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Correlations of high miRNA expressions with traditional proteins and prognosis of breast cancer

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ABSTRACT

Background: In this study, we aimed to analyze the correlations of high expressions of micro ribonucleic acids (miRNAs) with traditional proteins and prognosis of breast cancer.

Methods: The clinical data of 60 breast cancer patients treated from August 2018 to July 2019 were retrospectively analyzed. All the patients received radical mastectomy combined with postoperative adjuvant chemoradiotherapy and were followed up for 3 years after treatment. The 3-year survival was recorded. The surviving patients were included in a good prognosis group, and the deceased ones were assigned to a poor prognosis group. The levels of miRNAs (miR-182, miR-155, and miR-217) and traditional breast cancer proteins [estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (CerbB-2/HER-2), and cell proliferation factor-67 (Ki-67)] were measured. Receiver operating characteristic (ROC) curve was plotted to investigate the predictive value of miRNA levels for poor prognosis.

Results: The relative expressions of miR-182, miR-155 and miR-217 were negatively correlated with ER and PR ($r<0$, $P<0.05$), but positively correlated with positive Ki-67 expression ($r>0$, $P<0.05$). High relative expressions of miR-182, miR-155 and miR-217 and positive Ki-67 expression were risk factors for poor prognosis, and the positive expressions of ER and PR were protective factors ($OR<1$, $P<0.05$). The areas under the curves of the relative expressions of miR-182, miR-155 and miR-217 and combined detection for predicting poor prognosis all exceeded 0.70. The combined detection had the highest predictive value.

Conclusions: The high expressions of miR-182, miR-155 and miR-217 are correlated with the expressions of traditional breast cancer proteins ER, PR, and Ki-67, and may predict the prognosis of breast cancer patients.

Keywords: breast cancer, micro ribonucleic acid, prognosis, protein

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INTRODUCTION

Breast cancer results from the uncontrollable proliferation of mammary epithelial cells under the action of multiple carcinogenic factors, and patients often have breast lumps and nipple discharge. The incidence rate of breast cancer is about 24.2% among female cancer cases worldwide, seriously threatening women's health [1]. At present, breast cancer is mainly treated by surgeries, among which radical mastectomy can eliminate the tumor and improve the clinical symptoms of patients by resecting the whole breast and related lymph nodes, and postoperative adjuvant chemoradiotherapy can further remove the cancer cells and improve the clinical efficacy [2]. However, some patients still have a poor prognosis after treatment, resulting in a shorter survival time. Therefore, it is necessary to search for indicators that can effectively predict the prognosis of breast cancer patients.

Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (CerbB-2/HER-2), and cell proliferation factor-67 (Ki-67) all belong to traditional breast cancer proteins, and ER and PR are receptors regulating the growth and development of sexual organ tissues. As breast cancer is an estrogen-dependent tumor, the expressions of ER and PR in breast cancer tissues are of important significance for assessing the prognosis of patients and the efficacy of endocrine therapy [3]. CerbB-2/HER-2, a human proto-oncogene with the activity of transmembrane tyrosine kinase, can participate in cell proliferation signaling [4]. Ki-67 is a proliferating cell-associated antigen, which is an indispensable substance in cell proliferation [5]. Traditional breast

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cancer proteins are crucial for guiding treatment protocols and prognosis evaluation for breast cancer patients in clinical practice, but the indicators mentioned above are difficult to be assessed quantitatively.

Micro ribonucleic acids (miRNAs) are a class of non-coding RNA molecules that are extensively involved in cell proliferation, differentiation, and apoptosis, and their abnormalities can be detected in various cancers [6]. For instance, they are closely related to the proliferation and invasion of choriocarcinoma cells [7]. MiR-182 located on the human chromosome 7 forms a gene cluster with miR-96 and miR-183. It targets the transcription elongation factor A-like 7 gene and mediates the c-Myc/cyclin D1/nuclear factor-κB signaling, thus modulating the proliferation and colony-forming capacities of cancer cells [8]. MiR-155 can directly inhibit the anti-oncogene suppressor of cytokine signaling 1 to promote the proliferation and development of breast cancer cells [9]. Moreover, miR-155 overexpression can stimulate signal transducer and activator of transcription 3 and activate breast cancer cells through the Janus kinase pathway, thus promoting tumor development and increasing the risk of poor prognosis [10]. Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a major regulator of mitochondrial biogenesis and energy metabolism, involving fatty acid synthesis and reactive oxygen species levels, and it is able to regulate the expression of vascular endothelial growth factor, thereby providing nutrients to tumor cells [11]. MiR-217 is an upstream regulator of PGC-1α, which can bind the 3'-UTR of PGC-1α gene to facilitate PGC-1α expression and tumor development, increasing the risk of poor prognosis [12].

