Plasma microRNAs Predict Chemoresistance in Patients With Metastatic Breast Cancer

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Abstract

Background: MicroRNAs contribute to chemotherapy response in different types of cancer. We hypothesized that plasma miRNAs are potentially associated with chemotherapy response in patients with metastatic breast cancer. Patients and Methods: Fourteen candidate microRNAs were chosen from the literature, and their plasma levels were measured by quantitative polymerase chain reaction (PCR). Forty metastatic breast cancer patients were chosen as the training groups. The potential significant microRNAs were validated in another 103 plasma samples. Results: In the training set, we identified 3 microRNAs (miR-200a, miR-210, and miR-451) as significantly dysregulated miRNAs between sensitive group (partial response (and stable disease) and resistant group (progressive disease). Then, in the validation set, miR-200a (area under the curve ¼ 0.881, sensitivity ¼ 94.1%, specificity ¼ 76.7%) and miR-210 (area under the curve ¼ 0.851, sensitivity ¼ 88.2%, specificity ¼ 72.1%) showed high diagnostic accuracy for distinguishing sensitive group from resistant group. Furthermore, the plasma level of miR-200a was significantly associated with the stage in surgery (P ¼ .035), and the high level of miR-210 expression was associated with internal organ metastasis (liver, lung, and brain; P ¼ .024). Conclusions: Plasma miR-200a and miR-210 could be effective biomarkers for the prediction of chemotherapy resistance in metastatic breast cancer patients.

Keywords

metastatic breast cancer, miRNA, plasma, predictive, chemoresistance

Abbreviations

CBR, clinical benefit rate; ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; HER2, epithelial growth factor 2; HR, hormone receptor; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ORR, overall response rate; PD, progression disease; PgR, progesterone receptor; PR, partial response;; SD, stable disease.

Introduction

Breast cancer is the most common disease and the second leading cause of death for women worldwide.¹ In China, the incidence of breast cancer has rapidly increased.² About 30% of early-stage breast cancer develop metastasis and about 5% of patients are diagnosed with advanced distant metastasis.³ Improvement of treatment does not significantly change the prognosis of metastatic breast cancer. The 5-year survival rate of metastatic breast cancer patients is only 23%, which is much lower than 84% to 99% in early-stage without distant metastases.⁴

Chemotherapy plays an important role in the treatment paradigms for metastatic breast cancers. However, chemotherapy cannot eliminate the cancer cells. The resistant cells will cause...
cancer recurrence or metastasis, which impedes the success of chemotherapy. Hence, it is necessary to identify effective biomarkers for patients with drug-resistant risk, especially for those with metastatic breast cancers. Therefore, searching for highly sensitive, specific, non-invasive predictive biomarkers in peripheral blood of metastatic breast cancer patients is very urgent and practical.

The microRNAs (miRNAs) are a class of small noncoding RNA molecules (19-25 nucleotides), which play an important role in the regulation of proliferation, differentiation, apoptosis, and migration of cancer cells.\textsuperscript{5,6} The panel of miRNA expression is quite different between cancers and normal tissues.\textsuperscript{7} Previous studies showed that circulating miRNAs in peripheral blood may derive from cancer cells and reflect the pathological characteristics of primary tumors.\textsuperscript{8,9} The plasma or serum miRNAs are quite stable and suitable for biomarker screening.\textsuperscript{10-12} The origins of miRNAs in plasma are currently unclear. The blood cells and tumor cells may contribute to the miRNAs in peripheral blood. And tumor cells release amount of miRNAs by active\textsuperscript{13} or passive\textsuperscript{14} ways. Furthermore, tumor-derived exosomes and microvesicles in the peripheral blood contain many miRNAs.\textsuperscript{15} Some miRNAs have been reported as the promising features for predicting chemotherapy response in early stage of breast cancer patients.\textsuperscript{16,17} We hypothesized that miRNAs in plasma are promising noninvasive biomarkers in metastatic breast cancer.

In our study, 14 miRNAs, which were reported to regulate metastasis formation, epithelial mesenchymal transition, and stem cell characteristics in the previous studies,\textsuperscript{18-23} were chosen from literatures as the candidate biomarkers. Due to the small amount of microRNAs in the limited volume plasma, we used a serum-direct Multiplex RT-PCR (SdM-qRT-PCR) to quantify the plasma levels of the miRNAs in metastatic breast cancer patients.\textsuperscript{18} Here, we for the first time evaluated the potential of the microRNAs to predict chemotherapy resistance in metastatic breast cancer patients; miR-200a and miR-210 have been identified as new predictors.

