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Increased expression of miR-330-3p: a novel independent indicator of poor prognosis in human breast cancer

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Abstract. – OBJECTIVE: Previous study has reported that miR-330-3p was highly expressed in breast cancer (BC) patients. However, the effect of miR-330-3p in BC progression remains largely unclear. The purpose of this study was to investigate the clinical significance of miR-330-3p expression in BC.

PATIENTS AND METHODS: The expression of miR-330-3p was detected by quantitative Real-time PCR in BC tissues and matched normal breast tissues. The association of miR-330-3p expression with clinicopathological factors of BC patients was also analyzed by x2-test. Prognosis value of patients with BC was assessed by Kaplan-Meier method and Cox proportional hazards model, respectively.

RESULTS: Quantitative real-time PCR analysis showed that the expression level of miR-330-3p was significantly higher in BC specimens than that in corresponding noncancerous tissues ($p < 0.01$). The levels of miR-330-3p were positively correlated with the status of TNM stage ($p = 0.011$) and lymph node metastasis ($p = 0.006$). Kaplan-Meier analysis revealed that 5-year overall survival of BC patients with high miR-330-3p expression was shorter compared to those patients with low miR-330-3p expression ($p < 0.0001$). Moreover, univariate and multivariate regression analysis demonstrated that miR-330-3p was an independent prognostic factor in BC.

CONCLUSIONS: Our data suggest that miR-330-3p upregulation maybe concurrently associated with prognosis in patients with BC, suggesting that miR-330-3p may be a potential prognostic biomarker and therapeutic target for patients with BC.

Key Words:
miR-330-3p, Breast cancer, Prognosis.

Introduction

Breast cancer (BC) is one of the most common cancers and the leading cause of cancer-associated mortality among women, worldwide[1]. Over the past three decades, the incidence of BC has been increasing in China[2]. Although progress has been made in the diagnosis and treatment of BC, its prognosis and long-term survival remain poor for patients with metastases[3]. Two strategies could be used to enhance the outcome of BC patients: early detection and appropriate treatment[4]. Therefore, it is necessary to develop novel and more effective diagnostic and prognostic biomarkers for BC patients.

MiRNAs are small non-coding RNAs of about 21-25 nucleotides (nt) that regulate gene expression by binding to targeting mRNAs at their 3'-untranslated region (UTR)[5,6]. It has been confirmed that miRNAs have important regulatory roles in various biological processes, including cell differentiation, apoptosis, proliferation, and metastasis[7,8]. Various types of tumors revealed aberrant expression of miRNAs that can serve as either tumor suppressors or oncogenes. For instance, Chen et al[9] reported that miR-379-5p served as a tumor suppressor in hepatocellular carcinoma by targeting FAK/AKT signaling. Cheng et al[10] reported that down-regulation of miR-144-3p was associated with poor prognosis of human glioblastoma, and up-regulation of miR-144-3p suppressed glioblastoma cell proliferation and metastasis by targeting FZD7. However, Cai et al[11] suggested miR-155 as a tumor promoter by promoting the proliferation of prostate cancer cells. In BC, accumulating miRNAs were identified to participate in BC angiogenesis,

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invasion, and metastasis. Based on the important role of miRNAs in development of BC, more and more researchers focused on their clinical significance in BC.

MiR-330-3p, first discovered by Weber[12], has been reported to be abnormally expressed in several tumors. However, the expression trend was different in different types of tumor tissues. Down-regulation of miR-330-3p expression was found in melanoma[13] and glioblastoma[14], but over-expression of miR-330-3p was showed in prostate cancer[15] and non-small-cell lung cancer[16]. In BC, miR-330-3p was found to be significantly up-regulated[17]. Although miR-330-3p was reported to be associated with survival of BC patients, whether miR-330-3p expression could be an independent poor prognostic factor has not been investigated. In the present study, we aimed to further explore the prognostic value of miR-330-3p in BC.

Patients and Methods

Patient Data and Tissue Samples

A total of 233 patients who were admitted to Shandong Province Qianfoshan Hospital between 2008 and 2012 were included in the present study. The average age of breast cancer patients was 53.2 ± 14.8 years (range: 31-69). None of the patients recruited in this study had undergone preoperative chemotherapy or radiotherapy. The disease grade of all tissue samples was assessed and confirmed by two professional pathologists independently. All tissue samples were snap frozen in liquid nitrogen after removal from the patients and stored at -80°C. The clinicopathological characteristics of the patients and tumors are listed in Table I. Understanding and written consent of each subject were obtained. The study protocol was approved by the Institutional Review Board of Shandong Province Qianfoshan Hospital.

