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ORIGINAL ARTICLE

Detection and analysis of circulating large intergenic non-coding RNA regulator of reprogramming in plasma for breast cancer

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Abstract

Background: Previous studies have indicated that large intergenic non-coding RNA regulator of reprogramming (lincRNA-ROR) plays an important role in regulating tumor carcinogenesis and metastasis; however, whether circulating lincRNA-ROR could function as a potential biomarker for breast cancer (BC) diagnosis and monitoring is unknown. This study was conducted to investigate circulating lincRNA-ROR in plasma as a potential biomarker for BC diagnosis and monitoring.

Methods: We performed reverse transcription-quantitative-PCR to examine lincRNA-ROR expression levels in cell lines, 24 pairs of BC tissue samples, and 94 plasma samples from BC patients. Potential correlations between plasma lincRNA-ROR levels and clinicopathological characteristics were analyzed. A receiver operating characteristic curve was calculated to evaluate the diagnostic values for BC. Pearson correlation analysis of lincRNA-ROR in plasma samples and the corresponding tissues of the same patients was performed to explore tumor monitoring values.

Results: LincRNA-ROR expression was significantly increased in BC cell lines, tissues, and plasma (all $P < 0.01$). Plasma lincRNA-ROR levels were associated with estrogen receptors ($P = 0.042$) and lymph node metastasis ($P = 0.046$). The area under the receiver operating characteristic curve of plasma lincRNA-ROR was 0.844 (sensitivity 80.0%, specificity 56.7%), which was higher than carcinoembryonic and carbohydrate antigen 15-3 values. Moreover, plasma lincRNA-ROR levels were decreased in postoperative compared to preoperative samples ($P < 0.0001$). Plasma lincRNA-ROR levels moderately correlated with the corresponding tissue level in the same patients ($r^2 = 0.638$, $P < 0.0001$).

Conclusion: Plasma lincRNA-ROR may be a potential biomarker for BC diagnosis and a dynamic monitor.

Introduction

Breast cancer (BC) is a major cause of morbidity and mortality in women all over the world.[1] Despite the advances that have been made in treatment, BC continues to present a major clinical challenge as most cases are diagnosed at advanced stage, with consequent poor prognosis and limited treatment options. Thus, early detection and treatment of BC will contribute to improved clinical outcomes. The gold standard of BC diagnosis remains histological examination; however, procedures to obtain tissue samples are difficult, high risk to patients, and require consistent evaluation by expert pathologists. Furthermore, traditional markers, including carbohydrate antigen (CA) 15-3, CA

27.29, and carcinoembryonic antigen (CEA) are of limited utility at early BC screening because of a lack of sufficiently high diagnostic sensitivity and specificity.[2,3] Therefore, the field of circulating nucleic acids (CNAs) in plasma or serum is of great interest in the search for non-invasive biomarkers for the diagnosis and management of BC.[4]

Long non-coding RNAs (lncRNAs) are known as transcribed RNA molecules over 200 nt in length with no protein-coding potential that are often critical regulators of gene expression.[5,6] Previous studies have suggested that lncRNAs are functionally diverse and could act as guides, tethers, decoys and scaffolds.[7] lncRNAs have been demonstrated to play an important role in a series of biological processes, including cell proliferation, apoptosis, and angiogenesis. The aberrant expression of lncRNAs is associated with human cancers, and could promote tumorigenic and metastatic processes.[8] Large intergenic non-coding RNA regulator of reprogramming (lincRNA-ROR, linc-ROR), is a member of a subvariety of lncRNAs, sized 2.6 kb, and first discovered in induced pluripotent stem cells (iPSCs).[9] The original name is lincRNA-ST8SIA3, for regulator of reprogramming, and it is therefore also known as lincRNA-ROR. LincRNA-ROR is dramatically upregulated in pluripotent cells where it is modulated by the important pluripotency factors Oct4, SOX2, and Nanog. Increased expression of lincRNA-ROR plays an important role in the maintenance of iPSCs and embryonic stem cells (ESCs), likely by preventing the activation of cellular stress pathways, including the p53 response.[9] Moreover, lincRNA-ROR is a strong negative regulator of p53 in response to DNA damage.[10] LincRNA-ROR has been proven to contribute to bladder cancer cell proliferation, migration, and epithelial-to-mesenchymal transition (EMT) by targeting ZEB1.[11] Similarly, Wang *et al.* found that lincRNA-ROR promoted proliferation and invasion while suppressing apoptosis in gastric cancer stem cells.[12] LincRNA-ROR is also dramatically upregulated in BC tissues and cell lines, and plays a vital role in BC pathological processes, including invasion, metastasis, and EMT, as well as drug resistance.[13–16] Although lincRNA-ROR exhibits a carcinogenic effect in BC processes, it is unclear whether circulating plasma lincRNA-ROR could serve as a useful biomarker for BC. We hypothesized that lincRNA-ROR could serve as a cancer biomarker secreted into the blood, and the detection of circulating lincRNA-ROR in plasma could be a useful diagnostic or screening tool for BC.

