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Low expression of miR-409-3p is a prognostic marker for breast cancer

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Abstract. – OBJECTIVE: The present study aimed to clarify the expression pattern and prognostic role of miR-409-3p in breast cancer patients.

MATERIALS AND METHODS: miR-409-3p levels in breast cancer tissues were tested by qRT-PCR. The chi-square test and Fishers exact tests were used to examine the associations between miR-409-3p expression and the clinicopathological characters. Overall survival (OS) was estimated using the Kaplan-Meier method. The univariable and the multivariable Cox regression analyses were used to evaluate independent prognostic factors.

RESULTS: miR-409-3p expression in breast cancer specimens was observed to be decreased compared with matched normal breast tissues ($p < 0.01$). Additionally, low miR-409-3p expression in breast cancer tissues was significantly associated with the advanced TNM stage ($p = 0.004$), lymph node metastasis ($p = 0.001$), and poorer pathological differentiation ($p = 0.026$). The patients in the low miR-409-3p group had a shorter overall survival than those in the high miR-409-3p group ($p < 0.001$). Furthermore, the univariate and the multivariate analyses showed that miR-409-3p expression was an independent predictor of overall survival ($p < 0.05$).

CONCLUSIONS: Our results revealed that miR-409-3p was related to the prognosis of patients with breast cancer and might be a promising predictor of miR-409-3p recurrence.

Key Words:
miR-409-3p, Breast cancer, Prognosis.

Introduction

Breast cancer(BC) is one of the most common malignant tumors and the second leading cause of cancer-related death among women worldwide[1]. During recent decades, remarkable progress has been made and the disease can be treated by surgery, endocrine, cytotoxic, or targeted therapies. However, prevention and therapy of BC remain a major public health concern[2,3]. Early detection is one of the most important strategies for enhancing the survival of BC patients. Thus, it is of great significance to explore novel and efficient biomarkers for the diagnosis and prognosis of BC patients.

MicroRNAs (miRNAs) are highly conserved, 22 nucleotides long non-coding RNAs, that act as posttranscriptional regulators of gene expression by silencing their mRNA targets[4,5]. MiRNAs are known to mediate diverse biological processes, such as cell proliferation, differentiation, and apoptosis[6,7]. Accumulating evidence has also suggested that miRNAs participate in tumor oncogenesis, including during the processes of angiogenesis, invasion and metastasis[8]. More and more studies reported that miRNAs could be promising biomarkers for cancer diagnosis[9], prognosis[10] and prediction of treatment response[11].

A previous research[12] showed that miR-409-3p was significantly downregulated in BC tissues and cell lines and over-expression of miR-409-3p suppressed breast cancer cell growth and invasion by targeting Akt1. In the present work, we aimed to evaluate the clinical significance of miR-409-3p in BC patients.

Patients and Methods

Paients and Tissue Samples

All tissue samples were collected from patients who underwent surgery between 2007 and 2011 in the Department of Radiology, International Hospital of Zhejiang University. The tissues were frozen immediately after resection and

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stored in liquid nitrogen until use. All diagnoses were based on pathological evidence. Clinical information of BC patients were collected and summarized in Table I. The present investigation was approved by the Research Ethics Committee of International Hospital of Zhejiang University. Informed consent was obtained from all the patients. All specimens were handled and made anonymous according to the ethical and legal standards.

RNA Isolation and qRT-PCR

Total RNA isolation from tissues was performed using mirVana miRNA Isolation Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions. The reverse transcription was conducted using a TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The expression level of miR-409-3p was analyzed using the Hairpin-it miRNA qPCR Quantitation Kit (GenePharma, Pudong, Shanghai, China) following the manufacturer's instructions. All the primers were obtained from the TaqMan miRNA Assays. Cycling conditions were 1 cycle of 95°C for 1 min, 40 cycles of 95°C for 5 s, and 60°C for1 min. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method normalized to the individual U6 level.

Statistical Analysis

Data are presented as mean ± SD. Differences between groups were analyzed by Student's *t*-test (two-tailed), and analysis of variance (ANOVA) was used to find significant differences between groups. Multiple comparison between the groups was performed using S-N-K method. Survival curves were estimated by the Kaplan-Meier method. The Cox proportional hazards model was applied for univariate and multivariate analyses. In all cases, $p < 0.05$ was considered statistically significant. The collected data were analyzed by using the SPSS version18.0 (SPSS Inc., Chicago, IL, USA).

Results

miR-409-3p is Significantly Down-regulated in BC Tissues

We explored the expression levels of miR-409-3p in 190 pairs of BC tissues and adjacent non-tumor tissues. Compared with adjacent non-malignant tissues, BC tissues showed decreased expression levels of miR-409-3p ($p < 0.01$, Figure 1A). Notably, low miR-409-3p expression was significantly associated with a more aggressive tumor phenotype (Figure 1B, $p < 0.01$).

