

Correlation of microRNA-367 in the clinicopathologic features and prognosis of breast cancer patients

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Abstract

Breast cancer (BC) is a malignant tumor originating from cells of the breast. Notably, microRNAs have been recognized as biomarkers of BC metastasis. The present study is designed to evaluate the association between microRNA (miR)-367 expression and BC with the variance of clinicopathologic features and prognosis.

Initially, 63 BC patients were allocated in the BC group, while the other 40 healthy volunteers were recruited as the control group. miR-367 expression in the serum of patients and healthy controls was detected using real-time polymerase chain reaction. Furthermore, the relation between miR-367 in serum and clinicopathologic features and prognosis of BC patients was accessed.

miR-367 expression in serum of the BC group was evidently lower than that in the control group (all $P < .001$). Besides, miR-367 underexpression in the BC group was closely associated with the variance in tumor nodes metastasis advanced stage, tumor diameter, and lymph node metastasis of BC (all $P < .001$). In addition, compared with the control group, poorly expressed miR-367 BC group had short period of disease-free survival and overall survival (all $P < .001$).

Our study demonstrated that miR-367 expression is associated with BC clinicopathologic features and prognosis. This investigation may offer new insight for BC treatment.

Abbreviations: BC = breast cancer, DFS = disease-free survival, miR = microRNA, miRs = microRNAs, OS = overall survival, RT-qPCR = reverse transcription-quantitative polymerase chain reaction, TNM = tumor node metastasis.

Keywords: breast cancer, clinicopathologic features, microRNA-367, prognosis, serum

1. Introduction

Breast cancer (BC) is a malignant tumor that occurs in the epithelial tissue of the breast gland, which is the most prevalent cancer among the female globally.^[1] Breast cancer affects approximately 12% women worldwide, and incidence and mortality rates in developed countries are declining but growing in developing countries.^[2–4] At present, women give little attention to clinical inspection and examination of BC, and that's why it is often diagnosed in advanced stage.^[1] Breast cancer can be triggered by factors such as age, menarche history,

reproductive patterns, physical activity, breast characteristics, and body habitus.^[4] Surgery, molecular treatment, radiation therapy, and chemotherapy are considered as approaches for BC treatment.^[5] Although valuable progress was achieved in search of the therapy of BC, it remains a challenge in treating metastatic BC since it spreads to other organs and predicts poor prognosis and 5-year survival rate.^[6] In this context, novel therapeutic strategies and clinical diagnostic targets for BC are in urgent need. Toward this, we undertook microRNA (miR)-based approach to understand the underlying mechanism in BC development, in order to develop novel intervention strategies. Importantly, dysregulated microRNAs (miRs) are observed in many malignancies, and miRs are regarded as new biomarkers for many cancers, including breast cancer.^[7,8] Breast cancer is commonly categorized into 4 main subtypes (luminal A, luminal B, human epidermal growth factor receptor 2 positive, and basal). It was proved that miRs have been linked to all stages of breast cancer.^[3] As novel clinical diagnostic targets, miRs can be used to further subtyping of BC and predicting metastasis or therapeutic resistance.^[9] The potential of miRs as biomarker targets is facilitated due to their stability in blood and their ability to withstand repeated freezing and thawing cycles.^[10] As possible biological targets for BC, miRs can affect BC progression via chemical modification, blocking tumor promoters and restoring tumor inhibitors.^[11] As a key member of the miR-302/367 cluster, miR-367 can play a role in a number of cancers as it prevents cell senescence and death, maintains proliferation signal activation, sabotages growth inhibitors, and modulates cellular biological activities as well as angiogenesis.^[12] Kaid and her colleagues discovered that miR-367 may be a medical target, which can be used as a marker in aggressive

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

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embryonal central nervous system tumor and facilitates prognosis and early diagnosis.^[13] Furthermore, miR-367-3p expression levels are closely correlated with International Federation of Gynecology and Obstetrics (FIGO) stage and lymph node metastasis, and high expression of miR-367-3p allows to a high survival rate, oppresses cancer cell metastasis, and quenches the malignant behaviors of tumor in endometrial cancer.^[14] Although miR-367 is reported to regulate the pathological development in different human cancers, researches about the functions of miR-367 in BC progression need to be elucidated.

