European Review for Medical and Pharmacological Sciences 2015; 19: 4762-4765

Low miR-498 expression levels are associated with poor prognosis in ovarian cancer

J. CONG[1], R. LIU[2], X. WANG[1], J. WANG[1], H. WANG[2], J. HOU[1]

[1]Department of Gynaecology, Qingdao University Affiliated Yantai Yuhuangding Hospital, Yantai, Shandong, China.
[2]Department of Laboratory, Qingdao University Affiliated Yantai Yuhuangding Hospital, Yantai, Shandong, China.

Jianglin Cong and Riming Liu are equal contributors.

Abstract. – OBJECTIVE: Decreased expression levels of microRNA (miR)-498 were reported in several human cancers. However, the prognostic value of the miR-498 expression in ovarian cancer has not been assessed. In this study, we addressed this knowledge gap by evaluating an association of miR-498 expression levels with ovarian cancer prognosis.

PATIENTS AND METHODS: qPCR was used to detect expression levels of miR-498 in cancer specimens and matched adjacent normal tissue specimens. The log-rank test was used to analyze survival rate, whereas the Cox regression model was used fir multivariate analysis of potential prognostic factors.

RESULTS: Expression levels of miR-498 were significantly lower in ovarian cancer tissue specimens compared with matched normal adjacent tissue ($p < 0.001$). Decreased miR-498 expression levels correlated well with FIGO stage, tumour grade and lymph node metastases (respective $p = 0.001$, 0.015, and 0.017). Furthermore, patients with lower miR-498 expression had shorter overall and progression-free survival (both $p < 0.01$ vs. those with high miR-498 expression).

CONCLUSIONS: Decreased expression levels of miR-498 are associated with worse overall survival and poor prognosis in patients with ovarian cancer, highlighting potential usefulness of this miR for prognosis in patients with ovarian cancer.

Key words:
Ovarian cancer, microRNA-498, Recurrence-free survival, Overall survival.

Introduction

Ovarian cancer is one of the most common gynecologic malignancy and is the leading cause of deaths caused by gynaecological malignancies[1]. Ovarian carcinoma is usually diagnosed at later stages[2]. In recent years, modern treatment methods have been developed, including surgery and chemoradiotherapy. The quality of patients' lives with ovarian cancer has been improved. However, clinical prognosis in these patients still remains very poor. It has been reported that a five-year survival rate in patients with ovarian cancer range from 93% when diagnosed at the stage I to 25% at the stage IV[3]. This underscores the importance of timely diagnosis, also with the use of novel molecular biomarkers.

Micro RNA (miRNA) is endogenous small noncoding RNA whose function is to regulate gene expression at post-transcriptional level[4]. miRNA is often aberrantly expressed in human cancers and may function as regulators defining cancer progression[5]. miRNA plays an important role in cell apoptosis, differentiation and proliferation. Thus, abnormally low expression levels of miR-498 have been documented in colorectal and ovarian cancers. However, the association of these expression levels with the prognosis has not been previously studied. In this study, we addressed this knowledge gap by evaluating an association of miR-498 expression levels with ovarian cancer prognosis.

Patients and Methods

Patients and specimens

We obtained 175 specimens of ovarian cancer tissue and matched adjacent normal tissue from patients who underwent a curative resection at our Hospital between February 2008 and May 2014. The specimens were immediately frozen in liquid nitrogen after surgical removal and stored at -80° C until further use. None of the patients received preoperative chemotherapy or radiotherapy. All patients were followed up.

The study protocol was approved by the Research Ethics Committee of the Qingdao Univer-

Corresponding Author: Jianqing Hou, Md, e-mail: yn702@163.com

sity Affiliated Yantai Yuhuangding Hospital. Informed consents were obtained from all patients.

