacent normal tissues were also collected from breast cancer patients at the Xiangyang Hospital. Fresh surgical specimens were immediately frozen in liquid nitro-

Table 1. Clinical characteristics of 45 patients and the expression of MALAT1 in primary breast cancer tissues

Characteristics	Case	MALAT1 median (range)	*P*-value
Age (years)			0.869
<55	24	4.61 (1.12-7.97)	
≥55	21	4.43 (1.36-8.21)	
Menopausal status			0.475
Pre	20	4.24 (1.11-7.12)	
Post	25	4.77 (2.01-8.87)	
Tumor size			0.351
<3 cm	19	4.67 (2.14-8.32)	
≥3 cm	26	4.16 (1.24-8.03)	
Lymph node metastasis			0.008
No	24	3.57 (1.08-6.56)	
Yes	21	5.65 (2.48-8.55)	
Differentiation			0.106
Well	14	4.33 (2.11-7.55)	
Moderate	26	4.27 (1.45-7.91)	
Poor	5	4.89 (3.08-8.87)	
TNM stage			
I-II	27	3.98 (1.10-7.72)	0.034
III	18	5.42 (2.48-8.39)	
ER status			0.025
Negative	19	3.74 (1.28-7.09)	
Positive	26	5.63 (2.37-8.25)	
PR status			0.061
Negative	21	4.12 (1.21-7.85)	
Positive	24	5.06 (2.06-8.32)	
Her-2 status			0.873
Negative	31	4.59 (2.31-8.06)	
Positive	14	4.47 (2.02-8.73)	

ER: Estrogen receptor; PR: Progesterone receptor.

gen and were then stored at -80°C for further use.

For chemo-response study, another independent cohort of 178 serum samples were collected from the breast cancer patients who received standard cyclophosphamide based chemotherapy at the Xiangyang Hospital between 2009 and 2012. All the patients were pathologically confirmed and the clinical samples were collected before chemotherapy was started. They were classified according to the WHO criteria and staged according to the tumor-node-metastasis (TNM) classification. Tumor response status was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) and was assigned to patients with complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Overall survival (OS) was defined as the time from inclusion to death for any reason. Written informed consent was obtained from all patients according to local ethical regulations of the Ethics Committee of the Xiangyang Hospital.

Cell culture

The human breast cancer cell lines MCF7, MDAMB436, BT549, MDAMB468, and human breast epithelia cell line MCF10A were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) in 2014. All breast cancer cell lines were maintained in RPMI 1640 (Thermo Fisher Scientific, Wilmington, DE, USA). Normal breast epithelia cells were grown in DMEM/F12 1:1 medium. All cells were cultured with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA), 100 U/ml penicillin, and 100 g/ml streptomycin (Life Technologies, Grand Island, NY, USA) at 37°C in 5% CO_2 and 95% air. The cell authenticity was determined by short tandem repeat analysis technology (Cell IDTM System, Promega, Madison, WI).

RNA extraction

Total RNA was isolated from primary breast cancer tissues and breast cancer cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA); serum RNA used acid phenol according to the manufacturer's instructions. The extracted total RNA was eluted in 20 μl nuclease-free water and the RNA concentration was measured by Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA). The samples with A260 nm/A280 nm ratios between 1.8 and 2.0 were used for further experiments.

Quantitative real-time PCR (RT-qPCR) and RT-qPCR directly applied in serum (RT-qPCR-D)

For primary breast cancer tissues and cell lines, the cDNA was synthesized from 200 ng extracted total RNA using the PrimeScript RT reagent Kit (Takara Bio Company, Shiga, Japan)

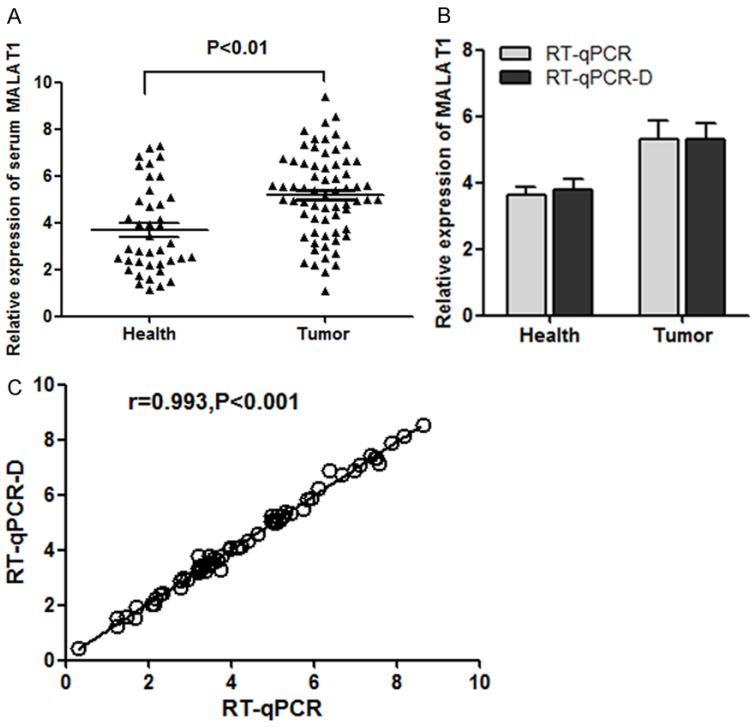


Figure 2. A significant increased expression of serum MALAT1 was also observed from 66 breast cancer patients by RT-qPCR-D. A. Serum MALAT1 expression was significantly increased in breast cancer patients compared with healthy controls by RT-qPCR-D. B. Relative serum levels of cell free MALAT1 among different groups detected by RT-qPCR-D and RT-qPCR. C. Correlation analysis between RT-qPCR-D and RT-qPCR in detecting serum cell free MALAT1 levels.

