Original Article
Serum *microRNA-34c* acts as a novel diagnostic biomarker in breast cancer patients

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Abstract: Background: *MicroRNA-34* (*miR-34*) is consisted of three miRNAs and has been reported to abnormal expression in various cancers. The aim of this study was to detect the expression of *miR-34c* and its diagnostic value in breast cancer. Methods: Quantitative Real-Time reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to detect the expression of *miR-34c* in 107 patients with breast cancer and 93 healthy controls. The relationship between *miR-34c* expression and clinicopathological characteristics in breast cancer was estimated through chi-square test. The diagnostic value of *miR-34c* was evaluated by building a receiver operating characteristic (ROC) curve. Results: The expression of *miR-34c* was down-regulated in the serum of patients with breast cancer compared with those of healthy controls. Its down-regulation was significantly correlated with stage (*P*=0.046), tumor grade (*P*=0.000) and lymph node status (*P*=0.025). Furthermore, the value of the area under the receiver-operating characteristic curve (AUC-ROC) was 0.854. The optimal cutoff value was 4.345, providing a sensitivity of 72.00% and a specificity of 88.80%. Conclusion: Our data indicated that *miR-34c* was increased, and it might be a potential independent diagnostic bio-marker in breast cancer.

**Keywords:** *MicroRNA-34c*, breast cancer, diagnosis

Introduction

Breast cancer is the most common malignant disease in many countries and is a leading cause of cancer-related death among women worldwide [1]. There were about 248,620 new cases be diagnosed in China in 2011 which made it was one of the major concerns for female health [2, 3]. The pivotal issue for patients with this cancer is to detect it at an early stage. Although there are a certain degree of improvements in cancer screening techniques, the diagnosis of breast cancer using biomarkers remains a major challenge [4]. An increasing number of studies have identified a diverse array of breast cancer-related molecules biomarker for monitoring patients with metastasis as non-invasive and sensitive circulating biomarkers, such as cancer antigen 15-3, cancer antigen 27.29, and carcinoembryonic antigen [5, 6]. However, the value of such biomarkers for breast cancer is limited, because of the heterogeneity of breast cancer

cells [7, 8]. Therefore, it is crucial to explore reliable clinical diagnostic biomarkers, especially using noninvasive or minimal invasive methods to detect breast cancer as early as possible and improve the quality of life and survival.

MicroRNAs (miRNAs) are an abundant class of small non-coding RNAs with 18-25 nucleotides in length [9]. These small molecules have been found to regulate gene expression either by translational repression or degradation of mRNA after targeting the 3' UTR [10]. miRNAs have been confirmed to be associated with many processes involved in cancer progression, such as proliferation, differentiation, apoptosis and tumorigenesis [11]. As their abnormal expression, miRNAs have been classified as oncogene and tumor suppressor in caners [12]. It has been also demonstrated that the possible use of miRNA expression profiles could distinguish normal from neoplastic tissues and lead to the identification of new diagnostic and/or prognostic markers in a wide
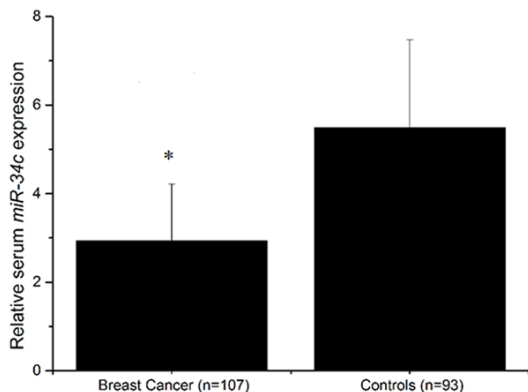
Figure 1. The expression for serum miR-34c in patients with breast cancer and healthy controls. Serum *miR-34c* was down-regulated in breast cancer patients compared to that in healthy controls (*P*<0.05).

array of human cancers including breast cancer [13-16]. *MicroRNA-34c* (*miR-34c*), a putative tumor-suppressor gene, has been demonstrated to be abnormally expressed in various malignant diseases. However, its clinical significance in breast cancer is still unclear, as well as the diagnostic value.

