

Original Article

Decreased expression of miR-204 is associated with poor prognosis in patients with breast cancer

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Abstract: The identification of biomarkers in breast cancer diagnosis and therapy is important in achieving early cancer diagnosis and improving patient outcomes. The aim of this study was to examine clinical significance of miR-204 expression in tissues from breast cancer patients. The relationship between miR-204 expression and clinicopathological characteristics was investigated. MiR-204 expression was significantly associated with TNM stage and metastasis. Patients with low miR-204 expression had poorer overall survival time and disease free survival time than those with high miR-204 expression. Furthermore, miR-204 expression was correlated with chemotherapeutic resistance of breast cancer patients. In conclusion, the miR-204 may be a potential diagnostic and prognostic biomarker of breast cancer.

Keywords: MiR-204, breast cancer, prognosis

Introduction

Breast cancer, with an incidence of more than 1,000,000 new cases and 370,000 deaths annually worldwide, remains a major challenge today [1]. The majority of breast cancer morbidity and mortality is due to incurable metastatic disease that is highly resistant to conventional therapies [2]. Early diagnosis and prognostic evaluation of breast cancer are crucial for timely and appropriate treatment. Therefore, it is urgent to find out early diagnosis marker of breast cancer.

MicroRNAs (miRNAs) are a class of approximately 22-nucleotide noncoding RNAs that play important role in diverse physiological and pathological processes [3]. Mounting evidence demonstrates that the dysregulation of miRNAs are involved in the progression of cancer, including breast cancer [4]. It is well documented that miRNAs are involved in diverse biological processes, including differentiation, proliferation, apoptosis, and tumorigenesis [5]. Depending on their specific gene targets, miRNAs may function as oncogenes and/or tumor sup-

pressors [6]. Recently, it is suggested that miRNAs could be promising biomarkers for cancer diagnosis and prognosis [7].

We recently demonstrated that miR-204 can be a promising biomarker for EBV positive nasopharyngeal carcinoma [8]. In the present study, we investigated whether miR-204 expression was associated with outcome of breast cancer patients.

Materials and methods

Patients and samples

This study was conducted with the approval of the Ethical and Scientific Committees of Guangzhou Medical University. A total of 129 female breast cancer patients who were diagnosed by histo-pathology in Cancer Center of Guangzhou Medical University from October 2006 to September 2011 were obtained. Specimens were formalin-fixed and embedded in paraffin by standard methodology after obtained during surgery. IHC of ER, PR, and HER-2 status were performed in the Pathology

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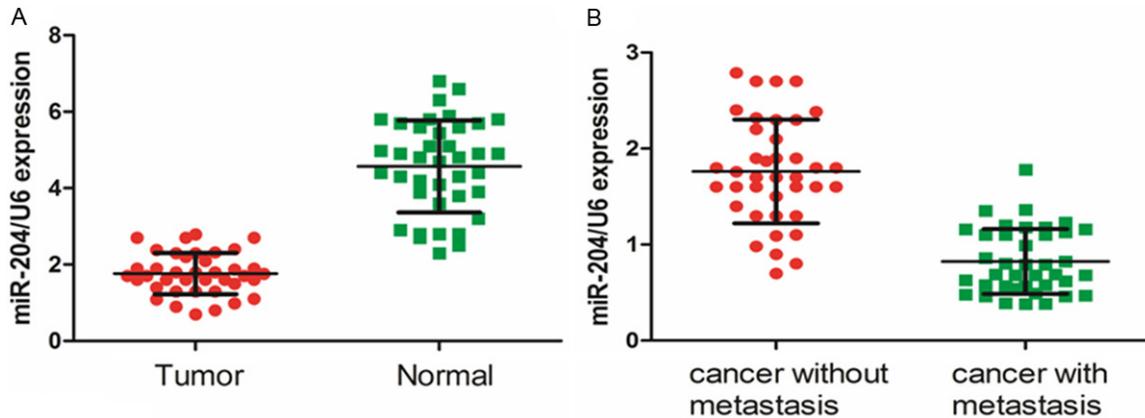


Figure 1. MiR-204 was down-regulated in breast tissues and was correlated with metastasis in breast cancer patients. A: The expression of miR-204 was detected by qRT-PCR in 39 pairs of human breast cancer tissues and adjacent normal breast tissues. B: Compared with breast cancer patients without metastasis, those patients with metastasis had lower level of miR-204.

Department of Cancer Center of Guangzhou Medical University. All the patients complete clinicopathological data were available and reviewed. The date of death and the date of relapse were used to calculate estimate overall survival (OS) and disease-free survival (DFS). All the patients underwent pre-operative neoadjuvant chemotherapy with an association of 5-Fluorouracil (5-FU), Epirubicin and Cyclophosphamide (FEC) for 4-6 cycles. Treatment response was assessed by the RECIST criteria [9]. Patients achieving complete (CR) or partial (PR) response were considered as responder; while disease stabilization (SD) or disease progression (PD) considered as non-responder. According to the clinical response of neoadjuvant chemotherapy, adjuvant FEC or Taxotere was selectively administered in the patients with PR/CR or PD/SD.