and amplified by RT-qPCR using Light Cycler 480 SYBR Green I Master (Roche, Germany) and GAPDH was served as the reference gene. For serum cell free MALAT1 detection, we used Zhang et al previously established RT-qPCR-D method without RNA extraction [16]. Briefly, prepare the 2× preparation buffer that contained 2.5% Tween 20 (EMD Chemicals, Gibbstown, NJ), 50 mmol/L Tris (Sigma-Aldrich), and 1 mmol/L EDTA (Sigma-Aldrich). First, 5 μl of serum were mixed with an equal volume of 2× preparation buffer. Subsequently, the above mixture was reverse transcribed (RT) in triplicates in a 20 μl reaction volume. Finally, the RT product was 10-fold diluted and centrifuged at 16,000 g for 5 min, and 5 μl supernatant solutions were used as cDNA template for qPCR. The reagents and reaction conditions were same as RT-qPCR. The premier sequences were as follows: MALAT1 (Forward): GGGTGTTTACGTAGACCAGAACC, (Reverse): CTTCCAAAAGCCTTCTGCCTTAG; GAPDH (Forward): GCACCGTCAAGGCTGAGAAC, (Reverse): ATGGTGGTGAAGACGCCAGT.

Breast cancer antigen15-3 (CA15-3) assay

Levels of CA15-3 were measured by electrochemiluminescence with Roche Cobas e601 Analyzer (Roche AG, Germany), and the upper limits were defined as 25 U/mL according to the manufacturer's recommendations.

Statistical analysis

Kolmogorov-Smirnov test was used to determine the normality of the distribution of data in each group. The differences between two groups were analyzed by the Mann-Whitney U-test. Correlation analyses were carried out using Spearman's rank correlation method. Receiver operating characteristic (ROC) curves were established to discriminate responding patients from non-responding patients. Area under the ROC curve (AUC) was used as an accuracy index for evaluating the predictive performance of MALAT1. A log-rank test was used to analyze the statistical differences in survival as deduced from Kaplan-Meier curves. Cox proportional-hazard regression analysis was performed to calculate HR and 95% CI for each covariable. All differences were regarded as statistically significant when $P<0.05$. MedCalc 9.3.9.0 (MedCalc, Mariakerke, Belgium) was used for ROC analysis, and other analyses were performed with GraphPad Prism 5.0 (GraphPad Software, La Jolla, California, USA).

Results

Breast cancer cell examination

RT-qPCR was used to detect the MALAT1 expression levels in breast cancer cells and tissues, normalized to GAPDH. The results showed that breast cancer cell lines of MCF7, MDAMB436, BT549 and MDAMB468 expressed enhanced levels of MALAT1 than the breast epithelia cell line MCF10A (**Figure 1A**).

Table 2. Clinical characteristics of 66 patients and the expression of MALAT1 in serum of breast cancer patients

Characteristics	Case	MALAT1 median (range)	*P*-value
Age (years)			0.775
<55	36	5.41 (2.12-7.87)	
≥55	30	5.13 (1.36-8.06)	
Menopausal status			0.851
Pre	38	5.24 (1.31-8.12)	
Post	28	5.37 (2.05-8.17)	
Tumor size			0.151
<3 cm	29	4.86 (1.26-8.04)	
≥3 cm	37	5.67 (2.38-8.42)	
Lymph node metastasis			0.012
No	41	4.62 (1.41-7.57)	
Yes	25	5.87 (2.68-8.89)	
Differentiation			0.080
Well	17	5.03 (1.21-8.21)	
Moderate	41	5.27 (1.44-8.53)	
Poor	8	6.01 (3.08-9.17)	
TNM stage			
I-II	44	4.57 (1.10-8.72)	0.005
III	22	5.92 (2.76-9.39)	
ER status			0.012
Negative	27	4.74 (1.26-8.19)	
Positive	39	5.83 (2.47-8.99)	
PR status			0.008
Negative	30	4.52 (1.38-7.85)	
Positive	36	6.06 (2.26-8.72)	
Her-2 status			0.793
Negative	43	5.49 (1.31-9.06)	
Positive	23	5.17 (2.02-8.73)	

ER: Estrogen receptor; PR: Progesterone receptor.

