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The expression and significance of five types of miRNAs in breast cancer

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Background: This study aimed to investigate the expression and significance of 5 types of miRNAs in breast cancer to provide a theoretical and practical foundation for using these miRNAs in the diagnosis and treatment of breast cancer, thereby improving medical services.

Material/Methods: Stem-loop real-time RT-PCR was used to detect the expression levels of miR-145, miR-21, miR-10b, miR-125a, and miR-206 in 35 cases of breast cancer and adjacent normal breast tissues, and to analyze the relationship of miRNAs expression with clinicopathological features of breast cancer. The expression levels of estrogen receptor (ER) and progesterone receptor (PR) were examined by immunohistochemistry. Fluorescence *in situ* hybridization was used for the detection of HER-2 and TOP 2A.

Results: The expression levels of miR-145, miR-125a, and miR-206 in breast cancer were lower than those in adjacent normal tissues. MiR-145 was negatively correlated with tumor size, lymph node metastasis, ER, HER-2, and TOP 2A ($P < 0.05$), regardless of age, menstruation, and PR. MiR-125a was correlated with negative node status, negative HER-2 status ($P < 0.05$), whereas tumor size, age, menstruation, ER, and PR were independent factors. MiR-206 expression was correlated with negative ER status, negative PR status, and negative HER-2 status ($P < 0.05$), regardless of age, menstruation, lymph node metastasis, and TOP 2A. MiR-21 and miR-10b expression in breast cancer tissues was significantly higher than that in adjacent tissues ($P < 0.05$). MiR-21 in post-menstrual patients with lymph node metastasis was highly expressed ($P < 0.05$), and had no correlations with tumor size, ER, PR, and TOP 2A expression. MiR-10b expression was positively correlated with breast cancer tumor size, lymph node metastasis, and TOP 2A status ($P < 0.05$), but had no correlations with age, menstruation, ER, PR, and HER-2.

Conclusions: MiR-145, miR-21, miR-10b, miR-125a, and miR-206 may play important roles in breast cancer development and invasion.

MeSH Keywords: **Breast Cancer • miRNAs • HER-2 • TOP 2A**

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Background

The morbidity of breast cancer is gradually increasing, and a growing number of young people are now at risk for this disease. In China, breast cancer continues to be the leading cause of death among women. With the improvement in diagnosis and development of drugs and radiotherapy technology, the survival rate of breast cancer continues to improve. However, the overall rate is not as good as expected. Therefore, early detection of the tumor and accurate analysis of molecular biology characteristics are still of great importance.

MicroRNA (miRNA) is a non-coding protein and single-strand small RNA. MiRNA is 18–25 nucleotides long, and it is more conservative during evolution. It mainly inhibits translation or induces the degradation of some related mRNAs through target mRNA recognition, leading to changes in objective mRNA at the protein expression level. Recent studies showed that some miRNAs have functions similar to that of oncogenes or anti-oncogenes, which may play an important role in the invasion and development of breast cancer.

MiR-125a, miR-206, and miR-145 are similar to anti-oncogenes, and they are related to the occurrence of tumors. Tavazoie [1] found that miR-206 expression decreases in highly metastatic breast cancer cell lines. After these cells recover miR-206 expression, their invasive and metastatic abilities decline. Thus, miR-206 plays a key role in adjustment during breast cancer proliferation. According to the research of Scott and GK [2], miR-125a can affect the protein levels of HER-2 and HER-3, thereby inhibiting the metastatic and invasive abilities of breast cancer cells. Moreover, Lee [3] showed that miR-145 expression increases after miR-145 precursors transfect breast cancer cell lines, but can dose-dependently inhibit cell growth.

MiR-21 and miR-10b are similar to oncogenes, and related to the invasion and metastasis of tumors [4,5]. *In vitro* and *in vivo* experiments showed that eliminating miR-21 gene fragments can inhibit the proliferation and invasion of MCF-7 and MDA-MB-231 cells [5]. In the 4T1 rat breast tumor metastasis model, miR-10b can effectively inhibit the metastasis of malignant breast cancer cells with high specificity. The possible mechanism for this effect may be the inhibition of the entry of 4T1 tumor cells into blood vessels [6]. However, the specific roles of miR-21 and miR-10b in the occurrence and development of breast cancer remain unclear.