Therefore, the correlations of miRNA expressions with traditional proteins and prognosis of breast cancer patients were analyzed in this study, aiming to provide valuable evidence for future treatment and prognostic evaluation.

METHODS

General data

This study was approved by the ethics committee of our hospital, and we obtained the patient's approval to study their samples. The clinical data of 60 breast cancer patients admitted to and treated in the hospital from August 2018 to July 2019 were retrospectively analyzed. The patients were aged from 43 to 52 years old, with a mean age of (46.81±2.86) years old. The body mass index was 18-24 kg/m2 and (20.90±1.03) kg/m2 on average. The tumor size ranged from 1.5 to 3.5 cm, with a mean size of (2.60±0.41) cm. As for the pathological type, there were 32 cases of infiltrating ductal carcinoma, 22 cases of infiltrating lobular carcinoma, and 6 cases of others. Regarding the clinical stage, 37 patients were in stage I-II and 23 patients were in stage III-IV. The inclusion criteria were set as follows: 1) patients with breast cancer meeting the diagnostic criteria in the Clinical Practice Guidelines in Breast Cancer by the Chinese Anti-Cancer Association and confirmed by postoperative pathological examinations [13], 2) those treated with modified radical mastectomy + postoperative adjuvant chemotherapy (TAC regimen), 3) female patients, 4) those with unilateral lesions, and 5) those with complete clinical data. The exclusion criteria included: 1) patients complicated with other malignant tumors, 2) those with a past history of breast cancer, or 3) those with immune dysfunction.

Treatment methods

All patients underwent modified radical mastectomy and postoperative adjuvant chemoradiotherapy. To be specific, chemotherapy was dominated by the TAC regimen. The chemotherapy was conducted for 21 d as a cycle and 6 cycles in total. Patients with ≥4 positive axillary lymph node metastases were subjected to radiotherapy. Moreover, endocrine therapy was applied to patients with positive ER and PR according to pathological results after operation.

MiRNA detection

Breast cancer tissues were fixed, dehydrated, embedded in paraffin, and sliced into 4-μm-thick sections. Meanwhile, cancer tissues with tumor cells accounting for over 50% were collected (2-5 pieces for each case) to prepare total RNA. Specifically, the sections were put into 1.5 mL centrifuge tubes (Shandong Longtewei Medical Instrument Co., Ltd., China), added and mixed with 1 mL of xylene, and placed at 50°C for 3 min to dissolve the paraffin. Finally, xylene was removed by centrifugation and eliminated again using 1 mL of absolute ethanol. After drying, the total RNA was extracted via TRIzol method according to the instructions of total RNA extraction kit (Otsuka Pharmaceutical Co., Ltd., Japan). Then the purified RNA was dissolved in 30 μL and 60 μL of 1 g/L DEPC water and subpackaged in 20 μL for detection, and the rest was stored at -80°C for standby. The detection was performed by a real-time quantitative polymerase chain reaction (qPCR) system (LightCycler 480 II, Roche Diagnostics, Switzerland) with RNA as the template. The initial RNA concentration before reverse transcription was 250 ng/ml. In addition, reverse transcription was implemented using miR-182, miR-155, miR-217, and the stem-loop structure of U6 [14] as the primers under the following conditions: 70°C for 5 min, 42°C for 50 min and

95°C for 5 min, so as to obtain the cDNAs of miR-182, miR-155, miR-217 and U6. Next, the specific PCR amplification primers for miR-182, miR-155, miR-217, and U6 as well as KAPA SYBR FAST qPCR kit (Beijing Microread Genetics Co., Ltd., China) were adopted to perform qPCR on the qPCR system under the following reaction conditions: pre-denaturation at 95°C for 3 min, 40 cycles of 95°C for 15 s and 60°C for 30 s, followed by 1 fluorescence detection. The number of cycles (Ct value) for the fluorescence signal reaching the set threshold in the reaction tube was recorded. The relative expressions of miR-182, miR-155 and miR-217 were expressed by the 2-ΔΔCt method.