In the present study, a series of experiments were conducted to evaluate circulating the association of miR-367 and BC metastases, markers for tumour recurrence and response to clinical treatments, likewise discuss the potential applications of miRNA for therapeutic metastatic breast cancer diagnosis, treatment, and basic research.

2. Materials and methods

2.1. Ethics statement

This study was approved and supervised by the Ethics Committee of our hospital. All patients signed the informed consent. All procedures were strictly conducted in accordance with the code of ethics.

2.2. Clinical subjects

From June 2015 to June 2018, 63 BC patients who received modified radical mastectomy in our hospital were enrolled as the BC group. Another 40 healthy volunteers in our hospital during the same period were selected as the control group. Inclusion criteria for these subjects were as follows:

- (1) all patients were pathologically diagnosed with BC;
- (2) no patient received radiotherapy or chemotherapy;
- (3) no patient was complicated with other malignant tumors;
- (4) no patient was complicated with any hematological or immune diseases;
- (5) no patient was infected with acute or chronic infections such as hepatitis, tuberculosis, and acute pneumonia;
- (6) no patients were affected by dysfunction of important organs such as heart, liver, and kidney.

The main clinical outcomes of this study were disease-free survival (DFS) and overall survival (OS), which were calculated using the life table method. DFS referred to the time from initial surgery to the first recurrence or metastasis of disease, or specific death of BC. OS implied the interval from initial surgery to death resulted from any cause.

2.3. Sample collection and RNA extraction

A total of 5 mL blood samples from each person in both groups were placed in ethylenediamine tetraacetic acid dipotassium anticoagulant tubes for 30 minutes, and then centrifuged at 4000 r/minutes (with a centrifugation radius of 20 cm) for 25 minutes. The supernatant was transferred into RNase-free Eppendorf centrifuge tubes, with 200 μ L in each tube. RNA was extracted using the Trizol method according to the instructions of the RNA extraction kits (Sangon Biotech Co., Ltd., Shanghai, China).

2.4. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Reverse transcription was performed using the TaqMan Micro-RNA reverse transcription kits (Sangon Biotech Co., Ltd., Shanghai, China). The information of miR-367 specific primers and U6 small nuclear RNA primers (as the internal reference) (all from Invitrogen Inc., Carlsbad, CA) is as follows: miR-367, F: 5'-GCAGAATTGCACTTTAGCAATG-3', R: 5'-GGTCCA GTTTTTTTTTTTTTTTTTCAC-3'; U6, F: 5'-CTCGCTTCGG CAGACA-3', R: 5'-AACGCTTCACGAA TTTGCGT-3'. Circulation conditions were: a cycle at 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, a cycle at 57°C for 30 seconds and a cycle at 72°C for 30 seconds. Then, an ABI 7500 fluorescence qPCR instrument (Applied Biosystems, Inc., Carlsbad, CA) was applied to quantify the samples for the calculation of the relative quantification, which meant the relative expression of miR-367 and U6. And $2^{-\Delta Ct}$ method was applied for the relative quantification, values of each sample. The formula was as follows: $\Delta Ct = C_{miR-367} - C_{U6}$.^[15] Each experiment consisted of 3 samples and was performed in 3 sessions.

2.5. Statistical analysis

Statistical Program for Social Sciences version 21.0 (IBM Corp. Armonk, NY) was employed for data analysis. The results were presented as mean \pm standard deviation. The *t* test was employed for comparisons between 2 groups. Count data were shown as %, and χ^2 test was applied for analysis of comparisons between 2 groups. Receiver operating characteristic curve was used to evaluate the diagnostic value of indices. Kaplan–Meier curve was employed for survival analysis, and log-rank test was used for comparisons of survival difference. The *P* value was attained using a two-tailed test, and *P* < .05 indicated a significant difference.