### Materials and Methods

**Study Cohort**

All the plasma were taken from the specimen bank of Beijing Cancer Hospital. We selected 40 metastatic breast cancer patients in the training stage and 103 metastatic breast cancer patients in the validation stage who were treated between June 2009 and June 2014. All the patients received chemotherapy with single-agent docetaxel or combination with docetaxel, who had response evaluation results. Eight age-matched healthy women with no history of cancer and in good health condition were recruited as controls at the same period with breast cancer patients. This study was approved by the ethics committee of Beijing Cancer Hospital (Beijing, China; 2016-KT45), and written informed consent was obtained from all the patients. This study strictly conformed to the principles outlined in the Declaration of Helsinki.

Before starting the treatment, plasma was obtained using the following procedures: 4 mL of peripheral blood from patients was collected in heparin-containing tubes and incubated at room temperature within 1 hour and then centrifuged at 2000 rpm for 15 minutes at room temperature. The supernatant was transferred to a microfuge tube and stored at −80°C for further use. Specimens that showed hemolysis were excluded for further miRNA detection.

**Patient Treatment and Assessment**

All the patients received single-agent docetaxel or combination with docetaxel every 21 days for 4 to 6 cycles. None of the Her2-positive patients received trastuzumab in this study because of economic reasons.

Demographic and clinic pathological details of patients were obtained from the medical records of the Department of Breast Oncology. The estrogen receptor (ER), progesterone receptor (PgR), and Her2 status were the result of the primary tumor. Estrogen receptor and PgR status were considered positive when 10% or more tumor cells exhibited nuclear staining for the receptors. Her2 positivity was defined as either as score of 3+ by Immunohistochemistry (IHC) or positivity by fluorescence in situ hybridization (FISH).

Treatment response was assessed by the RECIST criteria.\textsuperscript{19} Complete response (CR), partial response (PR), and stable disease (SD) were considered as sensitive(S) group. Progressive disease (PD) was considered as resistant(R) group.

**Quantitative RT-PCR of Plasma miRNAs**

Serum-direct Multiplex qRT-PCR (SdM-qRT-PCR) was performed following our previous study.\textsuperscript{18} Briefly, reverse transcription was performed using the plasma samples without RNA extraction and the Prime Script RT reagent Kit (Takara Bio Inc, Kyoto, Japan). The specific miRNAs RT primers were synthesized by Ribo Bio (Guangzhou, China). The 20-μL reverse transcription reaction mixture was incubated at 42°C for 30 minutes and 85°C for 15 seconds. Then, cDNA was obtained. The total of 2 μL cDNA was used per 25 μL qPCR reaction, including the SYBR Green I dye and miRNA-specific detection primers (Ribobio, Guangzhou, China). The quantitative real-time PCR reaction was performed at 95°C for 2 minutes in 40 cycles at 95°C for 15 seconds and 60°C for 60 seconds. To obtain reproducible results, plasma from each patient was split into 3 aliquots and performed the whole detection assay.

The relative expression of each miRNA was calculated from the following equation: relative expression = $2^{-\Delta C_t}$, where $C_t$ is the threshold cycle for a sample and $\Delta C_t = C_{\text{miRNA}} - C_{\text{miR-191}}$.

**Statistical Analysis**

The statistical analyses were performed using the SPSS software package, version 19.0. The differences of miRNAs expression levels between R group and S group were identified...
using an unpaired t test. Data were presented as mean (standard deviation). In the validation stage, the χ² or 2-tailed Fishers exact test was used to identify potential associations of plasma miRNAs and clinic pathological risk factors with PD. And the factors that might were associated with PD were analyzed by using the univariate and multivariate logistic regression analyses. $P \leq .05$ in all cases was considered statistically significant.

**Results**

*Characteristics of Patients and Study Design*

In total, 143 breast cancer patients were included in this study. All the patients received chemotherapy with single-agent docetaxel or combination with docetaxel. In the training stage, 20 patients achieved PR or SD, defined as sensitive (S) group and 20 patients achieved PD, defined as resistant (R) group. Eight age-matched healthy women were recruited as controls. The median age was 53 (35-70) years old. In the validation stage, 34 patients with PR, 52 patients with SD and 17 patients with PD were studied.