Detection of traditional breast cancer proteins

The protein expressions of ER, PR, CerbB-2/HER-2, and Ki-67 in each group of tissue samples were examined by immunohistochemistry. Specifically, all tissue samples were routinely embedded in paraffin after being fixed with 4% formaldehyde, then serially sliced into 4-μm-thick sections and observed under a 400× DM1000 LED biomicroscope (Leica Microsystems CMS GmbH, Germany) by randomly selecting 5 fields of view. The appearance of yellowish-brown or yellow particles in the nuclei indicated positive expression, and the number of positive cells among 200 tumor cells in each field of view was recorded. The judgment criteria for positive expression were as follows: no positive cells in staining for negative expression, and positive cells in staining for positive expression.

Grouping criteria and methods

Patients were followed up for 3 years after treatment, and the survival conditions and survival time during the follow-up period were recorded. The patients who died during the follow-up were included in the poor prognosis group, and those of surviving patients were assigned to the good prognosis group.

Statistical analysis

SPSS 25.0 software was used for statistical analysis. The measurement data were represented as (x ± s) and compared between groups through the independent-samples t-test. The count data were expressed as percentage and examined by the χ2 test. The Kaplan-Meier method was used to plot the survival curves of patients. The relationship between miRNA levels and traditional breast cancer protein expressions was explored by bivariate Spearman correlation analysis. Cox regression analysis was applied to assess the effects of the expressions of miRNAs and traditional breast cancer proteins on the prognosis of breast cancer patients. The receiver operating characteristic (ROC) curve was plotted and the area under the curve (AUC) was calculated to test the predictive value of miRNAs for poor prognosis. P<0.05 suggested a statistically significant difference.

RESULTS

Survival of patients

The breast cancer patients were followed up for 3 years after surgical treatment. Among them, 13 (21.67%) patients had a poor prognosis, and 47 (78.33%) patients had a good prognosis. The mean survival time of the patients was (2.07 ± 0.78) years. The survival analysis function is shown in Figure 1.

Baseline clinical data

Compared with the good prognosis group, the poor prognosis group exhibited elevated relative expressions of miR-182, miR-155, and miR-217, a lowered proportion of patients with positive expressions of ER and PR as well as an increased proportion of patients with positive expressions of CerbB-2/HER-2 and Ki-67 (P<0.05) (Table 1).

Relationship between miRNA levels and expressions of traditional breast cancer proteins

The results of bivariate Spearman correlation analysis showed that the relative expressions of miR-182, miR-155, and miR-217 were negatively correlated with ER and PR (r<0, P<0.05) but positively correlated with positive Ki-67 expression (r>0, P<0.05) (Table 2 and Figure 2).

Effects of miRNA levels and traditional breast cancer protein expressions on poor prognosis of breast cancer patients

The Cox regression analysis was conducted with the prognosis of breast cancer patients as a dependent variable ("0" = good prognosis, "1" = poor prognosis), and

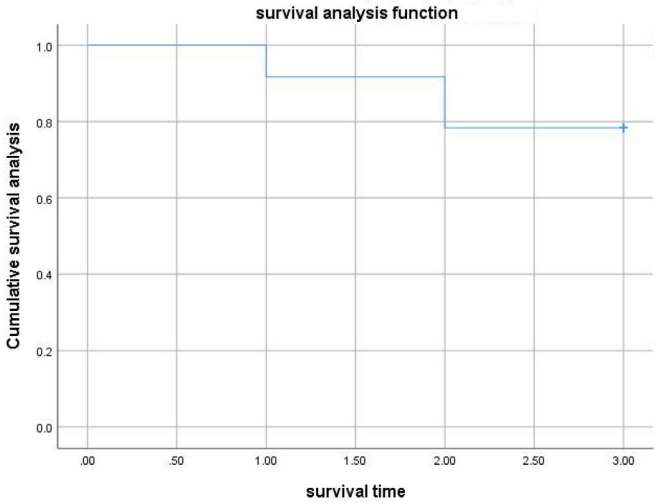

Figure 1. Survival analysis function (X axis: years)

