European Review for Medical and Pharmacological Sciences 2016; 20: 5143-5147

Down-regulation of long non-coding RNA MEG3 serves as an unfavorable risk factor for survival of patients with breast cancer

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Abstract. – OBJECTIVE: The downregulation of Long non-coding RNA MEG3 (MEG3) has been observed in breast cancer (BC). However, there is no previous study of the relationship between MEG3 and patient prognosis in BC. Accordingly, this study investigated the prognostic values of MEG3 in BC patients.

MATERIALS AND METHODS: we performed RT-qPCR to detect the expression of MEG3 in 207 paired BC tissues and adjacent noncancerous tissues. The association of MEG3 expression with clinicopathological factors or prognosis was statistically analyzed.

RESULTS: Our findings revealed that the MEG3 expression was significantly decreased in clinical BC tissues compared to adjacent normal tissues ($p < 0.01$). MEG3 level was significantly associated with differentiation grade ($p = 0.004$), TNM stage ($p = 0.011$) and lymph nodes metastasis ($p = 0.000$). Using Kaplan-Meier analysis, we found that patients with low MEG3 expression had significantly poor overall survival (OS) rate ($p < 0.001$) and progression-free survival (PFS) rate ($p < 0.001$). Moreover, multivariate Cox analysis revealed MEG3 expression was an independent poor prognostic factor for both 5-year OS ($p = 0.003$) and 5-year PFS ($p = 0.002$) in BC patients.

CONCLUSIONS: Our results indicated that MEG3 expression was an independent prognostic factor for patients with BC, which may serve as a novel biomarker in BC patients.

Key Words:
lncRNA, MEG3, Breast cancer, prognosis, quantitative real-time PCR

Introduction

Breast cancer (BC), the leading cause of cancer-related deaths in women worldwide, is a common and highly lethal malignancy[1,2]. Although progress has been made in the diagnosis and treatment of BC, the survival for most patients, particularly those with metastases, have not dramatically improved[3]. As finding molecular targets for BC treatment might help improve survival rate, more and more studies have attempted to identify biological factors which could be used for early diagnosis and prognostic evaluation of BC[4,5]. However, few molecules have been assayed as therapeutic or prognostic biomarkers.

long non-coding RNAs (lncRNAs) are more than 200 nucleotides in length and unable to be translated into proteins[6]. However, previous investigations[7,8] have demonstrated that lncRNAs play vital regulatory roles in cell proliferation, apoptosis, differentiation and migration. Accumulating evidence suggests that correlations exist between lncRNAs expression and clinical recurrence, metastasis development and survival[9,10]. lncRNA MEG3 (MEG3) is one of the few lncRNAs that are consistently down-regulated in malignancies of various tissue origins, including non-small cell lung cancer[11], cervical carcinoma[12], pituitary tumor[13] and breast cancer[14]. However, the prognostic value between MEG3 and BC are unknown. In this study, we focused on the lncRNA MEG3. Our data showed that MEG3 might be a new potential biomarker of BC.

Patients and Methods

Patients and Tissue Samples

This study collected 207 breast cancer patients who received surgical resection and 16 in Chinese PLA General Hospital from May 2009 to March 2011. Collected samples were flash frozen in liquid nitrogen following surgery. None of the patients recruited in this study had undergone preoperative chemotherapy or radiotherapy. The pathologic features and tumor

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Table I. Correlation between the expression level of lncRNA MEG3 with clinicopathological features.

Characteristic	Number	MEG3 expression		*p*-value
		Low	High	
Age (years)				0.282
<50	91	41	50	
≥50	116	61	55	
Tumor size (cm)				0.382
<3	136	70	66	
≥3	71	32	39	
ER				0.554
Positive	132	63	69	
Negative	75	39	36	
PR				0.858
Positive	119	58	61	
Negative	88	44	44	
HER-2				0.778
Positive	128	60	68	
Negative	79	42	44	
Differentiation grade				0.004
G1/G2	130	54	76	
G3	77	49	29	
TNM stage				0.011
I/II	141	61	80	
III		41	25	
Lymph nodes metastasis				0.000
No	155	64	91	
Yes	52	38	14	

stage were reviewed by two pathologists according to the World Health Organization Classification. Clinical information was obtained from patient charts and pathological reports. All the experiments were approved by Chinese PLA General Hospital. Written informed consent for the analysis of tissue specimens was obtained from all patients. Clinicopathologic characteristics of all patients enrolled in this study were summarized in Table I.

Real-time Quantitative Polymerase Chain Reaction (qRT-PCR)

Total RNA from tumor samples was extracted using the Trizol LS reagent (Invitrogen, Waltham, MA, USA) and quantified with Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). MEG3 expression levels were measured by qRT-PCR using an ABI7500 system and SYBR Green PCR Master Mix (Takara, Dalian, Liaoning, China) using the following cycling parameters, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 45 s. All quantifications were performed with U6 as the internal standard. The PCR primer sequences were as follows: MEG3 sense, 5'- CTGCCCATCTACACCTCACG -3' and reverse,

5'- CTCTCCGCCGTCTGCGCTAGGGGCT-3'; U6 reverse,

5'-CTCGCTTCGGCAGCACA-3' and

reverse, 5'-AACGCTTCAGGAATTTGCGT-3'.

