MicroRNA-124-3p Functions as a Marker in Diagnosing Breast Cancer

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Abstract

Background

Early diagnosis of breast cancer can reduce the high fatality rate. Thus novel diagnostic methods are urgently required for breast cancer patients. The research aimed at checking potential correlation for serum *microRNA-124-3p* (*miR-124-3p*) expression with breast cancer, and exploring its competence as a promising potential biomarkers for breast cancer.

Methods

Quantitative real-time PCR (qRT-PCR) assay detected *miR-124-3p* levels. Possible relationship between *miR-124-3p* degree and clinical features of breast cancer patients was measured by Chi-square analysis. Sensitivity, specificity and area under the curve (AUC) for serum *miR-124-3p* degree were settled adopting receiver operating characteristics (ROC) analyses.

Results

Relative degree for serum *miR-124-3p* notably reduced among breast cancer sufferers in comparison with healthy persons (P=0.000). Moreover, *miR-124-3p* degree held tight connection to clinical stage (P=0.034), histological grade (P=0.002), and lymph node metastasis (P=0.001). ROC curve analyses unveiled at the optimal cut-off of 0.935, serum *miR-124-3p* expression reached a sensitivity of 78.4% and a specificity of 84.8% in discriminating between breast cancers sufferers and healthy persons, achieving an area under the curve (AUC) of 0.872 (95% CI: 0.752-0.871).

Conclusion

Serum *miR-124-3p* degree might represent one non-invasive indicator in diagnosing breast cancer.

Background

Breast cancer represents one most widespread malignancy among females around the world [1]. The incidence of breast cancer varies across the world, usually lower in less-developed countries than that in the more-developed countries. It is of great importance for early detection together with curative resection to improve breast cancer prognoses [2, 3]. Currently, the pathological observation are the main approaches to diagnose this disease. With considerable advancements in identifying serum indicators, it is essentially required to find indicators holding satisfactory specificity and sensitivity through serum detection, which is beneficial for precise screening, target treatment and outcome prediction [4]. Until now, no satisfactory indicators have been established in screening the malignancy. Hence, finding promising novel indicators would held considerable clinical significance.

MicroRNAs (miRNAs) impose negative or positive influences on gene expressions via antisense complimentarily, thus affecting RNA stability and protein translations [5-10]. Changes in miRNA degrees

might alter biological courses such as cellular multiplication, differentiation, and apoptosis, which impose crucial influences on oncogenesis and tumour furtherance [11]. Reportedly, miRNAs take part in oncogenesis, assuming boosting or repressing functions [12-14]. According to existing evidence, miRNAs in serum or plasma were sufficient indicators in diseases [15-18]. As one member in miRNAs, *microRNA-124-3p* （*miR-124-3p*） could wield restraining effects against breast cancer, which negatively regulated the proliferation and invasion for malignant cells [19]. Nonetheless, diagnostic value for serum *miR-124-3p* in breast cancer is far from totally comprehended.

Through the present research, we detected relative degree for serum *miR-124-3p* in breast cancer, and evaluated possible connection for serum *miR-124-3p* to clinicopathological characteristics among cases with breast cancers. We also assessed the diagnostic performance of serum *miR-124-3p* in breast cancer.

Methods And Materials

Patients and serum samples

Our research acquired permission from the Ethic Committee of the 903rd Hospital of PLA. Enrolled subjects signed the informed consents.

Serum specimens came from 105 breast cancer sufferers receiving treatments in the 903rd Hospital of PLA. Histopathology for cases was examined. Cases would be removed from our study once they had other cancers at any sites or experienced chemotherapy or radiotherapy ahead of sampling. Detailed clinicopathological indexes for cases were manifested by **Table 1**. 97 normal serum samples from healthy volunteers was recruited as controls.