The clinical characteristics of all the patients are presented in Table 1.

**Selection of Candidate miRNAs**

Previous studies indicated that the role of microRNAs in cancer was associated with the progression of breast cancer. We found 14 microRNAs: miR-150, miR-145, miR-155, miR-21, miR-200a, miR-200b, miR-200c, miR-210, miR-203, miR-221, miR-375, miR-451, miR-34a, and miR-122, which were particularly associated with prognosis, drug resistance, and stem cell characteristics in the previous reports.20-25 MiR-16 and miR-191 were taken as reference controls as previously indicated.10,26 Thus, we selected the 16 miRNAs as the candidates to perform the integrative analyses in the plasma of metastatic breast cancer patients.

The endogenous control for detection serum miRNAs was determined as described in our previous study.18 By using GeNorm and NormFinder, miR-191 was chosen as the most stably expressed miRNA internal control (Supplementary data, Figure S1).

**Plasma microRNA Candidates for Breast Cancer Prognosis in the Training Set**

The expression level of 14 plasma miRNAs was measured by qPCR in the 40 breast cancer patients in training stage (Table 2). The difference between R group and S group was determined using an unpaired t test. We found 2 miRNAs (miR-200a ($P < .001$) and miR-210 ($P < .001$)) significantly increased and 1 miRNA (miR-451 ($P < .001$)) significantly decreased in the R group (Figure 1A). In addition, the expression of the 3 miRNAs in the plasma of metastatic breast cancer patients was significantly higher than that in the healthy controls (Figure S2). Furthermore, the receiver operating characteristic (ROC) curve analysis showed that area under curve (AUC) was 0.847

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**Table 1. Clinicopathological Characteristics of the Patients With Metastatic Breast Cancer.**

| Characteristic                        | Discovery Stage, n=40 (%) | Validation Stage, n=103 (%) |
|---------------------------------------|---------------------------|-----------------------------|
| Age (years), median (range)           | 53 (35-77)                | 54 (30-80)                  |
| ECOG 0,1                              | 36 (90.0)                 | 99 (96.1)                   |
| 2                                     | 4 (10.0)                  | 4 (3.9)                     |
| Histology                             |                           |                             |
| IDC                                   | 34 (85.0)                 | 97 (94.2)                   |
| ILC                                   | 2 (5.0)                   | 2 (1.9)                     |
| Others                                | 4 (10.0)                  | 4 (3.9)                     |
| AJCC stage in surgery                 |                           |                             |
| Stage I                               | 3 (7.5)                   | 10 (9.7)                    |
| Stage II                              | 15 (37.5)                 | 40 (38.8)                   |
| Stage III                             | 16 (40.0)                 | 37 (35.9)                   |
| Stage IV                              | 6 (15.0)                  | 9 (8.7)                     |
| Unknown                               |                           | 7 (6.8)                     |
| ER status                             |                           |                             |
| Negative                              | 12 (30.0)                 | 32 (31.1)                   |
| Positive                              | 25 (62.5)                 | 70 (68.0)                   |
| Unknown                               | 3 (7.5)                   | 1 (1.0)                     |
| PR status                             |                           |                             |
| Negative                              | 17 (42.5)                 | 36 (35.0)                   |
| Positive                              | 20 (50.0)                 | 66 (64.1)                   |
| Unknown                               | 3 (7.5)                   | 1 (1.0)                     |
| HR status                             |                           |                             |
| Negative                              | 11 (27.5)                 | 23 (22.3)                   |
| Positive                              | 26 (65.0)                 | 79 (76.7)                   |
| Unknown                               | 3 (7.5)                   | 1 (1.0)                     |
| HER2(IHC)                             |                           |                             |
| 0                                     | 12 (30.0)                 | 35 (34.0)                   |
| 1                                     | 6 (15.0)                  | 20 (19.4)                   |
| 2                                     | 8 (20.0)                  | 24 (23.3)                   |
| 3                                     | 10 (25.0)                 | 18 (17.5)                   |
| Unknown                               | 4 (10.0)                  | 6 (5.8)                     |
| TNBC                                  |                           |                             |
| Yes                                   | 4 (10.0)                  | 15 (14.6)                   |
| No                                    | 31 (77.5)                 | 83 (80.6)                   |
| Unknown                               | 5 (12.5)                  | 5 (4.9)                     |
| Histological grade                    |                           |                             |
| G1                                    | 27 (26.2)                 | 12 (30.0)                   |
| G2                                    | 49 (47.6)                 | 19 (47.5)                   |
| G3                                    | 23 (22.3)                 | 8 (20.0)                    |
| Unknown                               | 4 (3.9)                   | 1 (2.5)                     |
| Surgical Approach                     |                           |                             |
| Modified Radical                      | 38 (95.0)                 | 100 (97.1)                  |
| Mastectomy                            | 2 (5.0)                   | 2 (1.9)                     |
| Breast Conservation Surgery           |                           | 1 (1.0)                     |
| Other                                 |                           |                             |
| Liver metastasis                      | 21 (52.5)                 | 41 (39.8)                   |
| Lung metastasis                       | 15 (37.5)                 | 40 (38.8)                   |
| Brain metastasis                      | 4 (10.0)                  | 5 (4.9)                     |
| Bone metastasis                       | 22 (55.0)                 | 48 (46.6)                   |
| Lymph node metastasis                | 26 (65.0)                 | 64 (62.1)                   |
| Chest wall metastasis                 | 11 (27.5)                 | 21 (20.4)                   |
| Malignant pleural effusion            | 13 (32.5)                 | 23 (22.3)                   |
| Malignant pericardial effusion        | 2 (5.0)                   | 4 (3.9)                     |
| Soft tissue metastasis                | 2 (5.0)                   | 3 (2.9)                     |
| Other site metastasis                 | 2 (5.0)                   | 4 (3.9)                     |