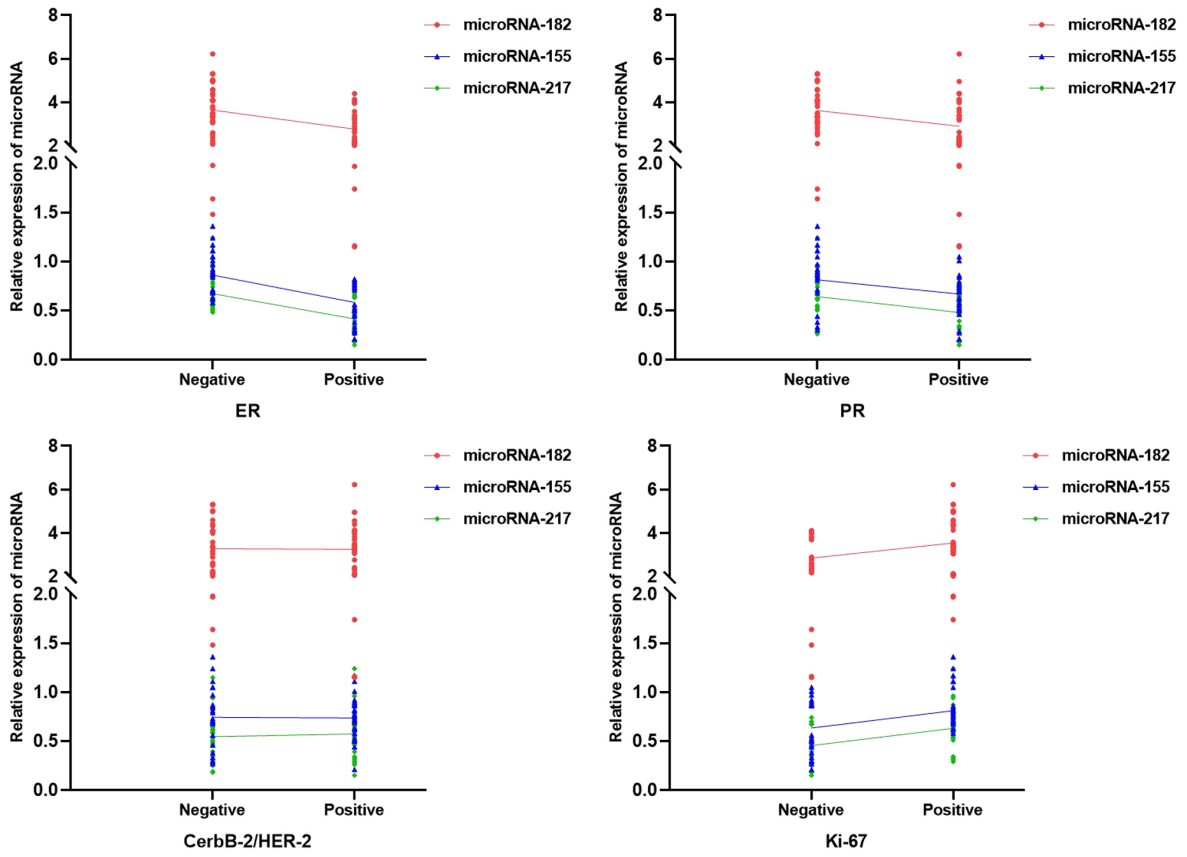

Figure 2. Correlations between miRNA levels and expressions of traditional breast cancer proteins.