Table 1 The correlation of serum *miR-124-3p* expression with clinicopathological parameters of breast cancer patients

Parameters	NO. of cases (n=105)	*MiR-124-3p* expression		c^2	*P* values
		Low (n=54)	High (n=51)		
Age (years)				0.351	0.554
≤60	67	33	34		
>60	38	21	17		
Tumor size				0.587	0.444
≤2	64	31	33		
>2	41	23	18		
Clinical stage				4.473	0.034
□-□	72	32	40		
□	33	22	11		
Histological grade				9.583	0.002
□-□	81	35	46		
□	24	19	5		
ER				0.561	0.454
Negative	62	30	32		
Positive	43	24	19		
PR				0.061	0.805
Negative	30	16	14		
Positive	75	38	37		
HER2				0.008	0.929
Negative	51	26	25		
Positive	54	28	26		
Lymph node metastasis				11.147	0.001
Absent	85	37	48		
Present	20	17	3		

ER: estrogen receptor; PR: progesterone receptor; HER-2: C-erbB-2.

RNA extraction and quantitative real-time PCR

Total RNA was separated for serum samples adopting Trizol reagent (Takara, Dalian, China) abiding by the producer's guidance. RNA was reverse transcribed into cDNA employing One Step PrimeScript miRNA cDNA Synthesis Kit (Takara, Dalian, China). Then, we implemented quantitative RT-PCR assays (qPCR). miRNA degree was evaluated applying Applied Biosytems 7500 Fast Real-time PCR System (Applied Biosystems, Carlsbad, USA) with SYBR Premix Ex Taq (Takara, Dalian, China) abiding by corresponding indications. The relative expressions for *miR-124-3p* were quantified using $2^{-\Delta\Delta Ct}$ approach [20], namely $\Delta Ct=Ct_{miRNA}-Ct_{endogenous\ control}$ in which Ct represents threshold cycle, or the amount of cycles where fluorescent signal surpass the threshold. *RNU6B* degree represented internal reference.

Statistical analysis

All the data syntheses were completed in SPSS 18.0 (SPSS. Chicago, IL, USA), and graphs were plotted by Origin 9.0. The statistical differences between two groups was checked through independent sample *t* test. analysis evaluated potential link for serum *miR-124-3p* degree to clinicopatholgical characteristics among included sufferers. Receive operating characteristic (ROC) curve calculated sensitivity and specificity for serum *miR-124-3p* expression and estimate the diagnostic capability in breast cancer. *P* under 0.05 meant the presence of statistical significance.

Results

Serum *miR-124-3p* expression among breast cancer and healthy controls

As shown in **Figure 1**, qRT-PCR revealed serum *miR-124-3p* level strongly lowered among breast cancer sufferers in comparison to healthy persons (0.669±0.296 vs 1.130±0.434). The median *miR-124-3p* degree among all 105 patients with breast cancer was 0.64. We classified enrolled cases into low (n=54) and high (n=51) *miR-124-3p* level classes using the median degree as a cutoff.

Correlation for serum *miR-124-3p* degree with clinicopathological traits among sufferers with breast cancer

According to the results of Chi-square test, serum *miR-124-3p* degree exhibited strong connection to clinical stage (*P*=0.034), histological grade (*P*=0.002), and lymph node metastasis (*P*=0.001), but not to age, cancer dimension, ER, PR, or HER-2 (all *P*>0.05).

Diagnostic efficacy for serum *miR-124-3p* in breast cancers

ROC curves was plotted to identify whether *miR-124-3p* could discriminate between breast cancer sufferers and healthy persons. The results unveiled at the optimal cut-off (0.935), serum level of *miR-124-3p* achieved a sensitivity of 78.4% and a specificity of 84.8% to discriminate the cases and controls, reaching an area under the curve AUC of 0.872 (95% CI: 0.752-0.871, **Figure 2**).