In the present study, we detected the *miR-34c* expression in breast cancer and analyzed its relationship with clinical factors of patients with breast cancer. Meanwhile, the ROC curve was established in order to identify whether *miR-34c* could discriminate breast cancer patients from healthy controls.

Materials and methods

Patients and sample collection

Serum samples in the discovery stage were collected from 107 female breast cancer patients enrolled at Linyi People's Hospital. All the serum was collected at diagnosis before the patients received surgery and other treatments. The tumor stage at primary diagnosis was classified according to the revised American Joint Committee on Cancer tumor-node-metastasis classification. 93 healthy people who were chosen from population that underwent routine physical examinations with no history of cancer and no clinically diagnosed diseases at Linyi People's Hospital were recruited as healthy controls. Written informed consent which conformed to the principles outlined in the Declaration of Helsinki was signed by all patients and healthy individuals in advance.

This study was approved by the Research and Ethical Committee of Linyi People's Hospital.

4 ml peripheral blood was severally sampled from patients with breast cancer and healthy controls and put into BD Vacutainer tubes immediately. Then the blood samples were stood for 30-60 min, and centrifuged at 2000 rpm for 15 min at room temperature. The supernatant was transferred to an EP tube and stored at -80°C for further use.

RNA extraction and qRT-PCR analysis

Total RNA was extracted from the serum specimens using TRIzol LS reagent (Invitrogen, San Diego, CA, USA) according to the manufacturer's instructions. The first chain of cDNA was synthesized by reverse transcription using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). RT-PCR reaction was conducted in a 7300 Real-Time PCR system (Applied Biosystems) according to the manufacturer's protocol. *U6* small nuclear RNA was taken as internal controls. The relative expression of *miR-34c* expression was calculated using $2^{-\Delta\Delta CT}$ method [17].

Statistical analysis

All the analyses were performed using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA) and the graphs were generated using Origin Pro 9.0. All data were presented as Mean ± SD. Differences between two groups were analyzed by students' t test. Chi-squared test was used to assess the correlation between the expression of serum *miR-34c* and clinical pathological factors of breast cancer patients. ROC curves were constructed to assess the diagnostic value of *miR-34c*. P value <0.05 was considered to be statistically significant.

Results

Expression of serum miR-34c was decreased in breast cancer

The expression of *miR-34c* in 107 patients with breast cancer and 93 healthy controls were analyzed by qRT-PCR analysis. The relative expression of *miR-34c* in breast cancer was 2.932±1.279, while that in healthy controls was 5.491±1.985. The statistical analysis showed that the expression of *miR-34c* was

Table 1. Association of serum *miR-34c* expression and clinicopathological characteristics of breast cancer patients

Characteristics	Cases (n=107)	MiR-34c expression		X²	P values
		Low (n=63)	High (n=44)		
Age (years)					
<50	69	41	28	0.024	0.878
≥50	38	22	16		
Stage					
I-II	71	37	34	3.990	0.046
III	36	26	10		
Tumor grade					
I-II	76	35	41	17.823	0.000
III	31	28	3		
Tumor diameter (cm)					
<2	65	34	31	2.953	0.086
≥2	42	29	13		
Lymph node status					
Negative	29	12	17	5.032	0.025
Positive	78	51	27		
ER status					
Negative	33	17	16	1.069	0.301
Positive	74	46	28		
PR status					
Negative	26	15	11	0.020	0.888
Positive	81	48	33		
HER-2/neu status					
Negative	67	35	32	3.263	0.071
Positive	40	28	12		

significantly lower in patients with breast cancer than that in healthy controls (*P*<0.05; **Figure 1**).

Relationship between serum miR-34c expression and clinicopathological characteristics of patients with breast cancer

Our investigation revealed that *miR-34c* expression was decreased in breast cancer. Therefore, to further investigate the association of *miR-34c* expression and the clinicopathological characteristics of patients, we performed a chi-square test. As shown in **Table 1**, the decreased *miR-34c* expression was proved to be associated with stage (*P*=0.046), tumor grade (*P*=0.000) and lymph node status (*P*=0.025). However, there was no relationship between *miR-34c* expression and other features, including age, tumor diameter, ER status, PR status and HER-2/neu status (*P*>0.05).