(continued)
Table 1. (continued)

| Characteristics                  | Discovery Stage, n=40 (%) | Validation Stage, n=103 (%) |
|----------------------------------|---------------------------|-----------------------------|
| Internal organ metastasis (liver, lung, brain) | 28 (70.0) | 70 (68.0) |
| More than 3 sites of internal organ metastasis | 24 (60.0) | 54 (52.4) |
| Line of chemotherapy             |                           |                             |
| First line                       | 17 (42.5)                 | 52 (50.5)                   |
| Second line                      | 17 (42.5)                 | 28 (27.2)                   |
| Third line and more              | 6 (15.0)                  | 21 (20.4)                   |
| Unknown                          |                           | 2 (1.9)                     |
| Clinical response                |                           |                             |
| PR                               | 13 (32.5)                 | 34 (33.0)                   |
| SD                               | 7 (17.5)                  | 52 (50.5)                   |
| PD                               | 20 (50.0)                 | 86 (83.5)                   |
| ORR (CR+PR)                      | 13 (32.5)                 | 34 (33.0)                   |
| CBR (CR+PR+SD)                   | 20 (50.0)                 | 86 (83.5)                   |

Abbreviations: CBR, clinical benefit rate; ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; HR, hormone receptor; HER2, epithelial growth factor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ORR, overall response rate; PD, progression disease; PR, progesterone receptor; PR, partial response; SD, stable disease.

Correlation Analysis of Plasma miR-200a and miR-210 Level With Clinicopatholgocial Characteristics of Breast Cancer

We assessed the association of plasma level of miR-200a and miR-210 with PD in the combined set and found miR-200a and miR-210 expression was correlated with the PD rate (P < .001, Table 3). Then, miR-200a/miR-210 with other clinicopatholgocial characteristics was further analyzed using univariate and multivariate logistic regression analysis to detect the association with chemotherapy response (Table 4). The levels of plasma miR-200a expression (odds ratio [OR] = 0.041 95% confidence interval [CI]: 0.010-0.169, P < .001) and miR-210 (OR = 0.062, 95% CI: 0.017-0.229, P < .001) were identified as independent factors for chemotherapeutic response.

Further Analysis of the Relation of the miRNAs in the Plasma With Other Clinicopathological Characteristics

The expression of miR-200a was significantly associated with the stage in surgery, and miR-210 was associated with internal organ metastasis, as shown in Table 5. Then, we analyzed the expression levels of miRNAs and found that the patients with stage IV disease at diagnosis had higher expression levels of miR-200a (Figure 4A), and the patients with internal organ metastasis had higher expression levels of miR-210 (Figure 4B). The consistent results confirmed the association of miRNA levels and the clinical characteristics (Figure 5).