The expression of miR-21, miR-10b, miR-125a, miR-206, and miR-145 in breast cancer tissues and para-carcinoma tissues was examined. The expression of estrogen receptor (ER) and progesterone receptor (PR) in breast cancer was analyzed by immunohistochemistry, whereas that of HER-2 and topoisomerase 2A (TOP 2A) was analyzed by fluorescence *in situ*

hybridization (FISH). The relationship between the expression levels of miRNAs and breast cancer clinical pathology was analyzed. Thus, this research aimed to investigate the function of miRNAs in the invasion and development of breast cancer.

Material and Methods

Patients and tissue samples

A total of 35 cases of breast cancer patients were selected. Cancer tissues were obtained from these patients, who were pathologically confirmed to have invasive ductal carcinoma from January 1 2013 to December 1 2013 in the Tumor Hospital of The Second Affiliated Hospital of XJTU. All patients were female, with an age range of 42–62 years (mean age, 51 years). These patients underwent modified radical mastectomy or reserved radical mastectomy, and had complete clinical pathology data. The diameter of tumors ranged from 1.6 cm to 4.5 cm (mean diameter, 2.2 cm). Two samples were maintained under aseptic conditions during the operation. One sample was cancer tissues, and the other sample was para-carcinoma normal gland tissues, which were more than 3 cm away from cancer tissues. The samples were immediately flushed with 0.9% RNA- and enzyme-free NaCl, rapidly immersed in RNA enzyme inhibitor, and incubated at 4°C overnight. The protective liquid was removed from the samples, which were stored in a refrigerator at –80°C. All samples were confirmed by pathology examination. The patients were not treated before the operation, and neither neoadjuvant chemotherapy nor endocrine therapy was conducted. Based on histological classification by the WHO (2003) standards, each patient was pathologically diagnosed with invasive carcinoma after their operation.

Reagent

Trizol reagent was purchased from Invitrogen (USA). M-MLV reverse transcriptase, RNA enzyme inhibitor, oligo dT, dNTPs, KOD-plus, and SYBR Mastermix were obtained from Takara (Japan), and primers were from Shanghai Shenggong Company (China). Breast cancer HER-2, neu (17q12), TOP 2A (17q21), and CSP 17 multicolor detection kits were purchased from Guangzhou Biping Medical Technology Company.

Analysis of miRNA expression (quantitative real-time PCR)

Fresh tissues (50–100 mg) were weighed, and total RNA was extracted. Total RNA (500 ng) was used for RT by miRNA stem-loop RT primers. The reaction was conducted in a 10-μL system at the following reaction conditions: 16°C for 30 min, 42°C for 15 min, and 85°C for 5 s. Real-time qPCR was performed with a 20-μL reaction system. The miRNA reaction detection system included 1 μL of RT products, 1× SYBR Green I Mastermix,

0.5 lxmol/L miRNA-specific F-primer and 0.5 lxmol/L universal R-primer. The conditions of immediate quantitative PCR were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The PCR products were analyzed by 8% PAGE. The circulation times in which real-time qRT-PCR reached the set-up threshold in every reaction tube (Ct data) were recorded.

According to some related studies, the expression of miRNA was analyzed with U6 as the internal reference. Table 1 shows the primer sequences obtained using qPCR. The $2^{-\Delta\Delta Ct}$ method was used to express the gene expression of tumor tissues relative to that of the paired normal tissues. Moreover, $\Delta\Delta Ct = (Ct \text{ miRNA-Ct U6})_{\text{tumor}} - (Ct \text{ miRNA-Ct U6})_{\text{normal}}$ was normal.

Immunohistochemical analysis of the expression of ER and PR in cancer tissues

Samples were fixed in formalin, embedded in paraffin, and sliced into 4-μm-thick sections. The expression of ER and PR was analyzed by immunohistochemical streptavidin-peroxidase method. The results show positive expression of EP and ER at ≥10%, and the cancer cell nucleus was brown.