Breast cancer tissue examination

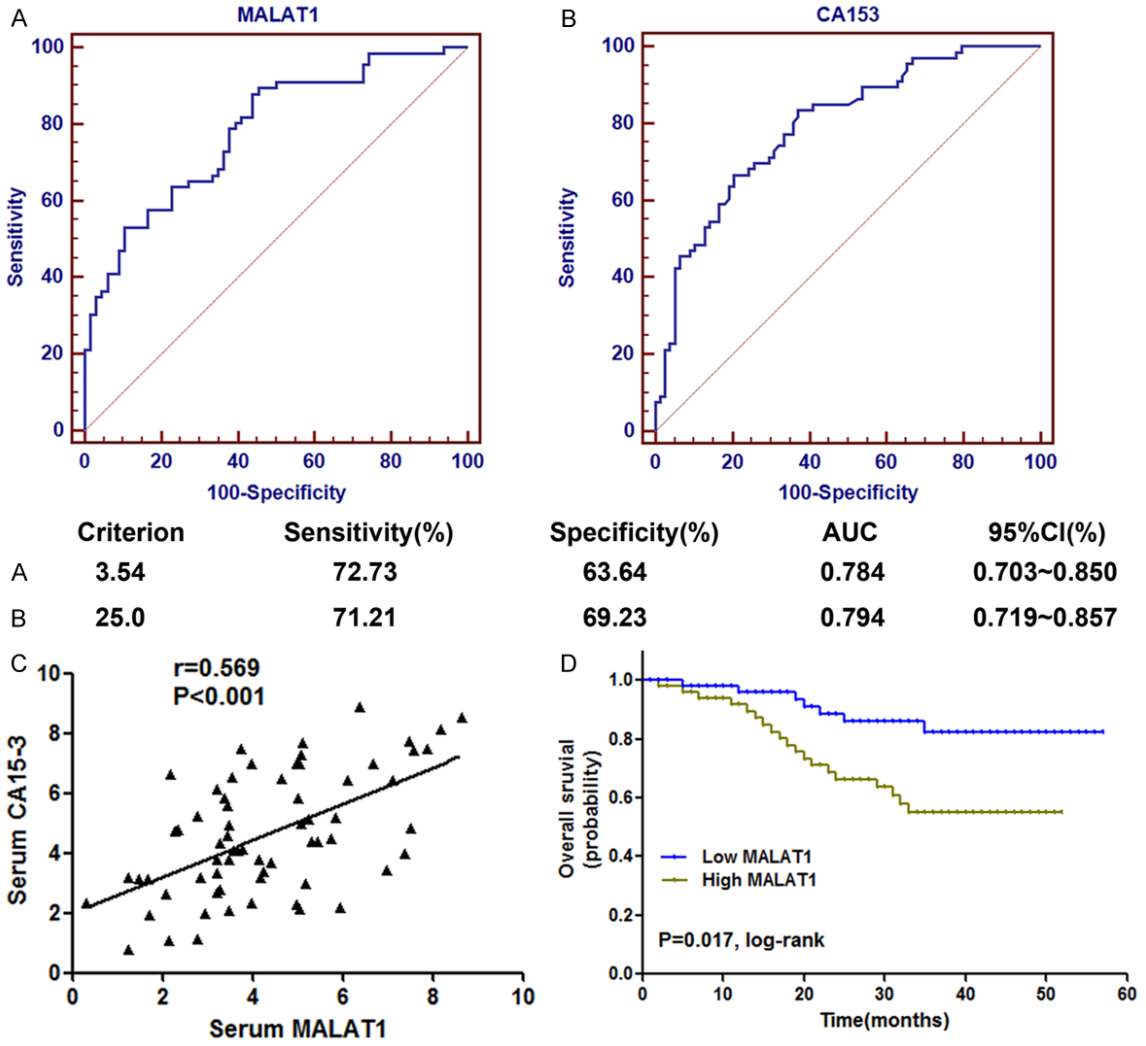
MALAT1 expression in primary breast cancer tissues: We then enrolled 45 patients to detect the expression of MALAT1 in primary tissues. There were 21 patients aging above 55 while 24 aging below 55, and the median age was 53.5 years old. According to the tumor node metastasis (TNM) staging criteria of breast cancer, 27 cases were thought to be stage I-II, 18 cases were stage III. The number of patients considered as well differentiation, moderately differentiation and poor differentiation were 14, 26 and 5, respectively. The expression level of MALAT1 was significantly increased in primary breast cancer tissues compared to paired noncancerous tissues by RT-qPCR (**Figure 1B**). Moreover, the breast cancer tissues in 53.3% (24 of 45) of cases had at least two-fold higher expression of MALAT1 compared with non-tumor tissues (**Figure 1C**).

Correlation between MALAT1 expression and clinical pathological factors: We then determined the association between MALAT1 and clinical pathological characteristics. As shown in **Table 1**, MALAT1 expression level was positively correlated with lymph node status, estrogen receptor (ER) and TNM stage, while no significant correlations were observed between MALAT1 expression and other clinicopathological factors including age, menopausal status, tumor size, tumor differentiation, progesterone receptor (PR) status and HER-2 status in 45 cases of breast cancer tissues.

