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Low expression of miR-597 is correlated with tumor stage and poor outcome in breast cancer

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Abstract. – OBJECTIVE: MicroRNAs (miRNAs) have been reported to play important roles in the progression of breast cancer (BC). In the present study, we aimed to explore the association between miR-597 expression level and prognosis of BC.

PATIENTS AND METHODS: The expression levels of miR-597 were measured using quantitative Real-time polymerase chain reaction (qRT-PCR) analysis. The association between miR-597 expression and clinicopathological factors was analyzed. Differences in BC patient survival were determined using the Kaplan-Meier method and log-rank test. The prognostic value of miR-597 was further verified using the Cox proportional hazards regression model.

RESULTS: Our data indicated that miR-597 was lowly expressed in BC compared with adjacent non-malignant tissues ($p<0.001$). Low miR-597 expression was observed to be closely associated with positive lymph node metastasis ($p=0.001$), higher TNM stage ($p = 0.003$), and poorer pathological differentiation ($p=0.006$). Furthermore, patients with lower levels of miR-597 expression had a shorter overall survival time than patients with higher miR-597 expression levels ($p=0.009$). In addition, multivariate Cox proportional hazards model analysis confirmed that miR-597 was an independent prognostic indicator of overall survival ($p=0.005$; HR 2.273; CI 95%, 1.117-4.291).

CONCLUSIONS: We showed, for the first time, that decreased miR-597 expression suggested unfavorable prognosis for BC patients.

Key Words:
miR-597, Breast cancer, Prognosis.

Introduction

Breast cancer (BC) is the most common type of cancer among women worldwide, accounting for about 27% (2.40 million) of all new cancer cases and 18% (893200) of the total cancer fatalities in 2015[1,2]. BC has been classified into four subtypes, namely luminal A, luminal B, basal-like, and ErbB2[3]. Although progress has been made in the diagnosis and treatment of BC, morbidity and mortality of BC still remain high[4]. In clinical practice, effective diagnostic and prognostic biomarkers are required for BC in order to improve 5-year survival rates. MicroRNAs (miRNAs) are a class of endogenous noncoding RNAs of 20-22 nucleotides[5]. Previous studies have revealed that miRNAs negatively regulate gene expression at the post-transcription level by mRNA degradation[6]. Thus, miRNAs modulate various cellular processes, such as cell proliferation, apoptosis, invasion, and cycle[7]. In addition, recent findings have showed that abnormal expression of miRNAs are associated with the development and progression of various cancers[8,9]. More important, some miRNAs have been identified to be associated with prognosis of patients with various tumors, such as miR-193a-3p[10], miR-497[11] and miR-940[12]. However, the function of most miRNAs in BC remains unclear. MiR-597 is located on the 8p23.1 chromosome; to our best knowledge, so far, its effect in tumors remains largely unknown. A study reported that miR-597 expression was significantly up-regulated in BC tissues[13]. However, the prognosis value of miR-597 in BC breast cancer has not been reported. Thus, we aimed at exploring the clinical significance of miR-597 in patients with BC.

Patients and Methods

Patients and Tissue Samples

Paired fresh BC and noncancerous breast tissues were collected from 190 patients at Daqing Oilfield General Hospital (Heilongjiang, People's Republic of China) between March 2009 and November 2011. Following surgical removal, the tissue samples were immediately frozen in liquid nitrogen and stored at -80°C. All BC samples were

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pathologically diagnosed by two experienced pathologists. None of the patients had received preoperative chemotherapy or radiotherapy. All patients had complete follow-up information until death. The clinicopathological features of patients are summarized in Table I. The research protocol was reviewed and approved by the Ethical Committee and Institutional Review Board of the Daqing Oilfield General Hospital. Informed consent was obtained from all patients.

RNA extraction and qPCR analyses

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was reverse transcribed to cDNA using a SYBR® Green PCR kit (TaKaRa, Otsu, Shiga, Japan). For miR-597, qRT-PCR reactions were performed using the SYBR Green PCR Master Mix of Hairpin-it™ miRNAs RT-PCR Quantitation Kit (GenePharma, Shanghai, China). PCR primers used were as follows: miR-597, forward 5'-ACACTCCAGCTGGGTGTGTCACTCGATGAC-3' and reverse 5'-TGGTGTCGTGGAGTCG-3'; GAPDH, forward 5′-AATGGGCAGCCGTTAGGAAA-3′ and reverse 5′-TGAAGGGGTCATTGATGGCA-3′. GAPDH was used as an internal control. The comparative $2^{-\Delta\Delta Ct}$ method was used for relative quantification and statistical analysis. All Real-time amplifications were measured in triplicate.

