

Mir-595 is a significant indicator of poor patient prognosis in epithelial ovarian cancer

Q.-H. ZHOU¹, Y.-M. ZHAO², L.-L. JIA², Y. ZHANG³

¹Department of Obstetrics and Gynecology, Taian City Central Hospital, Taian, Shandong, China

²Department of Obstetrics, Jinan Maternity and Child Care Hospital, Jinan, Shandong, China

³Department of Gynecology, Tengzhou Central People's Hospital, Zaozhuang Shandong, China

Abstract. – **OBJECTIVE:** MiR-595 has been demonstrated to be involved in tumorigenesis of several cancers. However, the clinical significance of miR-595 in epithelial ovarian cancer (EOC) remains unclear. The aim of this study was to explore the correlation of miR-595 expression with clinicopathologic features and prognosis in EOC patients.

PATIENTS AND METHODS: Quantitative Real-time PCR (qRT-PCR) was performed to evaluate the expression level of miR-595 in all participants. Correlations between miR-595 levels and clinicopathological factors were investigated. Association of miR-595 expression with overall survival was estimated by the Kaplan-Meier method. Univariate and multivariate Cox regression analyses were performed.

RESULTS: We observed that miR-595 was downregulated in EOC tissues in comparison with noncancerous ovarian tissues ($p < 0.05$). In addition, low miR-595 expression level was significantly associated with advanced FIGO stage ($p = 0.003$), distant metastasis ($p = 0.002$), and grade ($p = 0.014$). Furthermore, significantly shorter overall survival was observed in patients with lower expression of the miR-595. At last, multivariate analysis revealed that decreased expression of miR-595 was an independent predictor of overall survival of EOC patients.

CONCLUSIONS: Our data indicated that miR-595 expression might be a novel potential prognostic biomarker for EOC.

Key Words:

miR-595, Ovarian cancer, Prognosis.

Introduction

Ovarian cancer (OC) is the most common cause of gynecological cancer-associated death¹. Epithelial ovarian cancer (EOC) accounts for 90% of all ovarian malignancies, with more than 225,500 new cases diagnosed and nearly 142,000 deaths annually². Although current treatment strategies

significantly improved the quality of life in patients with EOC, the 5-year survival rate for advanced patients remains very low³. In addition, EOC progression is often asymptomatic and is detected at an advanced stage. Therefore, it is necessary for us to explore some precise prognostic markers to improve the survival of EOC patients.

MicroRNAs (miRNAs) are endogenous non-coding 19-23 nucleotide RNAs involved in post-transcriptional regulation of gene expression⁴. More and more evidence indicate that miRNAs were involved in various biological progressions, such as cell proliferation, apoptosis, development, and differentiation^{5,6}. More importantly, nowadays, growing data supports the idea that abnormal miRNAs expression plays critical roles in tumor initiation, clinical progression, and invasion⁷⁻⁹. Indeed, functional assay has suggested that miRNAs serve as either oncogene or tumor suppressor in carcinogenesis¹⁰. Because of the important role of miRNAs in tumor development, it has become a hot point to use miRNAs as tumor biomarker for predicting prognosis and diagnosis of tumors. For EOC, some miRNAs have been identified as independent prognostic factor for EOC patients, such as miR-613¹¹, miR-193b¹² and miR-145¹³.

Recently, a study by Tian et al¹⁴ reported that the expression levels of miR-595 were down-regulated in both OC tissues and cell lines. However, the clinical significance of miR-595 in EOC remains unknown. The aim of present study was to explore the prognostic of miR-595 in patients of EOC.

Patients and Methods

Patients

A total of 166 EOC tissues and adjacent non-tumor tissues from primary EOC patients

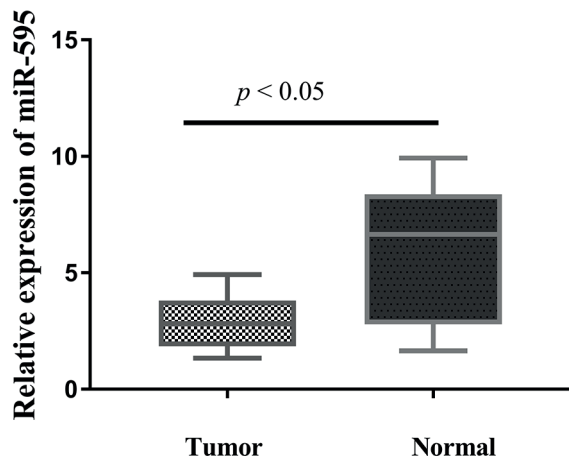


Figure 1. Expression level of miR-595 by qRT-PCR in 166 ovarian cancer tissues and paired non-tumor tissues. GAPDH was used as a loading control.