RNA isolation

RNA was extracted from formalin-fixed tissues, using TRIzol reagent, PureLink™ FFPE RNA Isolation Kit and mirVana PARIS kit (Life Technology, California, USA). RNA was diluted in RNase-free water and stored at -80°C before use.

Quantitative real-time PCR

For quantitative real-time PCR, the miRNA-specific TaqMan MicroRNA Assays (Applied Biosystems) for miR-204 was used as described by the manufacturer. U6 snRNA was used as an

endogenous control for miRNA detection. The expression of miR-204 was quantified by measuring cycle threshold (Ct) values and normalized using the $2^{-\Delta\Delta Ct}$ method relative to U6 snRNA.

Statistical analysis

All the statistical analyses were performed using SPSS13.0 for Windows (SPSS Inc., Chicago, IL, USA). Data are presented as mean \pm SD from at least three separate experiments. The distinct expression of miR-204 between tumor tissues and paracarcinoma tissues was examined by independent samples T-test. The relationships between miR-204 expression and clinicopathological parameters were examined by chi-square test. Overall survival (OS) or disease-free survival (DFS) curves were calculated by the Kaplan-Meier method and the log-rank test was used to determine the difference in OS or DFS rates between two groups. $P < 0.05$ was considered statistically significant.

Results

Down-regulation of miR-204 expression in human breast cancer tissues

In order to explore the role of miR-204 in breast carcinogenesis, the expression patterns of miR-204 in 39 pairs of human breast cancer tissues and adjacent normal breast tissues were analyzed using qRT-PCR. The result showed that the expression of miR-204 was

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Table 1. Clinicopathological variables and the expression of miR-204 in total breast cancer patients

Variables	miR-204			P Value*
	All case (n=129)	Low expression (n=65)	High expression (n=64)	
Age (years)				
≤50	49	23	26	0.540
>50	80	42	38	
ER status				
Positive	54	32	22	0.087
Negative	75	33	42	
PR status				
Positive	59	28	31	0.541
Negative	70	37	33	
HER-2 status				
Positive	49	27	22	0.402
Negative	80	38	42	
Tumor size (cm)				
≤2	44	21	23	0.664
>2	85	44	41	
TNM stage				
I-II	69	29	40	0.042*
III-IV	60	36	24	
Distant metastasis				
Yes	43	28	15	0.018*
No	86	37	49	

*means statistically significant (p<0.05).

reduced significantly, compared with adjacent normal breast tissues (**Figure 1A**, P<0.05). Interestingly, low miR-204 expression was significantly associated with a more aggressive tumour phenotype (**Figure 1B**, P<0.05).

The relationships between expression of miR-204 and clinical parameters in breast cancer patients

To further investigate the clinicopathological and prognostic significance of miR-204 levels in patients with breast cancer, the levels of miR-204 in a large cohort of 129 breast cancer tissues were examined by real-time PCR. The median value of all 129 breast cancer samples was chosen as the cut-off point for separating tumors with low-level expression of miR-204 from high-level expression miR-204 tumors. Thus, 65 (50.3%) breast cancer patients had low-level expression of miR-204, while 64 (49.7%) breast cancer patients had high-level expression of miR-204. The clinicopathologic

characteristics and miR-204 expression of the breast cancer patients involved in our study are shown in **Table 1**. There was no significant association between age, ER status, PR status, HER-2 status, tumor size and expression of miR-204. However, low expression of miR-204 correlated with late TNM stage (p=0.042) and higher metastasis (p=0.018).

Expression of miR-204 correlated with patients' overall survival (OS) and disease free survival (DFS)

We further evaluated whether miR-204 correlated with breast cancer patients' OS and DFS. The results showed that breast cancer patients' with low expression of miR-204 had poorer OS (**Figure 2A**, P<0.05) and DFS (**Figure 2B**, P<0.05). These results indicated that miR-204 could be a prognostic factor in breast cancer patients.

MiR-204 expression correlated with chemotherapeutic resistance of breast cancer patients

To determine whether the miR-204 expression levels were associated with chemotherapeutic efficacy, therapeutic response was evaluated by radiologic Response Evaluation Criteria in Solid Tumors (RECIST). According to RECIST, 76 patients (58.9%) responded to chemotherapy with PR or CR; 53 patients (41.1%) were not responsive with SD or PD. The result showed that miR-204 was significantly associated with therapeutic response, exhibiting lower expression in non-responsive patients (**Table 2**, P<0.05).