Discussion

Chemotherapy response predictive markers are quite important in the clinical practice. Growing body of evidence showed miRNAs played important role in chemoresistance of breast cancer patients.
Figure 1. Plasma levels and ROC analysis of miR-200a, miR-210, and miR-451 in the discovery set. A, The box plots showed the plasma levels of miR-200a, miR-210, and miR-451 in the discovery set composed of 20 resistant cases and 20 sensitive cases. B, ROC in the discovery set composed of 20 patients in R group and 20 in S group.

Figure 2. Plasma levels and ROC analysis of miR-200a, miR-210, and miR-451 in the validation set. A, The box plots show the plasma levels of miR-200a, miR-210, and miR-451 in the validation set composed of 17 resistant cases and 86 sensitive cases. B, ROC in the validation set composed of 17 patients in R group and 86 in S group.
cancer. Efforts have been made to evaluate circulating level of the microRNAs as biomarkers for prediction of prognosis or monitoring patient responses to therapy. Circulating level of miR-106b was found closely related to tumor size, metastasis, as well as shorter overall survival and progression-free survival. Fre`res et al analyzed the plasma miRNA signature and

Table 3. Correlation of miRNA Expression and Chemotherapy Response in the Combined Set.

| miRNAs      | CBR                  |                  |                  |
|-------------|----------------------|------------------|------------------|
|             | PD, n =37 (%)        | PR+SD, n = 106 (%)|                  |
| miR-200a    |                      |                  |                  |
| Lowa        | 3 (3.6)              | 81 (96.4)        |                  |
| Highb       | 34 (57.6)            | 25 (42.4)        |                  |
| P value     | <.001                |                  |                  |
| miR-210     |                      |                  |                  |
| Lowa        | 5 (6.1)              | 77 (93.9)        |                  |
| Highb       | 32 (52.5)            | 29 (47.5)        |                  |
| P value     | <.001                |                  |                  |

Abbreviations: CBR, clinical benefit rate; PD, progression disease; PR, partial response; SD, stable disease.
aExpression lower than the cut-off value.
bExpression equal to or higher than the cut-off value.

Figure 3. Plasma levels and ROC analysis of miR-200a, miR-210, and miR-451 in the combined set. A, The box plots showed the plasma levels of miR-200a, miR-210, and miR-451 in the combined set composed of 37 resistant cases and 106 sensitive cases. B, ROC in the combination set composed of 37 patients in R group and 106 in S group.

Table 4. The Correlation of Clinicopathological Characteristics and miRNA Expression With Chemotherapy Response.

| Clinical Characteristics | Univariate |          |          | Multivariate |          |          |
|--------------------------|------------|----------|----------|--------------|----------|----------|
|                          | OR         | P        | OR       | P            |          |          |
| Age                      | 1.015      | .443     | 5.992    | .057         |          |          |
| Stage at diagnosis       | 0.624      | .057     | 0.133    | .125         |          |          |
| ER status                | 1.244      | .057     | 0.885    | .933         |          |          |
| PgR status               | 1.006      | .988     | 1.577    | .690         |          |          |
| HR status                | 1.350      | .496     | 1.501    | .838         |          |          |
| HER2 status              | 0.702      | .483     | 1.060    | .908         |          |          |
| Histological grade       | 1.960      | .103     | 0.971    | .982         |          |          |
| Liver metastasis         | 1.006      | .987     | 0.490    | .622         |          |          |
| Lung metastasis          | 1.422      | .382     | 0.869    | .908         |          |          |
| Brain metastasis         | 1.237      | .796     | 2.310    | .7013        |          |          |
| Bone metastasis          | 0.655      | .271     | 0.669    | .650         |          |          |
| Lymph nodes metastasis   | 1.046      | .910     | 1.908    | .505         |          |          |
| Internal organ metastasis| 1.602      | .883     | 6.735    | .268         |          |          |
| More than 3 sites of internal organ metastasis | 1.027 | .944 | 1.867 | .560 |          |          |
| Line of chemotherapy     | 0.495      | .077     | 0.882    | .881         |          |          |
| miR-200a                 | 0.027      | <.001    | 0.065    | .001         |          |          |
| miR-210                  | 0.059      | <.001    | 0.016    | <.001        |          |          |

Abbreviations: ER, estrogen receptor; HR, hormone receptor; HER2, epithelial growth factor 2; PgR, progesterone receptor.
showed high diagnostic or predictive accuracy with breast cancers. Here, we for the first time showed miR-200a and miR-210 could predict metastatic breast cancer chemoresistance as new biomarkers. Compared with previous reports, we found that plasma miR200a and miR-210 has a good predictive performance for PD in the metastatic setting, which is a more complex group. All these studies may ultimately lead to better treatment options for breast cancer patients.