Relationship Between Clinicopathological Characteristics and miR-409-3p Expression in BC Patients

We next observed the correlation between miR-409-3p expression and BC clinicopathological features. BC samples were classified into the low expression group (n = 95) and the high expression group (n = 95) according to the median expression level of all BC samples (median expression value 3.65). As summarized in Table I, miR-409-3p was associated significantly with advanced TNM stage ($p = 0.004$), lymph node metastasis ($p = 0.001$), and poorer pathological

Table I. The relationship between miR-409-3p expression and clinicopathologic parameters

Variable	Category	No.	miR-409-3p expression		*p*
			Low	High	
Age	< 50 years	77	41	36	0.460
	≥ 50 years	113	54	59	
Tumor size	< 3 cm	88	38	50	0.081
	≥ 3 cm	102	57	45	
Lymph node metastasis	Yes	103	63	40	0.001
	No	87	32	55	
TNM stage	I/II	121	51	70	0.004
	III	69	44	25	
Pathological differentiation	Moderately and highly differentiated	115	50	65	0.026
	Poorly differentiated	75	45	30	
HER2 status	Over expressed	93	43	50	0.310
	Negative	97	52	45	

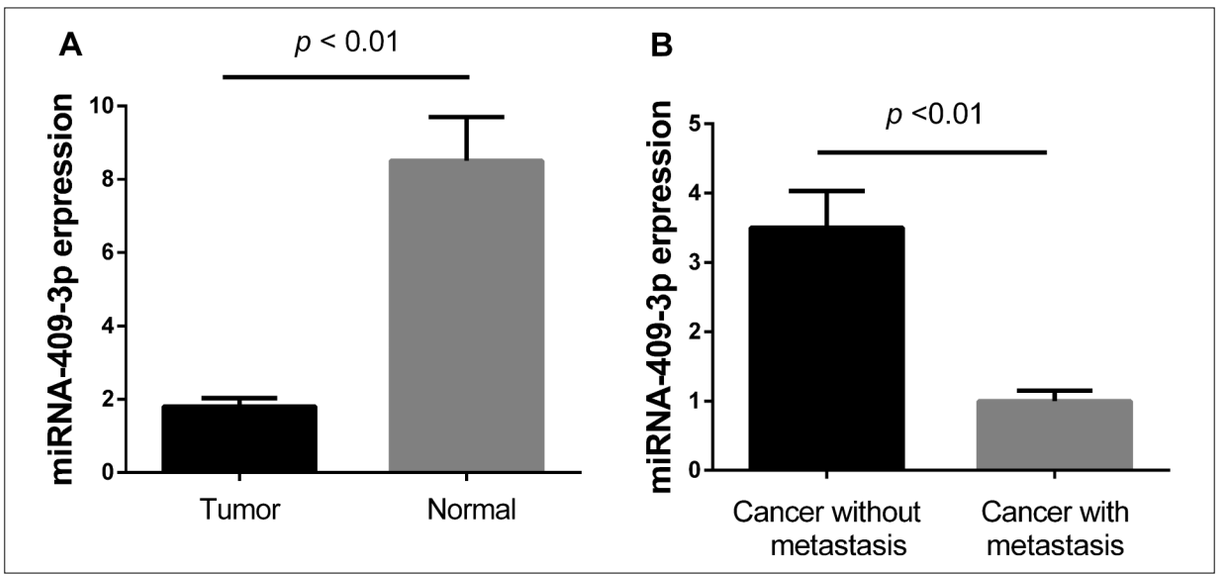

Figure 1. miR-409-3p expression in breast cancer tissue. ***A,*** Quantitative PCR analysis of relative miR-409-3p expression in tissues from 95 pairs of BC patients and adjacent normal tissues. ***B,*** Compared with BC patients without metastasis, those patients with metastasis had la ower level of miR-409-3p.

differentiation ($p = 0.026$). No significant associations were found between miR-409-3p level and patients' age, tumor size, or HER2 status.

Prognostic Values of miR-409-3p Expression in BC

Kaplan-Meier analyses were performed to investigate the association between the miR-409-3p expression and the prognosis of BC patients. The results showed that the patients with low miR-409-3p expression exhibited evidently poorer overall survival rates than those with high miR-409-3p expression ($p = 0.001$; shown in Figure 2). The univariate and the multivariate analyses were also performed to identify factors related to patient prognosis, as shown in Table II. The univariate Cox proportional hazard regression analysis revealed that TNM stage, lymph node metastasis, poorer pathological differentiation and miR-409-3p were predictive factors for prognosis. Furthermore, a multivariate analysis showed that miR-409-3p expression is an independent prognostic factor of overall survival in patients with BC ($p = 0.012$).