Discussion

To date, studies have shown that miRNA expression is correlated with clinical and biological features of tumors, which can be a potential biomarker for therapy and prognosis [10, 11]. MiR-204 is reported to down-regulated in sev-

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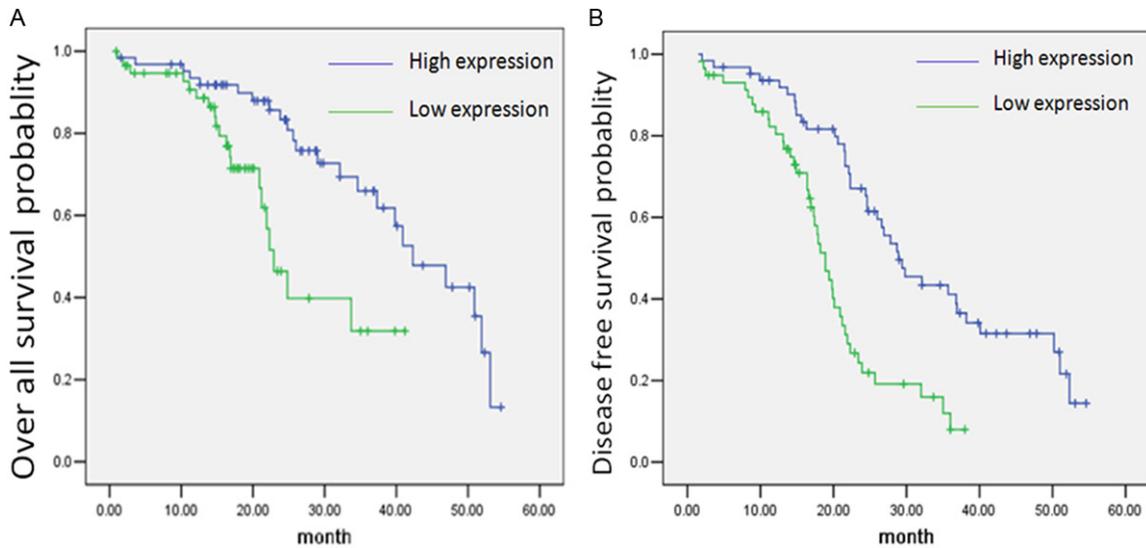


Figure 2. MiR-204 as a prognostic factor in breast cancer patients. A: Breast cancer patients with low miR-204 expression had poorer overall survival (OS) probability ($P < 0.05$). B: Breast cancer patients with low miR-204 expression had shorter disease free survival (DFS) probability ($P < 0.05$).

Table 2. Correlation of miR-204 level and response to chemotherapy

Treatment response	miR-204			P Value*
	All case (n=129)	Low expression (n=65)	High expression (n=64)	
CR+PR	76	32	44	0.024*
SD+PD	53	33	20	

CR, complete response; PR, partial response; SD, stable disease; PD, disease progression.

eral cancers, including intrahepatic cholangiocarcinoma [12], glioma [13], neuroblastoma [14], endometrial [15], and gastric cancer [16]. Loss of miR-204 contributes to cancer cell migration and invasion. Restoration of miR-204 inhibited epithelial to mesenchymal transition (EMT) by targeting the EMT inducer, including slug and SIRT1 [12, 16]. In addition, miR-204 suppressed the growth of cancer cell by modulating proliferation [17]. These literatures fully demonstrated that miR-204 functioned as a tumor suppressor in various cancers.

MiR-204 has been suggested to be involved in regulating breast cancer cell migration and invasion [18]. However, whether miR-204 is associated with breast cancer patients' prognosis, OS, DFS and chemotherapeutic response remains unknown. In the present study, we found that miR-204 was significant down-regu-

lated in breast cancer tissues than normal tissues. In addition, we analyzed the prognostic role of the expression of miR-204 in breast cancer patients. We found that low expression of miR-204 correlated with advanced clinical stage and more distant metastasis in breast cancer.

These results suggested that

loss of miR-204 may promote the invasive property of breast cancer. Consistent with our results, Zhang L et al. demonstrate that down-regulated miR-204 may be related to the onset of gastric cancer metastasis [16]. Ying Z et al. showed that miR-204 was reduced in glioma with aberrantly aggressive phenotype [13]. We further showed that breast cancer patients with low miR-204 expression had poorer OS and DFS. This result indicated that miR-204 expression could be a prognosis for breast cancer patients.

Last but not least, we found that miR-204 expression correlated with chemotherapeutic resistance of breast cancer patients. Patients with low miR-204 expression were less sensitive to chemotherapy. In a previous study, Ryan J et al. showed that miR-204 increased neuroblastoma cells' sensitivity to cisplatin by direct

targeting anti-apoptotic Bcl-2 [14]. This finding was subsequently supported by the result from Sacconi A et al. [19], which demonstrated that miR-204 targeted Bcl-2 and enhanced cisplatin responsiveness of gastric cancer. In agreement with these studies, we demonstrated that low miR-204 expression correlated with chemotherapeutic resistance of breast cancer patients. Taken together, our and others' findings suggest that miR-204 can be a suitable indicator for chemotherapeutic response.

In summary, our findings demonstrated for the first time that miR-204 was down-regulated in breast cancer. Breast cancer patients with low miR-204 expression had poorer OS and DFS. And low miR-204 expression was correlated with chemotherapeutic resistance of breast cancer patients. These results suggested that miR-204 could be employed as a new prognostic marker for breast cancer.

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