RNA Isolation, Reverse Transcription (RT), and Quantitative PCR (qPCR)

Total RNAs from BC tissues were extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instruction. cDNA was synthesized from 10 ng total RNA using a TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed using SYBR Premix Ex Taq™ II Kit (TaKaRa, Xianyang, Shanxi, China) on an MX3005P QPCR system (Eurofins MWG Operon, Ebersberg, Germany). The conditions for reverse transcription were as follows: 30°C for 10 min, 42°C for 1 h, and 95°C for 10 min. The PCR primer sets were provided by Ge-

Table I. Clinicopathological features and the expression of miR-330-3p in BC patients.

Characteristics	No. of patients	miR-330-3p expression		p
		Low	High	
Age (years)				0.319
≤ 50	114	51	63	
> 50	119	61	58	
Tumor size				0.314
≤ 2.0 cm	142	72	70	
> 2.0 cm	91	40	51	
ER status				0.729
Positive	140	66	74	
Negative	93	46	47	
PR status				0.229
Positive	101	44	57	
Negative	132	68	64	
Histological type				0.752
Ductal	139	68	71	
Lobular	94	44	50	
TNM stage				0.011
I-II	147	80	67	
III	86	32	54	
Lymph node metastasis				0.006
Positive	79	28	51	
Negative	154	84	70	

nepharma (Pudong, Shanghai, China). The relative quantification values for miR-330-3p were calculated by the $2^{-\Delta\Delta Ct}$ method and U6 was used as an internal reference.

Statistical Analysis

The statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). All data were collected and showed as the mean ± SEM. Statistical differences between groups were evaluated using the Student's paired two-tailed *t*-test. Categorical variables were compared by the x^2-test or Fisher' exact test. Survival curves were plotted by the Kaplan-Meier method and compared by the log-rank test. Cox proportional hazards models were used to explore the effect of miR-330-3p levels in univariate and multivariate analyses. A $p < 0.05$ means that the differences were statistically significant.

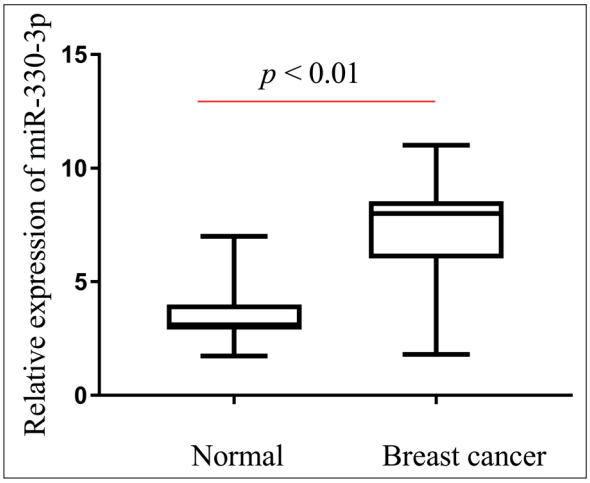

Figure 1. The miR-330-3p relative expression levels were determined by qRT-PCR in BC tissues and the adjacent non-neoplastic tissues.

Results

MiR-330-3p Was Significantly Elevated in BC Tissues

To determine whether miR-330-3p is correlated with the progression of BC, miR-330-3p levels in BC tissues and matched normal tissues were determined by qRT-PCR. As shown in Figure 1, the results showed that MiR-330-3p expression was significantly higher in BC tissues (8.321 ± 0.942) than that in adjacent normal tissues (3.428 ± 0.321) ($p < 0.001$). These data indicated miR-330-3p as a positive regulator in BC progression.

Association Between miR-330-3p and Clinicopathological Features of BC

To explore the clinical significance of miR-330-3p in BC patients, we divided 233 BC patients into two groups according to the median value of miR-339-5p expression level, including high-expression group (n = 121) and low-expression group (n = 112), respectively. As shown in Table I, the results of x^2-test indicated that high expression of miR-330-3p was positively associated with TNM stage ($p = 0.011$) and lymph node metastasis ($p = 0.006$). However, no significant difference was observed between miR-330-3p expression and patients' age, tumor size, ER status, PR status, and histological type ($p > 0.05$).

Correlations of miR-330-3p Expression and Survival of BC

Then, Kaplan-Meier survival analysis was performed to analyze the prognostic value of miR-330-3p in BC patients. As shown in Figure 2, the overall survival of patients with high miR-330-3p expression was significantly worse than that of

Table II. Univariate analysis of overall survival in BC patients.