In this study, we first measured the expression level of lincRNA-ROR in case-control studies consisting of cells, and tissue and plasma samples. LincRNA-ROR expression was obviously upregulated in BC samples. In addition, we investigated whether a correlation exists between plasma lincRNA-ROR expression and the clinicopathological features of BC patients. Finally, we further evaluated the diagnostic and dynamic monitor values of plasma lincRNA-ROR for BC patients. The results of this study indicate that plasma lincRNA-ROR may be a potential biomarker for BC diagnosis and clinical monitoring.

Methods

Ethics statement

The Ethical Review Committee of the Affiliated Hospital of Southwest Medical University approved this study. Written informed consent was obtained from each subject prior to blood and tumor sample collection. All experiments were performed in accordance with the guidelines and principles of the Declaration of Helsinki.[17]

Patients and samples

This case-control study included 24 pairs of BC tissues and adjacent normal tissues (5 cm away from tumor tissue), 94 BC plasma samples and 90 cancer-free control plasma samples, and 24 pairs of preoperative and postoperative (15 days after surgery) plasma samples. All samples in this study were consecutively recruited from BC patients and age and gender matched healthy volunteers from the Affiliated Hospital of Southwest Medical University between March 2015 and February 2016. To satisfy the inclusion criteria, all patients were confirmed with primary BC by pathological examination of surgical specimens and biopsies and were not subject to any preoperative radiotherapy or chemotherapy, other malignant disease, or acute injury before sample collection. The clinical information of BC patients was obtained from medical records, including patient age, menstrual history, tumor size, histological type, tumor stage, lymph node metastasis, estrogen receptor (ER), progesterone receptor (PR), HER2, and tumor growth fraction (Ki-67 antigen). The Union for International Cancer Control (UICC) classification was used to stage BC tumors. Patient age ranged from 30 to 70, with a median age of 50. The healthy volunteers selected had not been exposed to any other cancerous disease or injury, and their age ranged from 29 to 67, with a median age of 48.

Tissue samples were obtained from BC patients during surgery, and were then frozen in liquid nitrogen and stored at −80°C within 15 minutes. Whole blood samples were collected from each participant in EDTA-2K vacutainer tubes (Becton Dickinson, San Jose, CA, USA) and then immediately centrifuged at 3000 rpm for 15 minutes at room temperature. The supernatant was transferred into an EP tube (General Electric, Fairfield, CT, USA) and stored at −80°C.

Cell lines and cell culture

Breast cancer cell lines MCF-7, MDA-MB-231, SK-BR-3, BT549, and human mammary immortalized epithelial cell MCF10A were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). These five cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) (Sigma, St. Louis, MO, USA) containing inactivated 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 100 U/mL penicillin, and 100 mg/mL streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in 5% CO_2. Cells were digested and subcultured with 0.25% trypsin and then examined under an 80% microscopic view. After incubation for 72 h, the cells were separately collected for RNA isolation.