Statistical Analysis

All statistical analyses were performed using the SPSS 16.0 statistical software package (SPSS Inc., Chicago, IL, USA). Mir-597 expression levels in clinical samples were compared by Wilcoxon test. The associations between miR-597 expression and clinical characteristics were analyzed by Fisher's exact test. Survival curves were constructed and differences among groups were calculated using the Kaplan-Meier method. Significant variables in univariate analyses were used in multivariate analyses according to the Cox regression analyses. $p<0.05$ was considered significantly different.

Results

Expression of miR-597 in BC Tissues

In order to explore the role of miR-597 in BC development, we performed qRT-PCR to detect

Table I. The association between miR-597 expression and clinicopathological features of breast cancer.

Clinicopathological factors	Cases number	miR-597 expression		*p*-value
		Low (n = 93)	High (n = 97)	
Age (years)				0.634
<50	83	39	44	
≥50	107	54	53	
Tumor size (cm)				0.115
<2.5	85	47	38	
≥2.5	105	46	59	
Histological type				0.396
Ductal	67	30	37	
Lobular	123	63	60	
ER status				0.918
Negative	81	40	41	
Positive	109	53	56	
PR status				0.275
Negative	113	59	54	
Positive	77	34	43	
Lymph node metastasis				0.001
Yes	55	37	18	
No	135	56	79	
TNM stage				0.003
I/II	139	59	80	
III	51	34	17	
Pathological differentiation				0.006
Moderately and highly differentiated	147	64	83	
Poorly differentiated	43	29	14	

Table II. Association between miR-597 expression and clinicopathological parameters in breast cancer patients (n=190).

	Univariate analysis			Multivariate analysis		
Characteristics	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age (years)	1.423	0.783-1.944	0.417	-	-	-
Tumor size (cm)	1.944	0.893-2.241	0.094	-	-	-
Histological type	0.791	0.422-1.563	0.319	-	-	-
ER status	1.783	0.893-2.773	0.273	-	-	-
PR status	1.532	0.672-3.721	0.371	-	-	-
Lymph node metastasis	3.893	1.652-6.523	0.001	3.213	1.328-5.523	0.002
TNM stage	3.293	1.329-4.532	0.006	2.892	1.139-3.889	0.008
Pathological differentiation	4.213	1.832-7.832	0.004	3.781	1.562-6.221	0.006
miR-597 expression	2.783	1.342-5.321	0.003	2.273	1.117-4.291	0.005

the expression levels of miR-597. As shown in Figure 1, we observed that miR-596 expression was significantly down-regulated in BC tissue compared with adjacent normal tissues ($p<0.001$). These results were in line with He et al[13] findings.

Relationship Between miR-597 Expression and Clinical Features

To further explore the clinicopathological correlation of miR-597 expression in BC tissues, we divided patients into groups with high and low expression based on mean levels of relative quantity. The relationship between miR-597 expression and clinicopathologic features was summarized in Table I. Low miR-597 expression was observed to be closely associated with positive lymph node metastasis ($p=0.001$), higher TNM stage ($p=0.003$), and poorer pathological differentiation ($p=0.006$). However, we did not find any significant association between miR-597 levels and other clinicopathological features, such as, age, tumor size, histological type, ER status and PR status (all $p>0.05$).

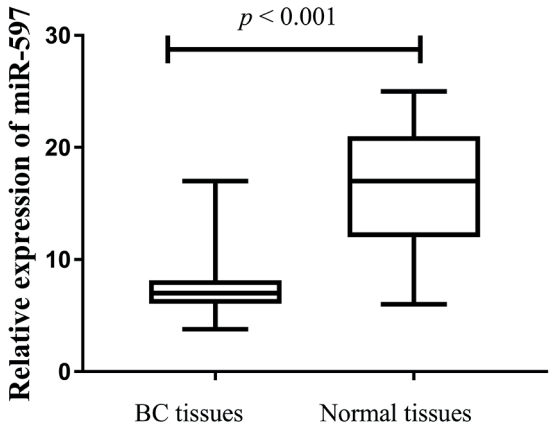

Figure 1. qRT-PCR analysis of the 190 clinical BC cases as well as their adjacent noncancerous tissues.