Table 1. Baseline data in the two groups

Data		Poor prognosis group (n=13)	Good prognosis group (n=47)	χ^2/t	P value
Age (x ± s, years)		47.85±3.19	46.77±4.02	0.892	0.376
Body mass index (x ± s, kg/m^2)		20.43±2.27	21.17±1.98	1.156	0.252
Tumor size (x ± s, cm)		3.57±1.01	2.11±0.93	4.919	<0.001
Pathological type [n (%)]	Infiltrating ductal carcinoma	7 (53.85)	25 (53.19)	0.598	0.742
	Infiltrating lobular carcinoma	4 (30.77)	18 (38.30)		
	Others	2 (15.38)	4 (8.51)		
Clinical stage [n (%)]	Stage I-II	5 (38.46)	32 (68.09)	2.631	0.105
	Stage III-IV	8 (61.54)	15 (31.91)		
Lymph node metastasis [n (%)]	Yes	7 (53.85)	37 (78.72)	2.076	0.150
	No	6 (46.15)	10 (21.28)		
Relative expression of miR-182 (x ± s)		4.46±1.04	2.94±0.96	4.964	<0.001
Relative expression of miR-155 (x ± s)		0.86±0.22	0.71±0.23	2.24	0.029
Relative expression of miR-217 (x ± s)		0.72±0.27	0.52±0.19	3.053	0.003
ER [n (%)]	Positive	2 (15.38)	24 (51.06)	5.279	0.022
	Negative	11 (84.62)	23 (48.94)		
PR [n (%)]	Positive	3 (23.08)	27 (57.45)	4.812	0.028
	Negative	10 (76.92)	20 (42.55)		
CerbB-2 / HER-2 [n (%)]	Positive	10 (76.92)	21 (44.68)	4.239	0.040
	Negative	3 (23.08)	26 (55.32)		
Ki-67 [n (%)]	Positive	11 (84.62)	25 (53.19)	4.190	0.041
	Negative	2 (15.38)	22 (46.81)		

Table 2. Relationship between miRNA levels and expressions of traditional breast cancer proteins

Indicator	ER	PR	CerbB-2/HER-2	Ki-67
Relative expression of miR-182	-0.392, P=0.014	-0.264, P=0.029	-0.128, P=0.191	0.428, P<0.001
Relative expression of miR-155	-0.406, P<0.001	-0.321, P=0.021	-0.168, P=0.168	0.328, P=0.026
Relative expression of miR-217	-0.746, P<0.001	-0.568, P<0.001	-0.117, P=0.207	0.573, P<0.001

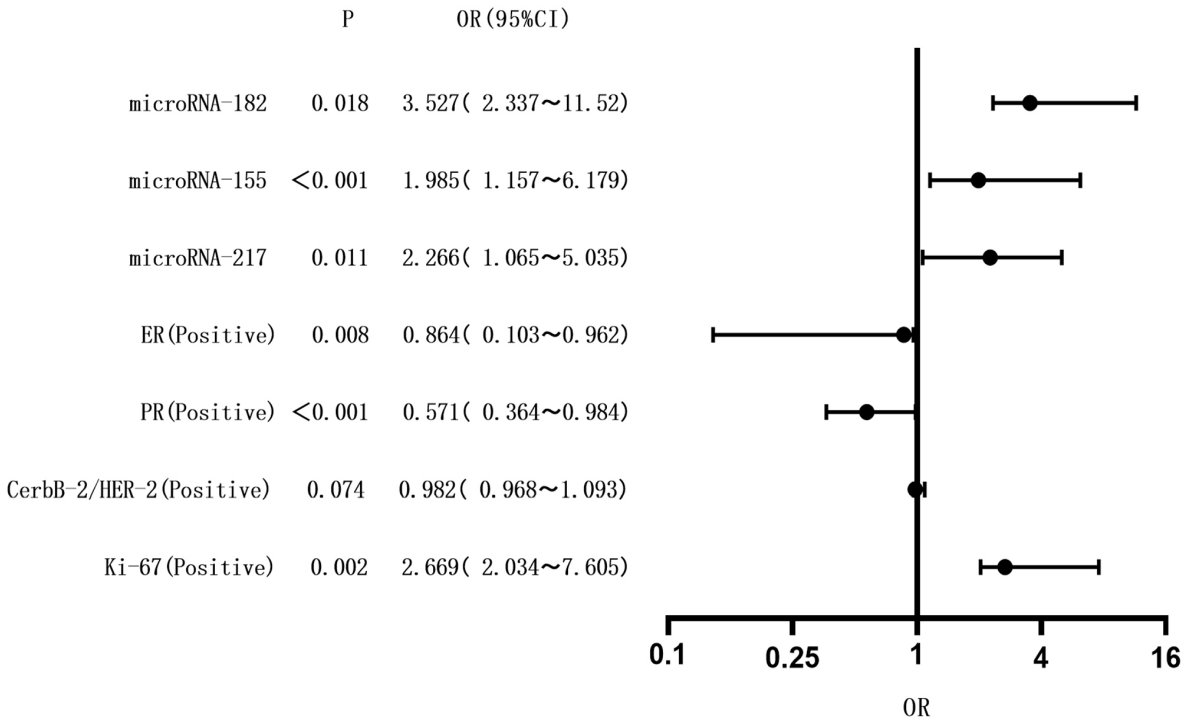

Figure 3. Forest plot of factors and characteristics based on multivariate logistic regression analysis.