Diagnostic value of serum miR-34c in breast cancer

The ROC curve was plotted to identify the value of *miR-34c* in distinguishing breast cancer from healthy controls. The result demonstrated that *miR-34c* had a high diagnostic value with an AUC of 0.854 combing with a sensitivity of 72.0% and a specificity of 88.8%, respectively. And the optimal cutoff value of *miR-34c* expression was 4.345 (**Figure 2**).

Discussion

Breast cancer is a heterogeneous disease because of complicated etiology including genetic and environmental factors. Its malignancy comprises multiple entities associated with distinctive histological and biological features, clinical presentations and behaviors and responses to therapy. From the molecular point of view, breast cancers can be divided into six molecular subtypes [18, 19]. Owing to a low survival of breast cancer patients, it is needed to identify new diagnostic biomarkers for predicting a therapeutic response and clinical outcomes.

With the widely use of various detecting techniques and further research of miRNAs, the relationship between miRNAs and the occurrence as well as development of multiplicate tumors attracts more attention. Potential effects of miRNAs makes it to be important molecular markers in the diagnosis and prognosis of cancers so that provide new therapy strategies in multiple diseases [20]. Circulating miRNAs were also identified by subsequent studies. For instance, the first discovered serum miRNA biomarker was *miR-21*, which was found in high serum levels associated with increased relapse-free survival in patients with diffuse large B-cell lymphoma [21]. In addition, it was showed that circulating miRNAs originated from tumor tissues. And they were present
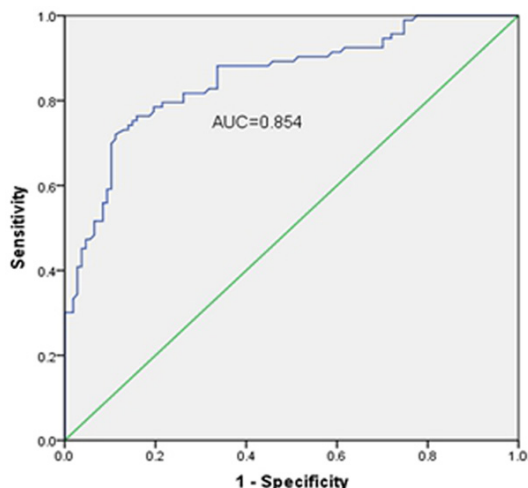
Figure 2. ROC curve for evaluating the accuracy of serum *miR-34c* to discriminate patients with breast cancer from healthy controls.

in a certain form in the blood and resistant to RNase activities [22]. Indeed, their sensitivity and specificity in diseases and noninvasive collection from serum have encouraged the pursuit of research on miRNA biomarkers.

MiR-34c, locates at chromosome 11q23, is a member of miRNA-34 family which is highly conserved among different species [23]. It has been reported to induce cell apoptosis and inhibit cell proliferation and invasion in a variety of tumor cells [24-26]. Moreover, it has been shown that *miR-34c* was downregulated in a number of different malignancies such as neuroblastoma, lung cancer, and colorectal cancer [27-30]. Although *miR-34c* had been vrigifed to abnormal expression in breast cancer via previous studies [31-34], its diagnostic role remained unknown. In the current study, we compared the expression level of *miR-34c* in serum from 107 breast cancers and 93 healthy controls. And the serum *miR-34c* expression was proved to be significantly lower in breast cancer patients than that in healthy controls. It revealed that *miR-34c* might be a tumor suppressor in breast cancer. This was also consistent with previous studies.

To further explore the role of *miR-34c* in breast cancer, we investigated its relationship with clinical factors of patients. The outcome manifested that the low expression of serum miR-34a in breast cancer was tightly related to stage, tumor grade and lymph node status which indicated that it was involved in the pro-

gression of the cancer. Moreover, ROC curve was built and indicated a high diagnostic value of *miR-34c* with an AUC of 0.854 corresponding with a high sensitivity and specificity.

In conclusion, the expression of *miR-34c* in breast cancer patients is lower than that in healthy controls. And it may participate in the development of breast cancer. ROC results display a significant diagnostic accuracy of *miR-34c*. The findings indicate that serum *miR-34c* appears to be potentially useful biomarkers for breast cancer detection. However, as the limitations of this study, some further studies with larger sample involving in validation and optimizing improvement should be conducted to confirm our results.

Disclosure of conflict of interest

None.

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