MicroRNA-411 represents an innovative bio-marker in breast cancer detection

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Abstract

Background: MicroRNA-411 (*MiR-411*) has been reported to play an important role in tumorigenesis. This study was aimed to investigate the diagnostic performance of serum *miR-411* in breast cancer.

Methods: The serum level of *miR-411* was determined in breast cancer patients using quantitative real-time PCR (qRT-PCR). Chi-square was applied to evaluate the association between *miR-411* expression and clinical characteristics. The diagnostic value of serum *miR-411* for breast cancer was estimated using receiver operator characteristic (ROC) analysis.

Results: Serum *miR-411* in patients with breast cancer was markedly decreased compared with healthy controls ($P < 0.001$). The level of *miR-411* was correlated with clinical stage ($P = 0.019$), histological grade ($P = 0.014$), and lymph node metastasis ($P = 0.036$). ROC curve showed that serum level of *miR-411* could discriminate between breast cancer patients and healthy controls, with the AUC of 0.796, combining with the sensitivity of 82.1% and the specificity of 83.2%. The cut-off value of *miR-411* for breast cancer diagnosis was 1.245.

Conclusions: *MiR-411* plays inhibitory roles in aggressive progression of breast cancer. Serum *miR-411* may be a potential non-invasive biomarker for breast cancer diagnosis.

Background

Breast cancer is one of the most common cancers among women, with severe mortality [1]. The mainly cause of breast cancer death is tumor metastasis [2]. Despite of great progress made in treatments, numbers of breast cancer patients die from the metastatic disease [3]. The therapeutic effects are significantly associated with tumor stage at diagnosis [4]. Early detection remains a major challenge for breast cancer [5]. Biomarkers detection provides an effective approach for early diagnosis of breast cancer. The commonly used biomarkers for breast cancer diagnosis include CEA (carcinoembryonic antigen), CA153 (carbohydrate antigen 153), HER-2/Neu, estrogen receptor (ER), progesterone receptor (PR), epidermal growth factor receptor (EGFR), and vascular endothelial growth factor receptor (VEGFR) [6]. Unfortunately, the low specificity and sensitivity limit their clinical application in breast cancer screening [7–9]. As a consequence, new biomarkers are urgently needed in early detection and screening of breast cancer.

MicroRNAs (miRNAs) are a novel class of small non-coding RNAs that play regulatory roles in gene expression at the post-transcriptional level [10, 11]. MiRNAs take part in regulation of various important cellular processes, such as differentiation, migration, and apoptosis [12]. Abnormal expression of miRNAs may contribute to pathological mechanisms of human disease, like cancer [13]. The expression profiles of miRNAs are stable in body fluids and archived tissue samples which can be detected by non-invasive methods, suggesting that detection of miRNAs may provide a promising way for cancer diagnosis [14–16]. MicroRNA-411 (*MiR-411*) is a common member of miRNA family, and its dysregulation has been reported to be associated with several types of cancer. For examples, Xia et al. reported that *miR-411*

served as an oncogene in hepatocellular carcinoma by promoting cell proliferation of the cancer cells [17]. However, until now, the serum level of *miR-411* and its diagnostic performance in breast cancer remained unidentified.

In the current study, we sought to investigate diagnostic performance of serum *miR-411* in breast cancer. The serum levels of *miR-411* in breast cancer patients were detected, as well as its association with clinical characteristics. In addition, receiver operating characteristic (ROC) analysis was performed to determine the diagnostic value of *miR-411* in breast cancer.

Methods

Patients and serum samples

The present study was approved by the the Ethical Committee of the hospital and all participants provided written informed consents in advance. 107 patients who were pathologically diagnosed with breast cancer at Harrison International Peace Hospital were enrolled in the study. None of the patients had received surgery, chemotherapy, or radiotherapy before blood collection. The control blood samples were collected from 95 age-matched healthy volunteers who were without malignancy history and inflammatory diseases. Clinicopathological features of patients were summarized in Table 1.