Breast cancer serum examination

MALAT1 expression in serum of breast cancer patients: To further validate the clinical role of MALAT1, we focus on the expression of MALAT1 in serum of breast cancer patients. Another set of 66 breast cancer patients were enrolled in our study to obtain serum samples. Among these patients, 36 patients were below the age of 55 and 30 were above the age of 55 with the median age of 54 years old. According to the TNM staging criteria of breast cancer, 44 cases were considered to be stage I-II, 22 cases were stage III. Seventeen patients were considered as well differentiation, 41 cases were moderately differentiation and 8 cases were poor differentiation. By using RT-qPCR-D, we found that MALAT1 expression was significantly increased in serum samples from breast cancer patients compared with healthy individuals (**Figure 2A**). Besides, the RT-qPCR assay also demonstrated a consistent result with RT-qPCR-D (**Figure 2B**). Moreover, there was a significantly linear correlation in serum MALAT1 levels between RT-qPCR-D and RT-qPCR ($r= 0.993$, $P<0.001$; **Figure 2C**). This suggests that the RT-qPCR-D assay is also applicable in detection of serum lncRNA expression in breast cancer patients.

Correlation between serum MALAT1 expression and clinical pathological factors: Subsequently, we determined the correlation between



	Criterion	Sensitivity(%)	Specificity(%)	AUC	95%CI(%)
A	3.54	72.73	63.64	0.784	0.703~0.850
B	25.0	71.21	69.23	0.794	0.719~0.857

Figure 3. The diagnostic and prognostic value of MALAT1 in breast cancer tissues. ROC curve was drawn to exhibit the diagnostic capacity of serum MALAT1 (A) and CA15-3 (B). (C) MALAT1 expression was positively correlated with CA15-3 expression in 66 serum samples from breast cancer patients. (D) Patients with high expression of serum MALAT1 was associated with shorter overall survival compared with the low expressing breast cancer patients.

serum MALAT1 expression and clinical pathological factors. Consistent with breast cancer tissue samples, serum MALAT1 was significantly associated with lymph node status, ER status and TNM stage. Furthermore, a significant association was also observed between serum MALAT1 expression and PR status of breast cancer patients. However, no significant correlations were observed between serum MALAT1 expression and other clinicopathological factors including age, menopausal status, tumor size, tumor differentiation and HER-2 status (**Table 2**).

The diagnostic and prognostic value of serum MALAT1: After validating the upregulation of circulating MALAT1 in serum of breast cancer patients, we sought to explore the clinical role of circulating MALAT1 in breast cancer. By performing the ROC curve analysis, we investigated the diagnostic value of circulating serum MALAT1 level in distinguishing breast cancer patients from healthy individuals. Our date showed that the area under the ROC curve (AUC) of MALAT1 was 0.784, and the diagnostic sensitivity and specificity were 72.73% and 63.64%, respectively (**Figure 3A**). This date was parallel to the common used biomarker in breast cancer, CA15-3. The AUC of CA15-3 was 0.794 with the diagnostic sensitivity and specificity reached 71.21% and 69.23%, respectively (**Figure 3B**). Besides, a positive cor-

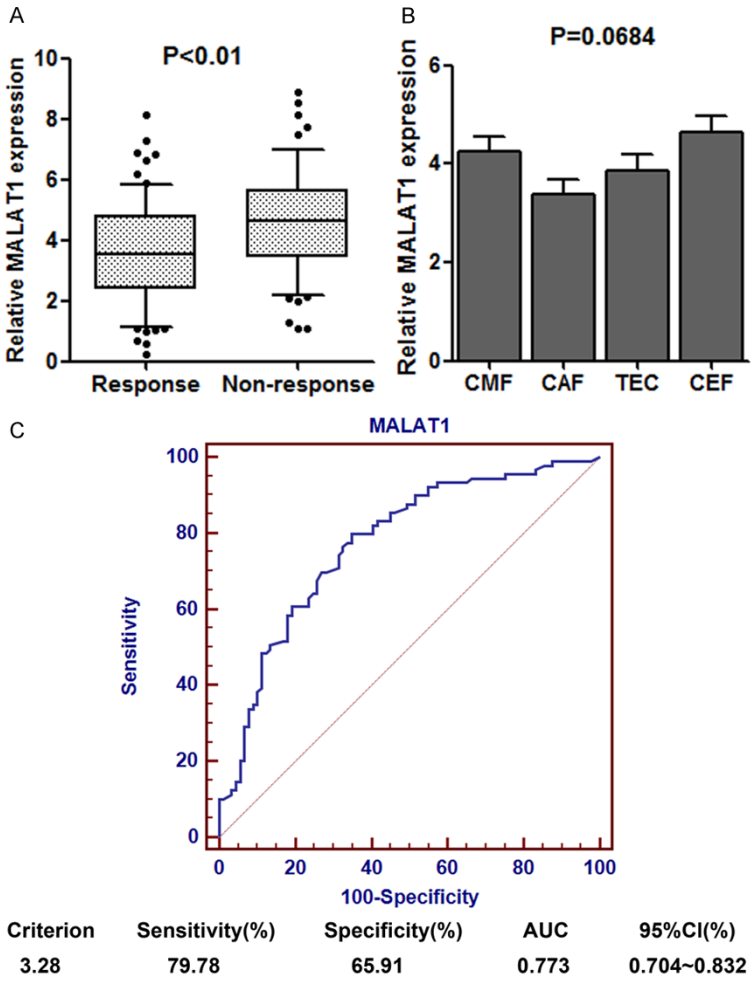


Figure 4. MALAT1 expression was associated with chemo-response to cyclophosphamide based treatment in breast cancer patients. A. Serum MALAT1 levels were significantly higher in non-responding group than in responding group among breast cancer patients receiving cyclophosphamide based chemotherapy. B. No significant difference was found of serum MALAT1 level among different cyclophosphamide based chemotherapy regimens. C. ROC curve was drawn to explore the capacity of serum MALAT1 in distinguishing responding and non-responding patients receiving cyclophosphamide based treatment.

relation was also found between MALAT1 and CA15-3 expression in the 66 serum samples, which further validates the clinical value of circulating MALAT1 in breast cancer (**Figure 3C**).