FISH to examine the expression of HER-2 and TOP 2A in cancer tissues

Samples were fixed in formalin, embedded in paraffin, and sliced into 4-μm-thick sections. Sections were initially observed under the 40× objective with a suitable filter, and counted under the 100× objective. The signals of gene-specific probe (GSP) HER-2 (red), GSP TOP 2A (green), and centromeric probe (CSP) 17 (cyan) were counted in 60 nuclei at the clear tumor areas. The sum of signals of GSP HER-2, GSP TOP 2A, and CSP 17 were recorded, and the data of GSP HER-2/CSP 17 and GSP TOP 2A/CSP 17 were calculated. The signals of HER and TOP 2A were analyzed according to the following criteria: a) the signal of GSP HER-2 and TOP 2A (red, green) combined to bunches; b) GSP HER-2 and TOP 2A/CSP 17 (red, green/cyan) ≥2.2 and 2; c) GSP HER-2 and TOP 2A/CSP 17 (red, green/cyan) <1.8 and 0.8; and d) GSP HER-2 and TOP 2A/CSP 17 (red, green/cyan) between 1.8–2.2 and 0.8–2.0. If “a” or “b,” the situation was gene amplification of HER-2/neu. If “c,” gene amplification of HER-2/neu did not occur. If “d,” new signal data should be counted in 20 other nuclei, or another researcher should recalculate the data. Researchers should indicate the consequence if the recalculated data were at the critical value. Under situations with weak signal or high background, signal judgment may be disturbed, so the experiment was repeated using another tissue slice.

Statistical analysis

Differences in expression were analyzed by SPSS 13.0. Given the limited number of samples in this study, the data demonstrate

Table 1. Primers used for real-time quantitative PCR.

Primer	Order (5'–3')
miR-125a-5p	
F-Primer	ATCCAGTGCGTGTCGTG
T-Primer	TGCTCCCTGAGACCTTTAA
miR-206	
F-Primer	ATCCAGTGCGTGTCGTG
T-Primer	TGCTTGGAAATGAAGGAAG
miR-21	
F-Primer	ATCCAGTGCGTGTCGTG
T-Primer	TGCTTAGCTTATCAGACTG
miR-10b	
F-Primer	ATCCAGTGCGTGTCGTG
T-Primer	TGCTTACCCTGTAGAACCGA
miR-145	
F-Primer	CAGTGCGTGTCGTGGAGT
T-Primer	AGGTCCAGTTTCCAGG
U6	
F-Primer	GCTTCGGCAGCACATATACTAAAAT
T-Primer	CGCTTACGAATTTGCGTGCAT

an abnormal distribution. All miRNA expression data are shown as the median and interquartile range (IQR). The nonparametric Wilcoxon test was used to rank matched-pair data, and the Mann-Whitney U test was used to compare data between the 2 groups. Differences were statistically significant at $P < 0.05$.

Results

Expression of miR-125a, miR-206, miR-145, miR-21, and miR-10b in breast cancer

Among the 35 samples, the relative quantity ($2^{-\Delta\Delta Ct}$) of miR-125a was below 1 (80%) in 28 cases and above 1 (20%) in 7 cases. The data ranged from 0.02 to 3.97 and the IQR was 0.57–0.97. The median was 0.79, which is 0.79 times that of normal tissue. The data were analyzed by Wilcoxon signed-rank text. Differences were statistically significant at $P < 0.05$. Among the 35 samples, the relative quantity ($2^{-\Delta\Delta Ct}$) of miR-206 was below 1 (86%) in 30 cases of breast cancer and above 1 (14%) in 5 cases. The data ranged from 0.06 to 1.82 and the IQR was 0.64–0.92. The median was 0.85, which is 0.85 times that of

Figure 1. HER-2 and TOP 2A expression in breast cancer tissues (100×). (A) Negative HER-2; (B) Positive HER-2-scattering distribution; (C) Positive HER-2-clustering distribution; (D) Negative TOP 2A; (E) Positive TOP 2A-scattering distribution; (F) Positive TOP 2A-clustering distribution.

normal tissue. The data were analyzed by Wilcoxon signed-rank test. Differences were statistically significant at $P < 0.05$.