RNA extraction and qPCR

The TRIzol (Invitrogen, Carlsbad, CA, USA) protocol was used to isolate total RNA from frozen specimen. The purity and concentration of obtained RNA was verified with NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The one step PrimeScript miRNA cDNA Synthesis Kit (Takara, Otsu, Shiga, Japan) was used to generate cDNA from 1 µl of total RNA. qPCR was done using the ABI 7500 Real Time PCR system (Applied Biosystems, Foster City, CA, USA). Primers for quantification of miR-498 levels were purchased from Applied Biosystems, and RNU6B RNA (Hs_RNU6B_2 miScript Primer Assay, Qiagen, Hilden, Germany) was used as an endogenous control.

PCR reactions were performed in triplicate. Relative amount of miR-498 was determined using the $2^{-\Delta CT}$ ($\Delta CT = C_{TmiR498} - C_{TU6\ RNA}$) method.

Statistical Analysis

Statistical analyses were carried out using the SPSS software (IBM, version 18.0). The chi-square and Fisher's exact tests, or unpaired *t*-test were used to evaluate the association between expression levels of miR-498 and other parameters. Survival was estimated using the Kaplan-Meier method. Differences between survival curves were analyzed using the log-rank test. The Cox regression model was used for the multivariate survival analysis. The $p < 0.05$ was considered as indicating a statistically significant difference.

Results

miR-498 expression

We evaluated expression levels of miR-498 in 175 ovarian cancer and matched normal adjacent tissue specimens. As shown in Figure 1, expression of miR-498 was significantly lower in ovarian cancer specimens ($p < 0.01$ vs. matched normal adjacent tissue).

miR-498 expression vs. clinicopathological characteristics

We next divided the patients with ovarian cancer into two groups according to median expression levels of miR-498, and analyzed the association between miR-498 expression and clinicopathological characteristics. Low miR-22 expression levels correlated well with FIGO stage ($p = 0.001$; Table I), tumour grade ($p = 0.015$; Table I), and lymph node metastases ($p = 0.017$; Table I). In contrast, we did not detect a significant correlation between miR-498 expression levels and age, histology, or residual tumour size (Table I).

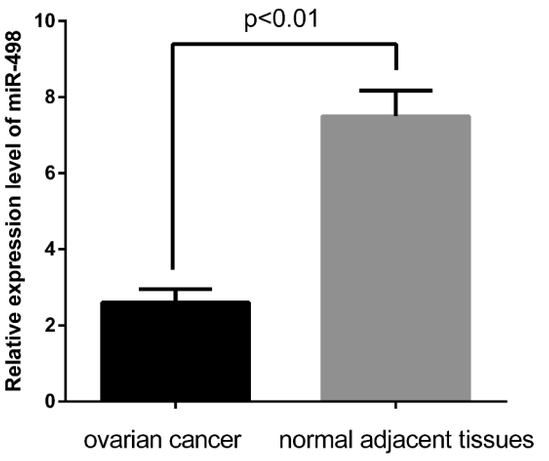

Figure 1. miR-498 expression levels in ovarian cancer and matched adjacent normal tissues.

Association between miR-498 expression levels, clinico-pathologic parameters, and survival

We then prepared Kaplan-Meier curves for overall and progression-free survival. It was obvious that patients with low expression levels of miR-498 had significantly worse overall ($p = 0.0056$; Figure 2) and progression-free ($p = 0.003$; Figure 3) survival.

Afterwards, univariate and multivariate analyses were performed to determine the significance of association between miR-498 expression levels and clinico-pathological parameters. Thus, multivariate analysis indicated that miR-498 expression, International Federation of Gynecology and Obstetrics (FIGO) stage, tumour grade, and lymph node metastasization were independent prognostic factors predicting the overall survival in patients with ovarian cancer (respective $p = 0.004$, 0.016, 0.028, and 0.023; Table II). The same was true for progression-free survival (respective $p = 0.003$, 0.007, 0.014, and 0.007; Table II).

Discussion

Aberrant miRNA expression has been detected in human cancers in recent years[6]. Some miRNAs

Table I. Correlation between miR-498 expression levels and clinico-pathological parameters.