the miRNAs and traditional breast cancer proteins (relative expressions of miR-182, miR-155, and miR-217, as well as expressions of ER, PR, CerbB-2/HER-2, and Ki-67) as independent variables. The assignment of variables is listed in Table 3. High relative expressions of miR-182, miR-155 and miR-217 and positive Ki-67 expression were risk factors for the poor prognosis of breast cancer patients [odds ratio (OR) >1, P<0.05], while the positive expressions of ER and PR were protective factors (OR<1, P<0.05) (Table 4 and Figure 3).

Table 3. Assignment of variables

Related factor	Variable type	Variable assignment
RE of miR-182	Continuous	-
RE of miR-155	Continuous	-
RE of miR-217	Continuous	-
ER	Categorical	"0" = negative, "1" = positive
PR	Categorical	"0" = negative, "1" = positive
CerbB-2/HER-2	Categorical	"0" = negative, "1" = positive
Ki-67	Categorical	"0" = negative, "1" = positive

RE- relative expression

Predictive value of miRNAs for poor prognosis of breast cancer patients

The prognosis of breast cancer patients was set as a state variable ("0" = good prognosis, "1" = poor prognosis) and the relative expressions of miRNAs in patients were used as test variables to plot the ROC curves. The AUCs of the relative expressions of miR-182, miR-155, and miR-217 and combined detection for predicting the poor prognosis of breast cancer patients were >0.70, with certain predictive value, and the combined detection had the highest predictive value (Table 5 and Figure 4).

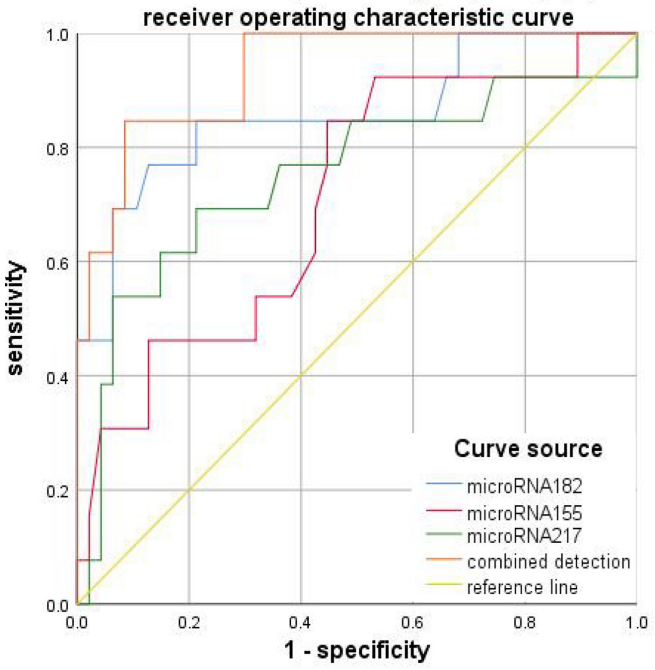

Figure 4. Predictive value of miRNAs for poor prognosis of breast cancer patients.

DISCUSSION

The mortality rate of breast cancer cases has gradually decreased with the development of treatment strategies and methods [15]. Modified radical mastectomy and postoperative adjuvant chemoradiotherapy are commonly applied in the treatment of breast cancer, which can effectively remove tumors and kill cancer cells, but

Table 4. Effects of miRNA levels and traditional breast cancer protein expressions on poor prognosis of breast cancer patients

Related factor	β	Standard error	Wald χ2	P value	Odds ratio	95% confidence interval
Relative expression of miR-182	3.274	1.574	4.891	0.018	3.527	2.337-11.52
Relative expression of miR-155	2.362	2.039	5.576	<0.001	1.985	1.157-6.179
Relative expression of miR-217	2.937	1.117	4.028	0.011	2.266	1.065-5.035
Positive ER	-1.996	3.084	4.639	0.008	0.864	0.103-0.962
Positive PR	-2.07	2.581	5.652	<0.001	0.571	0.364-0.984
Positive CerbB-2/HER-2	1.372	1.654	2.601	0.074	0.982	0.968-1.093
Positive Ki-67	2.571	2.516	4.964	0.002	2.669	2.034-7.605
Constant	41.684	1.245	6.601	<0.001	-	-