were surgically obtained between 2009 and 2012 in Taian City Central Hospital, China. All diagnoses were confirmed by histology. No patient had received chemotherapy, radiotherapy, and immunotherapy before surgery. All specimens were frozen in liquid nitrogen immediately after collection and stored at -80°C until use. The clinical and pathological characteristics for each patient were also collected. Permission to use the tissue sections for research purposes was obtained and approved by the Ethics Committee of the Taian City Central Hospital. Written informed consent was obtained from all patients before participation in the study.

RNA Isolation and Quantitative Real-time PCR

Total RNA was isolated using Invitrogen TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturers' instructions. Complementary DNA (cDNA) was randomly primed from 2 μg of total RNA using the Omniscript reverse transcription kit (Qiagen, Bayern, Germany). For analysis of the miR-595, Real time-PCR was performed with miScript SYBR Green PCR Kit and miScript Primer PCR Assay. GAPDH was used as endogenous controls. The primers used for qRT-PCR in this study were designed and purchased from Invitrogen, (Carlsbad, CA, USA). The comparative $2^{-\Delta\Delta\text{Ct}}$ method was used for relative quantification and statistical analysis. All the qRT-PCR reactions were run in triplicate.

Statistical Analysis

Statistical analysis was performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean $\pm$ standard deviation. χ^2 -test was applied to analyze the correlation between miR-595 expression and the clinicopathological features. Survival curves were calculated using Kaplan-Meier estimates, and differences between groups were tested by log-rank test. Cox regression model was used for univariate and multivariate analysis. A significant difference was considered statistically when p -value was <0.05 .

Results

miR-595 Expression was Down-Regulated in EOC

To analyze the expression of miR-595 in EOC, qRT-PCR was performed to determine levels of miR-595 in 166 paired invasive EOC specimens and pair-matched adjacent noncancerous tissues. As shown in Figure 1, we observed that the expression of miR-595 was significantly decreased in EOC tissues as compared with the adjacent tissues ($p < 0.05$). The results suggested that decreased miR-595 expression may be related to EOC pathogenesis.

Association Between Clinicopathological Characteristics and miR-595 Expression in EOC Patients

In order to further elucidate the significance of miR-595 in EOC, we analyzed the association between the expression of miR-595 and clinicopathological characteristics of EOC. The 166 patients with EOC were divided into two groups based on the median relative miR-595 expression value. As shown in Table I, it was found that low miR-595 expression level was significantly associated with advanced FIGO stage ($p = 0.003$), distant metastasis ($p = 0.002$), and grade ($p = 0.014$). However, there was no association between miR-595 levels and age, histology, tumor size or serum CA125 of EOC patients (all, $p > 0.05$).

Association Between miR-595 Expression and Patient Survival

Above results indicated that low expression of miR-595 was associated with advanced tumor development, thus, we wondered whether miR-595 could influence prognosis of EOC patients. Kaplan-Meier method confirmed our hypothe-

Table I. Clinicopathological variables and miR-595 expression in ovarian cancer.

Parameters	Group	Total	miR-595 expression		<i>p</i> -value
			High	Low	
Age (years)	< 55	77	40	37	0.859
	≥ 55	89	45	44	
Histology	Serous	75	34	41	0.169
	Non-serous	91	51	40	
Tumor size (cm)	≤ 10	108	57	51	0.580
	> 10	58	28	30	
Serum CA125	< 319	96	50	46	0.791
	≥ 319	70	35	35	
FIGO stage	I+II	101	61	40	0.003
	III+IV	65	24	41	
Grade	G1	94	56	38	0.014
	G2+G3	72	29	43	
Distant metastasis	Yes	51	14	37	0.002
	No	115	71	44	

sis. As shown in Figure 2, we found that patients with low miR-595 expression levels displayed lower overall survival rates than patients with high miR-595 expression levels ($p = 0.0005$). To further determine whether miR-595 was an independent prognostic factor for EOC, we performed Cox survival analyses. Univariate analysis showed that FIGO stage ($p = 0.002$), grade ($p = 0.004$), distant metastasis ($p = 0.001$) and miR-595 expression ($p < 0.001$) were associated with the prognosis of EOC patients (Table II). Moreover, decreased miR-595 expression ($p < 0.001$) was an independent poor prognostic factor for EOC patients through multivariate analysis (Table II).