Among the 35 samples, the relative quantity ($2^{-\Delta\Delta Ct}$) of miR-145 was below 1 (60%) in 21 cases of breast cancer and above 1 (40%) in 14 cases. The data ranged from 0.13 to 7.73 and the IQR was 0.51–1.72. The median was 0.78, which is 0.78 times that of normal tissue. The data were analyzed by Wilcoxon signed-rank test. The difference was statistically significant ($P < 0.05$).

Among the 35 samples, the relative quantity ($2^{-\Delta\Delta Ct}$) of miR-21 was above 1 (94%) in 33 cases of breast cancer and below 1 (6%) in 2 cases. The data ranged from 0.38 to 199.50 and the IQR was 4.69–27.34. The median was 8.58, indicating 8.58 times that of normal tissues. The data were analyzed by Wilcoxon signed-rank test. The difference was statistically significant ($P < 0.05$).

Among the 35 samples, the relative quantity ($2^{-\Delta\Delta Ct}$) of miR-10b was above 1 (63%) in 22 cases of breast cancer and below 1 (37%) in 13 cases. The data ranged from 0.15 to 15.72 and the IQR was 0.70–5.58. The median was 1.70, indicating

it was 1.70 times that of normal tissues. The data were analyzed by Wilcoxon signed-rank test. The difference was statistically significant ($P < 0.05$).

FISH to examine the expression of HER-2 and TOP 2A in breast cancer

Among the 35 patients, 16 cases (45.7%) exhibited HER-2 gene amplification, which was demonstrated as a red cluster or multiple red points, whereas 18 cases (51.4%) exhibited TOP 2A gene amplification, which was demonstrated as a green cluster or multiple green points (Table 1). Negative expression of HER-2 and TOP 2A was observed in the 17th chromosome (Figure 1).

Relationship between the expression of miR-145, miR-125a, miR-206, miR-21, and miR-10b and the clinical pathology of breast cancer tissues

The expression levels of miR-125a, miR-206, and miR-145 in breast cancer tissues were all lower than those in para-carcinoma tissues. Low expression of miR-125a in the metastatic lymph node group and high negative expression of HER-2 and TOP 2A

Table 2. The relationships miR-125a and miR-206 expression in breast cancer.

Clinic pathology reasons	Cases	Expression of miR-125a median (range)		P (two sides)	Expression of miR-206 median (range)		P (two sides)
Age							
<51	18	0.82	(0.65–1.75)	0.103	0.86	(0.74–0.92)	0.443
≥51	17	0.78	(0.13–0.91)		0.84	(0.31–0.94)	
Menstruation							
Pre-menopause	20	0.81	(0.63–1.29)	0.505	0.89	(0.76–0.95)	0.137
Post-menopause	15	0.78	(0.13–0.92)		0.74	(0.46–0.91)	
Size of tumor							
T ≤2cm	12	0.73	(0.35–0.92)	0.498	0.90	(0.77–1.15)	0.063
T >2 cm	23	0.85	(0.57–1.39)		0.84	(0.46–0.91)	
Lymph node							
Negative	18	0.91	(0.67–1.48)	0.049	0.84	(0.52–0.92)	0.318
Positive	17	0.65	(0.19–0.84)		0.89	(0.69–0.95)	
ER							
Negative	16	0.82	(0.66–1.55)	0.161	0.90	(0.77–1.16)	0.011
Positive	19	0.68	(0.13–0.92)		0.80	(0.15–0.89)	
PR							
Negative	19	0.82	(0.64–0.97)	0.133	0.89	(0.80–0.95)	0.031
Positive	16	0.72	(0.11–0.91)		0.66	(0.22–0.91)	
HER-2							
Negative	19	0.82	(0.65–1.74)	0.071	0.89	(0.84–0.95)	0.010
Positive	16	0.70	(0.12–0.91)		0.64	(0.12–0.89)	
TOP 2A							
Negative	17	0.97	(0.60–1.86)	0.062	0.89	(0.66–0.95)	0.335
Positive	18	0.73	(0.18–0.86)		0.85	(0.38–0.91)	

The expression level of miR-125a and miR-206 ($2^{-\Delta\Delta Ct}$) are shown by median and IQR. (Because of the sample cases is not enough, the data comes out as abnormal distribution. So we don't use $\bar{x} \pm s$ to explain it.)

in patients were unrelated to the tumor size, patient age, menstruation, negative ER, and PR expression ($P < 0.10$). MiR-206 was highly expressed in patients with small tumors, negative ER, negative PR, and negative HER-2 ($P < 0.10$). Thus, miR-206 was irrelevant to patient age, menstruation, metastatic lymph node, and TOP 2A expression (Table 2). MiR-145 was negatively correlated with the tumor size and lymph node metastasis ($P < 0.05$), and had no correlation with patient age, menstruation, and PR expression. However, miR-145 was correlated with negative ER, negative HER-2, and negative TOP 2A ($P < 0.05$) (Table 3).