Table 1
Association of serum *miR-411* level with clinicopathological factors

Factors	NO. of cases (n = 107)	miR-411 expression		χ^2	P values
		Low (n = 59)	High (n = 48)		
Age (years)					
< 55	61	31	30	1.071	0.301
≥ 55	46	28	18		
Clinical stage					
I-II	85	42	43	5.484	0.019
III	22	17	5		
Histological grade					
I-II	69	32	37	6.032	0.014
III	38	27	11		
Tumor diameter (cm)					
< 2	51	26	25	0.682	0.409
≥ 2	56	33	23		
Lymph node metastasis					
Negative	76	37	39	4.420	0.036
Positive	31	22	9		
ER status					
Negative	67	34	33	1.399	0.237
Positive	40	25	15		
PR status					
Negative	68	35	33	1.016	0.314
Positive	39	24	15		
HER-2/neu status					
Negative	75	40	35	0.331	0.565
Positive	32	19	13		
Note: ER: estrogen receptor; PR: progesterone receptor; HER-2: C-erbB-2.					

5 ml whole blood was collected from each participant in a serum separator tube containing EDTA. Samples were left to clot at room temperature for 30 min and then were centrifuged at 4,000 rpm for 10 min at 4°C. Serum specimens were stored at -80°C until further use.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from serum using the standard TRIZOL LS (Invitrogen, CA, USA) method according to the manufacturer's protocol. Total RNA concentration and integrity were determined with an ultraviolet spectrophotometer (Beckman, CA, USA) and a digital gel image analysis system (Bio-Rad, CA, USA).

Total RNA samples were reversely transcribed (RT) to cDNAs using primers specific to *miR-411* target. Specific cDNAs were then amplified by real-time quantitative RT-PCR using SYBR Premix Ex Taq (Takara, China) to investigate the relative expression of *miR-411*. The relative expression of miRNA was normalized to *U6* and calculated with the $2^{-\Delta\Delta Ct}$ method [18].

Statistical analysis

All statistical analyses were carried out with SPSS 18.0 software and GraphPad prism 5. Data were presented as mean $\pm$ SD. *MIR-411* expression levels were compared using Student's *t* test. The relationships between *miR-411* expression and clinicopathological factors were analyzed using chi-square test. Receiver operating characteristics (ROC) curves were established to evaluate the diagnostic value of serum *miR-411* in breast cancer. $P < 0.05$ was considered statistically significant.

Results

The expression level of serum miR-411 from breast cancer patients and healthy controls

QRT-PCR assay was carried out to measure the serum level of *miR-411* in 107 patients with breast cancer and 95 healthy controls. As shown in Fig. 1, the expression of serum *miR-411* were significantly lower in breast cancer than that in healthy controls ($P < 0.001$).

The association between serum miR-411 expression and clinicopathological characteristics

The patients were divided into high expression group ($n = 48$) and low expression group ($n = 59$), according to their average expression level of *miR-411*. To further determine the clinical significance of serum *miR-411* expression, chi-square test was performed. Results showed that *miR-411* was obviously associated with clinical stage ($P = 0.019$), histological stage ($P = 0.014$) and lymph node status ($P = 0.036$). No significant differences were found between *miR-411* and other clinicopathological features,

including age, tumor diameter, lymph node status, ER status, PR status and HER-2/neu status (all $P > 0.05$) (Table 1). It indicated that *miR-411* was implicated with the development and metastasis of breast cancer.

The diagnostic value of *miR-411* in breast cancer

ROC curves which were built based on serum levels of *miR-411* in breast cancer patients and healthy individuals were used to evaluate the diagnostic value of *miR-411* for breast cancer. The curve showed that serum *miR-411* could distinguish breast cancer patients from healthy individuals at the optimal cut-off value of 1.245, with the an area under the curve (AUC) of 0.796, combining with the sensitivity of 82.1% and the specificity of 83.2% (Fig. 2).

Discussion

Breast cancer is a severe threat to health among women worldwide, due to its high incidence rates and low overall survival [19]. Tumor metastasis may be responsible for the dismal clinical outcomes of breast cancer [3]. Unfortunately, the etiology of breast cancer remains unclear, and it is unable to determinate the key factor for tumor progression [20]. Early diagnosis is a pivotal approach to improve outcomes of breast cancer patients. Until now, the commonly used biomarkers for breast cancer diagnosis, such as CEA, CA153, HER-2/Neu, ER, PR, and EGFR, show limited diagnostic effectiveness [21]. Therefore, it is necessary to identify new diagnostic biomarkers for breast cancer patients to improve prognosis.

