

# Circulating microRNA-103a-3p could be a diagnostic and prognostic biomarker for breast cancer

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**Abstract.** Breast cancer (BC) is the most frequent cancer for women worldwide. Recently, a spectrum of cell-free circulating microRNAs (miR) has been recognized as promising biomarkers for BC diagnosis and prognosis, among which miR-103a-3p has been reported in several types of human cancer. However, the role of miR-103a-3p in BC remains unknown. A total of 112 patients with BC and 59 healthy controls were recruited into the present study. The expression level of serum miR-103a-3p was evaluated using reverse transcription-quantitative PCR. Receiver operating characteristic curves were utilized to calculate diagnostic accuracy. Survival curves were generated to analyze survival outcomes. It was found that circulating miR-103a-3p level was upregulated in patients with BC compared with that in healthy controls, and its expression was decreased following surgery. In addition, miR-103a-3p expression level was also associated with advanced clinicopathological features, including positive epidermal growth factor receptor 2 status, metastasis and an advanced TNM stage. The circulating serum miR-103a-3p level could be used to discriminate between patients with BC and the healthy controls prior to surgery using an area under curve [AUC, 0.697; 95% confidence intervals (CI), 0.615-0.778], and distinguish patients with BC and metastasis from those without metastasis (AUC, 0.936; 95% CI, 0.892-0.980). In addition, high expression level of miR-103a-3p was associated with worse survival outcomes in patients with BC. In conclusion, the present study suggests that miR-103a-3p could be a potential non-invasive diagnostic and prognostic biomarker for BC.

## Introduction

Breast cancer (BC) is the second most common malignancy worldwide and the most frequent cancer in women (1),

contributing to an estimated 25% of all new cancer cases and ~0.5 million cancer-related deaths each year (2). Despite progress in the current BC therapies, including surgery, radiotherapy, chemotherapy and endocrinotherapy, almost 30% patients with BC, diagnosed at early-stages, may develop distant metastasis, leading to death (3,4). So far, a number of clinicopathological features, including tumor size, histological grade, lymph node status, hormone receptor (HR) status and human epidermal growth factor receptor type 2 (HER2) status, have been used for the diagnosis and prognostic prediction in patients with BC (5); however, the value of these traditional markers in predicting the prognosis of BC is limited (6). Therefore, additional diagnostic or prognostic biomarkers for early surveillance in patients with BC is required.

MicroRNAs (miRNAs/miR) are a family of endogenous small non-coding RNAs, which are 18-23 nucleotides in length, and are considered to regulate numerous biological processes, including cell differentiation, proliferation, apoptosis and metastasis (7-9). To date, miRNA expression signatures have been found to play a tumor suppressive or oncogenic role in cancer using translational repression or target degradation and gene silencing (10). In addition, miRNAs have been shown to be promising biomarkers for BC as they can be readily detected in both tumor tissues and body fluids (as circulating miRNAs), including in plasma, serum or saliva (11-13).

Numerous studies have demonstrated that miR-103a-3p is an oncomiR in various types of cancer, including thyroid cancer (14), colorectal cancer (15), gastric cancer (16), oral squamous cell carcinoma (17), malignant mesothelioma (18) and salivary adenoid cystic carcinoma (19). In contrast, Ge *et al* (20) reported that downregulation of miR-103a-3p could inhibit the proliferation and invasion of prostate cancer. However, the role of miR-103a-3p in BC has not been elucidated.

The aim of the present study was to detect the expression level of serum miR-103a-3p in patients with BC, analyze the association between miR-103a-3p expression and clinicopathological features, and evaluate the ability of circulating miR-103a-3p to predict and diagnose BC.

## Materials and methods

**Clinical samples.** A total of 112 women with BC, who were admitted and received treatment at the Cangzhou Central Hospital (Hebei, China) between January 2009 and

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December 2014 were recruited into the present study. All the patients with BC underwent modified radical mastectomy or breast-conserving surgery. The serum samples were collected one day prior to and following surgery. Patients were included if they were i) histologically confirmed as having invasive ductal breast carcinoma (IDC) type; ii) had no other associated malignancies; iii) had complete follow-up clinicopathological information; and iv) who were disease-free and followed up for at least 5 years. Patients with any neoadjuvant treatment prior to surgery or with bilateral or inflammatory BC were excluded. In addition, a group of 59 age- and sex-matched healthy volunteers were enrolled as a control group, at the same institution between January 2013 and December 2014, and serum samples were also obtained during routine physical examinations. The mean age was  $54.1 \pm 9.8$  years for patients with BC and  $53.9 \pm 9.3$  years for healthy controls. The peripheral blood samples were collected from all participants in serum gel separator tubes. Each sample was centrifuged at  $3,000 \times g$  for 10 min at  $4^{\circ}\text{C}$  to separate serum, then stored at  $-80^{\circ}\text{C}$  until further use.