Discussion

Over the decades, survivals for breast cancer sufferers at early stages are lengthened [21]. Apart from timely diagnosis, adjuvant chemotherapy and endocrine therapy also considerably better cases' overall survivals. There are two traditional indicators in diagnosing this malignancy, CA15.3 and CEA, and the previous studies have demonstrated sensitivity and specificity for them lie at 15-45% and 10-30%, separately. They are unsatisfactory for screening of breast cancer. Recently, *miR-124-3p* was reported to be generally reduced in multiple tumors while its transfection suppresses the malignant cellular growth and migration [22-25]. For example, Gov E et al. reported that *miR-124-3p* was a plausible indicator in ovarian cancer [22]. Yuan and colleagues demonstrated *miR-124-3p* expression down-regulated amid bladder cancer tissues and cell lines, which played important role on malignant cellular multiplication, emigration and apoptosis [23]. Moreover, in the study of Margolin-Miller Y and colleagues, high *miR-124-3p* degree held strong connection to shortened progression-free survivals among ependymoma patients, which might represent a self-sufficient indicator for prognoses in this disease [24]. Zhang and colleagues claimed *miR-124-3p* declined among breast cancer tissues and breast cell lines. Its declines promoted malignant cellular advancement primarily through elevating degree for autophapy relevant protein, Beclin-1 [25].

Currently, explorations on indicators wield essential functions in comprehending mechanisms for varied illnesses. Despite their employment in diagnosing breast cancer, CEA and CA15.3 merely possess insufficient sensitivity and specificity, especially for cases at early stages. MicroRNAs could generate pivotal influences on tumorigenesis. It has been revealed half of them seat at tumour-relevant genomic positions/fragile loci, which indicated their involvement in tumour origination and development [26]. Currently, miRNAs have great potential as novel biomarkers in diagnosing, therapeutic monitor, and predicting outcomes among breast cancer sufferers.

In this research, serum *miR-124-3p* degree dramatically reduced among breast cancer sufferers in comparison to controls. Moreover, we found that serum *miR-124-3p* degree possessed tight connection to clinical stage, histological grade, and lymph node metastasis, suggesting its involvement in breast cancer advancement and metastasis. Our findings were consistent with the previous studies [25]. ROC curve analyses confirmed serum *miR-124-3p* degree had a high sensitivity and specificity in discriminating between breast cancer sufferers and healthy persons, reaching high AUC values.

Multiple shortcomings of this research should be noted. Firstly, *miR-124-3p* might assume repressing effects against breast cancer, but potential mechanisms for such activities remained unknown. Future experiments are still necessary to ascertain unspecified influences for *miR-124-3p* and its aims in the malignancy onset and development. Another limitation is the relatively small size. Larger-scale researches would be indispensable to confirm efficacy for serum *miR-124-3p* expression to diagnose breast cancer for making more definitive conclusions.

Conclusion

Taken together, serum *miR-124-3p* expression dramatically decreased among breast cancer sufferers in comparison to healthy persons. Low *miR-124-3p* degree exhibited strong connection to the cancer development and progression. Our findings offered primary evidence of *miR-124-3p* affecting breast cancer and its potential to be adopted in early diagnosing this malignancy.

Abbreviations

microRNA-124-3p (*miR-124-3p*)

Quantitative real-time PCR (qRT-PCR)

area under the curve (AUC)

receiver operating characteristics (ROC)

MicroRNAs (miRNAs)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of the 903rd Hospital of PLA and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Y.M. design of the work; Q.X. the acquisition, analysis, L.C. interpretation of data; L.W. the creation of new software used in the work; X.H. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