3. Results

3.1. Characteristics of patients

All BC patients were aged from 31 to 72 years old, with the average age of 51.38 ± 7.41 years old. Among 63 BC patients, 18 cases were premenopausal and the other 45 cases were postmenopausal. The 40 healthy controls were aged from 30 to 69 years old, with the average age of 52.18 ± 7.98 years old. Patients in the control group were all adult, non-pregnant, healthy women with no severe cardiopulmonary disease. There were no significant differences in general data such as age, medical history, and menstruation between 2 groups (all *P* > .05).

3.2. miR-367 expression is decreased in serum of BC patients

The results of RT-qPCR showed that the relative expression of miR-367 in the serum of the BC group was significantly lower than that of the control group (*P* < .001, *t* = 6.95) (Fig. 1). The patients were split into the miR-367 high expression group (>0.007 group) including 31 patients and the miR-367 low expression group (≤ 0.007 group) 32 patients according to the median of 0.007 for the relative expression of miR-367 in the serum of BC patients.

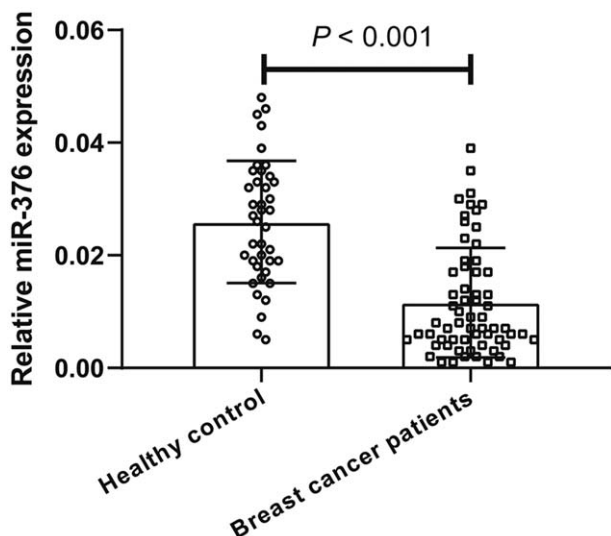


Figure 1. The relative expression of miR-376 in serum of both BC and healthy control groups. BC=breast cancer.

3.3. miR-367 overexpression is related to clinicopathologic features in BC patients

The miR-367 expression was importantly related to tumor node metastasis (TNM) stage, tumor diameter, and lymph node metastasis. It was found that miR-367 expression was remarkably higher in patients with high TNM stage, larger tumor diameter, and with lymph node metastasis than in those with low TNM stage, shorter tumor diameter, and with no lymph node metastasis (all $P < .05$). Surprisingly, miR-367 expression exhibited no correlation with age, menopausal status, pathological grade, estrogen receptor, progesterone receptor, or human epidermal growth receptor (Her)-2 (all $P > .05$) (Table 1).

3.4. miR-367 underexpression results in a short time of DFS of BC patients

Up to June 2020, during the 9 to 60 months of follow-up, 15 of 63 patients had disease recurrence or metastasis. Among 12 died patients, 5 patients died of recurrence or metastasis. Therefore, the DFS rate was 65.08% (41/63). Kaplan–Meier analysis, time table, and log-rank test were used to calculate the effects of miR-367 expression in serum on DFS of patients. It was found that DFS was decreased in the miR-367 low expression group (54.84%), as compared with the miR-367 high expression group (75.00%) ($P = .047$) (Fig. 2).

3.5. miR-367 overexpression is associated with high OS of BC patients

Of the 12 patients who died during follow-up, 3 patients were from the miR-367 high expression group, with an OS rate of 90.63%, while 9 patients were from the miR-367 low expression group, with an OS rate of 70.97%. The OS curve represented that there was a statistically significant difference between the BC patients with high and low miR-367 expression in serum ($P = .042$) (Fig. 3).