The expression of miR-21 and miR-10b in breast cancer tissues was obviously higher than that in para-carcinoma tissues. MiR-21 exhibited high expression in older, postmenopausal patients with various symptoms, such as lymph node metastasis and positive HER-2 ($P < 0.10$), but it was not related to the tumor size and expression levels of TOP 2A, ER, and PR. The

expression of miR-10b was related to the tumor size, lymph node metastasis, and positive TOP-2A ($P < 0.05$), but it was not associated with age, menstruation, ER, PR, and HER-2 (Table 3).

Discussion

Recent studies have already proved that the expression of miRNAs changes is closely related to various types of human cancer. Molecular therapy mediated by such changes is an important development in tumor treatment. However, the functions of miRNAs are complicated because they can effectively regulate and control hundreds of genes, including oncogenes and anti-oncogenes.

Studies indicated that miR-125a (has-miR-125a) is located at chromosome 19q13.41, including 2 miRNAs that are in the

Table 3. The relationships miR-10b, miR-21, and miR-145 and expression in breast cancer.

Clinic pathology reasons	Cases	Expression of miR-10b median (range)		P (two sides)	Expression of miR-21 median (range)		P (two sides)	Expression of miR-145 median (range)		P (two sides)
Age										
<51	18	1.93	(0.63–4.94)	0.869	5.79	(2.48–16.80)	0.065	0.70	(0.46–1.77)	0.717
≥51	17	1.03	(0.76–9.44)		12.63	(5.76–29.22)		0.93	(0.55–1.67)	
Menstruation										
Pre-menopause	20	2.67	(0.96–5.67)	0.301	5.69	(2.95–10.41)	0.011	0.71	(0.52–1.74)	0.841
Post-menopause	15	1.16	(0.56–3.29)		15.74	(6.38–52.29)		0.88	(0.47–1.63)	
Size of tumor										
T ≤2 cm	12	1.00	(0.50–2.89)	0.025	5.30	(3.56–46.99)	0.602	1.59	(0.80–2.61)	0.039
T >2 cm	23	2.59	(0.83–6.13)		9.39	(5.70–17.85)		0.70	(0.47–0.95)	
Lymph node										
Negative	18	0.89	(0.56–1.21)	0.001	5.42	(3.67–8.78)	0.002	1.55	(0.63–1.94)	0.033
Positive	17	5.58	(2.94–10.23)		27.34	(7.55–54.67)		0.65	(0.39–0.91)	
ER										
Negative	16	1.48	(0.77–5.37)	0.908	7.65	(3.96–33.06)	0.895	1.26	(0.70–1.74)	0.047
Positive	19	1.70	(0.64–6.13)		8.72	(4.71–21.48)		0.58	(0.34–1.63)	
PR										
Negative	19	1.72	(0.70–4.70)	0.585	12.00	(4.69–52.29)	0.233	0.93	(0.70–1.70)	0.329
Positive	16	1.19	(0.69–6.04)		6.71	(3.81–12.16)		0.58	(0.36–2.15)	
HER-2										
Negative	19	2.59	(0.83–5.70)	0.282	5.13	(1.84–17.85)	0.055	1.47	(0.65–1.82)	0.016
Positive	16	1.10	(0.61–3.55)		12.31	(6.01–30.15)		0.58	(0.36–0.92)	
TOP 2A										
Negative	17	1.03	(0.61–1.93)	0.039	5.13	(3.10–28.22)	0.248	0.95	(0.62–1.59)	0.033
Positive	18	3.62	(0.90–8.98)		9.06	(5.84–28.28)		0.48	(0.28–0.89)	