Clinical data, including age, tumor size, pathological type, lymph-node status, histological grading, metastasis and TNM stage, were also collected. The tumors were staged according to the 8th edition of the American Joint Committee on Cancer (21). Postoperative routine pathological examination, hormonal estrogen receptors (ER), progesterone receptors (PR), and HER2 were tested using immunohistochemistry by two pathologists in a blinded manner at Department of Pathology, Cangzhou Central Hospital (Cangzhou, China) independently. All enrolled patients provided written informed consent for the use of their tissue samples and clinical information in the present study. The Ethics Committee of Cangzhou Central Hospital (Hebei, China; approval no. 20210013) approved the study and was conducted in accordance with the Declaration of Helsinki.

**RNA extraction and reverse transcription-quantitative PCR (RT-qPCR).** Total RNA was extracted from the prepared serum samples using the RNA Isolation kit (Qiagen, Inc.), and the cDNA was synthesized using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RT-qPCR was subsequently performed using the TaqMan miR assay system (cat. no. A25576; Applied Biosystems; Thermo Fisher Scientific, Inc.) on an FTC-3000™ System (Funglyn Biotech Inc.). The thermocycling conditions for RT-qPCR were: Initial denaturation at  $95^{\circ}\text{C}$  for 1 min, followed by 40 cycles of  $95^{\circ}\text{C}$  for 10 sec and  $60^{\circ}\text{C}$  for 35 sec. Relative expression of miR-103a-3p was normalized to that of U6 using the  $2^{-\Delta\Delta C_q}$  method (22). The following primers (Shanghai GenePharma Co., Ltd) were used: miR-103a-3p forward, 5'-ATCCAGTGCGTGTCTGTG-3' and reverse, 5'-TGCTAGCAGCATTGTACAGG-3'; U6 forward, 5'-CGCTTCGGCAGCACATATAC-3' and reverse, 5'-TTCACGAATTTGCGTGTTCAT-3'.

**Statistical analysis.** The data are expressed as the mean  $\pm$  SD from at least three independent experiments. Statistical evaluations were performed using SPSS v20.0 (IBM Corp.). Differences between two groups were analyzed using an unpaired Student's t-test, while the expression of miR-103a-3p

in BC serum tissues before and after surgery was compared using a paired Student's t-test. Comparisons of multiple groups were performed using ANOVA followed by Tukey's post hoc test. Categorical data were compared using either a  $\chi^2$  test or a Fisher's exact test. Based on the median values of miR-103a-3p expression, patients with BC were classified into either miR-103a-3p low ( $n=56$ ) or high expression ( $n=56$ ) groups. Receiver operating characteristic (ROC) curves were utilized to calculate diagnostic accuracy. The survival outcomes, including overall survival (OS) and recurrence-free survival (RFS) times, were evaluated using Kaplan-Meier curves and compared using a log-rank test. OS time was calculated from the date of surgery to the date of the patient's death or to the date of last follow-up. RFS time was defined between the date of surgery to the date of BC recurrence. Prognostic factors were analyzed using Cox regression proportional hazards analysis.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Study population.** As shown in Table I, 112 patients with BC were recruited into the study, and a total of 59 age-matched healthy female volunteers were used as the control group. There were 81 (72.3%) patients with lymph-node involvement and 28 patients (25.0%) with distant metastasis. With respect to TNM stage, 54 patients with BC (48.2%) were at stage II, 30 patients (26.8%) at stage III, and 28 patients (25.0%) at stage IV. All other clinicopathological data are shown in Table I.

**miR-103a-3p is upregulated in patients with BC.** To primarily investigate the expression level of miR-103a-3p in BC tissues, sera from 112 patients with BC and 59 healthy controls were collected for RT-qPCR analysis. The results showed that miR-103a-3p expression was significantly upregulated in patients with BC compared with that in the controls ( $P < 0.001$ ; Fig. 1A). In addition, serum miR-103a-3p expression was significantly reduced in patients with BC following surgery ( $P < 0.001$ ; Fig. 1B). The miR-103a-3p expression level in patients with positive HER2 status was significantly higher compared with those who are HER2 negative ( $P < 0.001$ ; Fig. 2A). In addition, miR-103a-3p expression level in patients with BC and metastasis was significantly higher compared with that in those without metastasis ( $P < 0.001$ ; Fig. 2B). Comparison of miR-103a-3p expression level between patients with BC and different TNM stages showed statistically significant differences between stages II, III and IV (III vs. II,  $P < 0.001$ ; IV vs. II,  $P < 0.001$ ; IV vs. III,  $P < 0.001$ ; Fig. 2C). Furthermore, there was no significant difference between miR-103a-3p expression levels with respect to the molecular subtypes of BC (Luminal B vs. Luminal A,  $P = 0.732$ ; HER2 enriched vs. Luminal A,  $P = 0.840$ ; Triple negative vs. Luminal A,  $P = 0.501$ ; HER2 enriched vs. Luminal B,  $P = 0.667$ ; Triple negative vs. Luminal B,  $P = 0.341$ ; Triple negative vs. HER2 enriched,  $P = 0.829$ ; Fig. 2D).