Furthermore, Kaplan-Meier survival analysis was performed to investigate the prognostic role of MALAT1 in breast cancer patients. A median value of MALAT1 (5.25) in 66 serum samples from breast cancer patients was used to divided these patients into a high and a low group. Our results indicated that patients with high circulating MALAT1 expression were associated with shorter overall survival compared with low MALAT1 patients (**Figure 3D**). Besides, the 5-year survival rate was significantly lower in breast cancer patients who expressed high circulating MALAT1 expression (57.6%, 19/33), when compared with that of the patients expressing low levels of MALAT1 (75.8%, 25/33). Taken together, these date showed that the circulating MALAT1 expression level was a diagnostic and prognostic factor in breast cancer patients.

Serum MALAT1 level is associated with chemo-response to cyclophosphamide based treatment in breast cancer patients: Based on the above results, we further investigated the prognostic role of serum MALAT1 expression in 178 breast cancer patients receiving cyclophosphamide based treatment. Tumor response status was evaluated according to the RECIST version 1.0 criteria and was assigned to patients with responding (CR and PR) group and non-responding (SD and PD) group. By using RT-qPCR-D, we found that serum MALAT1 was significantly up-regulated in non-responding patients compared with responding patients (**Figure 4A**, P< 0.01). Moreover, no significant difference was found on MALAT1 expression among different cyclophosphamide based chemotherapy regimens, including CMF (cyclophosphamide + methotrexate + 5 FU), CAF (cyclophosphamide + adriamycin + 5 FU), TEC (docetaxel + epirubicin + cyclophosphamide), and CEF (cyclophosphamide + epirubicin + 5 FU) (**Figure 4B**, P>0.05). These indicate that the clinical prognostic value of MALAT1 was free of influence from different chemotherapy regimens. Subsequently, we explored the potential significance of MALAT1 in distinguishing responding and non-responding patients by performing ROC curve analysis. Our results showed

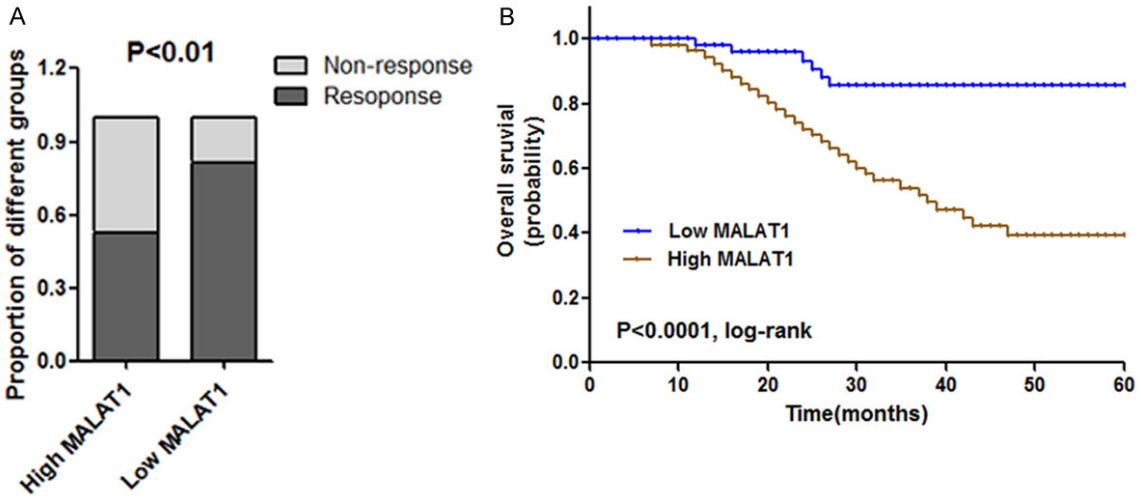


Figure 5. Serum MALAT1 is a prognostic factor in patients receiving cyclophosphamide based treatment. A. The proportion of breast cancer patients not responding to cyclophosphamide based chemotherapy was significantly higher in the high serum MALAT1 expressing group than in the low group. B. High serum MALAT1 expression was associated with short overall survival among breast cancer patients receiving cyclophosphamide based therapy.