The expression level of miR-145, miR-21, and miR-10b ($2^{-\Delta\Delta Ct}$) are shown by median and IQR. (Because of the sample cases is not enough, the data comes out as abnormal distribution. So we don't use $\bar{x} \pm s$ to explain it).

same precursor (miR-125a.5p and miR-125a.3p), and has the same origin as lin-4 in nematodes. MiR-125a can be detected as a phylogenetic mutation, which is associated with the occurrence of breast cancer. The possible mutation sites include rs10404453, rs12975333, and rs143525573. MiR-125a can also inhibit tumors by directly lowering the mRNA and protein levels of some genes, such as ERBB2 and RNA binding protein HuR [1].

MiR-206 is located at human chromosome 6p12.2, and is considered to be a type of miRNA related to skeletal muscles and the myocardium. It can boost muscle differentiation by lowering the P180 subunit of DNA polymerase and muscle transcription factors (e.g., Is1-3 and MyoR). MiR-206 can also

inhibit the proliferation of breast cancer cells, mainly by lowering the mRNA and protein expression levels of ER α and ER α co-regulatory proteins via the EGF/MAPK pathway [lowering steroid acceptor nuclear co-activators (e.g., SRC-1 and SRC-3) and transcription factor GATA-3] [7]. MiR-206 can also act on the protein Cx43 to inhibit the invasion and metastasis of cancer cells. MiR-206 can result in mutations at the rs6920648 locus, and is associated with the existence of breast cancer [8].

MiR-145, which comprises 23 basic groups, is situated at chromosome 5q32-33. The expression of miR-145 decreases in many types of tumor tissues, including breast cancer, colon cancer, and lung cancer. This phenomenon might be related to several factors, such as the location of miR-145, ability of miR-145

to easily cause deficiency in tumor cells, and genetic changes in the receptor's surface [9]. Similarly, miR-145 can inhibit hyperplasia in tumor cells by affecting target genes, such as c-Myc, IRS-1, OCT4, SOX2, and KLF4 [9].

MiR-21 is an miRNA located in the FRA17B fragile area of chromosome 17q23.2, and has an independent transcription unit. MiR-21 expression is remarkably abnormal in many types of tumor cells. Moreover, miR-21 controls the expression of many anti-oncogenes, including TPM1, programmed cell death 4, maspin, and bcl-2, to prompt the metastasis and hyperplasia of breast cancer cells [10].

MiR-10b is located between the HOXD4 and HOXD8 genes of 2p31.1, and its structure is 5'-UACCCUGUAGAACCGAAUUUGUG-3'. MiR-10b is related to many types of tumors [1]. Adjusting the Twist-miR-10b-HOXD 10-RhoC pathway is the main mechanism by which miR-10b inhibits the metastasis of breast cancer cells. MiR-10b also facilitates the metastasis of breast cancer cells by regulating the expression of the target genes uPAR and syndecan-1 [4,11].

Early diagnosis is essential to prevent and cure tumors. In this study, the relative transcript levels of miR-21 and miR-10b in patients' tumor tissues were higher than those in para-carcinoma normal tissues. By contrast, the relative transcript levels of miR-145, miR-125a, and miR-206 in patients' tumor tissues were apparently lower than those in para-carcinoma normal tissues. According to the literature [12–14], the relative transcript levels of miR-145, miR-125a, and miR-206 in serum are also visibly abnormal compared with those in serum from healthy females. Therefore, these miRNAs can be used as markers of breast cancer for early diagnosis.

Our results show that miR-145 and breast cancer tumor size, as well as miR-145 and lymph node metastasis, was negatively correlated in 35 cases of breast cancer. Although miR-125a and miR-206 were expressed at low levels in the large mass group and lymph node metastasis group, statistical analysis showed no significant correlation between the expression of miR-125a and miR-206 and breast tumor size, as well as between the expression of miR-125a and miR-206 and lymph node metastasis. We are currently further expanding the number of samples for analysis to determine the intrinsic correlations. Preliminary results show that miR-145, miR-125a, and miR-206 were similar to tumor suppressor genes, and may be involved in the suppression of breast cancer invasion and metastasis; these findings are consistent with those reported in the literature [2,3,15].