Decreased Expression of miR-597 in BC Tissues Predict Poor Prognosis

Next, we further analyzed the association between the expression levels of miR-597 and patients' survival by Kaplan-Meier analysis with the log-rank test. As shown in Figure 2, we found that patients with lower levels of miR-597 expression had a shorter overall survival time than patients with higher miR-597 expression levels ($p=0.009$). Further univariate proportional hazard model suggested that lymph node metastasis, TNM stage, pathological differentiation and miR-597 expression level were prognostic predictors (Table II). Furthermore, multivariate analysis was performed to analyze the factors that significantly associated with survival in the univariate analysis. As shown in Table II, the results confirmed that miR-597 was an independent prognostic indicator of overall survival ($p=0.005$; HR 2.273; CI 95%, 1.117-4.291).

Discussion

BC is the leading cause of cancer-related death among women worldwide[14]. Although the advances of treatment have been achieved in the past decades, therapeutic strategies for patients with BC remain not ideal. The dissatisfactory prognosis of BC patients is largely due to frequent recurrence and metastasis[15]. Recently, evidence showed that identification of new candidate molecules which can predict the diagnosis and prognosis of BC patients, could help guide

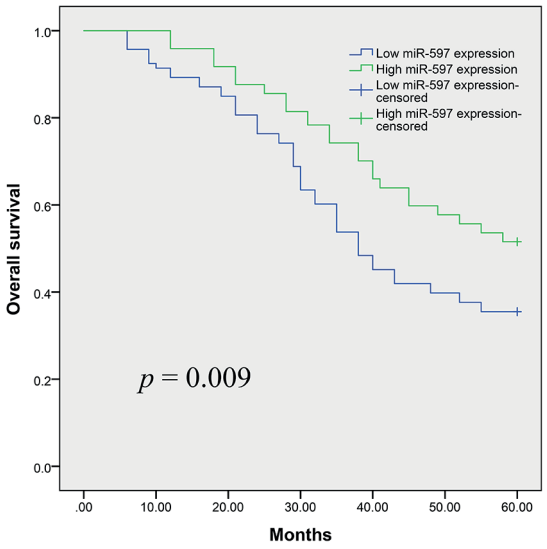

Figure 2. Kaplan-Meier curves for overall survival of 190 BC patients, divided according to miR-597 expression levels.

the effect of new appropriate therapies. Recently, a series of miRNAs have been reported to play an important role in progression of BC. Some potential mechanisms by which miRNAs exert its role in BC development have been revealed[16]. For instance, Chen et al[17] showed that up-regulation of miR-597 inhibited BC cell migration and invasion by targeting MYO10. Tuo et al[18] reported that long non-coding RNA UCA1 served as a tumor promoter by through decreasing tumor suppressive miR-143. Hu et al[19] found that forced expression of miR-601 suppresses cell growth and invasion by targeting PTP4A1 in BC. Further assay indicated that miR-601 was significantly associated with poor prognosis of BC patients. He et al[13] revealed that miR-597 was significantly downregulated in BC tissues and cell lines. Further *in vitro* assay showed that over-expression of miR-597 inhibited BC cell proliferation, migration and invasion through FOSL2. However, the prognostic value of miR-597 in BC patients remains unknown. In the present study, we examined miR-597 expression in BC samples using RT-PCR. Our results showed that miR-597 expression was significantly down-regulated in BC tissues compared with in normal breast tissues. Then, we explored the correlation of miR-597 expression and clinicopathological parameters and the results showed that low miR-597 expression was observed to be closely associated with positive lymph node metastasis, higher TNM stage, and poorer pathological differentiation. These results suggested that miR-597 was associated with advanced progression. Moreover, the results of Kaplan-Meier method revealed that BC patients with low miR-597 expression tend to have shorter overall survival than those with high miR-597 expression. Finally, multivariate Cox analysis proved that miR-597 was an independent prognostic indicator for BC patients.

Conclusions

Firstly we showed that miR-597 may become a good biomarker to predict the prognosis of BC patients. However, the mechanism was not explored in our study and further research is required in the future.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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