Discussion

Breast cancer is the second most common cancer with nearly 1.67 million new cases diagnosed in 2012[13]. Despite great advances in clinical treatments, including surgery, operative navigation system, chemotherapy, and radiotherapy. However, satisfactory therapeutic outcomes have not been achieved[14]. To develop novel therapeutic strategies to improve clinical outcome of patients with BC, it is very important to explore novel diagnostic and prognostic biomarkers of BC.

Growing evidence shows that miRNAs play important roles in a variety of biological process-

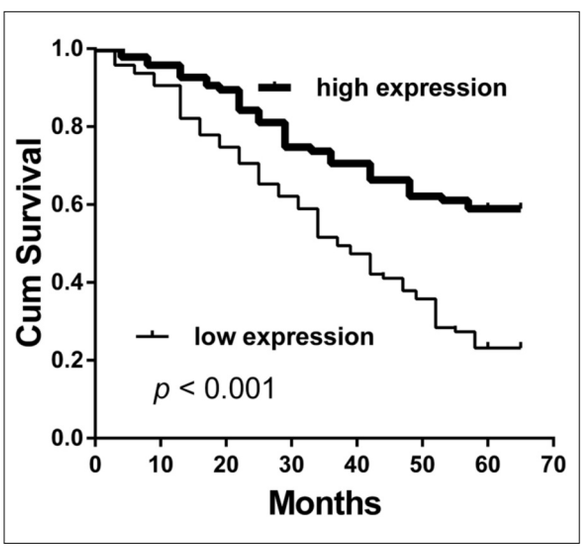

Figure 2. Kaplan-Meier postoperative survival curve for patterns of BC patients and miR-409-3p expression.

Table II. Univariate and multivariate analyses of prognostic parameters in patients with breast cancer by Cox regression analysis.

Variables	Univariate analyses			Multivariate analyses		
	HR	95% CI	*p*	HR	95% CI	*p*
Age	1.321	0.731-4.831	0.351	1.032	0.553-3.993	0.472
Tumor size	1.632	0.677-5.634	0.163	1.325	0.831-4.662	0.193
Lymph node metastasis	3.632	1.563-8.935	0.013	3.321	1.238-9.335	0.021
TNM stage	4.231	1.423-9.334	0.016	3.428	1.127-9.032	0.018
Pathological differentiation	3.668	1.832-8.839	0.003	3.023	1.462-7.872	0.005
HER2 status	1.744	0.421-3.346	0.127	2.129	0.539-4.331	0.103
miR-409-3p level	2.447	1.129-7.983	0.017	3.427	1.633-8.845	0.012

es and their dysregulation may be crucial to cancer initiation, progression, and treatment outcome[15,16]. For instance, Fu et al[17] showed that miR-206 remarkably repressed proliferation, migration, and invasion of breast cancer cells by direct targeting Cx43. Li et al[18] found that miR-17-5p modulates the cell cycle progression and apoptosis in human ovarian cancer by up-regulating YES1 expression. Yin et al[19] reported that miR-503 expression is associated with worse survival rate, suggesting that it may serve as a potential prognostic marker to identify patients at higher risk of recurrence. MiR-409-3p, one of the tumor suppressor miRNAs, has been reported to be down-regulated in different cancers including colorectal cancer, prostate cancer, and gastric cancer[20-22]. Recently, Zhang et al[12] found that miR-409-3p was significantly downregulated in BC tissues and cell lines and overexpression of miR-409-3p inhibited BC cell proliferation, migration and invasion *in vitro* and suppressed tumor growth *in vivo*. Those findings revealed that miR-409-3p might serve as a tumor suppressor in BC. However, until now, the prognostic significance of miR-409-3p in BC patients has not been reported.

In the present study, the results of PRC showed that miR-409-3p expression was significantly down-regulated in BC tissues compared with matched adjacent non-cancerous breast tissues. In malignant cases, lower expression miR-409-3p levels were significantly associated with advanced TNM stage, lymph node metastasis, and poorer pathological differentiation. Moreover, Kaplan-Meier analysis showed that BC patients with low miR-409-3p expression tend to have shorter overall survival. Finally, Cox regression analysis revealed that miR-409-3p expression were independent prognostic factors in patients with BC.

Conclusions

A decreased expression of miR-409-3p was noted in BC tissues. Our data indicated that miR-409-3P could be a useful prognostic biomarker for BC and a potential target for gene therapy. This work was a retrospective study with inappropriate clinical data, so further prospective clinical studies should be conducted in future to further confirm the reliability of the findings.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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