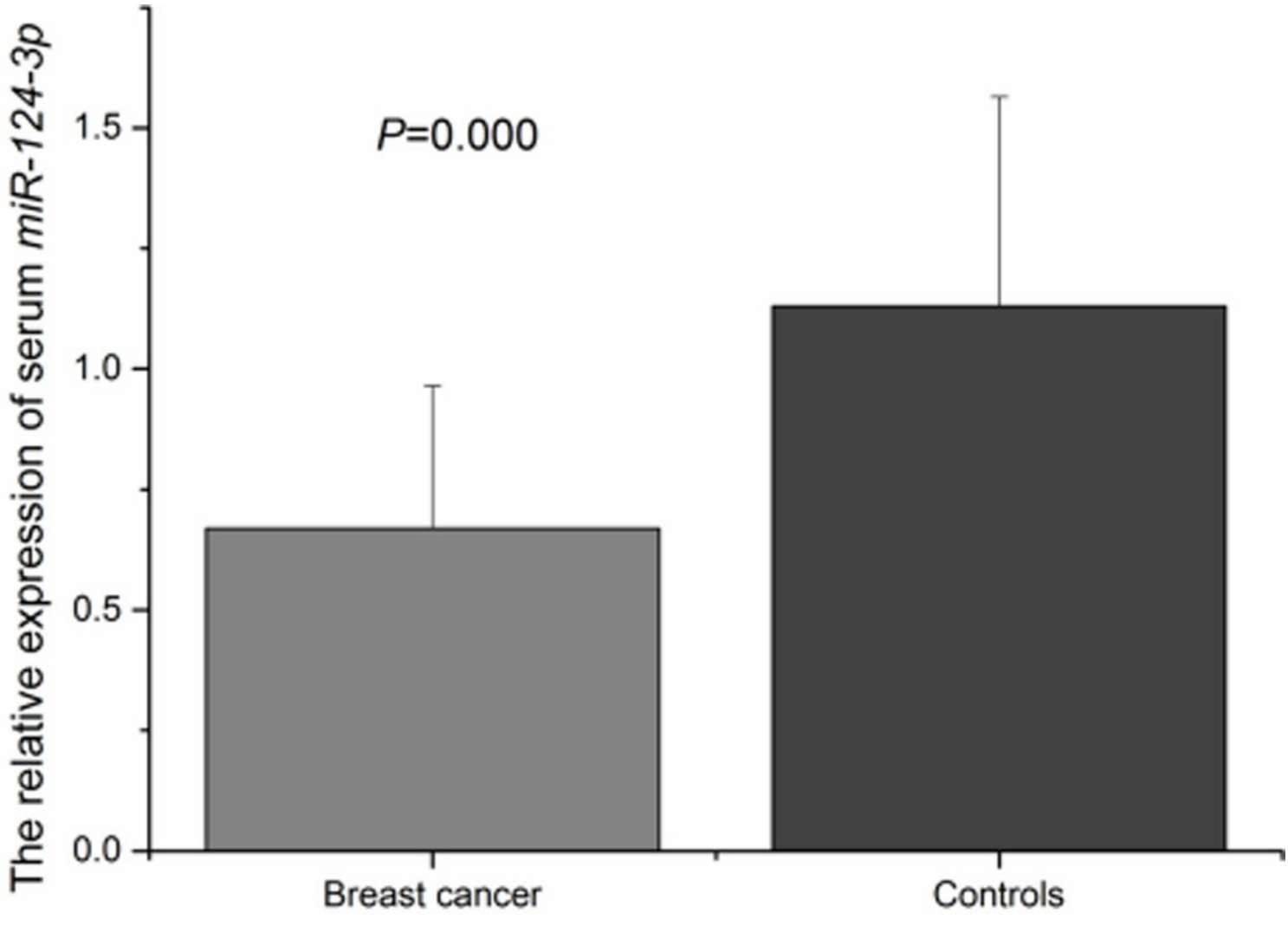

Figure 1

Serum miR-124-3p expression level in breast cancer patients and healthy controls. Serum expression level of miR-124-3p in patients with breast cancer was significantly lower than healthy controls (P=0.000).