Table 3. Multivariate Cox proportional hazards regression model analysis for 5-year overall survival in breast cancer patients receiving cyclophosphamide based treatment

Characteristics	Reference group	Multivariate analysis		
		RR	95% CI	*P*-value
Age	<55	0.999	0.489-2.013	0.998
	≥55			
Menopausal status	Pre-menopausal	1.632	0.779-3.257	0.231
	Post-menopausal			
Her-2 status	Negative	0.792	0.402-1.827	0.435
	Positive			
Tumor size	<3 cm	1.548	0.682-2.835	0.301
	≥3 cm			
Differentiation	Well	1.728	0.525-1.867	0.203
	Moderate			
	Poor			
ER status	Negative	1.975	0.614-2.726	0.124
	Positive			
PR status	Negative	1.834	1.201-2.998	0.076
	Positive			
Lymph node status	No	2.378	1.218-3.935	0.021
	Yes			
TNM stage	I-II	3.759	1.402-9.471	0.012
	III			
Serum MALAT1 level	High	2.594	1.205-4.383	0.017
	Low			

ER: Estrogen receptor; PR: Progesterone receptor.

that the AUC reached 0.773 (95% CI: 0.705-0.833), and the diagnostic sensitivity and specificity reached 79.78% and 65.91%, respectively, under the optimal cut-off value of 3.48 (**Figure 4C**). Collectively, we demonstrate that circulating MALAT1 is a chemotherapeutic indicator in breast cancer patients receiving cyclophosphamide based chemotherapy.

High circulating MALAT1 predicts poor survival in breast cancer patients receiving cyclophosphamide based treatment: Finally, we aimed to investigate the prognostic value of circulating MALAT1 in breast cancer patients receiving cyclophosphamide based treatment. When we stratified patients into a high (n=76) and a low (n=102) MALAT1 expressing group with the previously established optimal cut-off value (3.48), the proportion of patients not responding to chemotherapy was significantly higher in the high MALAT1 expressing group than in the low group (P<0.01, **Figure 5A**). More importantly, The 5-year survival rate of the breast cancer patients whose serum expressed high levels of MALAT1 was 39.5% (30/76), which was significantly lower than that of pa-

tients whose tumors expressed low levels of MALAT1 (84.3%, 86/102); this difference was statistically significant (P<0.001). By performing the Kaplan-Meier survival analysis, we revealed that patients with high level of circulating MALAT1 level had a significant shorter survival than did those with a high circulating MALAT1 expression level (P<0.0001, **Figure 5B**). Moreover, the Cox regression multivariate analysis indicated that serum MALAT1 expression level was an independent prognostic factor for overall survival of breast cancer patients who had received cyclophosphamide based chemotherapy (**Table 3**). To conclude, our date reveals that circulating MALAT1 is an independent prognostic indicator for chemoresponse to cyclophosphamide based treatment in breast cancer patients.

Discussion

Gene expression is a complex cellular process that is tightly regulated at several levels to ensure proper gene dosage, and this process is dysregulated in human malignancies. Currently, the major challenge to improve the clinical outcome of breast cancer patients is the chemotherapy failure caused by drug resistance. In our work, we focus on circulating biomarkers, which are urgently needed for increasing the early detection rate and decreasing the mortality rate in breast cancer. Our results validated the up-regulation of MALAT1 in breast cancer specimens, and enhanced MALAT1 was correlated with breast cancer invasion. More importantly, we firstly revealed that high expression of circulating MALAT1 is associated with poor chemoresponse to cyclophosphamide based treatment and predicts shorter survival after surgery in breast cancer patients receiving cyclophosphamide based chemotherapy.

MALAT1 is a highly abundant and conserved lncRNA, and is localized to the nuclear speckle subcompartment. Earlier studies indicated that MALAT1 modulates transcription and pre-mRNA processing of a large set of genes [17, 18]. MALAT1 is deregulated in several tumors, and is potentially of significant clinical importance. MALAT1 was found positively correlated with Gleason score, the level of prostate specific antigen (PSA), tumor stage and castration resistance in PCa. Gutschner et al showed that MALAT1-deficient lung cancer cells showed impaired migration and form fewer tumors in mice [19]. Wilusz et al found that MALAT1 functions as a precursor for the production of small RNAs and identified as a highly conserved small RNA [20]. However, its clinical role in tumorigenesis was only beginning to emerge in breast cancer. Xu et al reported that MALAT1 was down-regulated in breast cancer and MALAT1 knockdown induced epithelial-to-mesenchymal transition via the PI3K-AKT pathway in breast cancer cells [12]. However, another study demonstrated by Huang et al indicated that MALAT1 interacted with estrogen receptor and predicted poor survival in breast cancer [11]. Consistent with Huang et al, our study showed that MALAT1 is up-regulated in breast cancer and high expression level of MALAT1 is correlated with tumor progression in breast cancer patients.

Cell free RNA has been reported to be released and circulating in the blood of cancer patients [21]. Thus, the detection of these circulating RNA in plasma/serum may serve as a “liquid biopsy”, which allows real-time monitoring of disease progress, prognosis, or therapeutic response. Recently, serum RNAs have been directly amplified, negating the need for RNA extraction in detecting breast cancer and melanoma, often better reflecting their expression in tissues [22, 23]. More recently, Zhang et al developed a RT-qPCR based method to detect cell-free mRNAs in colorectal cancer where reverse transcription is performed directly in serum (RT-qPCR-D) [16]. They also demonstrated that the results obtained from RT-qPCR-D were significantly correlated with those detected by RT-qPCR. Moreover, because the direct assay bypassed extraction of circulating RNA from serum, minimizing human and mechanical errors, this method resulted in decreased inherent variability and had superior reproducibility. In our study, we detected the circulating MALAT1 expression from serum of breast cancer patients by using the RT-qPCR-D method. The results indicated that serum MALAT1 expression level was significantly increased in breast cancer patients and positively correlated with breast cancer progression, which is mostly consistent with the date from tissue detection by RT-qPCR. Moreover, the ROC curve analysis and Kaplan-Meier survival analysis suggest that circulating MALAT1 from serum can serve as preferable diagnostic and prognostic biomarker in breast cancer.