RNA extraction and quantitative real-time PCR

Total RNA was extracted from BC tissues and cells using Trizol Reagent (Takara, Tokyo, Japan). Total RNA in plasma was extracted using a Blood Total RNA Rapid Extraction Kit (BioTeke Corporation, Beijing, China). The reverse transcription (RT) reaction was conducted using a PrimeScript RT reagent Kit with gDNA Eraser (Takara), according to the manufacturer's instructions. RT reactions were performed at 42°C for two minutes, 37°C for 15 minutes, 85°C for five seconds, and 4°C for 10 minutes. LincRNA-ROR expression levels were detected by real-time PCR using SYBR Premix Ex Taq II (Takara) in a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The PCR conditions consisted of an initial polymerase activation step at 95°C for 30 seconds, 40 cycles of 95°C for five seconds, and 60°C for 30 seconds, followed by a dissociation cycle of 95°C for 15 seconds, 60°C for 60 seconds, 95°C for 15 seconds, and 60°C for 15 seconds. The relative expression levels of lincRNA-ROR were normalized by β-actin. The sequences of lincRNA-ROR and β-actin were as follows: lincRNA-ROR: 5′-CTCAGTGGGGAAGACTCCAG-3′ (forward), 5′-AGG AAGCCTGAGAGTTGGC-3′ (reverse); β-actin: 5′-TCCT CTCCCAAGTCCACACA-3′ (forward), 5′-GCACGAAGG CTCATCATTCA-3′ (reverse). Each reaction was performed in triplicate. The $2^{-\Delta\Delta CT}$ method was used to calculate relative expression levels.[18]

Detection of plasma cancer antigen 15-3 and carcinoembryonic antigen

Cancer antigen 15-3 and CEA plasma levels were detected using commercial kits via an electrochemiluminescence immunoassay method, which was implemented using a Roche cobas e 601, according to the manufacturer's instructions (Roche, Basel, Switzerland).

Statistical analysis

All statistical analyses were conducted using SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 5.0 (GraphPad Software, LaJolla, CA, USA). Data were presented with mean ± standard deviation (SD). Comparisons between two groups of continuous data with the Gaussian distribution were calculated by Student's *t*-test, while comparison between two groups was calculated by Mann–Whitney *U* test. The Wilcoxon test was used to compare preoperative and postoperative plasma samples. Sigmaplot version 12.5 (Systat Software, San Jose, CA, USA) was used to run a logistic regression analysis in order to control for potential confounders, and receiver operating characteristic (ROC) curves of lincRNA-ROR, CA15-3, and CEA were established to differentiate BC patients from healthy controls. All tests were two-sided and $P < 0.05$ was considered statistically significant.

Results

Expression of large intergenic non-coding RNA regulator of reprogramming (lincRNA-ROR) in breast cancer (BC) tissue and cell lines

Recent data indicated that lincRNA-ROR is involved in the maintenance of iPSCs and ESCs, and also could promote BC progression.[13,14] In order to validate lincRNA-ROR expression in BC, we first analyzed the expression level of lincRNA-ROR in 24 pairs of BC tissues and adjacent normal tissues by RT-quantitative-PCR (RT-qPCR). LincRNA-ROR expression in BC tissues was significantly higher than in adjacent normal tissues ($P < 0.01$) (Fig 1a). We further measured lincRNA-ROR expression in BC cell lines, and found that lincRNA-ROR expression was significantly upregulated in BC cell lines (MCF-7, MDA-MB-231, SK-BR-3, BT549) compared to immortalized mammary epithelial cell MCF10A (all $P < 0.001$) (Fig 1b). These results were consistent with those of previous studies and suggested that lincRNA-ROR overexpression may be a feature involved in breast tumorigenesis and cancer progression.

Expression of lincRNA-ROR in BC plasma and the correlation between BC patient characteristics

To evaluate lincRNA-ROR expression in BC patient's plasma, we detected plasma lincRNA-ROR levels in