**Association between serum miR-103a-3p expression level and clinicopathological features of BC.** The association between the miR-103a-3p expression level and the clinicopathological features in patients with BC was further analyzed. It was found

Table I. Association between miR-103a-3p expression level and clinicopathological features in patients with breast cancer.

Clinicopathological feature	Number (n=112)	miR-103a-3p expression		P-value
		Low (n=56)	High (n=56)	
Mean age $\pm$ SD, years		54.7 $\pm$ 10.3	53.1 $\pm$ 9.6	0.195 <sup>a</sup>
Tumor size, cm, n (%)				0.357 <sup>b</sup>
$\leq 2$	24 (21.4)	14 (25.0)	10 (17.9)	
$> 2$	88 (78.6)	42 (75.0)	46 (82.1)	
Pathological type, n (%)				0.844 <sup>c</sup>
IDC I	9 (8.0)	5 (8.9)	4 (7.1)	
IDC II	54 (48.2)	28 (50.0)	26 (46.4)	
IDC III	49 (43.8)	23 (41.1)	26 (46.4)	
Lymph-node status, n (%)				0.291 <sup>b</sup>
Negative	31 (27.7)	18 (32.1)	13 (23.2)	
Positive	81 (72.3)	38 (67.9)	43 (76.8)	
Histological grading, n (%)				0.638 <sup>c</sup>
I	10 (8.9)	6 (10.7)	4 (7.1)	
II	56 (50.0)	29 (51.8)	27 (48.2)	
III	46 (41.1)	21 (37.5)	25 (44.6)	
ER, n (%)				0.686 <sup>b</sup>
Negative	36 (32.1)	19 (33.9)	17 (30.4)	
Positive	76 (67.9)	37 (66.1)	39 (69.6)	
PR, n (%)				0.566 <sup>b</sup>
Negative	47 (42.0)	25 (44.6)	22 (39.3)	
Positive	65 (58.0)	31 (55.4)	34 (60.7)	
HER2, n (%)				0.018 <sup>b</sup>
Negative	40 (35.7)	26 (46.4)	14 (25.0)	
Positive	72 (64.3)	30 (53.6)	42 (75.0)	
Molecular subtype <sup>d</sup> , n (%)				0.968 <sup>c</sup>
Luminal A	36 (32.1)	19 (33.9)	17 (30.4)	
Luminal B	27 (24.1)	14 (25.0)	13 (23.2)	
HER2 enriched	10 (8.9)	5 (8.9)	5 (8.9)	
Triple negative	39 (34.8)	18 (32.1)	21 (37.5)	
Metastasis, n (%)				0.002 <sup>b</sup>
Absent	84 (75.0)	49 (87.5)	35 (62.5)	
Present	28 (25.0)	7 (12.5)	21 (37.5)	
TNM stage, n (%)				0.028 <sup>b</sup>
II	54 (48.2)	32 (57.1)	22 (48.2)	
III	30 (26.8)	16 (28.6)	14 (26.8)	
IV	28 (25.0)	8 (14.3)	20 (25.0)	

<sup>a</sup>Tested using an unpaired Student's t-test; <sup>b</sup>tested using  $\chi^2$  test; <sup>c</sup>tested using Fisher's exact test; <sup>d</sup>receptor classification. miR, microRNA; ER, estrogen receptor; PR, progesterone receptor; HER2, epidermal growth factor receptor 2; IDC, invasive ductal breast carcinoma.

that miR-103a-3p expression was significantly associated with HER2 status (P=0.018), metastasis (P=0.002) and TNM stage (P=0.028) (Table I).

