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Diminished miR-613 expression as a novel prognostic biomarker for human ovarian cancer

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Abstract. – OBJECTIVE: miR-613 may suppress ovarian cancer development and progression. We wished to assess the potential prognostic value of miR-613 expression analysis in ovarian cancer.

PATIENTS AND METHODS: A total of 236 pairs of human ovarian cancer and matched normal tissue specimens were collected. Resected tissue specimens were obtained during the operation and were snap-frozen in liquid nitrogen for subsequent RNA extraction. Total RNA was extracted from tissue specimens, and miR-613 expression was quantified using TaqMan qPCR. The miR-613 expression was normalized to expression of U6 as an internal standard.

RESULTS: miR-613 expression in ovarian cancer tissue specimens was significantly ($p < 0.001$) lower than that in matched normal adjacent tissue specimens. Furthermore, within ovarian cancer tissue specimens, 114 specimens exhibited lower levels of miR-613 ("low expression" group), whereas 122 specimens showed higher levels of this miR ("high expression" group). Low expression of miR-613 showed significant associations with the FIGO stage ($p < 0.001$), tumour grade ($p < 0.001$), and lymph node metastases ($p < 0.001$). Furthermore, low miR-613 expression was associated with lower progression-free and overall survival (respectively, $p < 0.01$ and < 0.0001). Finally, the multivariate analysis using the Cox proportional hazards model showed that miR-613 expression levels, FIGO stage, and lymph node metastases were independently associated with progression-free and overall survival in patients with ovarian cancer.

CONCLUSIONS: miR-613 expression levels are low in ovarian cancer tissue and correlate with progression-free and overall survival. Thus, miR-613 may be useful as a prognostic marker in ovarian cancer.

Key Words
Ovarian cancer, miR-613, qPCR, Progression-free survival, Overall survival.

Introduction

As one of the most common gynecologic malignancies, ovarian cancer accounts for the majority of female cancer-related deaths[1]. Even with the advanced diagnostic and treatment strategies, including targeted therapy, chemo-radiotherapy, and combination chemotherapy, the overall 5-year survival rate remains poor[2]. This is likely because the vast majority of patients are diagnosed at an advanced metastatic stage of the disease. Therefore, it is crucial to implement novel diagnostic and prognostic tools for ovarian cancer.

MicroRNA (miR) are short non-coding RNA which plays an important role in tumour progression and development[3]. There is accumulating evidence that many miR serve as tumour suppressors[4]. Thus, miR may be useful for diagnosis and prognosis of various cancers, including ovarian cancer. Illustrating this, anomalous expression of miR-503[5], miR-26b[6], and miR-494[7] were found to be associated with clinical prognosis. In line with this, miR-613 has been reported to act as a tumour suppressor to inhibit proliferation and invasive potential of ovarian cancer cells[8]. Also, miR-613 has been shown to counter papillary thyroid cancer[9]. There have been no studies about the potential prognostic value of miR-613 in ovarian cancer. To fill this knowledge gap, we examined here the association between miR-613 and the outcomes of ovarian cancer.

Patients and Methods

Patients and Tissue Specimens

The study protocol was approved by the Research Ethics Committee of The First People's Hospital of Shangqiu. Written informed consents

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were obtained from all patients. Patient data and tissue specimens were treated according to ethical and legal standards.

A total of 236 pairs of human ovarian cancer and matched normal tissue specimens were collected in the Department of Neurosurgery (The First People's Hospital of Shangqiu) between 2006 and 2013. Resected tissue specimens were obtained during the operation and were snap-frozen in liquid nitrogen for subsequent RNA extraction.

The median patient age was 44 years (range: 7-71 years). None of the patients received chemotherapy and radiotherapy before the surgery.

qPCR Analysis of miR-613 Expression

Total RNA was extracted from tissue specimens using the Trizol reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. miR-613 primers and probe were purchased from Applied Biosystems, Carlsband, CA, USA). The TaqMan qPCR was conducted using the ABI 7300 HT Sequence Detection system (Applied Biosystems, Carlsbad, CA, USA). The expression of miR-613 was normalized to expression of U6 as an internal standard. The $2^{-\Delta CT}$ ($\Delta C_T = C_T[miR613] - C_T[U6]$) method was utilized to quantify relative expression of miR-613.