Resistance to chemotherapy is one of the major causes for treatment failure in advanced breast cancer. Thus, predictive markers are required to increase the efficacy of chemotherapy and may also be helpful in monitoring therapy response in breast cancer. Cyclophosphamide is one of the most common used chemotherapeutic drugs given in combination with epirubicin, docetaxel and 5-Fluorouracil. There are currently no available molecular predictive markers for these combination regimens. The identification of cancer-specific lncRNAs is critical for understanding the roles of lncRNAs in tumorigenesis and may be important for defining novel therapeutic targets. Previous studies identified that HOTAIR is a predictive and prognostic biomarker for patients with advanced gastric adenocarcinoma receiving fluorouracil and platinum combination chemotherapy [24]. Besides, various reports also showed that MALAT1 may play important roles during chemoresistance in several cancers. Yang et al demonstrated that elevated MALAT1 is a novel predictor for prognosis and a potential therapeutic target for gastric cancer [25]. Cho et al found that MALAT1 long non-coding RNA is overexpressed in multiple myeloma and may serve as a marker to predict disease progression [26]. Although various researchers revealed the critical role of MALAT1 in breast cancer progression, however, the role of MALAT1 in breast cancer chemoresistance is largely unknown. To the best of our knowledge, this is the first study to investigate the clinical value of circulating MALAT1 in breast cancer chemoresistance. Our date indicated that serum MALAT1 level is significantly associated with cyclophosphamide response in breast cancer patients, and plays an important role in distinguishing the patients responding to cyclophosphamide treatment from the patients with no response. More importantly, high serum MALAT1 expression level was negatively associated with objective response in breast cancer patients receiving cyclophosphamide based therapy. These results revealed a diagnostic value of circulating MALAT1 for cyclophosphamide based chemotherapy in breast cancer patients.

Finally, we investigated the prognostic role of circulating MALAT1 in breast cancer patients receiving cyclophosphamide based chemotherapy. Breast cancer patients expressed high circulating MALAT1 levels showed a short 5-year survival rate than patients with low circulating MALAT1 levels. Additionally, the survival analysis indicated that overall survival of breast cancer patients with high level circulating MALAT1 who received cyclophosphamide based chemotherapy was significantly lower than that of patients with low-level circulating MALAT1. These data, together with our multivariate analyses suggest that high expression of circulating MALAT1 might be a significant independent predictor of poor prognosis for cyclophosphamide based chemotherapy among breast cancer patients. Thus, we conclude that overexpression of circulating MALAT1 may be involved in cyclophosphamide resistant phenotypes of human breast cancers.

In conclusion, our study showed that MALAT1 expression level is significantly increased in breast cancer specimens and associated with enhanced malignant potential. By using the RT-qPCR-D method, we reveal that cell-free MALAT1 from serum can serve as diagnostic and prognostic indicator for breast cancer patients. Importantly, serum MALAT1 is significantly associated with objective chemoresponse to cyclophosphamide and predicts poor survival in breast cancer patients receiving cyclophosphamide based treatment. Thus, serum MALAT1 may serve as a novel prognostic biomarker and therapeutic target in breast cancer patients. Suppression of MALAT1 could be a future direction to enhance chemosensitivity to cyclophosphamide based chemotherapy regimens.

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Disclosure of conflict of interest

None.

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References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermuller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dumais E, Patel S, Helt G, Ganesh M, Ghosh S, Piccolboni A, Sementchenko V, Tammana H and Gingeras TR. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 2007; 316: 1484-1488.

[3] Ponting CP, Oliver PL and Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.

[4] Tsai MC, Spitale RC and Chang HY. Long intergenic noncoding RNAs: new links in cancer progression. Cancer Res 2011; 71: 3-7.

[5] Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S and Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010; 464: 1071-1076.

[6] Ji P, Diederichs S, Wang W, Boing S, Metzger R, Schneider PM, Tidow N, Brandt B, Buerger H, Bulk E, Thomas M, Berdel WE, Serve H and Muller-Tidow C. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 2003; 22: 8031-8041.

[7] Luo JH, Ren B, Keryanov S, Tseng GC, Rao UN, Monga SP, Strom S, Demetris AJ, Nalesnik M, Yu YP, Ranganathan S and Michalopoulos GK. Transcriptomic and genomic analysis of human hepatocellular carcinomas and hepatoblastomas. Hepatology 2006; 44: 1012-1024.

[8] Guffanti A, Iacono M, Pelucchi P, Kim N, Solda G, Croft LJ, Taft RJ, Rizzi E, Askarian-Amiri M, Bonnal RJ, Callari M, Mignone F, Pesole G, Bertalot G, Bernardi LR, Albertini A, Lee C, Mattick JS, Zucchi I and De Bellis G. A transcriptional sketch of a primary human breast cancer by 454 deep sequencing. BMC Genomics 2009; 10: 163.