**Diagnostic value of miR-103a-3p in patients with BC.** To evaluate the diagnostic value of miR-103a-3p in patients with BC, the performance of serum miR-103a-3p level in distinguishing between patients with BC and the controls

was performed using ROC analysis. As shown in Fig. 3A, the optimal diagnostic cut-off value for miR-103a-3p was 3.01, and the AUC value for miR-103a-3p was 0.697 [95% confidence interval (CI), 0.615–0.778], with a sensitivity and specificity of 78.2 and 74.7%, respectively. Furthermore, ROC analysis also demonstrated that the optimal diagnostic cut-off value for miR-103a-3p was 3.4, and the AUC value for miR-103a-3p was 0.936 (95% CI, 0.892–0.980), with a sensitivity and specificity

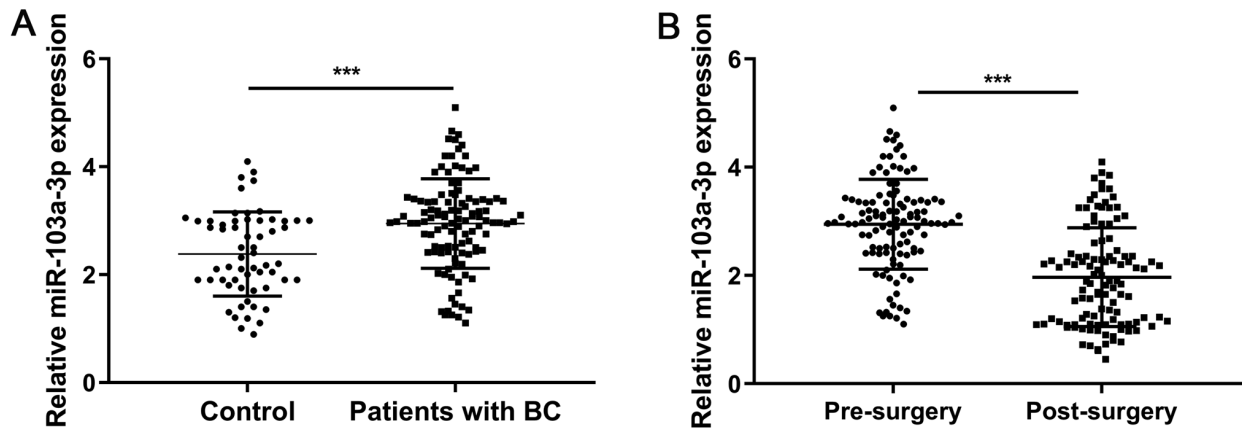


Figure 1. Upregulation of miR-103a-3p in serum from patients with BC. Reverse transcription-quantitative PCR analysis of serum miR-103a-3p expression in (A) patients with BC and healthy controls and in (B) patients with BC before and after surgery. \*\*\* $P<0.001$ . BC, breast cancer; miR, microRNA.