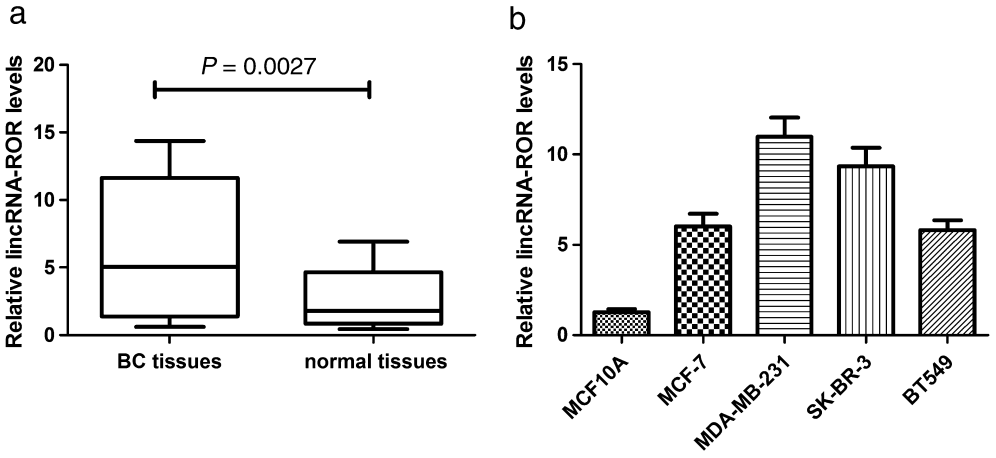

Figure 1 Validation of large intergenic non-coding RNA regulator of reprogramming (lincRNA-ROR) expression in clinical breast cancer (BC) tissues and cancer cell lines. The relative expression level of lincRNA-ROR was normalized to β-actin. LincRNA-ROR was significantly increased in (**a**) 24 human BC tissues compared to corresponding adjacent normal tissues ($P < 0.01$) and (**b**) human BC cell lines compared to the immortalized human mammary epithelial cell (MCF10A) (all $P < 0.001$).

a large-scale sample consisting of 94 BC patients and 90 healthy controls. Plasma lincRNA-ROR expression was significantly increased in BC patients compared to healthy controls ($P < 0.0001$) (Fig 2). Furthermore, the associations between plasma lincRNA-ROR and clinicopathological characteristics were examined in the 94 BC patients. A significant correlation was found between plasma lincRNA-ROR level and ER ($P = 0.042$) and lymph node metastasis ($P = 0.046$); however, no significant correlation between lincRNA-ROR levels and clinical characteristics of age, menstrual history, tumor size, histological type, tumor node metastasis stage, PR, HER2, or Ki-67 were observed. The correlations between plasma lincRNA-ROR and clinical characteristics are summarized in Table 1.

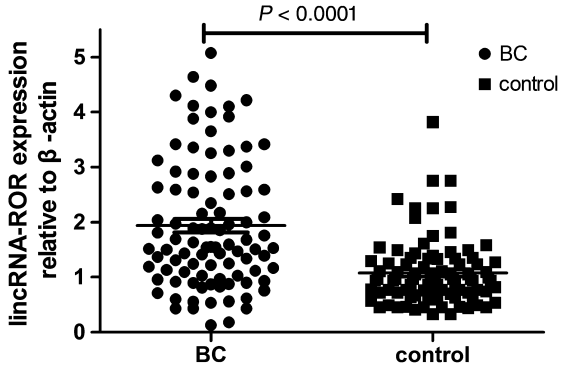

Figure 2 Evaluation of plasma large intergenic non-coding RNA regulator of reprogramming (lincRNA-ROR) level in 94 breast cancer (BC) patients and 90 healthy controls. The relative expression level of lincRNA-ROR was normalized to β-actin. Plasma lincRNA-ROR levels were significantly increased in BC patients compared to healthy controls ($P < 0.0001$).