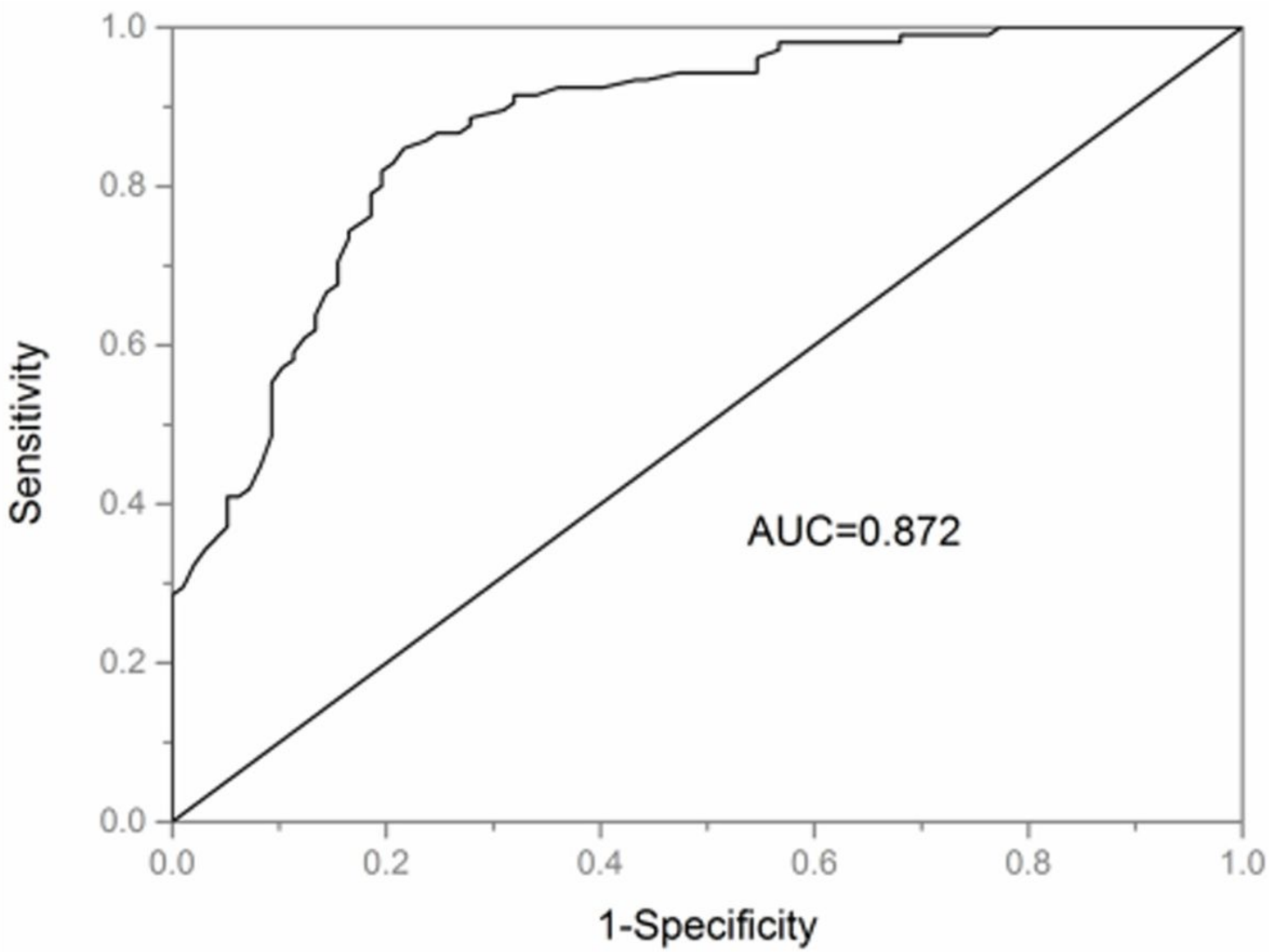

Figure 2

Receiver operator characteristic (ROC) analysis of miR-124-3p in breast cancer. The results showed miR-124-3p had a sensitivity of 78.4% and a specificity of 84.8% for distinguishing breast cancer patients from healthy controls with an area under the curve (AUC) of 0.812 (95% CI : 0.752-0.871).