Prognostic variables	Overall survival	
	HR (95% CI)	*p*-value
Age (≤ 50 *vs.* > 50)	1.542 (0.674-1.994)	0.423
Tumor size (≤ 2.0 *vs.* > 2.0)	1.783 (0.884-2.131)	0.289
ER status (Positive *vs.* Negative)	1.322 (0.398-1.994)	0.166
PR status (Positive *vs.* Negative)	1.563 (0.483-2.213)	0.193
Histological type (Ductal vs. Lobular)	1.883 (0.517-2.318)	0.113
TNM stage (III *vs.* I-II)	3.832 (1.432-5.231)	0.006
Lymph node metastasis (Positive *vs.* Negative)	4.873 (1.591-7.883)	0.002
miR-330-3p expression (High *vs.* Low)	3.891 (1.782-8.938)	0.001

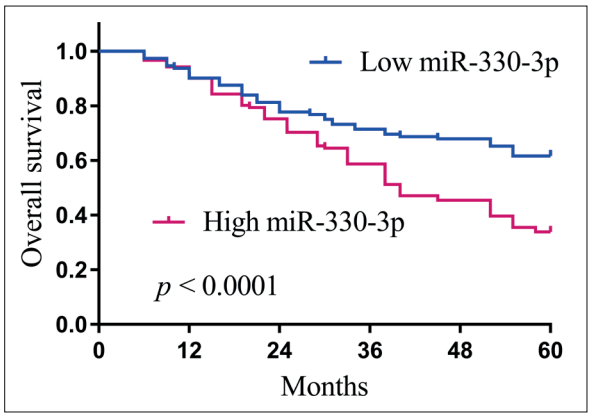

Figure 2. Kaplan-Meier curves estimating the 5-year overall survival rates according to the expression of miR-330-3p in patients with BC.

miR-330-3p-low patients ($p < 0.0001$). Also, univariate and multivariate analyses were performed to determine the relative risk of prognostic parameters. In univariate analyses, we observed that TNM stage, lymph node metastasis, and miR-330-3p expression were associated with prognosis of BC patients (all $p < 0.05$, Table II). Importantly, further results by multivariate analyses confirmed that miR-330-3p expression (HR = 3.213, 95% CI 1.446-7.329, $p = 0.001$) was an independent prognostic factor for the patients with BC.

Discussion

Even treated with combined tumor resection, chemotherapy, and radiotherapy, the outcome of BC patients with metastasis remains poor. Clinical outcomes vary significantly between patients and can be difficult to predict[18,19]. Currently, WHO BC grades were used for determining BC patient prognosis, but it is less useful for individual patients[20]. So evident discrepancies in survival times were often shown in patients at the same disease stage. Growing evidence indicated that miRNAs could be useful biomarkers for progression and prognosis of glioma[21,22]. Therefore, it is necessary for us to find useful miRNAs to predict prognosis.

Recently, the expression pattern and function of miRNA-330-3p have been explored in several tumors. For instance, Liu et al[16] reported that miR-330-3p was overexpressed in NSCLC and its forced expression by miR-330-3p mimics could promote NSCLC cell proliferation by targeting early growth response 2. Yao et al[14] showed that up-regulation of miR-330-3p promoted the malignant behavior of glioblastoma stem cells by down-regulating the expression of SH3GL2 involving in PI3K/AKT signaling pathways. In contrast, miR-330-3p expression was observed to be lowly expressed in colorectal cancer, and its overexpression suppressed the proliferation of colorectal cancer cells by targeting Cdc42[23]. Those results suggested that miR-330-3p may serve as either oncogenes or tumor suppressors in a cancer-specific manner. In 2017, Mesci et al[17] reported that miR-330-3p could promote cell metastasis by targeting CCBE1, and it could be used to predict survival of BC patients. However, evidence of the prognostic value of miR-330-3p expression in BC is still limited.

We examined the expression levels of miR-330-3p in BC specimens and adjacent normal tissues. MiR-330-3p expression was observed to be significantly up-regulated in BC tissues. Then, we found that the levels of miR-330-3p were positively correlated with the status of TNM stage and lymph node metastasis. Furthermore, we analyzed the association of miR-330-3p expression with prognosis of BC patients. Patients with higher miR-330-3p expression had a significantly shorter overall survival than patients with lower miR-330-3p expression. Those findings were in line with a previous study. In addition, we performed Cox proportional hazards model to further determine the prognostic value of miR-330-3p in BC patients. The results of multivariate analysis indicated that miR-330-3p was an independent predictor of survival in patients with BC.

Table III. UMultivariate analysis of overall survival in BC patients.

Prognostic variables	Overall survival	
	HR (95% CI)	*p*-value
TNM stage (III *vs.* I-II)	3.231 (1.231-4.342)	0.009
Lymph node metastasis (Positive *vs.* Negative)	3.417 (1.331-5.652)	0.006
miR-330-3p expression (High *vs.* Low)	3.213 (1.446-7.329)	0.001

However, our present work didn't perform cells experiments to explore the biological function and the potential mechanism by which miR-330-3p influenced the clinical outcome of BC patients. Future studies are needed to explore the specific role of miR-330-3p in BC progression.

Conclusions

We highlight the significance of miR-330-3p in BC progression, suggesting that miR-330-3p serves as a candidate cancer biomarker for the prognosis in BC patients.

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Conflict of Interest
The Authors declare that they have no conflict of interests.

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