Evaluation of plasma lincRNA-ROR as a promising novel biomarker for BC

In order to evaluate the diagnostic capabilities of plasma lincRNA-ROR, CEA, and CA15-3 for BC, we randomly selected 30 early stage patients (tumor diameter <3 cm, no lymph node or distant metastasis) from 94 BC patients and 30 age and gender matched cancer-free controls from 90 healthy volunteers to detect the expression levels of plasma lincRNA-ROR, CEA, and CA15-3. An ROC curve was performed to evaluate the diagnostic value. The optimal cut-off value for plasma lincRNA-ROR levels in BC patients was 1.205 and the area under the curve (AUC) value was 0.844, with a sensitivity of 80.0% and a specificity of 56.7%, suggesting that plasma lincRNA-ROR was suitable for differentiating BC patients from healthy individuals. Regarding conventional tumor markers of BC, at a cut-off value of 9.250 for CA15-3 expression, the AUC value was 0.663, with sensitivity and specificity of 73.3% and 60.0%, respectively; at a cut-off value of 2.405 for CEA expression, the AUC value was 0.516, with sensitivity and specificity of 66.7% and 50.0%, respectively. The AUC and the sensitivity of lincRNA-ROR were much higher than of conventional tumor markers, suggesting that plasma lincRNA-ROR levels are superior to CEA or CA15-3 levels for the early detection of BC. In addition, we further constructed binary logistic regression to evaluate the combined diagnostic values of lincRNA-ROR, CEA, and CA15-3; the AUC of combination was 0.894, with sensitivity of 86.7% and specificity of 83.3%, which showed improvement predictability over lincRNA-ROR alone (Fig 3). The ROC curve area is shown in Table 2, while sensitivity and specificity are shown in Table 3. These results indicated that plasma lincRNA-ROR could function as a promising

biomarker for BC screening, and a combination of these three plasma indexes could enhance diagnostic power.

Evaluation of plasma lincRNA-ROR as a biomarker for monitoring tumor dynamics in BC patients

Detecting the plasma lncRNA level could be an effective tool to reflect tumor dynamics, such as plasma leaks from tumor cells. To confirm this hypothesis and evaluate the possibility of the clinical application of plasma lincRNA-ROR, we first measured lincRNA-ROR expression levels in 24 paired preoperative and postoperative plasma samples from BC patients using RT-qPCR. LincRNA-ROR expression levels were remarkably reduced in postoperative compared to preoperative plasma (P = 0.0002) (Fig 4a). Moreover, to clarify the correlation of lincRNA-ROR expression between plasma and BC tissue, we measured the lincRNA-ROR expression level in 24 BC tumor tissues and paired preoperative plasma samples using RT-qPCR and analyzed the correlation between the two groups using Pearson correlation analysis. A moderate correlation was observed for lincRNA-ROR (r^2 = 0.638, P < 0.0001) (Fig 4b), consistent with our previous hypothesis. These findings indicated that lincRNA-ROR expression in plasma might reflect expression in the tumor, thus plasma lincRNA-ROR level may represent a new biomarker to monitor tumor status.

Table 1 Correlations of plasma lincRNA-ROR and clinical characteristics of BC patients

Characteristics	No. of patients (n = 94)	lincRNA-ROR levels (mean ± SD)	P
Age (years)			
< 50	54	1.96 ± 1.24	0.802
≥ 50	40	1.90 ± 1.07	
Menstrual history			
Premenopausal	66	1.92 ± 1.15	0.854
Postmenopausal	28	1.90 ± 1.26	
Tumor size			
≥ 2 cm	39	1.95 ± 1.07	0.549
< 2 cm	55	1.87 ± 1.12	
Histological type			
Invasive carcinoma	58	1.91 ± 1.14	0.135
Non-invasive carcinoma	36	1.68 ± 1.26	
TNM stage			
I–II	62	1.87 ± 0.88	0.747
III–IV	32	1.96 ± 1.24	
Lymph node metastasis			
Metastasis	54	2.14 ± 1.12	0.046
No metastasis	40	1.65 ± 1.18	
ER			
(+)	64	2.13 ± 1.18	0.042
(–)	30	1.62 ± 1.09	
PR			
(+)	56	1.99 ± 1.15	0.599
(–)	38	1.86 ± 1.20	
HER2			
(+)	69	1.97 ± 1.06	0.697
(–)	25	1.86 ± 1.47	
Ki-67			
≤ 10%	71	1.95 ± 1.14	0.854
> 10%	23	1.90 ± 1.26	

BC, breast cancer; CI, confidence interval; ER, estrogen receptor; lincRNA-ROR, large intergenic non-coding RNA regulator of reprogramming; PR, progesterone receptor; SD, standard deviation; TNM, tumor node metastasis.