Parameters	Number of patients	miR-498 expression level		p
		Low expression	High expression	
Age < 55 years	93	43	50	0.510
Age ≥ 55 years	82	42	40	
FIGO stage I-II	92	27	65	0.001
FIGO stage III-IV	83	58	25	
Histology: serous	81	39	42	0.917
Histology: nonserous	94	46	48	
Residual tumour size <1.0 cm	118	57	61	0.919
Residual tumour size ≥1.0 cm	57	28	29	
Tumour grade: well	59	10	49	0.015
Tumor grade: moderate	54	30	24	
Tumor grade: poor	62	45	17	
Negative lymph node metastasis	107	45	62	0.017
Positive lymph node metastasis	68	40	28	
Serum CA125 <319	95	43	52	0.340
Serum CA125 ≥319	80	42	38	

function as oncogenes, whereas others serve as tumour suppressors[7]. Aberrant miRNA expression could be used a biomarker for prognosis in cancer[8-11].

There have been three studies performed to date indicating that miR-498 serves as a tumour suppressor. Lower levels of miR-498 expression have been detected in colorectal cancer tissue and colorectal cancer cell lines; furthermore, the role of miR-498 was confirmed in cell culture experiment[12]. miR-498 was found to be down-regulated in non-small cell lung cancer, and decreasing of miR-498 expression promoted cancer proliferation[13]. In addition, miR-498 expression levels in human colorectal cancer were lower than in human ovary surface epithelial cells.

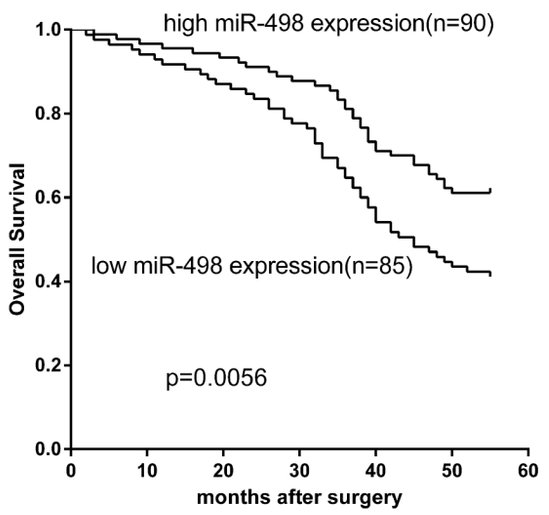

Figure 2. miR-498 expression and its association with overall survival in patients with ovarian cancer.

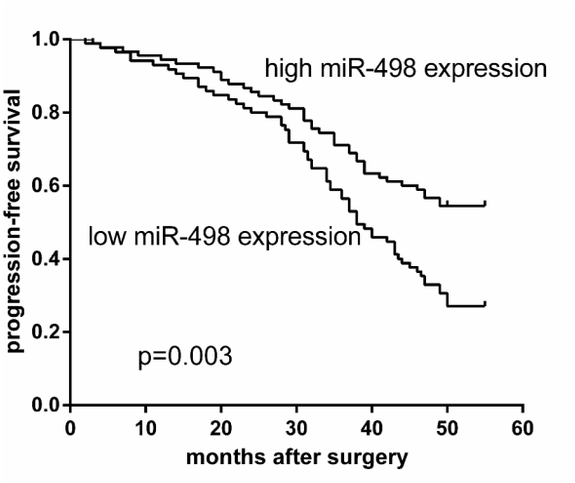

Figure 3. miR-498 expression and its association with progression-free survival in patients with ovarian cancer.

Table II. Multivariate Cox regression analysis of parameters determining disease-free and overall survival.

Parameters	Overall survival		Progression-free survival	
	HR (95%CI)	p	HR (95%CI)	p
Age	0.892 (0.564-1.854)	0.266	0.977 (0.547-1.928)	0.319
FIGO stage	4.226 (1.314-8.473)	0.016	4.874 (2.399-9.547)	0.007
Histology	1.545 (0.641-4.293)	0.852	1.441 (0.391-2.234)	0.716
Residual tumor size	2.438 (0.615-5.809)	0.124	2.323 (0.750-3.258)	0.118
Tumour grade	3.232 (2.241-9.127)	0.028	4.128 (2.237-8.761)	0.014
Lymph node metastases	1.921 (1.114-8.452)	0.023	4.289 (2.541-11.387)	0.007
Serum CA125	3.891 (0.525-8.231)	0.081	2.761 (0.804-5.092	0.092
MiR-498 expression level	2.751 (2.231-8.981)	0.004	2.519 (2.021-12.391)	0.003

miR-498 may also act as a tumour suppressor in ovarian cancer. Thus, FOXO3 was identified as a direct target of miR-498[14]. However to date, clinical significance and prognostic value of miR-498 in ovarian cancer has not been investigated.