Our results suggested that high level of miR-200a and miR-210 in plasma of metastatic breast cancer patients was associated with chemotherapy resistance. MiR-200 family consists of 5 members: miR-200a/miR-200b/miR-429/ miR-200c/ miR-141, which are classified into 2 categories according to their chromosomal locations at 1 and 12. MiR-200 family was reported to inhibit EMT and suppress the proliferation of stem cells. In breast cancer patients, San-Jian Yu et al reported that the level of miR-200a in lymph node metastasis group increased more than 7-fold when compared with that in nonmetastasis patients. The data suggested that the high level of miR-200a was associated with metastatic behavior in breast cancer. Additionally, Madhavan et al showed that the metastatic breast cancer patients with circulating tumor cells (CTC)-

Table 5. Expression of the Candidate miRNAs in the Plasma and Associations With Other Clinical Characteristics (P value).

| Clinical Characteristics | miR-200a | miR-210 |
|--------------------------|----------|---------|
| Age                      | .072     | .962    |
| ECOG                     | .736^a   | .496^a  |
| Stage IV at diagnosis    | .035     | .729    |
| ER status                | .636     | .803    |
| PgR status               | .900     | .378    |
| HR status                | .690     | .489    |
| HER2(IHC) status         | .761     | .724    |
| Histological grade       | .468     | .901    |
| Liver metastasis         | .886     | .596    |
| Lung metastasis          | .555     | .219    |
| Brain metastasis         | .307^a   | 1.000^a |
| Bone metastasis          | .289     | .771    |
| Lymph nodes metastasis   | .174     | .361    |
| Internal organ metastasis| .836     | .024    |
| More than 3 sites of internal organ metastasis | .951 | .206 |

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; HR, hormone receptor; HER2, epithelial growth factor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; PR, progesterone receptor.

^Fisher exact test.

Figure 4. Plasma levels in the triple negative breast cancer (TNBC). A, The box plots showed the plasma levels of miR-200a of 5 resistant cases and 14 sensitive cases of TNBC. B, The box plots showed the plasma levels of miR-210 of 5 resistant cases and 14 sensitive cases of TNBC.

Figure 5. Plasma microRNA analysis in the subgroup of patients with breast cancer. A, The box plots showed the plasma levels of miR-200a according the stage in surgery. B, The box plots showed the plasma levels of miR-210 in the patients with or without internal organ metastasis.
positive had higher level of miR200a than that in CTC-negative patients. Together with our findings of miR-200a in plasma, these consistent data indicated the prognostic value of the microRNA. We would further test the correlation of miR200a with the prognosis of the metastatic patients. In hypoxic microenvironment, miR-210 is a critical regulator for cell survival.\textsuperscript{25,34} High level expression of miR-210 was detected in many cancers including breast cancer.\textsuperscript{35} A meta-analysis from 511 breast cancer cases indicated that high level of miR-210 expression might predict poor survival in the patients.\textsuperscript{36} Toyama et al\textsuperscript{37} showed that the level of miR-210 in triple-negative breast cancers was significantly higher than that in estrogen receptor-positive/HER2-negative breast cancers. Moreover, high level of miR-210 was an independent factor for worse prognosis in breast cancer, especially in lymph node-negative triple-negative patients.\textsuperscript{37,38} In this study, we found that miR-210 in plasma was associated with drug resistance, and the higher expression of miR-210 was associated with internal organ metastasis. Collectively, our results showed that high level of plasma miR-200a and miR-210 was associated with chemotherapy resistance in metastatic breast cancer patients. These results provided important evidence for the application of circulating microRNAs for the prediction of response to breast cancer treatment. Larger prospective, multi-institutional studies to validate the potential role of plasma miRNAs as chemotherapy predictive markers for metastatic breast cancer are expected in the future.

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**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding.**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by Grants from National Basic Research Program of the Chinese Ministry of Science and Technology (973 Grant no.2013CB531202) and National Natural Science Foundation of China (No.81502586).

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**Supplemental Material**

Supplemental material for this article is available online.

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