Statistical Analysis

The SPSS software 16.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The Student's *t*-test was utilized to analyze the differences in quantitative outcomes, whereas the Fisher's test was used to test the differences in qualitative outcomes. The survival was calculated by the Kaplan-Meier method and the log-rank test. The progression-free survival time was calculated from the date of the initial surgery until the first evidence of local, regional, or distant tumour progression. The overall survival time was calculated from the date of the primary surgery to patient's death. The Cox regression model was used to perform multivariate analysis of miR-613 as a potential prognostic factor. Differences were considered significant at the $p < 0.05$.

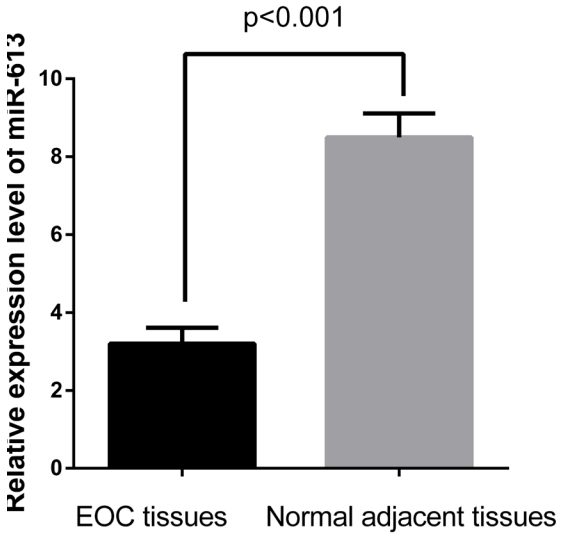

Figure 1. Expression of miR-613 in ovarian cancer (EOS) or normal adjacent tissue.

Results

miR-613 is Down-regulated in Ovarian Cancer Tissue Specimens

miR-613 expression in ovarian cancer tissue specimens was significantly ($p < 0.001$) lower than that in matched normal adjacent tissue specimens (Figure 1). Furthermore, within ovarian cancer tissue specimens, 114 specimens exhibited lower levels of miR-613 ("low expression" group), whereas 122 specimens showed higher levels of this miR ("high expression" group, Table I). This specimen separation was done using the median expression level of miR-613.

We next examined potential associations between miR-613 expression levels and clinic-pathological characteristics of patients with ovarian cancer. Low expression of miR-613 showed significant associations with the International Federation of Gynecology and Obstetrics (FIGO) stage ($p < 0.001$), tumour grade ($p < 0.001$), and lymph node metastases ($p < 0.001$), but no associations with other tested clinical characteristics, including histology, patient age, and the levels of CA125 (Table I).

Low Expression of miR-613 is Associated with Poorer Prognosis

We, then, evaluated the prognostic value of the miR-613 expression in ovarian cancer. First, we performed the Kaplan-Meier and log-rank analyses to test for differences in progression-free and overall survival in patients whose tissue specimens expressed miR-613 at low or high levels ("low expression" and "high expression" groups, defined above). We observed that low expression levels of miR-613 were associated with lower pro-

Table I. miR-613 expression vs. clinico-pathological characteristics.

Characteristics	Low miR-613 expression (n = 114)		High miR-613 expression (n = 122)		*p*
	Absolute number	%	Absolute number	%	
Age (years)					
≤ 55	60	52.6	58	47.5	0.434
> 55	54	47.4	64	52.5	
FIGO stage					
I-II	35	30.7	82	67.2	0.0001
III-IV	79	69.3	40	32.8	
Histology					
Serous	72	63.3	78	63.9	0.901
Non-serous	42	36.8	15	36.1	
Residual tumour size (cm)					
< 1.0	65	57	83	68	0.08
≥ 1.0	49	43	39	32	
Grade					
Well	18	15.7	56	45.9	0.006
Moderate	43	37.7	54	44.2	
Poor	53	46.4	12	9.8	
Lymph node metastases					
Negative	64	56.1	88	72.1	0.01
Positive	50	43.8	34	27.9	
Serum CA125					
< 319	54	47.4	67	54.9	0.246
≥ 319	60	52.6	55	45.1	

gression-free and overall survival (respectively, $p < 0.01$ and < 0.0001; Figures 2 and 3).