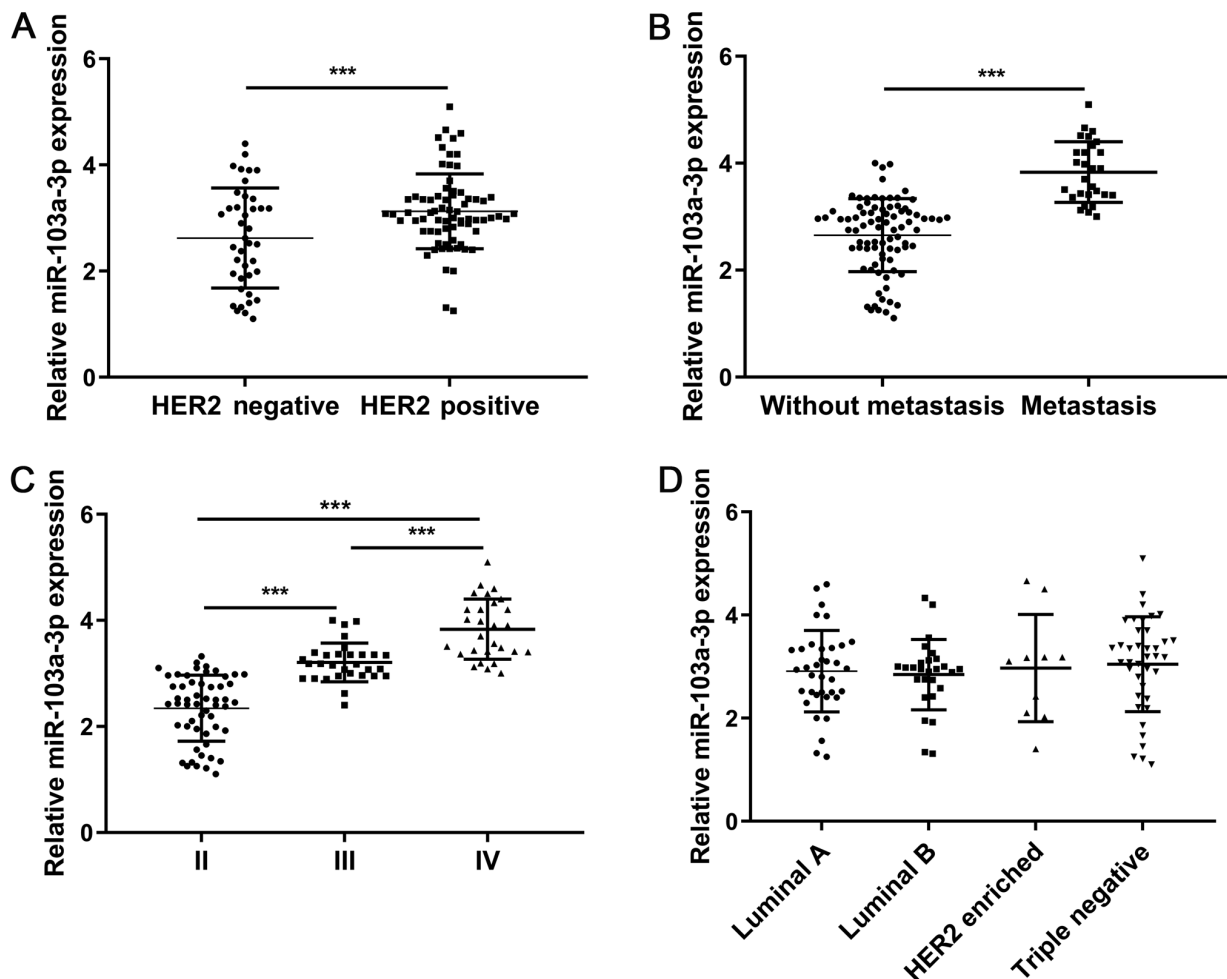


Figure 2. Reverse transcription-quantitative PCR analysis of miR-103a-3p expression in serum from patients with BC stratified by (A) HER2 status, (B) metastasis, (C) TNM stage and (D) molecular subtypes. Luminal B vs. Luminal A,  $P=0.732$ ; HER2 enriched vs. Luminal A,  $P=0.501$ ; HER2 enriched vs. Luminal B,  $P=0.667$ ; Triple negative vs. Luminal B,  $P=0.341$ ; Triple negative vs. HER2 enriched,  $P=0.829$ . \*\*\* $P<0.001$ . BC, breast cancer; miR, microRNA.

of 88.6 and 84.0%, respectively, in distinguishing patients with BC and metastasis from those without metastasis (Fig. 3B).

*Prognostic value of miR-103a-3p in patients with BC.* Next, using Kaplan-Meier curves to analyze OS time, the results

showed that patients with BC and a high expression level of miR-103a-3p was associated with worse OS ( $P=0.016$ ) (Fig. 4A) and RFS times ( $P=0.033$ ) (Fig. 4B) compared with that in patients with a low expression level of miR-103a-3p. Univariate Cox regression analyses demonstrated that HER2 status

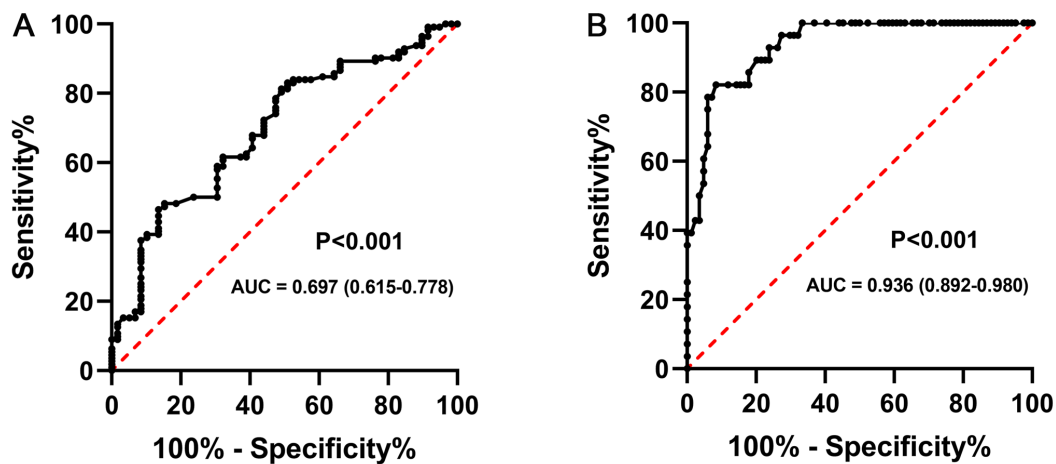


Figure 3. Diagnostic power of miR-103a-3p in patients with BC. Receiver operating characteristic curve indicates the ability of serum miR-103a-3p to distinguish between (A) patients with BC from the controls and (B) patients with BC and metastasis from patients with BC and without metastasis. BC, breast cancer; AUC, area under the curve; miR, microRNA.