Growing evidences have suggested that miRNAs may provide an effective tool for cancer diagnosis, due to its stable expression profiles in body fluids and tissues specimens, as well as its significantly association with tumor progression [22]. In breast cancer, a variety of dysregulated miRNAs were observed, suggesting their important functions in tumor development and progression. *MiR-4262* was proved to be a tumor oncogene in breast cancer that its over-expression might contribute to proliferation and invasion of the cancer cells [23]. *MiR-421* was down-regulated in breast cancer tissues specimens and cell lines, and its expression patterns showed negative link with metastasis, tumor stage and recurrence. *MiR-421* might be a tumor suppressor in breast cancer [24]. Given their functional roles in progression of breast cancer, miRNAs were considered as promising biomarkers for the disease. In the present study, we investigated the diagnostic significance of *miR-411* in breast cancer.

In this study, we found *miR-411* expression was decreased in serum samples collected from breast cancer patients compared with healthy controls. Moreover, the down-regulated serum *miR-411* levels were tightly correlated with advanced clinical stage, high histological grade, and positive lymph node metastasis. It suggested that *miR-411*, as a tumor suppressor, was involved in the progression of breast cancer. The conclusion was consistent with the previous investigations. It was reported that the expression of *miR-411* was significantly down-regulated in breast cancer patients, and recovery its expression might suppress growth, migration, and invasion of the cancer cells [25, 26]. However, the molecular mechanisms for the anti-tumor action of *miR-411* in breast cancer remained poorly known. Further researches were still needed.

Breast cancer diagnosis is a challenging research job. The cancer is characterized by heterogeneous, with diverse genetic alterations [27]. In the previous studies, various molecular biomarkers were confirmed for breast cancer. For instances, Zhang et al. reported that plasma long non-coding *H19* levels were significantly different between breast cancer patients and healthy individuals that might be a potential diagnostic biomarker for the disease [28]. The study carried out by Chen et al. demonstrated that serum levels of DAND5 were positively correlated with aggressive clinical characteristics and low survival rate of breast cancer patients, suggesting its predictive potential in the cancer [3]. Identification of genetic alterations might provide an effective approach for early diagnosis and prognosis evaluation of breast cancer. In this study, ROC curve analysis was performed to investigate the diagnostic performance of *miR-411* in breast cancer patients. The results revealed that serum *miR-411* expression could differentiate breast cancer patients from healthy controls with satisfactory sensitivity and specificity. Despite of the various identified molecular biomarkers for breast cancer, few of them were applied in clinic. Therefore, well-designed study with large sample size was still needed to investigate the application value of serum *miR-411* for breast cancer diagnosis.

Conclusions

In conclusion, serum *miR-411* levels in patients with breast cancer is down-regulated, and its decreased expression correlates with malignant tumor progression. Serum *miR-411* may be a potential biomarker for early detection of breast cancer.

Abbreviations

MicroRNA-411 (*MiR-411*)

quantitative real-time PCR (qRT-PCR)

receiver operator characteristic (ROC)

estrogen receptor (ER)

progesterone receptor (PR)

epidermal growth factor receptor (EGFR)

vascular endothelial growth factor receptor (VEGFR)

MicroRNAs (miRNAs)

quantitative real-time polymerase chain reaction (qRT-PCR)

area under the curve (AUC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Harrison International Peace Hospital 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

S.C., L.Z. design of the work; S.C., L.Z. the acquisition, analysis, S.C., L.Z. interpretation of data; S.C., L.Z. the creation of new software used in the work; S.C., L.Z. 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-411 levels in 107 breast cancer and 95 healthy controls. Serum expression level of miR-411 in breast cancer were strongly down-regulated compared with healthy controls. ***: suggested $P < 0.001$.

Figure 2

Receiver operating characteristics (ROC) curve constructed based on serum levels of miR-411 in breast cancer patients and healthy controls. The AUC value of the curve was 0.796, suggesting that miR-411 could discriminate between breast cancer patients and healthy individuals. The cut-off value of miR-411 for breast cancer diagnosis was 1.245, with the sensitivity of 82.1% and the specificity of 83.2%.