In our study, we demonstrate that expression of miR-498 is lower in ovarian cancer tissue than in matched normal adjacent tissue. Furthermore, decreased expression levels of miR-498 were associated with FIGO stage, tumour grade, and lymph node metastasization. This indicates that miR-498 may be involved in carcinogenesis and metastasization of ovarian cancer. As expression levels of miR-498 were associated with survival of patients with ovarian cancer and were shown by us as an independent prognostic factor for ovarian cancer, we believe that expression level of miR-498 has a potential to serve as a biomarker for prognosis in these patients.

Conclusions

Decreased expression levels of miR-498 are associated with worse overall survival and poor prognosis in patients with ovarian cancer, highlighting potential usefulness of this miR for prognosis in patients with ovarian cancer.

Conflict of Interest statement

The Authors declare no conflicts of interest.

References

1. Knutson KL, Karyampudi L, Lamichhane P, Preston C. Targeted immune therapy of ovarian cancer. Cancer Metastasis Rev 2015; 34: 53-74.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.
3. Holschneider CH, Berek JS. Ovarian cancer: epidemiology, biology, and prognostic factors. Semin Surg Oncol 2000; 19: 3-10.
4. Baffa R, Fassan M, Volinia S, O'Hara B, Liu CG, Palazzo JP, Gardiman M, Rugge M, Gomella LG, Croce CM, Rosenberg A. MicroRNA expression profiling of human metastatic cancers identifies cancer gene targets. J Pathol 2009; 219: 214-221.
5. Adams BD, Kasinski AL, Slack FJ. Aberrant regulation and function of microRNAs in cancer. Curr Biol 2014; 24: R762-776.
6. Gregory RI, Shiekhattar R. MicroRNA biogenesis and cancer. Cancer Res 2005; 65: 3509-3512.
7. Kent OA, Mendell JT. A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. Oncogene 2006; 25: 6188-6196.
8. Su YH, Zhou Z, Yang KP, Wang XG, Zhu Y, Fa XE. MIR-142-5p and miR-9 may be involved in squamous lung cancer by regulating cell cycle related genes. Eur Rev Med Pharmacol Sci 2013; 17: 3213-3220.
9. Xu HS, Zong HL, Shang M, Ming X, Zhao JP, Ma C, Cao L. MiR-324-5p inhibits proliferation of glioma by target regulation of GLI1. Eur Rev Med Pharmacol Sci 2014; 18: 828-832.
10. Yin WZ, Li F, Zhang L, Ren XP, Zhang N, Wen JF. Down-regulation of microRNA-205 promotes gastric cancer cell proliferation. Eur Rev Med Pharmacol Sci 2014; 18: 1027-1032.
11. Su ZX, Zhao J, Rong ZH, Geng WM, Wu YG, Qin CK. Upregulation of microRNA-25 associates with prognosis in hepatocellular carcinoma. Diagn Pathol 2014; 9: 47.
12. Gopalan V, Smith RA, Lam AK. Downregulation of microRNA-498 in colorectal cancers and its cellular effects. Exp Cell Res 2015; 330: 423-428.
13. Wang M, Zhang Q, Wang J, Zhai Y. MicroRNA-498 is downregulated in non-small cell lung cancer and correlates with tumor progression. J Cancer Res Ther 2015; 11 Suppl: c107-111.
14. Liu R, Liu F, Li L, Sun M, Chen K. MiR-498 regulated FOXO3 expression and inhibited the proliferation of human ovarian cancer cells. Biomed Pharmacother 2015; 72: 52-57.