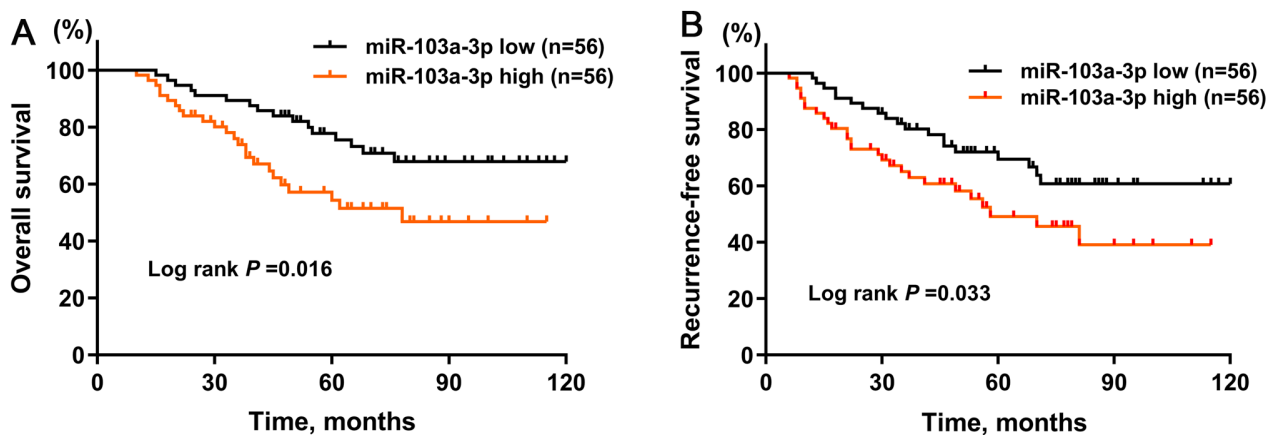


Figure 4. Prognostic power of miR-103a-3p in patients with BC. Kaplan-Meier curves of (A) overall survival and (B) recurrence-free survival in patients with BC stratified by miR-103a-3p expression level. BC, breast cancer; miR, microRNA.

[hazard ratio (HR), 2.141; 95% CI, 1.254-3.274;  $P=0.002$ ), metastasis (HR, 2.841; 95% CI, 1.542-3.984;  $P=0.001$ ), TNM stage (III vs. II, HR, 2.547; 95% CI, 1.564-3.854;  $P<0.001$ ; and IV vs. II, HR, 2.951; 95% CI, 1.785-3.641;  $P<0.001$ ) and high miR-103a-3p expression (HR, 1.774; 95% CI, 1.452-2.051;  $P=0.005$ ) were independent indicators for poor OS time in patients with BC (Table II). Multivariate Cox regression analyses showed that HER2 status [HR, 1.952; 95% CI, 1.112-2.874;  $P=0.012$ ), metastasis (HR, 2.412; 95% CI, 1.214-3.174;  $P=0.005$ ), TNM stage (III vs. II, HR, 2.471; 95% CI, 1.384-3.641;  $P=0.001$ ; and IV vs. II, HR, 2.814; 95% CI, 1.541-3.285;  $P<0.001$ ) and high miR-103a-3p expression (HR, 1.612; 95% CI, 1.314-1.854;  $P=0.023$ ) were independent indicators for poor OS time in patients with BC (Table II). In addition, univariate Cox regression analyses showed that HER2 status (HR, 2.325; 95% CI, 1.653-3.018;  $P=0.001$ ), metastasis (HR, 3.145; 95% CI, 2.521-3.954;  $P<0.001$ ), TNM stage (III vs. II, HR, 2.154; 95% CI, 1.621-2.963;  $P<0.001$ ; and IV vs. II, HR, 2.335; 95% CI, 1.841-3.115;  $P<0.001$ ) and high miR-103a-3p expression (HR, 1.684; 95% CI, 1.351-1.997;  $P=0.002$ ) were independent indicators for poor RFS (Table III). Multivariate Cox regression analyses revealed that HER2 status (HR,

2.010; 95% CI, 1.532-2.991;  $P=0.009$ ), metastasis (HR, 2.888; 95% CI, 2.113-3.208;  $P=0.001$ ), TNM stage (III vs. II, HR, 1.997; 95% CI, 1.554-2.563;  $P=0.003$ ; and IV vs. II, HR, 2.117; 95% CI, 1.609-3.695;  $P<0.001$ ) and high miR-103a-3p expression (HR, 1.333; 95% CI, 1.241-1.763;  $P=0.029$ ) were independent indicators of poor RFS time (Table III). Taken together, the results indicated that miR-103a-3p was an independent unfavorable prognostic factor in patients with BC.