The multivariate analysis using the Cox proportional hazards model showed that miR-613 expression levels, FIGO stage, and lymph node metastases were independently associated with progression-free and overall survival in patients with ovarian cancer (Table II).

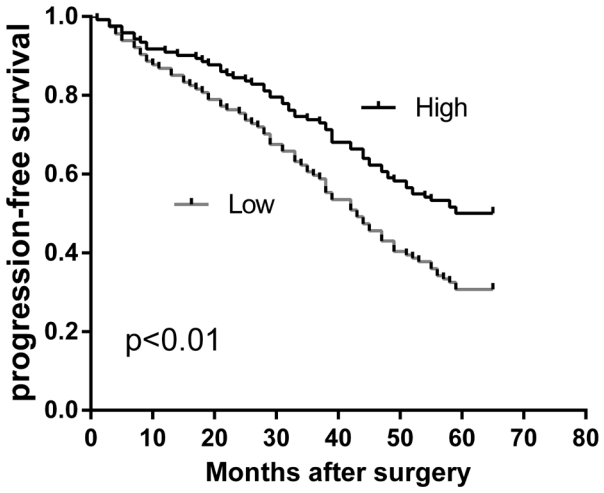

Figure 2. Post-surgery overall survival in patients with low vs high expression of miR-613.

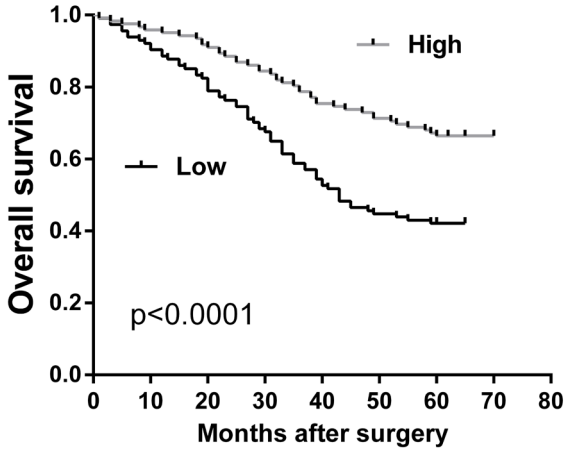

Figure 3. Post-surgery progression-free survival in patients with low vs high expression of miR-613.

Table I. Multivariate Cox proportional hazard model analysis of progression-free and overall survival in 236 patients with ovarian cancer.

Determinants	Progression-free survival		Overall survival	
	HR (95% CI)	p	HR (95% CI)	p
Age (>55 vs. ≤55 year)	1.128 (0.826-2.242)	0.328	1.026 (0.419-1.745)	0.286
FIGO stage	4.512 (2.621-9.913)	0.008	3.261 (1.392-8.715	0.024
Histology	1.521 (0.314-2.619)	0.729	1.661 (0.342-4.142)	0.631
Residual tumour size	2.329 (0.841-3.284)	0.127	2.416 (0.931-5.932)	0.114
Tumour grade	4.217 (2.146-8.812)	0.017	3.226 (2.119-9.257)	0.013
FIGO stage (II vs. I)	2.362 (1.842-3.262)	0.026	1.954 (0.782-2.762)	0.327
Lymph node metastases	4.129 (2.315-11.714)	0.006	1.626 (1.172-10.924)	0.0017
Serum Ca125	2.519 (0.945-7.215)	0.091	3.128 (0.946-6.331)	0.087
MiR-613 expression level	2.417(2.771-10.871)	0.003	2.215(1.671-8.882)	0.006

Discussion

miR post-transcriptionally regulates gene expression, including that of genes involved in the development and progression of cancer[10]. Recent studies highlight the role of miR in cancer cell proliferation and migration[11,12]. Here we assessed the prognostic value of expression of miR-613 in human ovarian cancer. We demonstrate that this miR may be used as a biomarker for ovarian cancer.