Each sample was analyzed in triplicate, and the relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

All statistical analyses were performed by using the SPSS 16.0 statistical software package (SPSS, Chicago, IL, USA). Differences between groups were analyzed using the Student's *t*-test or chi-square test. The Kaplan-Meier method was used to estimate survival; the log-rank test was used to test differences between the survival curves. The Cox proportional hazard regression model was performed to identify independent prognostic factors. The *p*-values lower than 0.05 were considered statistically significant.

Results

Down Regulation of MEG3 in BC Tissues

We first explored the expression of MEG3 in BC tissues. As shown in Figure 1, the qRT-

Table II. Univariate cox proportional hazards regression analysis of MEG3 expression and clinicopathologic parameters in patients with breast cancer.

Variables	OS		PFS	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (years)	1.642 (0.773-2.841)	0.417	1.231 (0.519-2.238)	0.312
Tumor size	2.236 (0.891-4.473)	0.198	1.936 (0.772-4.014	0.127
ER	1.556 (0.521-2.898)	0.492	1.325 (0.449-2,491)	0.377
PR	2.153 (1.033-4.673)	0.183	1.787 (0.814-4.056)	0.213
HER-2	3.238 (1.183-5.361)	0.429	2.891 (0.911-4.891)	0.382
Differentiation grade	3.918 (1.882-8.916)	0.008	3.279 (1.273-8.136)	0.005
TNM stage	2.327 (1.219-6.635)	0.012	2.773 (1.482-8.239	0.007
Lymph nodes metastasis	2.835 (1.023-9.338)	0.002	3.319 (1.672-11.329)	0.001
MEG3 expression	3.361 (1.762-8.037)	0.006	4.238 (2.336-9.922)	0.003

PCR analyses showed that the expression level of MEG3 in BC tissues was significantly lower than that in the matched adjacent non-tumor tissues ($p < 0.01$).

Association between MEG3 Expression and Clinicopathological Characteristics

We further analyzed the association between MEG3 expression levels and clinicopathological characteristics of BC. The MEG3 expression levels were classified as high or low in relation to the median value. As shown in Table I, The results showed that MEG3 level was significantly associated with differentiation grade ($p = 0.004$), TNM stage ($p = 0.011$) and lymph nodes metastasis ($p = 0.000$). but no significant correlations between miR-92a expression levels and other clinicopathological variables, including age, tumor size, ER, PR and HER-2 (All $p > 0.05$).

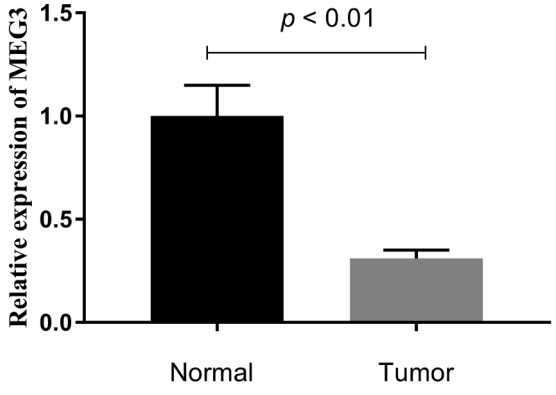

Figure 1. LncRNA MEG3 expression was significantly lower in cancer tissues than in adjacent normal tissue ($p < 0.01$).

Prognostic Values of MEG3 Expression in BC

To assess the prognostic value of MEG3 expression for BC, we performed Kaplan-Meier analysis and log-rank test. From the Kaplan-Meier survival curves, we found that the 5-year OS of low MEG3 expression group was significantly shorter than that of high MEG3 expression group (Figure 2, $p < 0.001$). Moreover, the 5-year PFS of low MEG3 expression group was also significantly shorter than that of high MEG3 expression group (Figure 3, $p < 0.001$). Moreover, MEG3 expression was associated with both OS and PFS in univariate Cox proportional hazards regression analysis (Table II). Finally, in a multivariate Cox model, we found that MEG3 expression was an indepen-

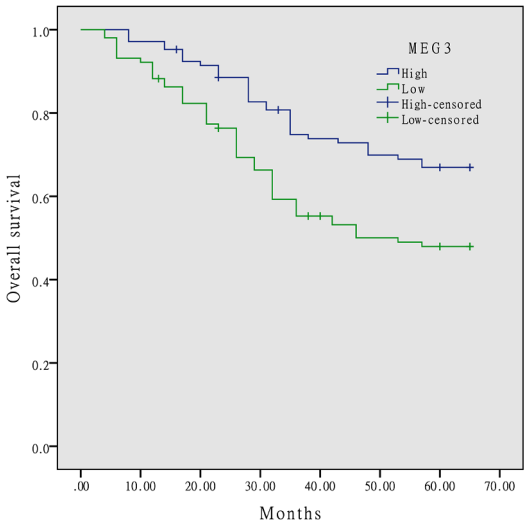

Figure 2. Patients with low MEG3 expression exhibited significantly shorter OS times than those with high MEG3 expression ($p < 0.001$).