## Discussion

BC is an aggressive cancer and commonly diagnosed at a late stage, with a risk of developing metastasis (23). Recently, a spectrum of miRNAs has been determined to be of great importance during the progression of BC, which may benefit BC diagnosis and prognosis (24). For example, Li *et al* (25) identified a panel of five plasma miRNAs (let-7b-5p, miR-122-5p, miR-146b-5p, miR-210-3p and miR-215-5p) to detect BC with high sensitivity and specificity. Zhang *et al* (26) screened a panel of 3 miRNAs (miR-199a, miR-29c and miR-424) for differentiating patients with BC from controls, with the highest diagnostic accuracy.

Table II. Univariate and multivariate analyses of prognostic factors associated with overall survival.

Variable	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years		0.952		
≤50	Ref			
>50	1.125 (0.895-1.425)			
Tumor size, cm		0.184		
≤2	Ref			
>2	1.235 (0.821-1.841)			
Pathological type		0.115		
IDC I	Ref			
IDC II	1.851 (0.912-2.541)	0.252		
IDC III	1.998 (0.925-2.845)	0.184		
Lymph-node status		0.098		
Negative	Ref			
Positive	1.965 (0.841-2.862)			
Histological grading		0.118		
I	Ref			
II	1.541 (0.852-2.415)	0.102		
III	1.815 (0.841-2.984)	0.215		
ER		0.521		
Negative	Ref			
Positive	1.276 (0.862-1.961)			
PR		0.181		
Negative	Ref			
Positive	1.452 (0.951-1.864)			
HER2		0.002 <sup>b</sup>		0.012 <sup>b</sup>
Negative	Ref		Ref	
Positive	2.141 (1.254-3.274)		1.952 (1.112-2.874)	
Molecular subtype <sup>a</sup>		0.841		
Luminal A	Ref			
Luminal B	1.241 (0.865-1.874)	0.546		
HER2 enriched	0.985 (0.741-1.324)	0.214		
Triple negative	1.141 (0.741-1.685)	0.623		
Metastasis		0.001 <sup>b</sup>		0.005 <sup>b</sup>
Absent	Ref		Ref	
Present	2.841 (1.542-3.984)		2.412 (1.214-3.174)	
TNM stage		<0.001 <sup>b</sup>		<0.001 <sup>b</sup>
II	Ref		Ref	
III	2.547 (1.564-3.854)	<0.001 <sup>b</sup>	2.471 (1.384-3.641)	0.001 <sup>b</sup>
IV	2.951 (1.785-3.641)	<0.001 <sup>b</sup>	2.814 (1.541-3.285)	<0.001 <sup>b</sup>
miR-103a-3p expression		0.005 <sup>b</sup>		0.023 <sup>b</sup>
Low	Ref		Ref	
High	1.774 (1.452-2.051)		1.612 (1.314-1.854)	

<sup>a</sup>Receptor classification; <sup>b</sup>P<0.05. miR, microRNA; HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; HER2, epidermal growth factor receptor 2; IDC, invasive ductal breast carcinoma.

Various studies have associated miR-103a-3p in tumor progression. As reported, miR-103a-3p expression levels were increased in gastric cancer tissues and enhanced overexpression

of miR-103a-3p promoted gastric cancer cell proliferation (16). Zhang *et al* (14) reported that miR-103a-3p was overexpressed in thyroid cancer tissues and knocking down its expression

Table III. Univariate and multivariate analyses of prognostic factors associated with recurrence-free survival.

Variable	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, years		0.558		
≤50	Ref			
>50	1.010 (0.874-1.351)			
Tumor size, cm		0.009		
≤2	Ref			
>2	1.351 (0.885-1.652)			
Pathological type		0.652		
IDC I	Ref			
IDC II	1.415 (0.886-2.412)	0.141		
IDC III	1.652 (0.904-2.214)	0.384		
Lymph-node status		0.196		
Negative	Ref			
Positive	1.741 (0.652-2.819)			
Histological grading		0.225		
I	Ref			
II	1.521 (0.854-1.912)	0.852		
III	1.662 (0.910-2.041)	0.102		
ER		0.274		
Negative	Ref			
Positive	1.112 (0.991-1.421)			
PR		0.206		
Negative	Ref			
Positive	1.352 (0.928-1.657)			
HER2		0.001 <sup>b</sup>		0.009 <sup>b</sup>
Negative	Ref		Ref	
Positive	2.325 (1.653-3.018)		2.010 (1.532-2.991)	
Molecular subtype <sup>a</sup>		0.524		
Luminal A	Ref			
Luminal B	1.041 (0.925-1.354)	0.256		
HER2 enriched	0.912 (0.825-1.319)	0.741		
Triple negative	1.085 (0.952-1.351)	0.230		
Metastasis		<0.001 <sup>b</sup>		0.001 <sup>b</sup>
Absent	Ref		Ref	
Present	3.145 (2.521-3.954)		2.888 (2.113-3.208)	
TNM stage		<0.001 <sup>b</sup>		<0.001 <sup>b</sup>
II	Ref		Ref	
III	2.154 (1.621-2.963)	<0.001 <sup>b</sup>	1.997 (1.554-2.563)	0.003 <sup>b</sup>
IV	2.335 (1.841-3.115)	<0.001 <sup>b</sup>	2.117 (1.609-3.695)	<0.001 <sup>b</sup>
miR-103a-3p expression		0.002 <sup>b</sup>		0.029 <sup>b</sup>
Low	Ref		Ref	
High	1.684 (1.351-1.997)		1.333 (1.241-1.763)	