Table 1

The correlation analysis between miR-367 expression and clinicopathological features of BC patients.

Parameters	Total	miR-367 expression		P
		High (n = 31)	Low (n = 32)	
Age (yr)				.382
<50	25	14 (56.00%)	11 (44.00%)	
≥50	38	17 (44.74%)	21 (55.26%)	
Menstrual state				.093
Premenopausal	22	14 (63.64%)	8 (36.36%)	
Postmenopausal	41	17 (41.46%)	24 (58.54%)	
TNM stage				.024
I/II	40	24 (60.00%)	16 (40.00%)	
III	23	7 (30.43%)	16 (69.57%)	
Histological grade				.052
G1/G2	37	22 (59.46%)	15 (40.54%)	
G3	26	9 (34.62%)	17 (65.38%)	
Tumour size (cm)				.031
<3	34	21 (61.76%)	13 (38.24%)	
≥3	29	10 (34.48%)	19 (65.52%)	
Lymph node metastasis				.046
Yes	31	12 (38.71%)	20 (64.52%)	
No	32	20 (62.50%)	12 (37.50%)	
ER status				.166
Negative	31	18 (58.06%)	13 (41.94%)	
Positive	32	13 (40.63%)	19 (59.38%)	
PR status				.513
Negative	36	19 (52.78%)	17 (47.22%)	
Positive	27	12 (44.44%)	15 (55.56%)	
Her-2 status				.532
Negative	33	15 (45.45%)	18 (54.55%)	
Positive	30	16 (53.33%)	14 (46.67%)	

BC=breast cancer, ER=estrogen receptor, Her=human epidermal growth receptor, miR= microRNA, PR=progesterone receptor, TNM=tumor node metastasis. The P with bold values showed significantly different.

4. Discussion

As the most common neoplastic disease and main cause of mortality in women, BC showed a high survival rate. Still, reducing BC incidence and mortality remains a healthy priority for public.^[16] Aberrant expression of miRs was observed in various phases of a mass of malignancies, and miRs were reported to control cell behaviors including growth, activation,

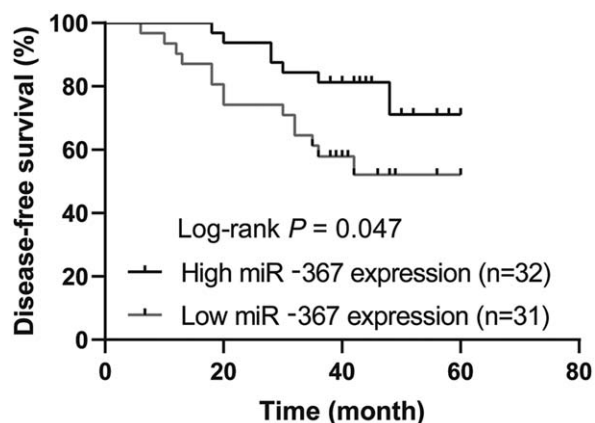


Figure 2. The relation between serum miR-367 expression and DFS in BC patients. BC=breast cancer, DFS=disease-free survival.

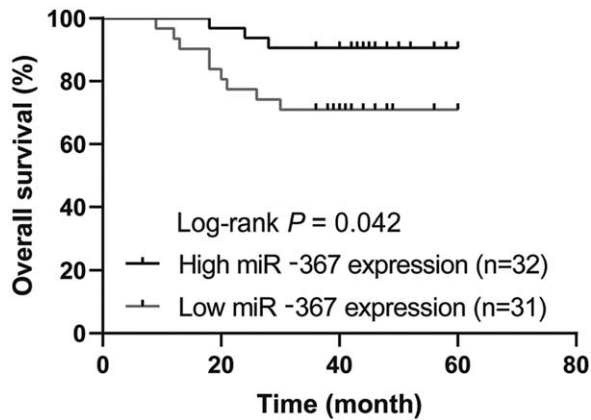


Figure 3. The relation between serum miR-367 expression and OS in BC patients. BC=breast cancer, OS=overall survival.