[9] Praz V, Jagannathan V and Bucher P. CleanEx: a database of heterogeneous gene expression data based on a consistent gene nomenclature. Nucleic Acids Res 2004; 32: D542-547.

[10] Ying L, Chen Q, Wang Y, Zhou Z, Huang Y and Qiu F. Upregulated MALAT-1 contributes to bladder cancer cell migration by inducing epithelial-to-mesenchymal transition. Mol Biosyst 2012; 8: 2289-2294.

[11] Huang NS, Chi YY, Xue JY, Liu MY, Huang S, Mo M, Zhou SL and Wu J. Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 (MALAT1) interacts with estrogen receptor and predicted poor survival in breast cancer. Oncotarget 2016; 7: 37957-37965.

[12] Xu S, Sui S, Zhang J, Bai N, Shi Q, Zhang G, Gao S, You Z, Zhan C, Liu F and Pang D. Downregulation of long noncoding RNA MALAT1 induces epithelial-to-mesenchymal transition via the PI3K-AKT pathway in breast cancer. Int J Clin Exp Pathol 2015; 8: 4881-4891.

[13] Edwards BK, Noone AM, Mariotto AB, Simard EP, Boscoe FP, Henley SJ, Jemal A, Cho H, Anderson RN, Kohler BA, Eheman CR and Ward EM. Annual Report to the Nation on the status of cancer, 1975-2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast, or prostate cancer. Cancer 2014; 120: 1290-1314.

[14] Wang H, Zhang X, Wang L, Zheng G, Du L, Yang Y, Dong Z, Liu Y, Qu A and Wang C. Investigation of cell free BIRC5 mRNA as a serum diagnostic and prognostic biomarker for colorectal cancer. J Surg Oncol 2014; 109: 574-579.

[15] Tsujiura M, Ichikawa D, Komatsu S, Shiozaki A, Takeshita H, Kosuga T, Konishi H, Morimura R, Deguchi K, Fujiwara H, Okamoto K and Otsuji E. Circulating microRNAs in plasma of patients with gastric cancers. Br J Cancer 2010; 102: 1174-1179.

[16] Zhang X, Yang X, Zhang Y, Liu X, Zheng G, Yang Y, Wang L, Du L and Wang C. Direct serum assay for cell-free bmi-1 mRNA and its potential diagnostic and prognostic value for colorectal cancer. Clin Cancer Res 2015; 21: 1225-1233.

[17] Tripathi V, Shen Z, Chakraborty A, Giri S, Freier SM, Wu X, Zhang Y, Gorospe M, Prasanth SG, Lal A and Prasanth KV. Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. PLoS Genet 2013; 9: e1003368.

[18] Tano K, Mizuno R, Okada T, Rakwal R, Shibato J, Masuo Y, Ijiri K and Akimitsu N. MALAT-1 enhances cell motility of lung adenocarcinoma cells by influencing the expression of motility-related genes. FEBS Lett 2010; 584: 4575-4580.

[19] Gutschner T, Hammerle M, Eissmann M, Hsu J, Kim Y, Hung G, Revenko A, Arun G, Stentrup M, Gross M, Zornig M, MacLeod AR, Spector DL and Diederichs S. The noncoding RNA MALAT1 is a critical regulator of the metastasis phenotype of lung cancer cells. Cancer Res 2013; 73: 1180-1189.

[20] Wilusz JE, Freier SM and Spector DL. 3' end processing of a long nuclear-retained noncoding RNA yields a tRNA-like cytoplasmic RNA. Cell 2008; 135: 919-932.

[21] Khan S, Jutzy JM, Aspe JR, McGregor DW, Neidigh JW and Wall NR. Survivin is released from cancer cells via exosomes. Apoptosis 2011; 16: 1-12.

[22] Asaga S, Kuo C, Nguyen T, Terpenning M, Giuliano AE and Hoon DS. Direct serum assay for microRNA-21 concentrations in early and advanced breast cancer. Clin Chem 2011; 57: 84-91.

[23] Ono S, Oyama T, Lam S, Chong K, Foshag LJ and Hoon DS. A direct plasma assay of circulating microRNA-210 of hypoxia can identify early systemic metastasis recurrence in melanoma patients. Oncotarget 2015; 6: 7053-7064.

[24] Zhao W, Dong S, Duan B, Chen P, Shi L, Gao H and Qi H. HOTAIR is a predictive and prognostic biomarker for patients with advanced gastric adenocarcinoma receiving fluorouracil and platinum combination chemotherapy. Am J Transl Res 2015; 7: 1295-1302.

[25] Yang H, Liu Z, Yuan C, Zhao Y, Wang L, Hu J, Xie D, Wang L and Chen D. Elevated JMJD1A is a novel predictor for prognosis and a potential therapeutic target for gastric cancer. Int J Clin Exp Pathol 2015; 8: 11092-11099.

[26] Cho SF, Chang YC, Chang CS, Lin SF, Liu YC, Hsiao HH, Chang JG and Liu TC. MALAT1 long non-coding RNA is overexpressed in multiple myeloma and may serve as a marker to predict disease progression. BMC Cancer 2014; 14: 809.