<sup>a</sup>Receptor classification; <sup>b</sup>P<0.05. miR, microRNA; HR, hazard ratio; ER, estrogen receptor; PR, progesterone receptor; HER2, epidermal growth factor receptor 2; IDC, invasive ductal breast carcinoma.

could inhibit cell proliferation, migration and invasion, and promote thyroid cancer cell apoptosis. In addition, knocking down miR-103a-3p expression in oral squamous cell carcinoma

could repress cell proliferation and induce apoptosis (17). Analysis of the clinical samples in the present study revealed that miR-103a-3p was upregulated in patients with BC and

was also significantly associated with advanced features of BC, including positive HER2 status, metastasis and a more advanced TNM stage. High expression of miR-103a-3p was also associated with poor survival outcomes. miR-103a-3p may represent a potential diagnostic biomarker and therapeutic target in patients with BC at different TNM stages. Notably, tumor metastasis is the main obstacle to prognosis in patients with BC. It was found that serum miR-103a-3p expression was markedly elevated in patients with BC and tumor metastasis, which furthers the understanding into the potential role of miR-103a-3p during BC metastasis.

Numerous studies have discussed the critical role of circulating miRNA expression as a non-invasive biomarker for early detection of numerous types of cancer (27-29), as serum samples are stable, and easily accessible for testing using RT-qPCR. However, some studies have recently reported that hemolysis during blood collection or sample processing can alter the levels of certain proposed miRNAs, such as miR-106a, miR-16 and miR-17 (30). To avoid having misleading results, it is vital to investigate whether hemolysis could affect the expression level of each miRNA in future studies. Notably, neither miR-103a-3p or U6 have been reported to be affected by hemolysis (30,31). Recently, circulating miR-103a-3p has become an important area as a potential non-invasive biomarker for the diagnosis and prognosis of multiple types of cancer. For example, Zhang *et al* (15) established a panel of seven miRNAs in plasma, including miR-103a-3p, to predict the occurrence of colorectal cancer. In addition, Weber *et al* (18) demonstrated that the combination of mesothelin and miR-103a-3p in plasma could mutually enhance the diagnostic performance in the detection of malignant mesothelioma. However, the diagnostic function of miR-103a-3p has not been elucidated in BC. In the present study, the results indicated that circulating serum miR-103a-3p expression could discriminate patients with BC from control subjects prior to surgery. In addition, miR-103a-3p expression had a high ability to distinguish patients with BC and metastasis from those without metastasis. Traditionally, some important predictive or prognostic biomarkers, including tumor size, tumor grade, lymph node involvement, ER status and HER2 status have been used for patients with BC (32). However, tumor tissue is required for the evaluation of all the aforementioned biomarkers, which limits their clinical applications. In the present study, miR-103a-3p was detected easily and stably in peripheral blood, and could be a new prognostic and predictive marker in patients with BC. In addition, miR-103a-3p may be a potential therapeutic target in patients with BC due to its association with tumor metastasis and stage. For patients with BC and a high expression level of miR-103a-3p, more precision treatment should be utilized to reduce the rate of tumor metastasis and improve patient prognosis as well.

There are some limitations in the present study. First, there is a lack of an external cohort to validate the diagnostic and prognostic ability of miR-103a-3p. In addition, the biological role of miR-103a-3p and its underlying mechanism during BC initiation and progression remain to be clarified.

In conclusion, the results from the present study demonstrate that miR-103a-3p was upregulated in patients with BC, and miR-103a-3p could act as a promising diagnostic and prognostic biomarker in patients with BC. Further

studies are warranted to validate the diagnostic and prognostic value of miR-103a-3p with larger sample sizes, and to investigate the biological roles of miR-103a-3p in BC growth and metastasis.

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## Availability of data and materials

All data generated and/or analyzed during the study are available from the corresponding author upon reasonable request.

## Author's contributions

HL and HBC conceived and designed the present study. QZB and WZ collected clinical samples and analyzed the data. HL and HBC wrote the manuscript. HL and HBC confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

All enrolled patients provided written informed consent for the use of their tissue samples and clinical information in the study. The Ethics Committee of Cangzhou Central Hospital (Hebei, China; approval no. 20210013) and was conducted in accordance with the Declaration of Helsinki.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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