As this is a newly discovered miR, the role of miR-613 in tumour progression remains poorly understood. Expression of miR-613 was found down-regulated in esophageal squamous cell carcinoma which may be important in both diagnosis and prognosis of this cancer[13]. miR-613 was also down-regulated in papillary thyroid cancer, whereas miR-613 overexpression inhibited proliferation of papillary thyroid carcinoma by targeting the FN1 gene[9]. It was also shown that miR-613 functions as an anti-oncogene by controlling the Wnt pathway in gastric cancer[14]. Collectively, these studies demonstrate that miR-613 appears to be involved in different types of cancer. A very recent report[8] demonstrated that miR-613 is down-regulated in human ovarian cancer cell lines and tissue specimens. In addition, it was demonstrated that overexpression of miR-613 inhibits ovarian cancer cell proliferation and invasion by regulating KRAS. There have been no previous reports of the prognostic value of miR-613 in patients with ovarian cancer, and our study aimed to fill this knowledge gap. Also, our results confirm that miR-613 expression is significantly decreased in ovarian cancer tissue, but we demonstrated associations between miR-613 expression and clinicopathological characteristics in patients with ovarian cancer. Thus, low miR-613 expression was associated with the FIGO stage, tumour grade, and lymph node metastases. Furthermore, the Kaplan-Meier analysis revealed that patients with low miR-613 expression had significantly shorter progression-free and overall survival. The univariate and multivariate analyses confirmed that expression of miR-613 is a potential prognostic biomarker for human ovarian cancer.

Conclusions

The miR-613 expression is low in ovarian cancer tissue. Expression levels of this miR correlate with progression-free and overall survival. Thus, miR-613 may be useful as a prognostic marker in ovarian cancer.

Conflicts of interest

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) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
3. Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annu Rev Pathol 2014; 9: 287-314.
4. Chen CZ. MicroRNAs as oncogenes and tumor suppressors. N Engl J Med 2005; 353: 1768-1771.
5. Yin ZL, Wang YL, Ge SF, Guo TT, Wang L, Zheng XM, Liu J. Reduced expression of miR-503 is associated with poor prognosis in cervical cancer. Eur Rev Med Pharmacol Sci 2015; 19: 4081-4085.

6. Xia M, Duan ML, Tong JH, Xu JG. MiR-26b suppresses tumor cell proliferation, migration and invasion by directly targeting COX-2 in lung cancer. Eur Rev Med Pharmacol Sci 2015; 19: 4728-4737.
7. Sun HB, Chen X, Ji H, Wu T, Lu HW, Zhang Y, Li H, Li YM. miR494 is an independent prognostic factor and promotes cell migration and invasion in colorectal cancer by directly targeting PTEN. Int J Oncol 2014; 45: 2486-2494.
8. Fu X, Cui Y, Yang S, Xu Y, Zhang Z. MicroRNA-613 inhibited ovarian cancer cell proliferation and invasion by regulating KRAS. Tumour Biol 2015 Dec 2. [Epub ahead of print].
9. Yang Z, Yuan Z, Fan Y, Deng X, Zheng Q. Integrated analyses of microRNA and mRNA expression profiles in aggressive papillary thyroid carcinoma. Mol Med Rep 2013; 8: 1353-1358.
10. Gregory RI, Shiekhattar R. MicroRNA biogenesis and cancer. Cancer Res 2005; 65: 3509-3512.
11. Li ZB, Li ZZ, Li L, Chu HT, Jia M. MiR-21 and miR-183 can simultaneously target SOCS6 and modulate growth and invasion of hepatocellular carcinoma (HCC) cells. Eur Rev Med Pharmacol Sci 2015; 19: 3208-3217.
12. Huang Y, Yang YB, Zhang XH, Yu XL, Wang ZB, Cheng XC. MicroRNA-21 gene and cancer. Med Oncol 2013; 30: 376.
13. Guan S, Wang C, Chen X, Liu B, Tan B, Liu F, Wang D, Han L, Wang L, Huang X, Wang J, Yao B, Shi J, Chen P, Nesa EU, Song Q, Cheng Y. MiR-613: a novel diagnostic and prognostic biomarker for patients with esophageal squamous cell carcinoma. Tumour Biol 2015 Oct 24. [Epub ahead of print].