and differentiation in varying degrees. So, miRs are recognized as reliable biomarkers of prognosis and treatment of tumors.^[12] Strongly expressed miR-367 was conducive to the elevation of the sensitivity to therapeutic drug in ovarian cancer.^[17] In this study, we were inspired to explore the possible association of miR-367 in biological processes of BC, and found that previous studies have indicated that several miRNAs such as miR-15a, miR-21, miR-205, miR-342-3p, and miR-520a-5p were associated with a few molecular and clinicopathologic features and poor prognosis in BC patients.^[18] Murray et al noted that miR-367-3p is also a reliable diagnostic and prognostic factor with high specificity and susceptibility in testicular germ cell tumors.^[19] It was noticeable to find that miR-367 is downregulated in individuals with colorectal cancer, and this downregulation may be responsible for the poor survival rate of these patients.^[20] Furthermore, when highly expressed miR-367-3p is combined with its target gene and other tumor suppressors to form a positive feedback loop in glioma microenvironment, neoplasm is repressed and survival rate is improved.^[21] In this study, according to the results of RT-qPCR, miR-367 expression in serum of BC patients is lower than that in the normal subjects. All in all, it was indicated that miR-367 is beneficial to a wide range of tumor alleviation.

A previous study provided the evidence that the age, tumor size, lymph node status, clinical stage, histological grade, pathological types, and operation method play an important role in judging the prognosis of triple-negative breast cancer.^[22] Cserni and his colleagues indicated that TNM means the characteristics of local lymph nodes including location and counts, primary neoplasm (such as size and ambient structure) and the prognosis of distant metastatic tumors.^[23] TNM stage is an important system in the monitoring, comparison, evaluation, and prognostic description of BC during the follow-up.^[24] Besides, since some oncogenes, including exosomes, growth cytokines, and antigens, are all flowed into local lymph nodes, making them hotbed of neoplastic cell proliferation and development. Lymph node metastasis is commonly seen in many malignancies and linked to a bad prognosis.^[25] The combination of miR-367-3p and chemotherapy can exert effective outcomes in hepatocellular carcinoma by negatively regulating androgen receptor and blocking cancer cell metastasis.^[26] Moreover, in gastric cancer cells where miR-367 is poorly expressed, miR-367 is necessarily to lead to the changes of tumor differentiation,

distant metastasis, and TNM stage, and thus to serve as a pivotal inhibitor in gastric cancer growth and dissemination.^[27] It was found from a previous research that miR-367 was underexpressed in glioma, resulting in a short time of OS,^[28] representing the positive relation between miR-367 and prognosis. In addition, in BC patients who received appropriate therapy, DFS and OS are greatly promoted.^[29] This study indicated that actively expressed miR-367 contributes to a better prognosis with the involvement of the improved DFS and OS, implying the better prognosis of BC. However, there are still some limitations in our study. First, the sample size of this study was limited. Second, a recent study revealed that miRs may tend to be increasingly reliable and sensitive for cancer cell recognition and detection,^[30] so this paper was encouraged to investigate probe novel miR-based strategies for BC mitigation. Third we failed to figure out the potential interaction between miR-367 and some other clinicopathologic features like age, menopausal status, pathological grade, estrogen receptor, progesterone receptor, and human epidermal growth receptor. In the future, if possible, we will further explore the underlying mechanism of other targets of miR-367 using a big sample size of BC patients. More attention will be paid on seeking reliable therapeutic targets of BC.

5. Conclusions

In summary, our study supported that miR-367 expression is associated with clinicopathologic features and prognosis of BC. Although our findings provide therapeutic implication in BC treatment, the experiment results and effective application into clinical practice need further validation.

Author contributions

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