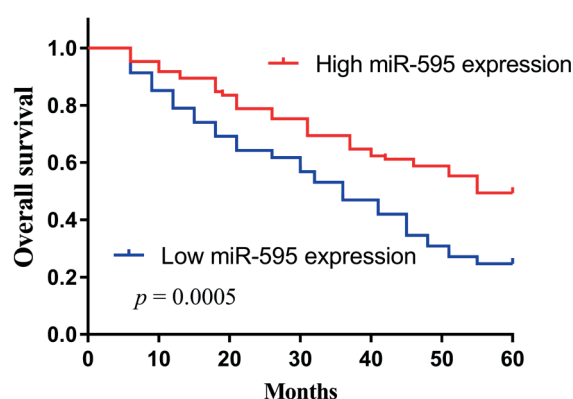


Figure 2. Kaplan-Meier curves for overall survival after surgery according to expression of miR-595 in 166 ovarian cancer patients. Patients with low miR-595 expression had poorer overall survival compared with high miR-595 group ($p = 0.0005$).

Discussion

EOC is one of the most common causes of cancer-related death among women in China¹⁵. Because of lack of early, safe and noninvasive detection methods, patients of EOC often experienced poor prognosis and high malignancy¹⁶. Thus, improving the EOC mortality rate necessitates the discovery of novel molecular markers that can function as prognostic markers for EOC.

The abnormal expression and biological function of miR-595 have been reported in several studies. For instance, Guled et al¹⁷ reported that miR-595 expression was up-regulated in malignant mesothelioma, suggesting that miR-595 may be a positive regulator in progression of this malignancy. Chen et al¹⁸ found that aberrant expression of miR-595 was involved in the regulation of neuroblastoma cells SH-SY5Y autophagy by targeting ULK1 expression. Another study by Hao et al¹⁹ indicated that high expression of miR-595 leads to down-regulation of SOX7, which in turn suppressed nasopharyngeal cancer progression. Importantly, Tian et al¹⁴ found that miR-595 expression was downregulated in the OC tissues and cell lines. Especially, for the lymph node metastases tissues, the significant low expression was observed in those tissues. Furthermore, functional experiment showed that miR-595 increased the sensitivity of OC to cisplatin by targeting ABCB. Those results highlighted the role of miR-595 in progression of OC. However, whether miR-595 could serve as a promising biomarker for prognosis of EOC remains unknown.

Table II. Univariate and multivariate analysis of survival in ovarian cancer patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.251	0.773-1.782	0.561	-	-	-
Histology	1.563	0.672-1.983	0.423	-	-	-
Tumor size	1.773	0.581-2.033	0.083	-	-	-
Serum CA125	1.423	0.611-1.893	0.132	-	-	-
FIGO stage	3.842	1.778-5.763	0.002	3.231	1.556-4.784	0.006
Grade	3.458	1.993-6.321	0.004	3.021	1.445-5.231	0.009
Distant metastasis	3.671	1.642-5.893	0.001	2.893	1.332-4.893	0.001
miR-595 expression	4.893	2.231-8.832	< 0.001	4.231	1.893-7.229	< 0.001

In line with Tian et al¹⁴ findings, our results of RT-PCR also showed that miR-595 expression was significantly down-regulated in EOC tissues compared with matched normal tissues. In addition, clinical information revealed that high miR-595 expression level was significantly associated with advanced FIGO stage ($p = 0.003$), distant metastasis ($p = 0.002$), and grade ($p = 0.014$). Furthermore, the Kaplan-Meier analysis suggested that patients with high levels of miR-595 expression tended to live a longer life than those with low levels. Of note, multivariate analyses indicated miR-595 low expression could be an independent prognostic factor in patients with EOC.

Conclusions

We, for the first time, provided evidence that decreased expression of miR-595 was correlated with poor prognosis in patients with EOC, suggesting that miR-595 was a potential new molecular marker for predicting the prognosis of EOC.

Conflict of interest

The authors declare no conflicts of interest.

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