Table 5. Predictive value of miRNAs for poor prognosis of breast cancer patients

Factor	AUC	Cut-off value	95% CI	P value	Specificity	Sensitivity	Youden index
Relative expression of miR-182	0.858	3.720	0.725-0.990	<0.001	0.766	0.846	0.612
Relative expression of miR-155	0.709	0.770	0.550-0.867	0.022	0.511	0.846	0.357
Relative expression of miR-217	0.750	0.535	0.577-0.924	0.006	0.638	0.769	0.407
Combined detection	0.933	-	0.865-1.000	<0.001	0.915	0.846	0.761

some patients still die after treatment [16]. The 5-year survival rate of breast cancer patients is 82.6% [17] exceeding that (78.33%) in the present study. It might be related to the severity of the disease in the patients included in this study, suggesting a high risk of poor prognosis. Therefore, indicators that can effectively predict the poor prognosis of breast cancer patients should be explored.

As single-stranded non-coding RNAs with about 22 nucleotides, miRNAs can regulate the degradation and translation of target genes by targeting them, thus participating in various biological processes such as cell proliferation, development, and apoptosis [18]. Meanwhile, miRNAs are fairly effective in assessing the invasion and metastasis of breast cancer [19]. In this study, the relative expressions of miR-182, miR-155, and miR-217 in the poor prognosis group were higher than those in the good prognosis group, and the high expressions of miR-182, miR-155, and miR-217 were risk factors for poor prognosis, implying a close relationship between these miRNAs and poor prognosis of breast cancer. Similarly, miR-182 has previously been identified as a potential prognostic biomarker for breast cancer [20]. Bajaj et al. also reported that miR-182 overexpression was correlated with worse clinical and pathological tumor characteristics in locally advanced triple negative breast cancer [21]. Khalighfard et al. found that detecting miR-155 during treatment may be a feasible diagnostic tool for monitoring breast cancer patients [22]. Circulating miR-155 also has a verified potential in the diagnosis of breast cancer [23]. Moreover, miR-217 plays important roles as oncogene or tumor suppressor in different cancers [24]. Thus, detecting the levels of these three mRNAs is potentially valuable for breast cancer patients.

In this study, the relative expressions of miR-182, miR-155, and miR-217 displayed negative correlations with ER and PR and positive correlations with positive Ki-67 expression, suggesting close relationships of miRNA levels with the expressions of these traditional breast cancer proteins. The growth of breast cancer cells is dependent on the hormone receptors ER and PR, both of which are crucial for assessing the efficacy of endocrine therapy for breast cancer [25]. Additionally, positive Ki-67 expression indicates high malignancy and active proliferation of tumor cells, leading to a higher risk of poor prognosis [26]. Both miR-21 and miR-206 have been closely correlated with ER and PR expressions in breast cancer [27]. Furthermore, miR-19b, miR-22, miR-222, miR-378a, and miR-181a levels have been reported to be affected by the status of ER, PR, and Ki-67 in breast cancer [28]. However, miR-182, miR-155 and miR-217 have seldom been correlated with the expressions of these proteins. Thus, the findings herein provide novel insights into future research regarding breast cancer.

Nevertheless, this study is limited. First, the sample size is small. Second, only three miRNAs are tested. We will include more cases and miRNAs to confirm our results.

CONCLUSIONS

In conclusion, the high expressions of miR-182, miR-155 and miR-217 are correlated with the expressions of traditional breast cancer proteins ER, PR, and Ki-67, and may predict the prognosis of breast cancer patients.

ABBREVIATIONS

AUC – area under the curve

ER – estrogen receptor

HER-2 – human epidermal growth factor receptor-2

Ki-67 – cell proliferation factor 67

miRNA / miR – micro ribonucleic acid

OR – odds ratio

RE – relative expression

RNA – ribonucleic acid

ROC – receiver operating characteristic

PR – progesterone receptor

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AUTHORS' CONTRIBUTION

JC - study design and data analysis; HZ - data analysis; XL - data collection; MH - writing.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

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