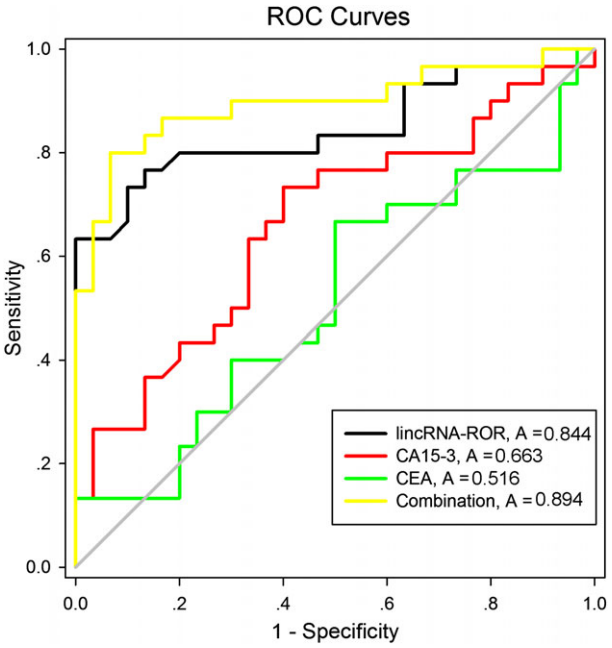

Figure 3 Evaluation of the diagnostic value of plasma large intergenic non-coding RNA regulator of reprogramming (lincRNA-ROR) compared to conventional tumor marker cancer antigen (CA) 15-3 and carcinoembryonic antigen (CEA) for breast cancer (BC). The area under the curve (AUC) and the sensitivity of lincRNA-ROR were much higher than in conventional tumor markers.

Table 2 The ROC curve area of lincRNA-ROR, CA 15-3, CEA, and combined index for BC diagnosis

Test variables	ROC curve area	95% CI	P
lincRNA-ROR	0.844	0.738–0.951	< 0.0001
CA 15-3	0.663	0.523–0.803	0.030
CEA	0.516	0.366–0.667	0.830
Combination	0.894	0.807–0.982	< 0.0001

BC, breast cancer; CA, cancer antigen; CEA, carcinoembryonic antigen; CI, confidence interval; lincRNA-ROR, large intergenic non-coding RNA regulator of reprogramming; ROC, receiver operating characteristic.

Table 3 Sensitivity and specificity of lincRNA-ROR, CA 15-3, CEA, and combined index for BC diagnosis

Test variables	Cut-off	Sensitivity	95% CI	Specificity	95%CI
lincRNA-ROR	1.205	0.800	0.614–0.923	0.567	0.374–0.745
CA15–3	9.250	0.733	0.541–0.877	0.600	0.406–0.773
CEA	2.405	0.667	0.472–0.827	0.500	0.313–0.687
Combination	1.876	0.867	0.693–0.962	0.833	0.653–0.944

BC, breast cancer; CA, cancer antigen; CEA, carcinoembryonic antigen; CI, confidence interval; lincRNA-ROR, large intergenic non-coding RNA regulator of reprogramming.

Discussion

Increasing studies have demonstrated that CNAs could be detected in the human peripheral circulation system, such as blood, urine, breast milk, and cerebrospinal fluid. Changes in CNA levels have been associated with tumor burden and malignant progression, and the detection of CNAs in plasma or serum could serve as a "liquid biopsy," which would be useful as non-invasive diagnostic biomarkers for human cancer.[19–22] Circulating microRNAs (miRNAs) have been identified as reliable biomarkers for cancer screening, diagnosis, and prognosis, including breast cancer.[21,23] In addition to miRNAs, many other classes of non-coding RNAs are associated with tumor physiological function and malignant progression, and these are also detectable in the serum and plasma of cancer patients.[24] The detection or identification of circulating RNAs using genome microarrays and quantitative RT-PCR could represent potential biomarkers for cancer.[25] Most researchers have focused on the role of lncRNAs in cancer progression and found aberrant expression in different cancer tissues and cells. Moreover, dysregulated lncRNAs could be secreted into the blood or urine and reflect the pathological process of cancer. Previously, our research group detected the presence of dysregulated lncRNAs, such as HOTAIR and H19, in the peripheral blood of cancer patients.[26,27] These lncRNAs could serve as biomarkers for cancer screening and monitoring. Hence, the detection of lncRNAs in blood represents a feasible strategy for the assessment of cancer progression.