MiR-10b expression is correlated with the tumor size and lymph node metastasis. As indicated in the literature, miR-10b expression may indicate the invasion and development of

breast cancer [12]. MiR-21 is related to patient age, menstruation, and lymph node metastasis. Combined with the findings of Lee [16], the results show that miR-21 may be correlated with old patients with lymph node metastasis. Thus, miR-21 can offer new techniques for the treatment of older patients.

Breast cancer can be divided into 3 types – hormone receptor-positive breast cancer, HER-2-positive breast cancer, and triple-negative breast cancer (TNBC)] – based on ER, PR, and HER-2 expression. This classification is significant in estimating cancer prognosis and choosing different individual treatment methods.

HER-2-positive breast cancer is highly malignant, and can reappear and transfer easily. Herceptin-targeted treatment has made significant progress for this type of cancer. Lee [16] showed that miR-21 is related to the metastasis of breast cancer lymph nodes and positive HER-2 expression. Moreover, miR-21 is an independent prognostic factor of breast cancer. Combined with Cong's research [17], the results showed that miR-21 is over-expressed in breast cancer cells resistant to trastuzumab, which causes the loss of PTEN expression. Thus, miR-21 may have some connections with Herceptin resistance. A previous study [2] showed that miR-125a is negatively associated with HER-2, indicating that the HER-2 gene may be under the regulation and control of miR-125a; therefore, miR-125a can distinctly inhibit the expression of oncogenes HER-2 and HER-3. MiR-125a expression is up-regulated when the HDAC inhibitor entinostat is used to treat HER-2-positive breast cancer cells, and has been shown to be an important mechanism of the functions of entinostat [18]. Thus, miR-21 and miR-125a are significant to developing new anti-HER-2 drugs and overcoming Herceptin resistance in drugs.

TNBC is characterized by high malignancy, high possibility of reappearance and metastasis, and poor treatment methods. In this study, miR-145 and miR-206 were found to be related to negative ER and negative HER-2 ($P < 0.05$). MiR-145 was highly expressed in negative PR ($P > 0.05$), whereas miR-206 was negatively correlated with negative PR ($P < 0.10$). Therefore, miR-145 and miR-206 may affect TNBC. *In vitro* experiments showed that the metastasis of cancer cells could be inhibited by leading miR-145 and miR-206 into metastatic breast cancer cells. MiR-145 and miR-206 are expected to be the new target spots in TNBC treatment. Besides inhibiting TNBC development, miR-206 can also inhibit positive ER lines, and is considered as the switch of breast cancer when it is transferred to the base type from lumen-A [19]. Therefore, miR-206 has a significant contribution in the treatment of breast cancer.

Drug resistance is an important cause of tumor treatment failure. Studies proved that miRNAs are related to drug

resistance of tumors. TOP 2A codes DNA topoisomerase α , and is one of the components of the nuclear matrix. TOP 2A can adjust the copy, transcription, recombination, and repair of DNA, which is not only a hyperplasia index of tumor cells, but also a treatment target of anthracycline chemotherapy drugs. In breast cancer tissues, tumors with high expression of TOP 2A always demonstrate highly malignant behaviors. This situation can be improved after using anthracycline alone or anthracycline combination chemotherapy. In this research, miR-21 was found to be positively related to TOP 2A, whereas miR-125a and miR-145 were negatively associated with TOP 2A. These findings show that miR-145 and miR-125a might inhibit the TOP 2A gene, and were connected with anthracycline drug resistance. Therefore, miR-21, miR-125a, and miR-145 may contribute to the chemotherapy effect of epirubicin, and are important in guiding the development of chemotherapy for breast cancer.

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Conclusions

Therefore, miR-125a, miR-206, miR-145, miR-21, and miR-10b are highly expected to be novel tumor markers for the diagnosis and prognosis of breast cancer. MiR-21 and miR-125a could contribute to the treatment of HER-2-positive breast cancer cells, whereas miR-145 and miR-206 could accelerate the development of treatment methods for TNBC. MiR-125a and miR-145 may play a key role in the effects of epirubicin chemotherapy. However, the detailed mechanism and whether these miRNAs can be used as treatment targets in clinical applications should be verified in future investigations.

Conflict of interest statement

All authors have no financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.