Table III. Multivariate analysis for prognostic factors of breast cancer.

Variables	OS		PFS	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (years)	2.231 (0.823-3.044)	0.362	1.582 (0.771-2.831)	0.443
Tumor size	2.741 (1.032-3.883)	0.237	2.074 (0.873-3.219)	0.317
ER	1.735 (0.854-3.219)	0.466	1.455 (0.692-2.993)	0.359
PR	2.673(1.193-4.982)	0.213	2.239 (0.872-4.663)	0.291
HER-2	3.081 (1.345-5.013	0.419	2.232 (1.013-4.477)	0.318
Differentiation grade	3.773 (1.239-9.337)	0.005	3.228 (1.873-8.235)	0.002
TNM stage	2.813 (1.177-7.738)	0.011	3.147 (1.653-6.989)	0.006
Lymph nodes metastasis	3.552 (1.763-9.018)	0.004	3.119 (2.019-8.881)	0.001
MEG3 expression	2.237 (0.892-6.631)	0.003	2.673 (1.139-7.742)	0.002

dent poor prognostic factor for both 5-year OS (p = 0.003, Table III) and 5-year progression-free survival (p = 0.002, Table III) in BC patients.

Discussion

BC is a serious disease threatening the health of women. So it is urgent for us to seek new potential biomarkers for its prognosis and therapy to improve clinical strategies of BC. Recent studies have attributed a crucial role played by lncRNAs in the progression of numerous diseases, particularly cancer. Furthermore, some hot lncRNAs have been thoroughly investigated, such as MALAT-1[15], HOTAIR[16] and BANCR[17]. For MEG3 in BC, it is still unclear about how MEG3 participates in the biological processes of BC. Thus, the aim of this study was to explore the associations of MEG3 expression with clinicopathologic features and prognosis of BC patients.

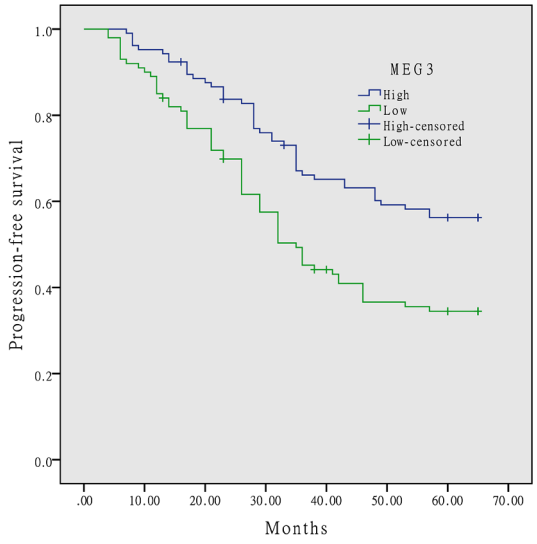

Figure 3. Patients with low MEG3 expression exhibited significantly shorter PFS times than those with high MEG3 expression (p < 0.001).

In the present work we confirmed that the expression level of MEG3 was decreased in clinical BC tissues as compared to those adjacent normal tissues. Furthermore, the statistical analysis revealed that MEG3 expression is correlated with differentiation grade, TNM stage and lymph nodes metastasis in BC. Kaplan-Meier survival and log-rank test analysis demonstrated that decreased expression of MEG3 was correlated with shorter OS and PFS. Moreover, the multivariate analysis confirmed that MEG3 in tissues was an independent factor for affecting the survival time of BC patients. To our knowledge, this is the first study to analyze the expression and clinical significance of MEG3 in BC.

MEG3 is located at chromosome 14q32, where the allelic loss is commonly implicated in BC[18]. Previous research reported dysregulated MEG3 expression in many human malignancies, and MEG3 functions as a tumor suppressor. Luo et al[19] found that MEG3 played a critical role in regulating cell proliferation, apoptosis and migration by targeting of Bcl-2 in prostate cancer cells. Sun et al[20] reported that MEG3 could be a poor prognostic biomarker in BC and knockdown of MEG3 expression by siRNA could promote BC cells proliferation apoptosis *in vitro*. Braconi et al[21] reported that ectopic expression of MEG3 induced apoptosis in hepatocellular cancer PRC/PRF/5 cells. Consistent with these finding, Sun et al[14] showed that downregulated long non-coding RNA MEG3 suppressed proliferation, migration and invasion by depending on p53's transcriptional activity. Additional studies are required to

determine if MEG3 expression could affect the survival of BC patients. Our observation provides the opportunity to consider potential clinical applications of MEG3 as a prognostic marker.

Conclusions

All the evidence above suggested that MEG3 is a novel molecular correlated with BC progression, it may be an independent prognostic indicator for BC patients.

Conflict of interest

The authors declare no conflicts of interest.

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