LincRNA-ROR is a subvariety of lncRNA that reportedly could function as an oncogenic factor involved in tumorigenesis and tumor progression, such as in gallbladder and breast cancers. Moreover, lincRNA-ROR is also associated with miRNPs and may function as a competing endogenous RNA (ceRNA) for specific miRNAs to interfere in their function. Wang *et al.* demonstrated that lincRNA-ROR was elevated in the cytoplasm and acted as a ceRNA for miRNA-145 to upregulate its target genes, including OCT4, SOX2, KLF4, and Nanog, enhancing EMT and metastasis.[28–32] In addition, Gao *et al.* reported that lincRNA-ROR may act as a ceRNA, effectively regulating Nanog expression by "sponging" miR-145 and could be regarded as a prognostic marker in pancreatic cancer.[33] Subsequently, Eades *et al.* further identified that lincRNA-ROR could promote tumor invasion by competitively inhibiting miR-145, which results the in upregulation of small GTPase ARF6.[15] ARF6 is an important actor in the metastatic process, which has an essential role for

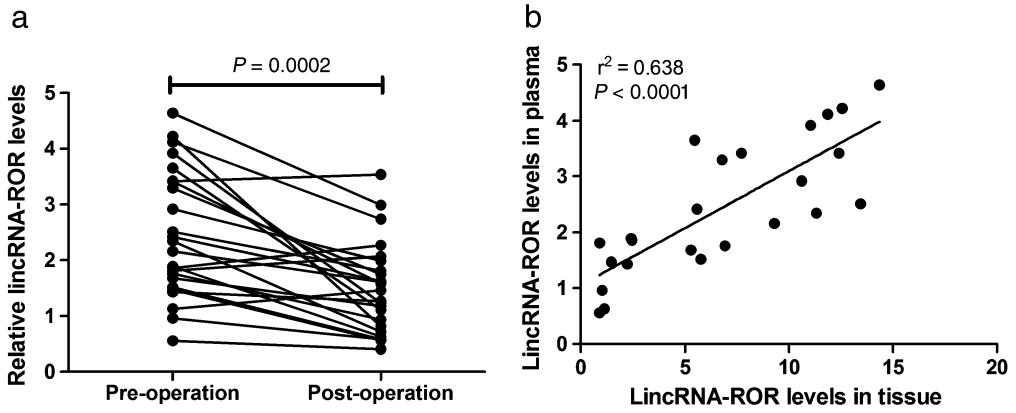

Figure 4 Evaluation of plasma large intergenic non-coding RNA regulator of reprogramming (lincRNA-ROR) as a biomarker for monitoring tumor dynamics in breast cancer (BC) patients. (**a**) Comparison of plasma lincRNA-ROR levels between preoperative and postoperative samples from BC patients. The plasma lincRNA-ROR expression levels were remarkably reduced in postoperative compared to preoperative plasma ($P < 0.001$). (**b**) Pearson's correlation scatter plot of lincRNA-ROR levels in tumor tissues and plasma. The expression levels of plasma lincRNA-ROR were moderately correlated with tissue lincRNA-ROR levels ($r^2 = 0.638$, $P < 0.0001$).

BC cell invasion: the overexpression of ARF6 modulates E-cadherin localization and affects cell-cell adhesion.[34] Recently, Hou *et al.* discovered that lincRNA-ROR was dramatically upregulated in BC tissues and cell lines, and lincRNA-ROR could enhance BC cell migration and invasion by functioning as a molecular sponge for miRNA-205.[13] Competitive binding of lincRNA-ROR to miRNA-205 prevented the degradation of miRNA-205 target genes, including *ZEB1* and *ZEB2. ZEB2* is an inducer of EMT, which exerts important roles in the regulation of EMT and promotes BC metastasis. In the current study, we found that lincRNA-ROR expression was significantly upregulated in BC cell lines, tissues, and plasma (all $P < 0.001$), and that plasma lincRNA-ROR level was associated with lymph node metastasis ($P = 0.046$). These findings were consistent with those of previous studies and support the tumor-promoting role of lincRNA-ROR in BC.

The risk and prognosis of BC are associated with the status of hormone receptors.[35] LncRNA expression levels are also significantly related to hormone receptors in BC patients.[17,36,37] LincRNA-ROR is reportedly overexpressed in triple-negative (ER-, PR-, HER2-) breast cancer and could serve as a biomarker or therapeutic target for improving survival.[15] However, are lincRNA-ROR plasma levels associated with hormone receptor status? In this study, we examined ER, PR, and HER2 status in BC patients and found that increased plasma lincRNA-ROR expression was associated with ER positive; however, no association between plasma lincRNA-ROR and PR, HER2, and Ki-67 levels was found. These results suggest that lincRNA-ROR expression is involved in ER status and that plasma lincRNA-ROR is a potential biomarker for reflecting cancer progression.

Traditional serum biomarkers have widely been used in the diagnosis of BC, including CA 15-3 and CEA. In order to evaluate the diagnostic capabilities of plasma lincRNA-ROR, we compared the diagnostic capabilities of lincRNA-ROR to those of conventional tumor markers. Based on an analysis of ROC curves, we found that lincRNA-ROR presents a high diagnostic capability for detecting BC (AUC 0.844, sensitivity 80.0%, specificity 56.7%). The diagnostic capability and the sensitivity of plasma lincRNA-ROR were much higher than that of conventional tumor markers CEA and CA 15-3. This suggests that plasma lincRNA-ROR could serve as a promising marker for BC detection. In addition, the combination of these three plasma biomarkers could improve the diagnostic capability of differentiating BC patients from healthy controls over lincRNA-ROR alone. Plasma lincRNA-ROR expression may be a reliable molecular marker for BC diagnosis or screening, and the combined application of various indexes may increase the detection rate of BC.

Why do cancer patients have such large quantities of CNAs? What mechanism is responsible for the shedding of tumor nucleic acids in the bloodstream? There is much debate over these questions. To date, the source of CNAs in blood and the mechanisms of how they provide long-term circulation in the bloodstream remain enigmatic. The generally supported notion is that tumor cell apoptosis or necrosis is the major source of free CNA,[24] while another explanation is that an active release of free CNAs by all living cells may be a plausible mechanism.[38,39] Moreover, most researchers have found that nucleic acids released into the circulation were surprisingly stable in spite of the fact that increased amounts of RNases circulate in the blood of cancer patients.[40,41] Nucleic acids could be protected from degradation by its packaging into exosomes, which are shed from cellular surfaces into the bloodstream. The CNAs of lncRNAs can be secreted into the blood or urine via exosomes and thus serve as cancer biomarkers.[42,43] Recently, Takahas *et al.* found that lincRNA-ROR could be released into the local microenvironment from hepatocellular carcinoma cells via exosomes and function as a mediator of intercellular signaling communication.[44] Evidence has demonstrated that lincRNA-ROR can be detected in the peripheral circulation system. In this study, we presumed that the release of lincRNA-ROR in the plasma might be a consequence of tumor initiation, and the detection of plasma lincRNA-ROR might be helpful to monitor tumor evolution in real time and prompt accurate clinical treatment decisions. To investigate whether plasma lincRNA-ROR levels could reflect tumor dynamics, we compared plasma lincRNA-ROR levels in 24 paired plasma samples obtained before and 15 days after surgery and evaluated the correlation between lincRNA-ROR expression levels in plasma and corresponding tissues of the same patients. Plasma lincRNA-ROR levels were significantly reduced in postoperative compared to preoperative samples ($P < 0.0001$), and plasma lincRNA-ROR expression was moderately correlated with tissue lincRNA-ROR expression ($r^2 = 0.638$, $P < 0.0001$). The results clearly showed that plasma lincRNA-ROR levels could reflect tumor status and that plasma lincRNA-ROR expression is available as a novel biomarker for tumor recurrence and to monitor patient status.

To summarize, we have clearly demonstrated that plasma lincRNA-ROR could be used as a diagnostic or monitoring biomarker for BC patients. However, further large-scale prospective studies are required to evaluate the possibility of using plasma lincRNA-ROR as a non-invasive biomarker for BC detection and clinical patient management. Furthermore, if possible, many other lncRNAs with high sensitivity and specificity for BC in plasma should be detected for translation into the clinical setting.

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Disclosure

No authors report any conflict of interest.

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